

CD14 expression and soluble CD14 after segmental allergen provocation in atopic asthma

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CD14 expression and soluble CD14 after segmental allergen provocation in atopic asthma. J.C. Virchow Jr, P. Julius, H. Matthys, C. Kroegel, W. Luttmann. ©ERS Journals Ltd 1998.
ABSTRACT: Allergic asthma is associated with the recruitment of activated inflammatory cells after allergen challenge. Surface expression of CD14 has been proposed as a marker of cell activation and differentiation.

We therefore measured CD14 expression on activated macrophages and granulocytes as well as soluble CD14 (sCD14) concentrations in peripheral blood and bronchoalveolar lavage (BAL) following segmental allergen provocation (SAP) with individually standardized doses of allergen in eight patients with allergic asthma. Two segments of the right lung were challenged with allergen. Two segments of the left lung, into which saline was instilled, served as controls. CD14 expression on macrophages and granulocytes was determined by flow-cytometry and concentrations of interleukins and sCD14 were analysed by enzyme-linked immunosorbent assay (ELISA) 10 min and 18 h after challenge.

Soluble CD14 concentrations remained unchanged in BAL fluid after saline challenge and 10 min after SAP, but increased significantly 18 h after SAP. Although macrophage numbers increased 18h after SAP, CD14 expression on these cells did not change. Unlike macrophages, granulocyte numbers correlated with sCD14 levels 18 h after SAP while their CD14 expression decreased significantly. Furthermore, sCD14 correlated with interleukin (IL)-13 concentrations 18 h after SAP.

An increase in soluble CD14 can be observed 18 h but not 10 min after segmental allergen provocation suggesting local release of this surface antigen. Our findings imply that CD14-mediated cell activation following segmental allergen provocation could play a role in asthmatic inflammation.

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CD14, a 53 kDa surface glycoprotein, has been described as a myelomonocytic differentiation antigen and activation marker on monocytes, macrophages, granulocytes and B-lymphocytes [1, 2]. CD14 serves as a receptor for lipopolysaccharide (LPS) [3] and binding of LPS to CD14 induces macrophage activation with release of cytokines such as interleukin (IL)-6, IL-8 and tumour necrosis factor (TNF- α) [2, 4]. *In vitro* CD14 expression on macrophages and monocytes is enhanced by TNF- α , IL-1, IL-6 [2] and downregulated by IL-4 [5, 6], while results for interferon (IFN)- γ [7, 8] are equivocal. Although CD14 expression on alveolar macrophages has been described in normal as well as diseased lung [9], the *in vivo* regulation of CD14 remains unclear.

In vitro, CD14 is constitutively released from monocytic cell cultures as soluble CD14 (sCD14) [10, 11]. Its levels increase concomitantly with the cell-surface expression of CD14 [2]. Shedding of CD14 is decreased by IL-4 [10, 12]. IL-13, a cytokine with similar features to IL-4 has been shown to downregulate sCD14 release on a transcriptional level [13]. A reduction of sCD14 levels by IFN- γ as reported by LANDMANN *et al.* [10] remains to be confirmed [12]. Addition of LPS and TNF- α to *in vitro* cultures causes a dose-dependent increase in sCD14 [12]

while sCD14 potentiates LPS-induced release of TNF- α and IL-6 from monocytic cells [14].

In vivo elevated levels of sCD14 have been reported in bronchoalveolar lavage (BAL) fluid in pulmonary diseases such as tuberculosis, sarcoidosis, allergic alveolitis and idiopathic pulmonary fibrosis [2, 9, 15–18]. Furthermore, DUBIN *et al.* [19] have recently shown an increase in LPS binding protein and sCD14 24 h following segmental allergen challenge. Several of the cytokines implicated in the regulation of CD14, such as TNF- α and IL-6, are released following CD14-mediated activation of macrophages and have been found in elevated concentrations following segmental allergen provocation (SAP) in allergic asthma [20]. Since activation of macrophages is a feature of asthma [21], we examined CD14 expression on alveolar macrophages and granulocytes as well as sCD14 concentrations in blood and BAL-fluid in sensitized subjects using the model of SAP. Furthermore, concentrations of IL-4, IL-13, and IL-5 which have been implicated in the differential regulation of allergic reactions as well as TNF- α and IFN- γ were measured to elucidate a possible role for these cytokines in the regulation of CD14 expression and sCD14 release *in vivo*.

