

Allergen Challenge Differentially Affects the Number of Circulating Monocyte Subsets

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Abstract

Peripheral blood monocyte (PBM) subsets play different roles in inflammatory response and tissue remodelling. The aim of this study was to investigate how allergen challenge affects the number of circulating PBMs in Dermatophagoides pteronyssinus (Dp) allergic patients (Dp-APs). Among 34 Dp-APs challenged, in 22 patients significant bronchoconstriction was demonstrated [responders (Rs)], while in 12, only upper respiratory symptoms were seen [non-responders (NRs)]. Twelve healthy, non-atopic subjects were used as controls (HCs). Expression of CD14, CD16 and CCR4 was evaluated by flow cytometry on the whole-blood samples before (T_0), 6 h (T_6) and 24 h (T_{24}) after the challenge. Plasma concentrations of CCL2, CX3CL1 and CCL17 were evaluated using ELISA. At T_0 , the mean percentage of CD14⁺⁺ CD16⁺ PBMs in Rs (35.4%; 95%CI 26.9–43.9%) was significantly greater than in HCs (14.6%; 95%CI 7.3–21.8%; $P = 0.006$) and in NRs (17.5%; 95%CI 9.6–25.4%; $P = 0.001$). The baseline number of CD14⁺⁺ CD16⁺ PBMs correlated with airway hyper responsiveness (AHR) ($r = -0.507$; 95%CI -0.834 to -0.432 , $P < 0.001$). At T_{24} , the number of CD14⁺⁺ CD16⁺ PBMs significantly decreased in Rs but not in NRs and the numbers inversely correlated with plasma CCL17 concentration. Changes in the number of circulating CD14⁺⁺ CD16⁺ cells after Dp challenge correlated with AHR ($r = 0.706$, 95%CI 0.43–0.861; $P < 0.001$). In all subjects, the greatest expression of CCR4 was found on CD14⁺⁺ CD16⁺ PBMs. Expansion of CD14⁺⁺ CD16⁺ monocytes in the peripheral blood with subsequent mobilization of those cells after allergen challenge may facilitate the development of AHR in Dp-APs.

Introduction

In the respiratory system, mononuclear phagocytes play an important role in the regulation of the inflammatory response to antigen challenge [1, 2]. Alveolar macrophages (AMs) of asthmatic patients are characterized by a decreased inhibitory effect on T cell proliferation [2]. Moreover, in animal asthma models, AMs have been shown to play a role in the development of asthma and airway hyper responsiveness (AHR) [3]. Peripheral blood monocytes (PBMs) migrate to the peripheral tissues spontaneously and in response to inflammatory mediators [4, 5]. Different chemotactic factors and different receptors are responsible for the spontaneous migration and stimulated extravasation of monocytes [4, 5].

Application of different monoclonal antibodies demonstrated that PBMs represent a heterogeneous population of cells differing in expression of surface receptors and in

profile of secreted mediators [4]. When PBMs are divided according to their expression of the lipopolysaccharide receptor CD14 and the low affinity immunoglobulin G receptor CD16, three major subpopulations can be distinguished [6, 7]. Those include CD14⁺⁺ CD16[–] PBMs also referred to as 'classical' monocytes, CD14⁺⁺ CD16⁺ PBMs called 'intermediate' monocytes and CD14⁺ CD16⁺⁺ PBMs called 'non-classical' monocytes [7]. The CD14⁺⁺ CD16⁺ PBMs express high level of CD163 and at least under certain conditions may release predominantly anti-inflammatory mediators such as interleukin-10 (IL-10) [6, 8]. However, other laboratories demonstrated strong pro-inflammatory potential of those cells [9]. Moreover, analysis of gene expression profiles demonstrated that CD14⁺⁺ CD16⁺ cells express many mediators crucial for tissue remodelling and angiogenesis indicating potential role of CD14⁺⁺ CD16⁺ cells in those processes [10]. Therefore, quantitative differences in

the number of PBM subsets infiltrating peripheral tissues may affect the outcome of the inflammatory response [11]. We have already demonstrated that in asthmatic patients, elevated numbers of CD14⁺⁺ CD16⁺ PBMs are found being the greatest in patients with severe asthma [6]. However, glucocorticoid therapy preferentially affects the number of circulating non-classical monocytes. During systemic glucocorticoid therapy of asthma exacerbation, clinical improvement was associated with decrease in the number of CD14⁺ CD16⁺⁺ PBMs [6].

