

Airway inflammation and altered alveolar macrophage phenotype pattern after repeated low-dose allergen exposure of atopic asthmatic subjects

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Summary

Background The alveolar macrophage (AM) constitutes an important link between pulmonary innate and adaptive immunity due to its antigen-presenting capacity and ability to express different immunomodulating mediators. The role of AMs in the pathogenesis of allergic inflammation has yet to be fully determined.

Objective To investigate clinical effects and any change in the AM phenotype pattern after inhalation of sub-clinical doses of allergen by asthmatic patients.

Methods Eight subjects with allergic asthma underwent repeated low-dose allergen provocations equivalent to 10% of PD₂₀. AMs recovered with bronchoalveolar lavage (BAL) were characterized by flow cytometric analysis of adhesion molecules, co-stimulatory molecules and markers for AM population activation and heterogeneity.

Results An allergic airway inflammation, sub-clinical in six out of eight subjects, was obtained after low-dose allergen provocations, as determined by increased airway methacholine reactivity, increased BAL fluid total cell and eosinophil counts and increased serum ECP levels. The AMs showed a post-challenge altered phenotype pattern with a decreased expression of CD11a, CD16, CD71 and HLA class I and an increased expression of CD11b and CD14. The AMs were positive for CD83 and a weak post-challenge increase in the CD83 expression was found.

Conclusion Repeated low-dose allergen exposure induces an allergic airway inflammation in asthmatic subjects. The inflammation is associated with an altered AM phenotype pattern, consistent with an influx of monocytes and a hypothetical increased accessory cell function in the airways, possibly contributing to the development and sustenance of airway inflammation in asthma.

Keywords: accessory function, allergic inflammation, asthma, low-dose allergen provocation, macrophage phenotype

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Introduction

The alveolar macrophage (AM) has an important antigen surveying function in the lung. In addition to its antigen-engulfing and -presenting ability, the AM has a large capacity to express various immunomodulating mediators in response to different antigenic stimuli. The pulmonary

macrophage population consists of functionally different subsets [1,2], probably representing various differentiation stages. The net effect of AM function in the healthy lung is thought to be immunosuppressive, providing an inhibitory effect on local, potentially harmful immunoreactivity [reviewed in 3,4].

Under certain *in vitro* conditions, however, AMs stimulated with various antigens or cytokines have been shown to exert an immunostimulatory activity [5–7]. Furthermore,

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Furue with coworkers [8], have demonstrated an altered AM accessory function associated with induction of type-2 T-helper (TH2) cell cytokine production (interleukin [IL]-4, IL-5) in patients with idiopathic pulmonary fibrosis. It has been suggested that failure of AM immunosuppressive mechanisms or a switch in AM function towards an accessory activity, could contribute to the development of the allergic airway inflammation with T-cell activation, secretion of TH2 type cytokines and eosinophil infiltration seen in allergic asthma [9–11]. Recent studies show that AMs from atopic asthmatics have a significantly higher capacity to stimulate IL-5 production by peripheral CD4⁺ T cells, as compared with AMs from atopic nonasthmatics, suggesting an important role for AMs in the development of asthma in atopic individuals [12,13]. AMs recovered from asthmatics are known to express an activated phenotype [14–16] and show an altered expression of pro-inflammatory mediators compared with AMs from healthy individuals [17–21]. The mechanisms behind a hypothetical immunomodulating function by AMs in allergic inflammation remain, however, to be clearly elucidated.

Traditionally, allergic inflammation in asthmatics has been characterized and studied using allergen challenge models that provoke an acute asthmatic reaction as determined by a 20% fall in forced expiratory volume in 1 s (FEV₁). Recently, provocation models using sub-clinical doses of allergen administered on repeated occasions have been taken in use [22–25]. One advantage of using provocation models that are closer to simulating a natural antigen exposure is the possibility to study the development and sustenance of sub-clinical allergic inflammation in the airways.