Materials and methods

Patients

Eight mild, allergic asthmatics, six males, and two females, mean age 27 ± 5 yrs with a duration of asthma of more than 2 yrs (mean duration 9.9 ± 4.6 yrs) were studied. All patients suffered from allergic asthma as defined elsewhere [22]. There was a history of intermittent wheeze, chest tightness, cough and sputum production, either spontaneously or exercise-induced, and a bronchial hyperreactivity as determined by a modified bronchoprovocation test with carbachol [23]. All patients had previously been diagnosed as asthmatic by an independent physician, and all had a positive skin-prick test to either birch pollen ($n=2$), rye pollen ($n=4$), or house-dust mite allergen ($n=2$) extracts (Allergopharma, Reinbek, Germany). Furthermore, they all had elevated total immunoglobulin (Ig)E levels (687.6 ± 868.7 $\text{kU} \cdot \text{L}^{-1}$) as well as specific IgE levels of >0.35 $\text{kU} \cdot \text{L}^{-1}$ (32.1 ± 33.6 $\text{kU} \cdot \text{L}^{-1}$) (Kabi Pharmacia CAP System, Uppsala, Sweden) to their respective allergen as well as a history of reversible bronchoconstriction after inhalation of these particular allergens. Only one patient had a low total IgE level but a clear history of allergen induced bronchoconstriction and an elevated specific IgE concentration. There was no history or clinical evidence suggesting a respiratory tract infection prior to or at the time of the segmental allergen challenge in any of the patients. All patients were nonsmokers. Baseline forced expiratory volume in one second (FEV₁) expressed as a percentage of the predicted value [24] was 92.9 ± 7.4 . All patients received inhaled β_2 -agonist therapy on an as needed basis. Cromoglycate ($n=3$) and inhaled corticosteroids ($n=2$) were withheld for 7 days prior to entry into the study. Further patient characteristics are listed in table 1. All patients gave their written informed consent. The study protocol was approved by the Ethics Committee of the University of Freiburg.

Inhaled allergen provocation

Prior to the segmental allergen challenge, all patients underwent an inhaled allergen challenge to establish dual bronchial reactions and to determine the individual provocative dose of allergen causing a 20% fall in FEV₁ (PD₂₀) for the respective allergen. All allergen preparations used in this study tested negative for LPS in the

limulus amoebocyte lysate assay [25]. Breathing at tidal volume, patients inhaled 2 mL saline from a jet nebulizer (Pari Boy; Pari Werke, Starnberg, Germany) which generated aerosol only during inspiration. The number of breaths required to inhale 2 mL saline was counted individually. Subsequently, each patient was challenged, starting with a 1:10,000 dilution (corresponding to 1 milli biological units (mBU)·mL⁻¹) of the original allergen solution (Abello, Bornheim, Germany) from which a total of 10 tidal volume manoeuvres were inhaled. FEV₁ was recorded after 5, 10 and 20 min. The allergen concentration was then repeatedly increased tenfold until a 20% decrease in FEV₁ was observed. FEV₁ was recorded at 30 min, 60 min and then hourly for 9 h. Patients were included in the study, if a second decline in FEV₁ of $>15\%$ from baseline consistent with a late asthmatic reaction was observed 5–9 h after allergen challenge. The individual PD₂₀ (mBU of allergen) was derived by extrapolation of the relationship between dose of allergen inhaled and FEV₁ to the point where a decline in FEV₁ $>20\%$ was recorded. A tenfold increased dose of allergen was then used for the subsequent SAP. Inhaled allergen challenge and SAP were separated by at least 3 weeks.

Segmental allergen challenge

Bronchoscopy was performed as described previously [20] using an Olympus BF 1T20D bronchoscope (Olympus, Hamburg, Germany) via the oral route. As a control, 2.5 mL of saline was instilled into the inferior lingular bronchus (B5 left). Furthermore, in six patients 2.5 mL of saline was instilled into one segment of the lower left lobe, which was lavaged after 10 min as described below. According to the inhaled allergen provocation, individually standardized doses of allergen (rye pollen, birch pollen or house-dust mite allergen) diluted in 2.5 mL saline were instilled into the medial basal segment of the right lower lobe (B7 right) and into the medial segment of the right middle lobe (B5 right). The right lower lobe bronchus was lavaged 10 min after endoscopic allergen deposition using aliquots of 25 mL which were aspirated using gentle hand suction. The patients were then extubated applying constant suction on the bronchoscope to avoid contamination of the left bronchial system.

After 18 h, the bronchoscope was re-inserted and the left lingular bronchus, into which 2.5 mL diluent had already been instilled, was lavaged; subsequently, the me-

Table 1. – Patient characteristics

Sex	Age yrs	Duration of asthma yrs	FEV ₁ baseline L	FEV ₁ % pred	IVC % pred	IgE $\text{kU} \cdot \text{L}^{-1}$	Allergen	Specific IgE $\text{kU} \cdot \text{L}^{-1}$	Allergen dose mBu	Medication
M	26	13	4.0	100	102	736	Rye	100.0	575	ICS, β
M	23	3	3.7	82	94	173	Rye	26.2	1240	CR, β
M	24	14	4.5	93	91	2000	Rye	18.7	1900	CR, β
M	21	7	4.5	105	93	246	Birch	3.8	1750	CR, β
F	29	12	2.8	93	95	2100	<i>D. pt.</i>	18.0	12630	ICS, β
M	25	9	3.9	92	68	107	<i>D. f.</i>	66.0	14600	β
F	38	5	2.8	93	92	99	Birch	19.6	8200	β
M	29	16	3.0	85	90	39	Rye	4.2	4500	β

FEV₁: forced expiratory volume in one second; % pred: percentage of predicted value; IVC: inspiratory vital capacity; IgE: immunoglobulin E; mBu: milli biological units; *D. pt.*: *Dermatophagoides pteronyssinus*; *D. f.*: *Dermatophagoides farinae*; ICS: inhaled corticosteroid; β : beta-2-agonist *p.r.n.*; CR: cromoglycate; M: male; F: female.

dial segment of the right middle lobe bronchus was identified and lavaged using the same technique as described above.