Allergic asthma patients exposed to a relevant allergen develop immediate bronchoconstriction [early asthmatic reaction (EAR)], which usually lasts <60 min and is dependent on mediators secreted by mast cells [12]. Subsequently, in majority of patients, airway inflammation dependent on the infiltration of the airways by inflammatory cells such as T cells, eosinophils and monocytes develops [13, 14]. At that time, the histological changes in the bronchial mucosa resemble those seen in patients with persistent asthma [13, 14]. This accumulation of inflammatory cells in the airways in a substantial proportion of patients leads to development of prolonged narrowing of the airways also referred to as late asthmatic reaction (LAR) [13, 14]. Prolonged airway inflammation in turn induces tissue remodelling and leads to AHR [13, 14].

In the current study, we have examined the potential association between individual subsets of PBMs and clinical and immunological parameters of HDM-APs (house dust mite, HDM). Moreover, the quantitative changes of individual PBM subsets in response to bronchial allergen challenge were evaluated.

Methods

Patients. The study was performed on 34 non-smoking, allergic to dust mite *Dermatophagoides pteronyssinus* (Dp) patients (Dp-APs), mean age 25 years (95% CI 18–36 years), who experienced rhinitis/conjunctivitis symptoms upon exposure to house dust. Twenty-two of 34 Dp-APs reported also asthma symptoms but they had never been regularly treated for asthma before. All patients had a baseline FEV₁ above 70% of the predictive value, positive skin prick tests to Dp extract and serum concentration of anti-Dp IgE > 0.7 kU/l. Twelve non-smoking, healthy, non-atopic subjects (HCs), mean age 25 years (95% CI 18–31 years) were included as a control group. Bronchial challenges were not performed in HC for ethical reasons. All subjects signed an informed consent. The study was approved by the local Ethics Committee.

Skin testing. All persons were skin tested using prick methodology with a screening panel of aeroallergens (Allergopharma, Reinbek Germany) as described before [15].

Bronchial challenges. Histamine and allergen bronchial challenge tests were performed as described before [15]. Allergen challenge was performed 24 h after histamine challenge.

Briefly, all patients inhaled doubling concentrations of histamine starting from a concentration of 0.062 mg/ml. The procedure was continued until either at least 20% fall of FEV₁ or histamine concentration 32 mg/ml was reached. The FEV₁ values obtained after inhalation of 0.9% solution of NaCl were used as the reference. Bronchial reactivity was expressed as a concentration of histamine causing 20% drop of FEV₁ (PC₂₀).

During allergen challenge, increasing doses (0.8, 4, 20, 100, 500 and 2500 SBE) of aqueous Dp extract (Allergopharma) were administered until at least 20% fall of FEV₁ or a cumulative dose 5000 SBE was reached. Forced expiratory manoeuvres were performed 15 min after inhalation of each dose of the allergen extract. The FEV₁ was measured every 15 min during the first hour, every 60 min during the next 11 h and then after 24 h. The sensitivity to allergen challenge was evaluated as a dose of allergen causing 20% drop of FEV₁ (PD₂₀).

Exhaled nitric oxide measurements. Concentration of nitric oxide in the expired air was evaluated 'on-line' using a chemiluminescence analyzer NOATM 280i (Sievers, Boulder, CO, USA). The measurements were performed as described before [16]. Briefly, each patient exhaled against the fixed expiratory resistance of 16 cm H₂O, which resulted in a constant flow of 50 ml/s. A plateau of NO concentration in the exhaled air at the selected exhalation rate was automatically selected by the computer software according to the American Thoracic Society recommendations. The measurements were repeated three times and the mean value expressed as fraction of expired NO (FeNO) was used for analysis.

Flow cytometry analysis. Samples of EDTA anticoagulated venous blood for flow cytometry and cytokine analyses were collected before (*T*₀), 6 h (*T*₆) and 24 h (*T*₂₄) after allergen challenge. Flow cytometry analysis was performed on the whole-blood samples using combinations of the following labelled monoclonal antibodies anti-CD14 FITC or anti-CD14 PE, anti-CD16 FITC or anti-CD16 PE-Cy5 and anti-CCR4 PE (all from BD PharMingen, Erembodegen, Belgium) as described before [6]. Labelled, isotype-matched antibodies were used as negative controls. After 30 min of incubation at room temperature, erythrocytes were lysed using FACS Lysing Solution (BD PharMingen). The remaining white cells were analysed using FACSCalibur cytometer (BD Immunocytometry Systems, San Jose, CA, USA) as described before [6]. Individual PBM subsets were given names according to the nomenclature proposed by Ziegler-Heitbrock *et al.* [7].

Immune assays. Serum concentration of total (tIgE) and specific anti-Dp IgE (sIgE) were evaluated using UniCap (Phadia, Uppsala, Sweden).

Plasma concentrations of CCL2, CX3CL1 and CCL17 were evaluated using Quantikine ELISA kits (R&D Diagnostics, Minneapolis, MN, USA).