In the present study, we aimed to characterize the AM phenotype pattern and to study clinical effects as well as effects on serum ECP levels, blood and bronchoalveolar lavage (BAL) cellular profiles, using a new low-dose allergen provocation regime, repeatedly exposing patients with mild allergic asthma to inhaled allergen in doses equivalent to 10% of the allergen dose causing a 20% fall in FEV₁ (PD₂₀).

Methods

Subjects

Patient characteristics and clinical data before and after low-dose allergen provocations are shown in Table 1.

Eight atopic patients, one male and seven females, aged 24–41 years (mean 30) with a history of mild allergic seasonal asthma, participated in the trial. Seven had never smoked whereas one had stopped smoking 5 years ago. In the study of AM phenotypes, the ex-smoking subject (patient 1) was excluded due to high macrophage autofluorescence.

All patients were sensitive to birch and grass pollen and two of the subjects to dog dander (patients 6 and 8). The patients allergic to animal dander did not have pets of their own and were asked to avoid animal contact during the study period. All subjects were in a stable phase of their disease, with values of FEV₁ ranging from 81 to 101% of the predicted normal value (median 93%). None was on regular asthma therapy, except for β_2 -agonists as needed. All were free from symptoms of airway infections for at least 4 weeks prior to the study and showed no signs of airway disease on pulmonary X-rays. Written consent was

Table 1. Patient characteristics and individual values of FEV₁ (% of predicted) and airway sensitivity to methacholine (PD₂₀, μ g) before and after low-dose allergen provocations

Patient	Age	Sex	RAST (kU/L)	Total IgE (kU/L)	Allergen, dose in trial (SQ)	FEV ₁ Before	FEV ₁ After	PD ₂₀ Before	PD ₂₀ After	PD ₂₀ 3 weeks after	
1	36	F	3	56	Birch, 9.0	81	81	92	74	54	
2	25	F	4	99	Birch, 84.0	88	79	220	60	770	
3	29	F	3	29	Birch, 14.0	92	90	82	33	56	
4	32	M	5	ND	Birch, 9.0	101	101	98	76	125	
5	41	F	4	79	Grass, 8.4	101	97	550	ND	800	
6	24	F	3	170	Birch, 113.0	80	81	112	70	115	
7	26	F	4	25	Grass, 42.0	101	98	220	26	150	
8	29	F	4	130	Birch, 45.0	94	95	360	220	550	
Median:	29		4	79		93	92	Geometric mean:	172	64*	196
Upper and lower quartiles:	26–34		3–4	29–130		81–101	81–98	Range:	82–550	26–220	54–800

**P* < 0.05 compared with before value; F = female, M = male, ND = not determined, PD₂₀ = the dose provoking a 20% fall in FEV₁

obtained from all subjects and the study received approval from the local Ethics Committee.

Study design

The study was performed outside the pollen season. Patients were administered allergen (birch or grass pollen, Table 1) for which they had a positive history and a positive skin prick test or RAST result. The allergen extracts used in the trial were standardized and freeze dried and all were obtained from Aquagen (ALK, Copenhagen, Denmark). The choice of allergen dose was based on a previous screening allergen inhalation challenge where cumulative allergen doses were administered to determine the PD₂₀ value [26]. The screening allergen provocation was performed 3–13 months before starting the low-dose study.

The low-dose allergen provocation trial was started by performing bronchoscopy with BAL. After 2–3 weeks, the low-dose allergen provocations were initiated. The patients inhaled a dose of allergen equivalent to 10% of the PD₂₀ in the morning and at the same time every day, for 7 week days. The same dose was given every day and varied from 8.4 SQ to 113.0 SQ between the individuals (Table 1). FEV₁ and peak expiratory flow rate (PEFR), were recorded before and 15 min after the allergen inhalation. The patients recorded PEFR values twice daily and were instructed to make additional recordings if any airway symptoms occurred. A second bronchoscopy and BAL was performed the day after the last allergen provocation. Peripheral blood was drawn in connection to bronchoscopies, as well as on days 3 and 5 of allergen provocations and 3 weeks after ending the provocations. To determine the non-specific bronchial responsiveness, methacholine challenges were performed before starting the provocations and after the final allergen dose, as well as 3 weeks after the low-dose allergen provocations were completed.