Analysis of blood and bronchoalveolar lavage leukocyte and lymphocyte subsets

Venous blood was drawn into sterile plastic containers containing ethylenediamine tetra-acetic acid (EDTA) (Sarstedt, Nümbrecht, Germany) prior to the first and second bronchoscopies. Peripheral blood differential cell counts were performed using an automated cell counter from the standard hospital service. Bronchoalveolar lavage (BAL) samples were filtered through a two-layer sterile gauze into sterile plastic vials (Falcon, Oxnard, USA), centrifuged at 4°C and 500×g for 10 min. The supernatant was removed and stored at -70°C. Nucleated cells were differentiated and results expressed as total number of cells per millilitre of recovered fluid. Specific binding of monoclonal antibodies to CD14 was analysed according to standard methods for triple immunofluorescence with a flow-cytometer (FACScan; Becton Dickinson, Heidelberg, Germany). Briefly, erythrocytes were lysed by adding 20 mL of hypotonic saline (NaCl 0.2%) to 3 mL blood for 30 s. Then 20 µL of either whole blood or cells from BAL were incubated in the presence of saturating concentrations of fluorescein-conjugated anti-CD14 monoclonal antibody (Dako, Hamburg, Germany) in the dark on ice for 30 min. The cells were washed twice with phosphate buffered saline (PBS) containing 2% foetal calf serum (FCS). Cytofluorometric analysis were performed on 10,000 cells from each sample by using laser excitation at 488 nm. Nonspecific fluorescence was detected by incubating cells with mouse IgG of the same isotype, but with irrelevant antigen specificity. The specific mean fluorescence (SMF) for each population expressed as fluorescence intensity (FI) was determined by subtracting the nonspecific fluorescence from the mean fluorescence measured with anti-CD14 antibodies.

Determination of sCD14, IFN-γ, TNF-α, IL-4, IL-5 and IL-13

The sCD14 concentrations were measured according to the recommendations of the manufacturer (IBL, Hamburg, Germany). IL-13 was quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) (Bender and Co., Vienna, Austria) according to the manufacturer's instructions. Antibody and standard cytokine for IFN-γ quantification were kindly provided by H. Galati (Hoffmann La Roche, Basel, Switzerland). Briefly, ELISA plates (Maxisorp, Nunc, Denmark) were coated with 5 µg·mL⁻¹ of anti-human IFN-γ antibody in coating buffer (0.1 M NaHCO₃, pH 8.2) over 24 h at room temperature, washed with distilled water and saturated with 1% bovine serum albumin (BSA)/Tris (pH 7.5) for 2 h. Standards and samples (200 µL per well) and 50 µL peroxidase-conjugated monoclonal anti-IFN-γ antibody at a concentration of 0.3 µg·mL⁻¹ were added overnight at 4°C. After thorough washing with distilled water 200 µL tetramethylbenzidine (TMB) solution (20 mM TMB and 50 mM H₂O₂ dissolved in 30 mM citrate buffer, pH 4.1) was added to each well. After 15 min at room temperature, the reaction

was stopped (100 µL of 0.1 M H₂SO₄) and absorption was measured at 450 nm. IL-4, IL-5 and TNF-α were assessed in BAL fluid using monoclonal anti-human cytokine antibodies (Clone: IL-4I, TRFK5, and MAb1; PharMingen, San Diego, CA, USA) as capture-antibody and biotin-conjugated monoclonal anti-human cytokine antibodies (Clone: MP4-25D2, JES1-5A10, and MAb11; PharMingen) in a concentration of 2 µg·mL⁻¹ as described previously [26]. The results were obtained from a standard curve established with human recombinant IL-4 (PBH, Hannover, Germany), IL-5 (kindly provided by H. Galati) and TNF-α (PharMingen). Measurements were performed in duplicate and are expressed as means from both determinations.

Statistical analysis

Results are expressed as arithmetic mean±SD. Differences between groups were analysed using the Wilcoxon matched pairs test. Differences with p-values of less than 0.05 were considered statistically significant. Relationships are expressed using Spearman's rank correlation.