Statistical analysis. The results are expressed as mean with 95% confidence interval (95%CI). Statistical analysis was performed using ANOVA test. *Post hoc* analysis was performed using Student's *t*-test. A probability value of $P < 0.05$ was taken to indicate statistical significance. Linear correlation by Pearson was used to estimate correlations between studied parameters.

Results

Allergen challenge resulted in the development of significant bronchoconstriction in 22 [responders (Rs)] of 34 Dp-APs. Those 22 patients reported both rhinitis and asthma symptoms. In 12 Dp-APs, no asthmatic response could be demonstrated [non-responders (NRs)]. Those patients had never reported asthma symptoms before the study. The baseline clinical and immunologic characteristics of the studied patients are presented in Table 1. There was no difference in age and sex distribution between Rs, NRs and HCs. All Dp-APs had comparable levels of serum tIgE, baseline lung function results and FeNO. The greatest mean eosinophil count was seen in Rs (415 cells/mm³; 95%CI 295–534 cells/mm³) being significantly greater than in NRs (214 cells/mm³; 95%CI 143–321 cells/mm³; $P = 0.02$) and in HCs (158 cells/mm³; 95%CI 96–219 cells/mm³; $P < 0.001$). The mean bronchial reactivity to histamine was signifi-

cantly less in NRs (PC₂₀ = 14.1 mg/ml; 6.2–32 mg/ml) than in Rs (PC₂₀ = 2.3 mg/ml; 0.7 to 4.8 mg/ml; $P < 0.0001$).

Elevated number of CD14++ CD16+ PBMs is present in Dp-APs who develop asthmatic response to allergen challenge

At T_0 , there was no significant difference in the mean number of PBMs between Rs, NRs and HCs ($P = 0.184$) (table 1). However, the mean number of CD14++ CD16+ PBMs was significantly elevated in Rs (156 cells/mm³; 95%CI 114–198 cells/mm³; $P = 0.001$) in comparison to HCs (40 cells/mm³; 95%CI 29–50 cells/mm³). Similarly, the mean percentage of CD14++ CD16+ PBMs was significantly elevated in Rs (35.4%; 95%CI 26.9–43.9%; $P = 0.01$) in comparison to HCs (14.6%; 95%CI 7.3–21.8%). In NRs, neither the mean number of CD14++ CD16+ PBMs (64 cells/mm³; 95%CI 36–92 cells/mm³) nor the mean percentage of CD14++ CD16+ PBMs (15.5%; cells/mm³; 95%CI 9.6–25.4%) was different from those of HCs ($P = 0.08$ and $P = 0.56$, respectively). The mean baseline numbers of CD14++ CD16– or CD14+ CD16++ PBMs did not differ between HCs, NRs and Rs (Table 1).

Allergen challenge differentially affects the number of individual PBM subsets

During a 24-h observation period after allergen challenge, no significant changes in the total number of PBMs in either of the studied groups could be demonstrated (Fig. 1). However, in Rs, significant decrease in the mean number of CD14++ CD16+ PBMs was

Table 1 Patients characteristics.

	HCs	NRs	Rs
Age (years)	25; 18–31	23; 18–34	26; 18–40
Sex (M/F)	5/7	4/8	9/13
FEV ₁ (% predicted)	114; 95–132	113; 101–125	108; 102–115
Histamine PC ₂₀ (mg/ml)	ND	14.1; 6.2–32	2.3; 1.07–4.8 ^c
Allergen PD ₂₀ (SBE)	ND	>5000	1233; 778–1956
FeNO (ppb)	14; 10–18	52; 32–85 ^a	78; 55–110 ^a
Total IgE (kU/l)	28; 5–52	190; 96–377 ^a	232; 123–438 ^a
Dp IgE (kU/l)	<0.35	2.2; 0.9–6.5	17.6; 9.6–32.2 ^b
Eosinophils (cells/mm ³)	158; 96–219	214; 143–321 ^a	415; 295–534 ^{a,b}
Monocytes (cells/mm ³)	391; 260–488	372; 312–442	434; 380–487
CD14++ CD16– (cells/mm ³)	294; 263–324	273; 240–308	235; 189–280
CD14++ CD16+ (cells/mm ³)	40; 29–50	64; 36–92	156; 114–198 ^{a,b}
CD14+ CD16++ (cells/mm ³)	18; 14–22	17; 11–23	20; 15–25
CCL2 (pg/ml)	107; 85–129	108; 70–145	101; 82–120
CX3CL1 (pg/ml)	253; 225–282	357; 300–415 ^a	359; 293–424 ^a
CCL17 (pg/ml)	52; 37–68	96; 82–110 ^a	159; 105–209 ^{a,b}

HCs, healthy, non-atopic subjects; NRs, non-responders; Rs, responders. The results are expressed as means with 95%CI.