Bronchial provocation tests

All bronchial provocations were performed by use of a dosimeter controlled jet nebulizer Spira Electro 2 (Respiratory Care Center, Hameenlinna, Finland).

Methacholine challenges were performed by administration of increasing doses of methacholine to obtain the PD₂₀ value. After recording of the baseline FEV₁, the subject inhaled saline followed by methacholine during a specified number of breaths, with dose increments every third minute. FEV₁ was measured 3 min after each dose of methacholine. The logarithmic methacholine doses were plotted against the percentage of the post-saline FEV₁.

PD₂₀ values were calculated from the cumulative dose–response curves by linear interpolation.

Blood analyses

The percentage and number of eosinophils were established by routine differential counting using a Coulter STKS (five part differential count).

Serum ECP levels were measured with a commercially available fluoroimmunoassay (Pharmacia ECP CAP System FEIA, Pharmacia Diagnostics AB, Uppsala, Sweden). The sera were collected and handled according to the manufacturer's instructions and stored at –20°C until analysed. Serum ECP levels > 2.3 µg/L were considered as positive.

Total serum IgE concentrations were measured using Pharmacia IgE CAP System (Pharmacia).

BAL and isolation of alveolar macrophages

Bronchoscopy with bronchoalveolar lavage was performed as previously described [27] using a flexible fibre optic bronchoscope (Olympus BF Type P20; Olympus Optical Co. Ltd, Tokyo, Japan). The patients were premedicated with morphine–scopolamine and administered inhaled β₂-agonist 10 min before the bronchoscopy. The bronchoscope was wedged in a sub-segment in the right middle lobe where five aliquots of 50 mL sterile, 37°C PBS solution were instilled. The fluid was gently aspirated after each instillation, pooled and collected in a sterile siliconized bottle kept on ice. The BAL fluid was strained through a single layer of Dacron nets (Type AP32, Millipore, Bedford, Ireland). Cells were centrifuged at 400 g for 10 min at +4°C and resuspended in RPMI 1640 (Sigma Aldrich Co., St Louis, MO, USA). Total cell counts and assessments of cell viability by trypan blue cell exclusion were performed using a Bürker chamber (Superior Marienfeld, Bad Mergentheim, Germany). Cytospins were prepared by cyto centrifugation of aliquots of cell suspension equivalent to 60 000 cells per slide for 3 min at 20g in a cyto centrifuge (Cytospin 2, Shandon, Runcorn, UK). The slides were stained in May–Grünwald Giemsa for evaluation of cell differentials and in toluidine blue for assessment of metachromatic cell counts.

To allow separate studies of BAL fluid AMs and lymphocytes, the cells were separated using anti-CD2-(pan T-cell) labelled magnetic beads (Dynabeads, Dynal, Oslo, Norway) which provide a negative selection of AMs resulting in a purity of >95%. Briefly, total BAL fluid cells were mixed with beads in a concentration of 15 × 10⁶ beads/millilitre. The mixture was incubated under gentle tilt rotation for 30 min at +4°C and the CD2-positive cell/bead population was separated using a magnet according to the manufacturer's instructions. The negative fraction containing AMs was washed in PBS and the cell count was determined.

Immunofluorescence labelling of cells and flow cytometry

Immunolabelling of AMs was performed using primary

Table 2. Description of monoclonal antibodies used for the study of alveolar macrophage antigen expression