Results

Cellular composition in peripheral blood

The total cell number in peripheral blood increased significantly 18 h after segmental allergen provocation (8,325±2,070 cells·µL⁻¹) compared to baseline (5,937±1,031 cells·µL⁻¹) (p=0.02). This was accompanied by a small but statistically significant increase in the number of eosinophils (254±157 cells·µL⁻¹ at baseline to 343±174 cells·µL⁻¹ 18 h after SAP; p<0.02). There was no significant change in the relative number of neutrophils, monocytes, or eosinophils, while the relative number of lymphocytes decreased significantly from 27.7±4.5 to 21.4±8.0% (p=0.05).

Cellular composition in bronchoalveolar lavage

The total cell numbers in the segments lavaged 10 min after allergen challenge (90.0±47.8 cells·µL⁻¹) and in the control segment lavaged 10 min after saline instillation (107.0±101.9 cells·µL⁻¹) were similar. The total cell number in BAL fluid 18 h after allergen challenge was 413.4±263.8 cells·µL⁻¹, while 260.7±201.0 cells·µL⁻¹ were present in the BAL fluid 18 h after saline instillation, which was not statistically different. The cellular findings are shown in table 2.

CD14 expression on macrophages and granulocytes

There was no obvious difference in the expression of CD14 on macrophages after saline or allergen challenge (300.5±132.1 FI 10 min after saline challenge; 377.1±125.2 FI 10 min after SAP; 358.1±108.9 FI 18 h after saline challenge; 363.1±47.0 FI 18 h after SAP). In contrast, CD14 expression on granulocytes was significantly lower 18 h following segmental allergen provocation (21.9±6.3 FI) as compared to segments lavaged 18 h after saline challenge (56.2±32.9 FI, p<0.02) (fig. 1) while CD14 expression in the control segment lavaged 10 min

Table 2. – Absolute and relative numbers of cells in bronchoalveolar lavage fluid after segmental allergen provocation

	Macrophages	Neutrophils	Eosinophils	Lymphocytes
Absolute number cells· μL^{-1}				
C 10 min	75.4±75.6	0.5±0.7	3.1±5.1	27.2±25.6
C 18 h	202.2±197.3	12.4±13.9	7.8±8.4	29.2±17.9
P 10 min	66.7±45.4	0.8±1.3	2.5±3.6	28.1±35.1
P 18 h	126.3±108.9	39.5±38.2	106.5±134.3	68.7±46.1
Relative number %				
C 10 min	71.2±11.6	0.7±1.0	1.4±2.1	26.7±10.8
C 18 h	69.5±13.8	9.9±9.2	4.6±4.9	16.0±11.7
P 10 min	70.6±16.6	0.6±1.0	2.0±1.5	26.7±16.9
P 18 h	39.0±23.7	9.0±7.3	32.6±20.9	19.5±7.2

Values are presented as mean±SD. Saline challenged control segments were lavaged after 10 min (C 10 min) and after 18 h (C 18 h). Allergen challenged segments were lavaged 10 min after challenge with 10 times the provocative dose of allergen causing forced expiratory volume in one second to fall by 20% (P 10 min) and 18 h after challenge (P 18 h). Statistical comparisons are given in the text.

after saline instillation (59.4±51.8 FI) and the segment lavaged 10 min after allergen challenge (52.7±34.1 FI) was similar to the segment lavaged 18 h after saline. There was no change in the CD14 expression on peripheral blood granulocytes before and 18 h after segmental allergen provocation.

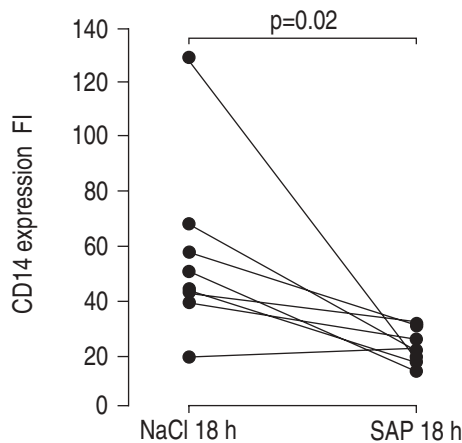


Fig. 1. – Expression of CD14 on granulocytes in bronchoalveolar lavage 18 h after endobronchial saline challenge (NaCl 18 h) and 18 h following segmental allergen provocation (SAP 18 h). FI: fluorescence intensity.

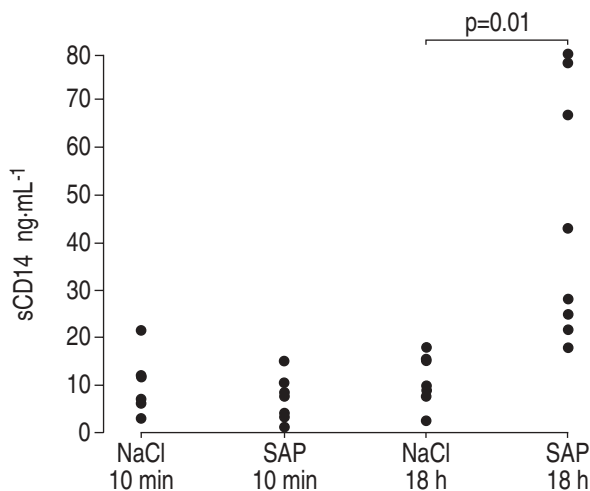


Fig. 2. – Concentrations of soluble CD14 (sCD14) in bronchoalveolar lavage 10 min (NaCl 10 min) and 18 h (NaCl 18 h) after endobronchial saline challenge as well as 10 min (SAP 10 min) and 18 h (SAP 18 h) following segmental allergen provocation.