^aSignificantly more than in HCs.

^bSignificantly more than in NRs.

^cSignificantly less than in NRs.

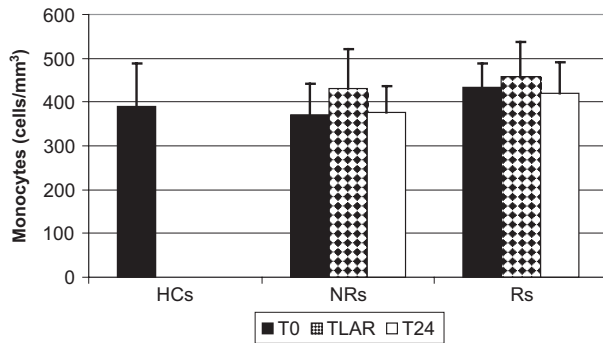


Figure 1 Number of circulating monocytes after intrabronchial allergen challenge. The Dp-APs were divided according to the type of asthmatic response to the allergen challenge: non-responders (NRs), responders (Rs).

seen which at T_{24} (107 cells/mm³; 95%CI 81–132 cells/mm³) was significantly less than at T_0 ($P = 0.003$) (Fig. 2). No significant change in the number of circulating CD14⁺⁺ CD16[–] or CD14⁺ CD16⁺⁺ cells was seen. In NRs, no statistically significant change of any of the PBM subsets could be demonstrated after allergen challenge (Fig. 2).

The frequency of CD14⁺⁺ CD16⁺ PBMs correlates with bronchial reactivity

Analysis of associations between the number of CD14⁺⁺ CD16⁺ PBMs and clinical or immunological parameters of the studied patients is presented in Table 2. The percentage of CD14⁺⁺ CD16⁺ PBMs at T_0 correlates with bronchial reactivity to histamine expressed as log_{PC20} ($r = -0.685$; 95%CI -0.834 to -0.432 ; $P < 0.001$) (Table 2). Similarly, the absolute number of CD14⁺⁺ CD16⁺ PBMs at T_0 correlates with log_{PC20} of histamine ($r = -0.507$; 95%CI -0.782 to -0.069 ; $P = 0.027$) (Fig. 3A). No other clinical or immunological parameters correlated with the number or percentage of CD14⁺⁺ CD16⁺ PBMs at T_0 , T_6 or T_{24} . No correlation between the number of other PBM subsets and any of the clinical or immunologic parameters was found. However, the change in the number of circulating CD14⁺⁺ CD16⁺ cells over a 24-h observation period (from T_0 to T_{24}) correlated with bronchial hyperreactivity ($r = 0.706$; 95%CI 0.43–0.861; $P < 0.001$) (Fig. 3B).

Allergen challenge is associated with increase in plasma CCL17 and CCL2 but not CX3CL1

To evaluate potential mechanisms for changes in individual monocyte subsets after allergen challenge, we performed serial evaluations of plasma CCL2, CX3CL1 and CCL17 concentration. The baseline concentrations of CX3CL1 (359 pg/ml; 95% CI 293–424 pg/ml) and

CCL17 (159 pg/ml; 95% CI 105–209 pg/ml) in Rs were significantly greater than in HCs (253 pg/ml; 95% CI 225–282 pg/ml, $P < 0.001$ and 52 pg/ml; 95% CI 37–68 pg/ml, $P < 0.001$, respectively). (Table 1). Moreover, in NRs, plasma concentration of CCL17 (96 pg/ml; 95%CI 82–110 pg/ml) was significantly greater than in HCs ($P < 0.001$) but was significantly less than in Rs ($P = 0.01$), while baseline plasma concentration of CX3CL1 in NRs (357 pg/ml; 95%CI 300–415 pg/ml) was greater than in HCs ($P = 0.003$) but did not differ from that of Rs ($P = 0.982$). The baseline plasma concentrations of CCL2 did not differ significantly between Rs, NRs and HCs. In Rs, allergen challenge resulted in increase in plasma CCL17 and CCL2 concentrations which at T_{24} were 296 pg/ml; 95%CI 180–398 pg/ml ($P < 0.001$) and 131 pg/ml; 95% CI 109–154 pg/ml ($P < 0.001$), respectively. No significant difference was seen between the mean plasma concentrations of CX3CL1 at T_0 , T_6 or T_{24} (Fig. 4). In NRs, plasma concentration of neither of the chemokine studied changed over a period of 24-h observation after allergen challenge (not shown). Moreover, at T_{24} , only plasma concentration of CCL17 correlated inversely with the number of circulating CD14⁺⁺ CD16⁺ cells ($r = -0.58$, 95% CI -0.835 to -0.12 , $P = 0.018$).