Monoclonal antibody	Clone	Antigen description/antibody reactivity	Source
Antihuman-			
CD11a	MHM24	Leukocyte function associated -1 (LFA-1) protein, adhesion molecule	DAKO
CD11b	2LPM19c	C3 bi receptor, adhesion molecule	DAKO
CD11c	KB90	Protein 150,95, adhesion molecule	DAKO
CD44	F10-44-2	Hyaluronic acid receptor, adhesion molecule	Serotec
CD54	B-C14	Intercellular adhesion molecule-1 (ICAM-1)	Serotec
CD58	BRIC-5	LFA-3, adhesion molecule	Serotec
CD14	TUK4	LPS receptor, myelomonocytic differentiation antigen	DAKO
CD16	3G8	FcγRIII, IgG receptor/Expressed on mature/activated macrophages	Coulter
CD83	HB15A	Glycoprotein member of the Ig super-family/mature dendritic cells	Serotec
RFD1	RFD1	HLA class II related antigen/dendritic cells	Serotec
CD80	BB-1	B7-1, ligand for CD28, co-stimulatory molecule	Serotec
CD86	BU63	B7-2, ligand for CD28, co-stimulatory molecule	Serotec
CD71	Ber-T9	Transferrin receptor/proliferating/mature macrophages	DAKO
HLA class I	W6/32	HLA class I, labels peptide products of the HLA-A, -B, -C loci	DAKO
HLA class II	CR3/43	HLA class II, labels products of the HLA-DP, -DQ, -DR sub-regions	DAKO

unconjugated, FITC- or phycoerythrin-conjugated monoclonal mouse antibodies labelling adhesion molecules, co-stimulatory molecules and markers for AM activation and heterogeneity (Table 2). Isotype-matched mouse immunoglobulin controls were used to compensate for non-specific background fluorescence. The results are shown as mean fluorescence intensity (MFI) values corrected for background fluorescence, or the percentage of cells within the macrophage gate positive for the marker. For each analysis, 3×10^5 AM were incubated with normal rabbit serum (DAKO) for 10 min. A saturating amount of primary antibody was added and the cells were incubated for 30 min in the dark at 4 °C, followed by two washes in cold PBS (centrifugation for 5 min, at 300g). Cells stained with unlabelled primary antibodies, were incubated with secondary FITC-labelled, F(ab')₂ rabbit antimouse IgG for 30 min. The cells were washed twice and eventually fixed in 1.0% paraformaldehyde. The flow cytometric analyses were performed using a FACS-Calibur flow cytometer (Becton Dickinson, San Jose, CA, USA). Before each analysis the instrument was calibrated using FACSComp software and Calibrite™ beads (Becton Dickinson). The MFI value was standardized using fixed instrument settings throughout the study and controlled using standard Flow-set beads (Coulter_3e2 Fluorospheres, Coulter Corporation, Miami, FL, USA). Macrophages were identified using forward and sideward scatter characteristics and 10 000 events were collected within the macrophage gate.

Statistical analyses

The data are demonstrated as medians with upper and lower quartile values, unless otherwise stated.

Comparisons of data retrieved before and after provocations were made using Wilcoxon matched pairs analyses. Correlations were calculated using Pearson Product-Moment correlation test. A *P*-value <0.05 was considered significant.

Results

Clinical parameters, blood and BAL fluid data

No patient experienced any significant early or late phase reaction during the study. Two patients developed slight airway obstruction which resulted in a modified provocation regime (3 provocation days for patient 2 and 6 days for patient 8).

The bronchial responsiveness to methacholine increased significantly (*P* < 0.05) from a geometric mean PD₂₀ value of 172 µg (range 82–550) before to 64 µg (range 26–220) after the allergen exposure period. After 3 weeks, no increase in airway responsiveness could be detected (Table 1). The post-challenge FEV₁ values were not significantly changed compared with prechallenge baseline values (Table 1). While no significant changes in the proportions of peripheral blood eosinophils were detected during or after the period of low-dose allergen inhalations, the serum ECP values were significantly increased on days 3, 5 and the day after provocations as compared with baseline ECP values (*P* < 0.05, all; Table 3). After 3 weeks, no increase in serum ECP levels was found.