Soluble CD14

A significantly higher concentration of sCD14 was observed in the allergen challenged segment 18 h after allergen instillation (45.1±26.1 ng·mL⁻¹) as compared to the control segment lavaged 18 h after saline challenge (10.6±5.2 ng·mL⁻¹, p=0.01) while sCD14 concentrations in the segments lavaged 10 min after saline (10.2±6.5 ng·mL⁻¹) and 10 min after allergen challenge (6.3±5.0 ng·mL⁻¹) were similar (fig. 2).

Concentrations of IFN- γ , TNF- α , IL-4, IL-5 and IL-13

IFN- γ concentrations measured at 10 min (45.0±62.8 pg·mL⁻¹) and 18 h (64.8±66.5 pg·mL⁻¹) after allergen challenge and 18 h (64.0±146.0 pg·mL⁻¹) after saline instillation were not significantly different. In contrast, the

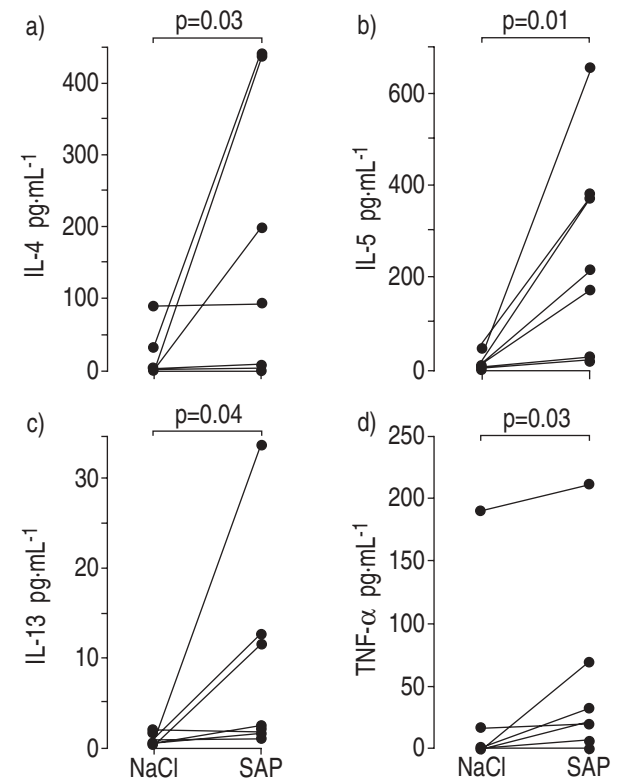


Fig. 3. – Concentrations of: a) interleukin (IL)-4; b) IL-5; c) IL-13; and d) tumour necrosis factor (TNF)- α in bronchoalveolar lavage 18 h (NaCl) following endobronchial saline challenge and 18 h (SAP) after segmental allergen provocation.

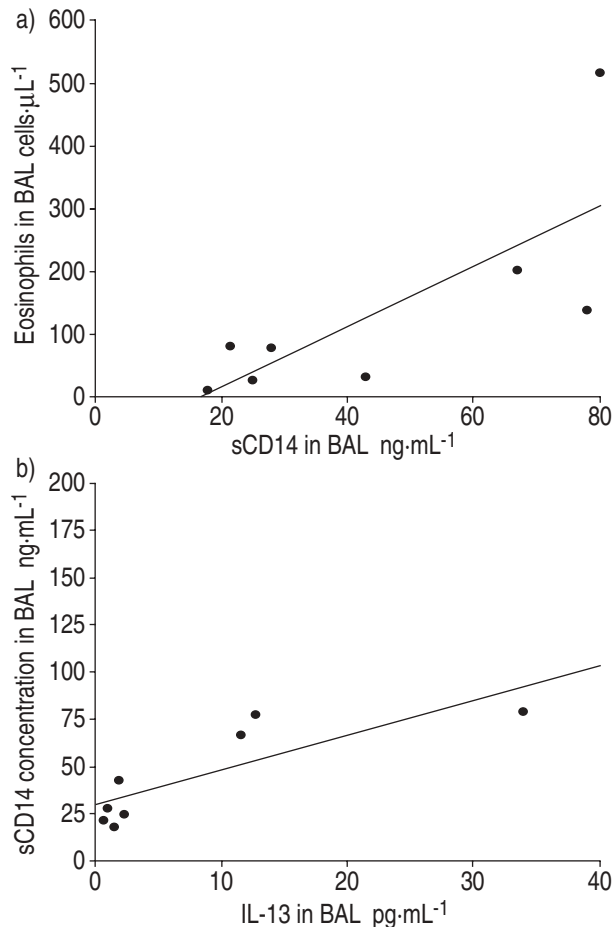


Fig. 4. – Correlation between: a) the number of eosinophils and the concentration of soluble CD14 (sCD14) in bronchoalveolar lavage (BAL) 18 h after segmental allergen provocation ($\rho=0.81$, $p=0.02$); and b) between the concentration of sCD14 and interleukin-13 (IL-13) in BAL 18 h after segmental allergen provocation ($\rho=0.83$, $p=0.01$).