Individual PBM subsets differ in the expression of CCR4

Subsequently, we analysed the expression of chemokine receptor CCR4 on individual PBM subsets. The mean expression of CCR4 on CD14⁺⁺ CD16⁺ PBMs (8.32 FU; 95%CI 7.85–8.78) was significantly greater than on CD14⁺ CD16⁺⁺ PBMs (7.11 FU; 95%CI 6.8–7.42 FU; $P = 0.001$) which in turn was significantly greater than on CD14⁺⁺ CD16[–] PBMs (6.08 FU; 95% CI 5.74–6.42 FU; $P = 0.001$) (Fig. 5). There was no significant difference in expression of CCR4 on individual PBM subsets between HCs, NRs and Rs.

Discussion

This is the first study which demonstrates different changes in the number of individual PBM subsets in allergic asthma patients in response to bronchial allergen challenge. Moreover, in our report, three monocyte subsets separated based on staining with anti-CD14 and anti-CD16 antibodies have been analysed. Previous studies focused mainly on two monocyte subpopulations divided solely on the basis of the presence or absence of CD16 [17–20]. Elevated number of circulating CD16⁺ monocytes has been reported in asthma [17] but also in other inflammatory diseases such as rheumatoid arthritis [18], tuberculosis [19] or sepsis [20]; however, no further subdivision of the population has been performed. Distinguishing CD14⁺⁺ CD16⁺ from CD14⁺ CD16⁺⁺

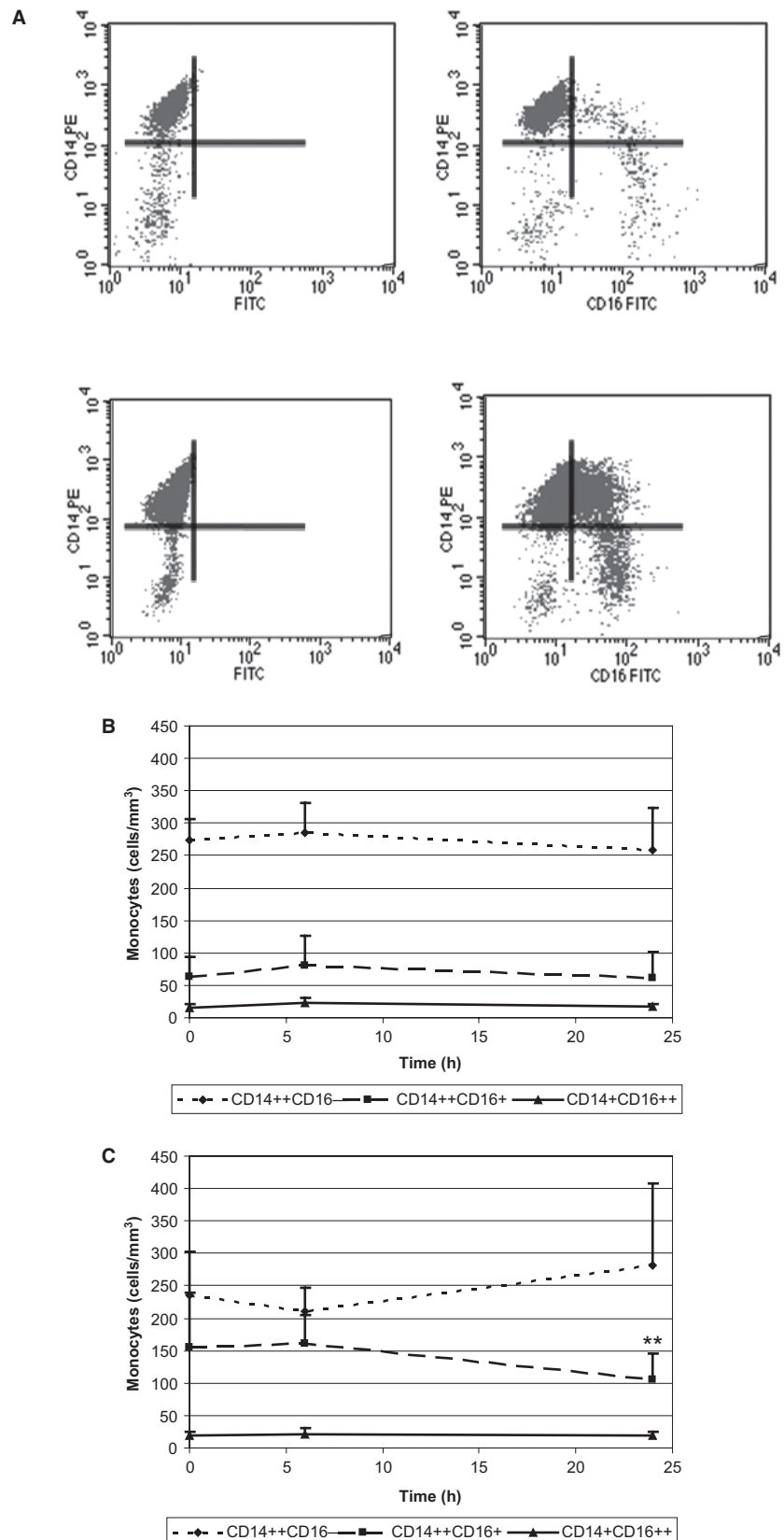
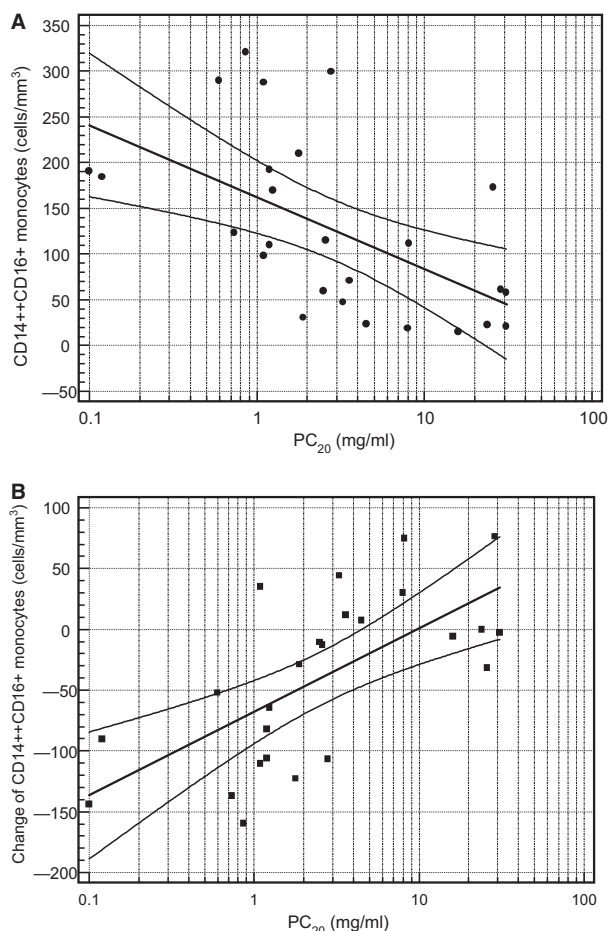