The BAL fluid recovery and cell viability did not differ in samples retrieved before and after low-dose allergen

Table 3. Serum concentrations of ECP and peripheral blood proportions of eosinophils, before, during and after low-dose allergen provocations

Patient	ECP ($\mu\text{g/L}$) Before	ECP ($\mu\text{g/L}$) Day 3†	ECP ($\mu\text{g/L}$) Day 5†	ECP ($\mu\text{g/L}$) 24 h after	ECP ($\mu\text{g/L}$) 3 weeks after	EOS (%) Before	EOS (%) Day 3†	EOS (%) Day 5†	EOS (%) 24 h after	EOS (%) 3 weeks after
1	16.0	23.0	23.0	20.0	9.0	4.7	4.2	4.9	6.0	5.5
2	9.9	30.0	ND	14.0	9.4	3.2	10.0	ND	9.3	1.5
3	2.9	5.8	5.3	5.2	5.0	3.2	2.6	2.0	2.7	1.6
4	3.7	11.0	20.0	11.0	7.4	4.2	10.1	5.7	4.3	7.7
5	9.2	16.0	ND	ND	28.0	3.4	4.7	5.0	3.6	4.9
6	4.0	4.3	9.1	8.3	13.0	2.3	1.5	1.4	1.7	1.3
7	3.9	8.7	4.4	6.2	3.5	2.3	8.6	8.5	9.7	6.8
8	3.4	11.0	26.0	36.0	12.0	1.8	2.2	5.3	4.7	1.9
Median:	4.0	11.0*	14.6*	11.0*	9.2	3.2	4.5	5.0	4.5	3.4
Upper and lower quartiles:	3.6–9.6	7.3–19.5	5.3–23.0	6.2–20.0	6.2–12.5	2.3–3.8	2.4–9.3	2.0–5.7	3.2–7.7	1.6–6.2

* $P < 0.05$ compared with baseline value, ND = not determined, †blood samples were drawn before allergen dosing

challenge. The total number of cells, as well as the proportion (data not shown) and concentration of eosinophils recovered by BAL were significantly increased after the allergen challenge ($P < 0.05$, respectively, Fig. 1, Table 4). A significant positive correlation was detected between the post-challenge BAL fluid proportion of eosinophils and the blood proportion of eosinophils on day 5 of provocations ($r = 0.81$, $P < 0.05$) and the day after finishing the provocations ($r = 0.72$, $P < 0.05$).

The BAL fluid concentration of macrophages showed a tendency to increase ($P = 0.09$) after provocations. A significant, negative correlation was detected between the post-challenge BAL fluid proportion of macrophages and post-challenge FEV₁ values ($r = -0.76$, $P < 0.05$).

No changes could be detected in BAL fluid lymphocyte or neutrophil counts.

AM phenotype data

The AM phenotype pattern showed significant alterations after low-dose allergen exposure (Table 5). The MFI for AM expression of the adhesion molecules CD11a ($P < 0.05$) and CD54 ($P = 0.07$) was decreased, while the MFI for CD11b was significantly increased ($P < 0.05$). No change was detected in the expression of CD11c, CD44 or CD58.

To study the heterogeneity within the AM population, markers for different AM maturation stages and dendritic cells were used. The AM showed a weak decrease of the post-challenge expression of CD16 (MFI, $P < 0.05$), while both the proportion of AMs expressing the myelomonocytic differentiation antigen, CD14, as well as the MFI of CD14 expression was increased ($P = 0.07$ and $P < 0.05$, respectively). The post-challenge proportion of AMs expressing CD14

	Before	After
Total cell count ($\times 10^6$)	23.6 (20.6–27.1)	30.8 (22.0–38.5) *
Total cell concentration ($\times 10^6/\text{L}$)	133.6 (114.5–157.2)	160.8 (113.4–198.4)
Macrophages ($\times 10^6/\text{L}$)	111.0 (101.3–128.0)	143.6 (96.2–186.0)
Lymphocytes ($\times 10^6/\text{L}$)	14.6 (3.8–22.3)	10.8 (7.1–16.0)
Neutrophils ($\times 10^6/\text{L}$)	1.4 (0.8–2.0)	1.9 (1.5–2.6)
Eosinophils ($\times 10^6/\text{L}$)	0 (0–0.4)	1.1 (0.2–2.0) *
Basophils ($\times 10^6/\text{L}$)	0 (0–0)	0 (0–0.3)
MAST cells (no. in 10 visual fields, $\times 16$ magnification)	1.0 (0.5–3.0)	3.0 (0.5–8.0)

* $P < 0.05$. Data are shown as medians and with upper and lower quartiles.