concentrations of TNF- α , IL-4, IL-5, and IL-13 in unconcentrated BAL fluid were significantly higher 18 h after allergen provocation compared to the segments lavaged 18 h after saline challenge (fig. 3).

Correlations

There was no correlation between the expression of CD14 on BAL macrophages and the concentration of sCD14 measured in the BAL fluid 18 h after SAP. However, sCD14 levels measured 18 h after segmental allergen provocation correlated with eosinophil as well as neutrophil numbers (sCD14 *versus* eosinophils: $\rho=0.81$, $p=0.02$) (fig. 4a), (sCD14 *versus* neutrophils: $\rho=0.85$, $p=0.01$). Furthermore, a close correlation was observed between sCD14 and IL-13 18 h following segmental allergen provocation ($\rho=0.83$, $p=0.01$) (fig. 4b). There was no significant correlation between sCD14 and the other analysed cytokines.

Discussion

CD14, a myelomonocytic differentiation antigen, has been described on a variety of cells implicated in the pathogenesis of bronchial asthma. The gene for CD14 maps

to a region in chromosome 5 encoding for several cytokines and receptors related to the pathogenesis of asthma [27, 28]. CD14 mediated binding of LPS and lipopolysaccharide binding protein (LBP) can induce macrophage activation, which has been described as a feature of bronchial asthma [21]. Since inhalation of LPS in patients with asthma can increase bronchial hyperreactivity [29] and domestic endotoxin exposure has been related to asthma severity [30], both CD14 expression and soluble CD14 concentrations could be relevant to the pathogenesis of bronchial asthma.

In this study, using the model of SAP, we observed a substantial increase in the concentration of sCD14 in BAL fluid of allergen challenged segments as compared to sham challenges. Despite a large accompanying increase in macrophages, no significant change in CD14 cell surface expression was observed in this cell population as compared to controls. To our knowledge this is the first report of an elevated CD14 expression on granulocytes (eosinophils and neutrophils) in BAL as compared to CD14 expression on peripheral blood granulocytes in patients with asthma. A number of cytokines present in BAL fluid following segmental allergen provocation [20, 31] can activate eosinophils as well as neutrophils. Interestingly, however, CD14 expression in this cell population decreased following SAP despite a large increase in eosinophil and neutrophil numbers 18 h after allergen provocation. This observed reduction in CD14 expression was accompanied by elevated concentrations of sCD14 18 h following SAP.

Our study extends previous observations of elevated concentrations of sCD14 in various other pulmonary diseases [9, 15]. In fact, mean sCD14 concentrations in BAL of allergic asthmatics 18 h after segmental allergen provocation were similar to those reported for active sarcoidosis [9] and slightly higher than in patients with tuberculosis [9, 15].

Elevated levels of sCD14 following allergen provocation have been detected recently [19]. The authors of this study observed an increase of sCD14 as well as LPS 24 h after SAP. In the study of DUBIN *et al.* [19], low levels of LPS in BAL fluid were insufficient to stimulate cytokine release from macrophages. Following SAP, the increase in sCD14 resulted in functional activity of the LBP-LPS complex, which can bind to sCD14 with subsequent release of cytokines from macrophages [19]. The authors were unable to relate the observed increase in sCD14 to the expression of CD14 on inflammatory cells in BAL fluid obtained 24 h after SAP. Similar to our observation of a correlation between eosinophils and sCD14 levels, a close correlation between the concentration of LBP and the percentage of eosinophils recovered in BAL fluid was observed.

Activated macrophages have been implicated as a source of sCD14 [10]. However, DUBIN *et al.* [19] rejected macrophages as the source of sCD14 and hypothesized that the observed increase in sCD14 in their study must be caused by plasma extravasation following allergen provocation. In contrast to their study, we have used two different time-points for BAL fluid collection following allergen provocation. Our observations of unchanged sCD14 levels in BAL fluid 10 min after allergen provocation strongly suggest that the observed increase in sCD14 must take place between 10 min and 18 h following allergen provocation.