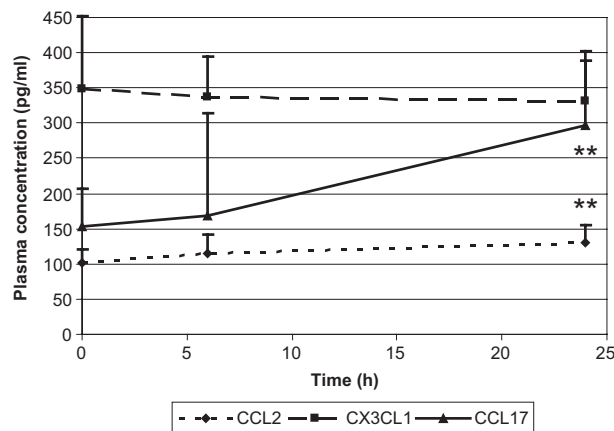
Figure 2 (A) Representative flow cytometry analyses of peripheral blood monocytes from a healthy subject (upper panel) and a patient with asthma (lower panel). The cells were stained with anti-CD14 PE antibodies and with anti-CD16 FITC (right column) or with an isotype FITC control antibody (left column). (B, C) Changes in the number of individual subsets of PBMs after intrabronchial challenge with *Dermatophagoides pteronyssinus* extract. The Dp-APs were divided according to the type of asthmatic response to the allergen challenge: (B) non-responders (NRs), (C) responders (Rs). **Significantly less than at T_0 ($P = 0.003$).

Table 2 Correlations between the percentage of CD14++ CD16+ cells and selected clinical and immunologic parameters.