Table 4. Bronchoalveolar lavage fluid cell count data, before and after low-dose allergen provocations

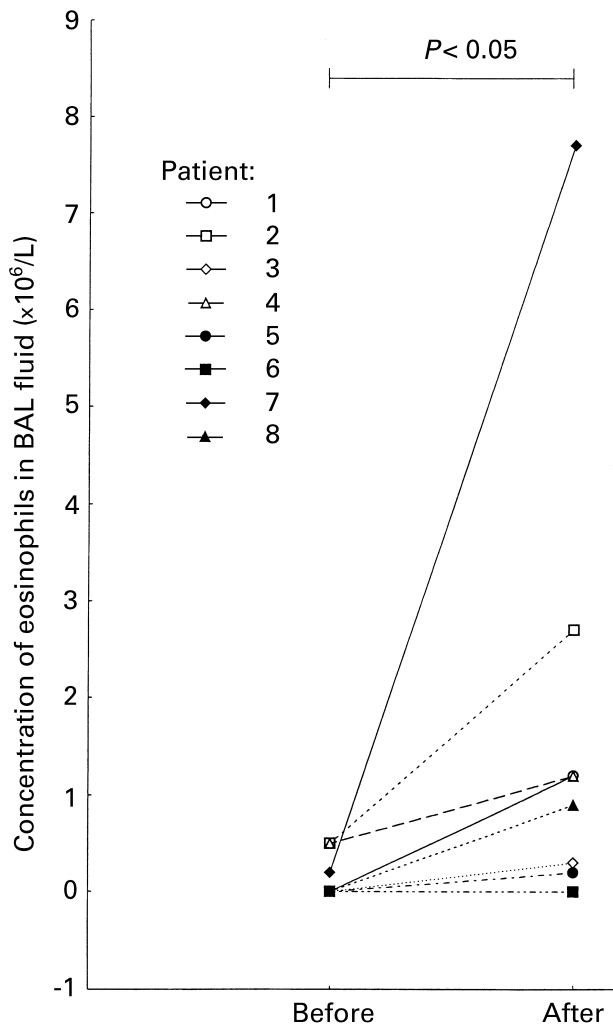


Fig. 1. Low-dose allergen provocation induced increase in BAL fluid eosinophil concentrations

showed a positive correlation with the post-challenge BAL fluid cell concentration ($r = 0.88$, $P < 0.05$) and the proportion of AM ($r = 0.92$, $P < 0.05$) and a negative correlation with the post-challenge FEV₁ values ($r = -0.81$, $P < 0.05$) (Fig. 2).

The expression of the antigen-presenting cell-associated marker CD83 within the AM population, investigated in four subjects, showed a tendency to increase after low-dose allergen provocations (Fig. 3), while no change in the expression of RFD1 could be detected.

The AM expression of the co-stimulatory molecule CD86 showed a specific pattern with one large population of cells weakly positive and one small population showing a more intense stain for the marker. No further evaluation of the two populations was performed in the present study. The post-challenge CD86 expression showed no significant change.

Table 5. Alveolar macrophage phenotype data. Mean fluorescence intensity (MFI) values for cells within the macrophage gate, before and after low-dose allergen provocations

	<i>n</i>	MFI	
		Before	After
CD11a	7	19.9 (16.4–21.2)	12.7 (10.4–16.0)*
CD11b	7	81.4 (72.0–87.1)	98.0 (80.2–107.8)*
CD11c	7	17.6 (16.9–20.3)	16.9 (13.5–20.1)
CD44	7	44.8 (41.8–66.8)	48.9 (38.1–53.4)
CD54	7	37.2 (27.8–43.2)	26.8 (19.3–30.6)
CD58	4	28.7 (14.5–28.9)	24.7 (21.0–25.6)
CD14	7	1.3 (0.7–1.7)	2.8 (2.2–5.4)*
CD16	7	23.0 (20.9–31.6)	22.2 (12.9–24.3)*
CD83	4	3.6 (1.3–9.5)	12.7 (7.6–16.4)
RFD1	7	64.6 (34.6–71.9)	57.5 (35.2–64.4)
CD86	7	4.8 (3.7–6.7)	4.2 (3.0–5.9)
Intense CD86	7	22.0 (0–24.2)	16.2 (0–23.1)
CD80	6	1.8 (1.6–2.5)	2.8 (2.2–3.5)
CD71	7	30.9 (19.4–55.6)	22.1 (14.3–41.2)*
HLAI	6	218.4 (165.8–235.5)	140.8 (111.1–193.7)*
HLAII	7	169.8 (99.6–293.2)	146.5 (118.1–216.1)