This questions the assumption of DUBIN *et al.* [19] that sCD14 is derived exclusively from the circulation. Ten minutes after allergen provocation a marked swelling of the bronchial mucosa could be observed through the bronchoscope. This reaction has been attributed to increased vascular permeability [32] allowing immediate exudation of proteins with a molecular mass as large as 340 kDa [32]. Albumin, with a molecular mass of 69 kDa, which is slightly larger than that of sCD14 (48–54 kDa), is already present at baseline conditions and increases significantly immediately after allergen challenge [33]. As yet, there is no evidence suggesting that extravasation of sCD14 is regulated differently to other plasma proteins. Therefore, our findings of unchanged sCD14 levels 10 min after segmental allergen challenge but significantly increased concentrations 18 h after allergen provocation would be compatible with the hypothesis that sCD14 could also be produced locally in the bronchoalveolar compartment.

CD14 has been described as a glycosylphosphatidylinositol-anchored protein in neutrophils [34]. To our knowledge, this is the first report addressing the contribution of granulocytes to the local sCD14 pool. Although CD14 expression on granulocytes was significantly lower compared to macrophages, we found a markedly upregulated expression of CD14 on endobronchial granulocytes compared to granulocytes from peripheral blood of the same patients prior to allergen provocation. CD14 expression in this cell population decreased significantly 18 h after segmental allergen provocation. One of the possible explanations for this observation could be shedding of CD14 from the surface of these cells.

Endotoxin-activity has been reported in allergen-extracts used for SAP [35]. Therefore, we cannot fully exclude the contribution of exogenous endotoxins to a number of features observed following segmental or inhaled allergen provocation such as increased IL-2 and TNF- α concentrations, as well as neutrophil infiltration [20, 36]. However, since *in vivo* administration of LPS induces a sharp increase in serum TNF- α at 90 min which returns to baseline within 4 h [37] it appears unlikely that the observed effects of our study are solely related to endotoxin.

IL-13 which is produced locally following SAP [31] has been shown to downregulate CD14 messenger ribonucleic acid (mRNA) *in vitro* [13]. This is in contrast to our observation of a correlation between IL-13 and sCD14 concentrations in BAL fluid 18 h after segmental provocation, challenging the *in vivo* relevance of *in vitro* data [13].

In conclusion, our study is the first to provide evidence about the endobronchial regulation of soluble CD14 over time following segmental allergen provocation. Our results suggest that in addition to macrophages, granulocytes are a possible source of this soluble surface marker which can be measured in markedly elevated concentrations 18 h but not 10 min after segmental allergen challenge. Interleukin-13, which has been implicated in the regulation of CD14 expression *in vitro* [13], might also be related to soluble CD14 levels *in vivo*. The observation of elevated soluble CD14 levels as a feature of the inflammatory response following segmental allergen provocation and their relationship to inflammatory cells implicated in the pathogenesis of asthma suggests that CD14-mediated mechanisms could augment allergic inflammation in the absence of allergen.

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References

- Goyert SM, Ferrero E, Rettig WJ, Yenamandra AK, Obata F, Le Beau MM. The CD14 monocyte differentiation antigen maps to a region encoding growth factors and receptors. *Science* 1988; 239: 497–500.
- Ziegler-Heitbrock HW, Ulevitch RJ. CD14: cell surface receptor and differentiation marker. *Immunol Today* 1993; 14: 121–125.
- Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* 1990; 249: 1431–1433.
- Dentener MA, Bazil V, Von Asmuth EJ, Ceska M, Buurman WA. Involvement of CD14 in lipopolysaccharide-induced tumor necrosis factor- α , IL-6 and IL-8 release by human monocytes and alveolar macrophages. *J Immunol* 1993; 150: 2885–2891.
- Lauener RP, Goyert SM, Geha RS, Vercelli D. Interleukin 4 down-regulates the expression of CD14 in normal human monocytes. *Eur J Immunol* 1990; 20: 2375–2381.
- Ruppert J, Friedrichs D, Xu H, Peters JH. IL-4 decreases the expression of the monocyte differentiation marker CD14, paralleled by an increasing accessory potency. *Immunobiology* 1991; 182: 449–464.
- Firestein GS, Zvaifler NJ. Down regulation of human monocyte differentiation antigens by interferon gamma. *Cell Immunol* 1987; 104: 343–354.
- Ishizuka T, Hirata I, Adachi M, *et al.* Effects of interferon-gamma on cell differentiation and cytokine production of a human monoblast cell line, U937. *Inflammation* 1995; 19: 627–636.
- Striz I, Zheng L, Wang YM, Pokorna H, Bauer PC, Costabel U. Soluble CD14 is increased in bronchoalveolar lavage of active sarcoidosis and correlates with alveolar macrophage membrane-bound CD14. *Am J Respir Crit Care Med* 1995; 151: 544–547.
- Landmann R, Fisscher AK, Obrecht JP. Interferon-gamma and interleukin-4 down-regulate soluble CD14 release in human monocytes and macrophages. *J Leukoc Biol* 1992; 52: 323–330.
- Bufler P, Stiegler G, Schuchmann M, *et al.* Soluble lipopolysaccharide receptor (CD14) is released *via* two different mechanisms from human monocytes and CD14 transfectants. *Eur J Immunol* 1995; 25: 604–610.
- Schutt C, Schilling T, Grunwald U, Schonfeld W, Kruger C. Endotoxin-neutralizing capacity of soluble CD14. *Res Immunol* 1992; 143: 71–78.
- Cosentino G, Soprana E, Thienes CP, Siccardi AG, Viale G, Vercelli D. IL-13 down-regulates CD14 expression and TNF- α secretion in normal human monocytes. *J Immunol* 1995; 155: 3145–3151.
- Labeta MO, Durieux JJ, Fernandez N, Herrmann R, Ferrara P. Release from a human monocyte-like cell line of two different soluble forms of the lipopolysaccharide receptor, CD14. *Eur J Immunol* 1993; 23: 2144–2151.
- Hoheisel G, Zheng L, Teschler H, Striz I, Costabel U. Increased soluble CD14 levels in BAL fluid in pulmonary tuberculosis. *Chest* 1995; 108: 1614–1616.
- Martin TR, Rubinfeld G, Steinberg KP, *et al.* Endotoxin, endotoxin-binding protein, and soluble CD14 are present