	<i>R</i>	<i>P</i>	95% CI
FEV ₁	0.114	0.535	-0.213 to 0.354
FEV ₁ at T _{EAR}	-0.332	0.142	-0.668 to 0.116
Log _{Hist} PC20	-0.685	<0.001	-0.834 to -0.432
Log _{Dp} PD20	0.256	0.263	-0.198 to 0.619
Log _{Total} IgE	-0.358	0.098	-0.621 to 0.082
Log _{Dp} IgE	-0.142	0.480	-0.495 to 0.252
Log _{FeNO}	-0.064	0.727	-0.404 to 0.066
Eosinophils	-0.008	0.967	-0.367 to 0.353

**Figure 3** Expression of CCR4 on individual PBM subsets. (A) Representative flow cytometry analysis. (B) The mean expression of CCR4 on individual PBM subsets.

among CD16+ monocytes is justified not only from morphological but also from functional point of view [7]. Interestingly, in the current study, we have demonstrated that in allergic asthma patients, elevated number of circulating CD16+ monocytes is because of expansion of the CD14++ CD16+ subset. The current study extends our previous observations reporting on elevated number

**Figure 4** Changes in plasma concentration of CCL2, CCL17 and CX3CL1 in patients who responded with a significant bronchoconstriction to allergen challenge (Rs). **Significantly greater than at T₀ (*P* < 0.01).

of CD14++ CD16+ monocytes in more severe asthmatic patients [6]. By demonstration of direct correlation between the number of CD14++ CD16+ monocytes and the degree of bronchial hyperreactivity, it further supports our previous observations linking elevated numbers of circulating CD14++ CD16+ monocytes with severe asthma [6]. It has already been demonstrated that the degree of bronchial reactivity to histamine or metacholine correlates with asthma severity measured as symptom scores, treatment required to control symptoms and diurnal variability of lung function parameters [21, 22]. Interestingly, it has been demonstrated that CD14++ CD16+ cells are potent producers of many pro-inflammatory cytokines while stimulated with viral nucleic acids indicating their possible role in regulation of the inflammatory response [9]. Surprisingly, however, we have not been able to demonstrate any direct correlation between the number of CD14++ CD16+ cells and any of the conventional parameters reflecting intensity of airway inflammation such as FeNO or peripheral blood eosinophilia. Therefore, we cannot provide evidence that CD14++ CD16+ monocytes significantly affect the intensity of allergic inflammation in response to allergen challenge. However, it cannot be excluded that in asthmatic patients, those cells may affect AHR through modulation of inflammatory response to respiratory infections including viral infections. Dysfunction of the airways seen in asthmatic patients depends not only on airway inflammation but also on structural changes in the airways referred to as remodelling. Although airway inflammation leads to development of bronchial reactivity, in asthmatic patients, successful therapy with inhaled corticosteroids has only mild effect on AHR [23, 24]. Anti-inflammatory effects of corticosteroids in asthma are associated with dramatic depletion of inflammatory cells,

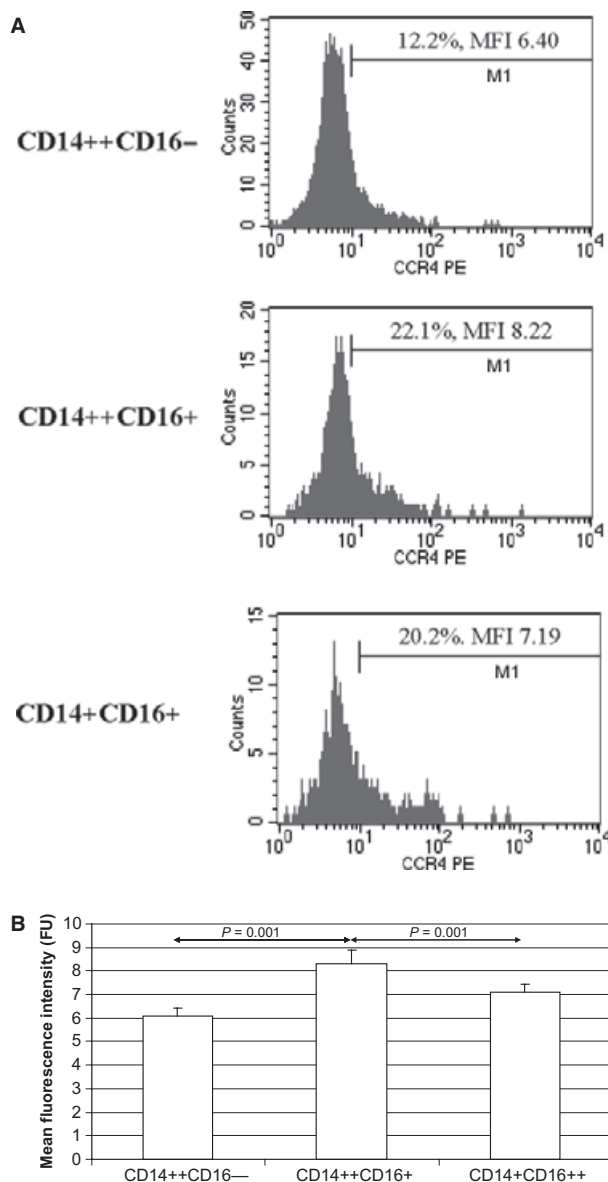


Figure 5 Correlation between airway hyper responsiveness expressed as PC20 of histamine and: (A) baseline number of CD14++ CD16+ PBMs; (B) change of CD14++ CD16+ PBM number over a period of 24 h after the allergen challenge.