* $P < 0.05$

The AM expression of CD80 was weak and did not change with allergen provocations.

The transferrin receptor, CD71, as well as HLA class I, showed a decreased post-challenge AM expression ($P < 0.05$ both). No change could be detected in the expression of HLA class II.

Discussion

In this study we show that sub-clinical, low-dose allergen exposure of individuals with mild, allergic asthma is associated with increased airway reactivity, increased BAL fluid total cell and eosinophil counts, increased serum ECP levels and a changed AM phenotype pattern. The provocation model with inhalations of allergen in a dose equivalent to 10% of PD₂₀, during 7 days, thus results in airway inflammation characterized by accumulation of eosinophils and a shift towards a monocyte-like AM phenotype.

Six out of eight subjects were free from symptoms throughout the study, while two subjects developed slight airway obstruction during the provocation period, calling for interruption of the provocations. In both the patients, the debut of obstruction (day 3 for patient 2 and day 6 for patient 8) coincided with high levels of serum ECP and blood eosinophil counts (Table 3). These findings indicate that activation of peripheral inflammatory cells could be connected to the development of airway symptoms.

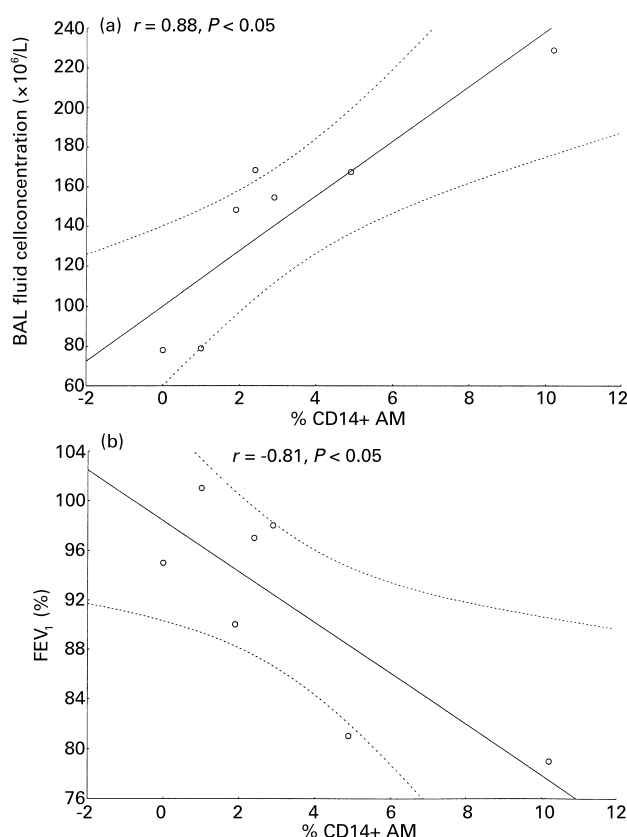


Fig. 2. Relationship between the percentage of alveolar macrophages (AMs) positive for CD14 and (a) the total BAL fluid cell concentration and (b) FEV₁ (% of predicted)

Investigating the AM phenotype pattern we found an altered AM expression of adhesion molecules after low-dose allergen provocations. Our findings are consistent with an increased post-challenge proportion of monocytes in the AM population [28,29]. This conclusion is supported by the increased AM expression of CD14, a marker commonly used to discriminate between macrophages and monocytes [30,31], as well as the decreased AM expression of CD71 and HLA class I, antigens that are upregulated during monocyte differentiation [32]. An increased proportion of monocytes in the airways is a feature of asthma [33,34]. In the present study we show that an increased recruitment of monocytes to the alveoli occur at an early, sub-clinical phase of allergic airway inflammation.