mainly eosinophils and lymphocytes from the airway tissues [24]. However, some structural changes in the airways are resistant to corticosteroid therapy [24]. It has been recently demonstrated that the degree of bronchial responsiveness to histamine but not to metacholine correlates with airway remodelling [25]. It is therefore tempting to speculate that the disequilibrium between individual PBM subsets may participate in the development of airway remodelling and AHR. The CD16+ monocytes play a role in tissue remodelling and angiogenesis [8, 10, 26]. Analysis of transcriptomes demonstrated that among all PBM subsets, the

CD14++ CD16+ cells are characterized by the greatest expression of genes involved in tissue remodelling and angiogenesis such as *TGFB1* or *CD105* [10]. Moreover, the Th-2 type cytokines such as IL-4 and IL-13, which are abundantly produced in allergic asthmatics, induce differentiation of monocytes into profibrotic and angiogenic macrophages, which in turn play a crucial role in remodelling of the lungs leading to pathological fibrosis [27].

Further insights into the potential role of individual PBM subsets in asthma are provided by analysis of their kinetics after allergen challenge. Decrease in the number of CD14++ CD16+ PBMs after allergen challenge may reflect different chemotactic potential of those subsets. In fact, different chemotactic potential of CD14++ CD16- and CD14++ CD16+ cells in response to inflammation induced by infectious agents has already been demonstrated [4]. In a steady state, elevated number of CD14++ CD16+ PBMs can be explained by relatively less trafficking of CD14++ CD16+ than CD14++ CD16- cells into inflammatory tissues. In stable asthmatic patients, we found decreased expression of CD16 on bronchial macrophages, which may reflect preferential influx of CD16- PBMs into the airways in asthmatics as compared to non-asthmatic subjects [28]. However, during acute asthma attack such as that seen after allergen exposure, preferential sequestration of CD14++ CD16+ PBMs may occur. It has been demonstrated that acute skin injury results in preferential accumulation of CD16+ monocytes [29]. Chemokines are crucial in directing individual cell migration into inflammatory sites. Surprisingly, we were not able to correlate plasma concentration of two major monocyte chemotactic chemokines CCL2 and CX3CL1 with the number of circulating monocyte subsets. However, an inverse correlation between CCL17 and the number of circulating CD14++ CD16+ monocytes 24 h after allergen challenge supports the concept of involvement of CCL17 and its receptor CCR4 in monocyte activation/migration. Among all chemokines, CCL17 and CCL22 which are ligands of the CCR4 are crucial for the attraction of cells which fuel Th-2 type immune response [30]. In fact, the key role of CCR4 in migration of T cells into airways of asthmatic patients has already been demonstrated [30]. However, the role of CCR4 in migration of monocytes has not been investigated. There is also little information concerning the expression of CCR4 on individual subsets of PBMs. Elevated expression of CCR4 on PBMs has been demonstrated in rheumatoid arthritis patients but the study did not address the expression of CCR4 on individual PBM subpopulations [31]. The CCR4-dependent activation of macrophages may play a role in inflammatory response and tissue remodelling [32]. In an experimental model of bleomycin-induced pulmonary fibrosis, CCR4 played a crucial role in activation of pul-

monary macrophages which in turn led to pulmonary fibrosis. Although in that experimental model, genetic modification leading to the absence of CCR4 did not significantly affect inflammatory cell recruitment to the lungs in response to bleomycin challenge. Interestingly, lung macrophages in the CCR4 knockout mice differed morphologically from those in the wild-type mice. Unfortunately, our study cannot prove if CCR4 selectively affects migration of some monocyte subsets or influences activation and/or maturation of monocytes. However, strong increase in plasma concentration of CCL17 and expression of CCR4 on some CD14++ CD16+ PBMs whose number decreases after allergen challenge strongly suggest a possible cause–effect relationship. Further studies are warranted to elucidate whether this is a direct effect of CCL17 on the CCR4 receptor present on CD14++ CD16+ cells or the effect is indirect with possible involvement of other cells and mediators.

In summary, our study for the first time demonstrates different kinetics of three monocyte subsets in response to allergen challenge linking CD14++ CD16+ cells with the pathogenesis of AHR. Moreover, it shows that in a steady state of chronic diseases such as asthma expansion of the CD14++ CD16+ cells in peripheral blood may facilitate migration of those cells during acute exacerbation. Further studies are warranted to understand the role of individual monocyte subsets and CCR4 and its ligands in the pathophysiology of allergic asthma, which may help in successful application of new therapeutic options in asthma.

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