The airway expression of the cell-bound and soluble form of the myelomonocytic differentiation and activation antigen CD14 [reviewed in 35] has been postulated as an important feature of airway inflammation. The gene encoding CD14 is localized in a region of chromosome 5, also encoding other mediators involved in the pathogenesis of allergic inflammation [36,37]. Furthermore, the CD14

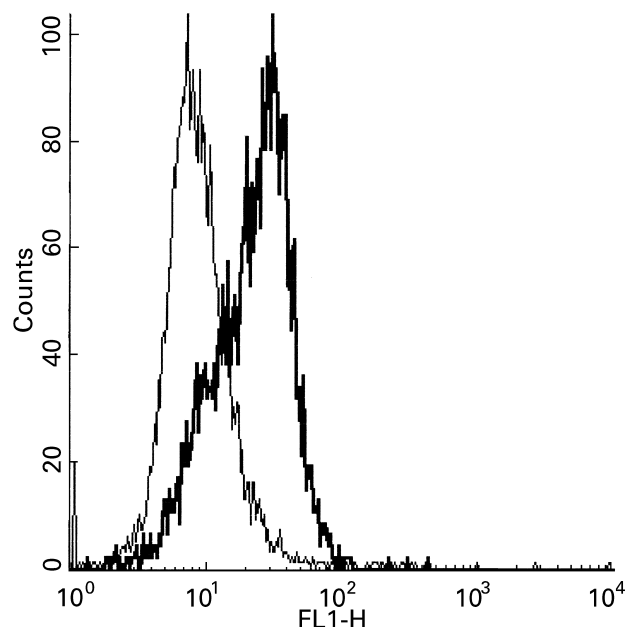


Fig. 3. Flow cytometry profiles of one representative patient demonstrating the low-dose allergen provocation induced increase in CD83 expression in the alveolar macrophage population. The x-axis shows the relative fluorescence on a logarithmic scale and the y-axis shows the number of events

antigen functions as a receptor for lipopolysaccharide (LPS) and LPS binding protein complexes [38] and the binding of LPS to CD14 is known to activate macrophages and induce their expression of different cytokines [39]. We found an increased AM CD14 expression after low-dose allergen provocations, associated positively with BAL fluid total cell counts and proportion of macrophages, and negatively with post-challenge FEV₁ values. These findings suggest an association between AM CD14 expression and degree of inflammation, as well as airway function and further implicate CD14 as a marker for airway inflammation.

One interesting finding in our study is, although analysed in four subjects only, the tendency of an increased expression of CD83 within the AM population. To our knowledge, CD83 expression by alveolar macrophages has not previously been reported. CD83, a member of the immunoglobulin (Ig) super-family, is commonly used as a marker for mature dendritic cells (DCs). Due to its structure and pattern of expression it is believed to serve an important role in antigen presentation or cell interactions [40]. In a study by van den Heuvel *et al.* [41], it was shown that monocytes from atopic subjects are capable of developing into DC with a more potent accessory capacity as compared with monocyte-derived DCs from normal controls. This finding indicates an increased accessory potency of DCs in atopic subjects, already present at monocyte level.

Hypothetically, the increased expression of CD83 we found within the AM population after low-dose allergen provocations, could indicate an increased accessory cell capacity in the airways, potentially contributing to T-cell activation and the development of allergic inflammation.

Additional functional properties of the AMs such as antigen-presenting capacity and cytokine production are currently under investigation.

In conclusion, we have shown that low-dose allergen exposure of individuals with mild, allergic asthma is associated with an allergic airway inflammation and an influx of monocytes to the airways. We found an association between alveolar macrophage CD14 expression and airway cellularity as well as airway function, implicating CD14 as a marker for airway inflammation. Furthermore, we found a post-challenge increase of CD83 expression within the AM population, which hypothetically indicates an increased accessory cell capacity in the airways, potentially contributing to the development and sustenance of airway inflammation in asthma.

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