- in bronchoalveolar lavage fluid of patients with adult respiratory distress syndrome. *Chest* 1994; 105: 55S–56S.
17. Pforte A, Schiessler A, Gais P, *et al.* Increased expression of the monocyte differentiation antigen CD14 in extrinsic allergic alveolitis. *Monaldi Arch Chest Dis* 1993; 48: 607–612.
 18. Pforte A, Schiessler A, Gais P, *et al.* Expression of CD14 correlates with lung function impairment in pulmonary sarcoidosis. *Chest* 1994; 105: 349–354.
 19. Dubin W, Martin TR, Swoveland P, *et al.* Bronchoalveolar lavage, segmental allergen challenge, macrophage, interleukin-6, endothelial cell, asthma and endotoxin-lipopolysaccharide binding protein and soluble CD14 in bronchoalveolar compartment. *Am J Physiol Lung Cell Mol Physiol* 1996; 14: L736–L744.
 20. Virchow JC Jr, Walker C, Hafner D, *et al.* T-cells and cytokines in bronchoalveolar lavage fluid after segmental allergen provocation in atopic asthma. *Am J Respir Crit Care Med* 1995; 151: 960–968.
 21. Chanez P, Bousquet J, Couret I, *et al.* Increased numbers of hypodense alveolar macrophages in patients with bronchial asthma. *Am Rev Respir Dis* 1991; 144: 923–930.
 22. Anonymous. International consensus report on diagnosis and treatment of asthma. National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland, USA. *Eur Respir J* 1992; 5: 601–641.
 23. Cockcroft DW, Killian DN, Mellon JJ, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977; 7: 235–243.
 24. Ruhle KH, Matthys H. Kritische Auswahl von Sollwerten für ein Computerprogramm zur Routinelungenfunktionsdiagnostik. *Pneumologie* 1976; 153: 223–233.
 25. Warren HS, Glennon ML, Wainwright N, *et al.* Binding and neutralization of endotoxin by Limulus antilipopolysaccharide factor. *Infect Immun* 1992; 60: 2506–2513.
 26. Luttmann W, Herzog V, Virchow JC Jr, Matthys H, Thierauch KH, Kroegel C. Prostacyclin modulates granulocyte/macrophage colony-stimulating factor release by human blood mononuclear cells. *Pulmonary Pharmacology* 1996; 9: 43–48.
 27. Zhang DE, Hetherington CJ, Tan S, *et al.* Sp1 is a critical factor for the monocytic specific expression of human CD14. *J Biol Chem* 1994; 269: 11425–11434.
 28. Postma DS, Bleecker ER, Amelung PJ, *et al.* Genetic susceptibility to asthma, bronchial hyperresponsiveness co-inherited with a major gene for atopy. *N Engl J Med* 1995; 333: 894–900.
 29. Michel O, Duchateau J, Sergysels R. Effect of inhaled endotoxin on bronchial reactivity in asthmatic and normal subjects. *J Appl Physiol* 1989; 66: 1059–1064.
 30. Michel O, Ginanni R, Duchateau J, Vertongen F, Le Bon B, Sergysels R. Domestic endotoxin exposure and clinical severity of asthma. *Clin Exp Allergy* 1991; 21: 441–448.
 31. Kroegel C, Julius P, Matthys H, Virchow JC Jr, Luttmann W. Endobronchial secretion of interleukin-13 following local allergen challenge in atopic asthma: relationship to interleukin-4 and eosinophil counts. *Eur Respir J* 1996; 9: 899–904.
 32. Salomonsson P, Gronneberg R, Gilljam H, *et al.* Bronchial exudation of bulk plasma at allergen challenge in allergic asthma. *Am Rev Respir Dis* 1992; 146: 1535–1542.
 33. Fick RB Jr, Metzger WJ, Richerson HB, *et al.* Increased bronchovascular permeability after allergen exposure in sensitive asthmatics. *J Appl Physiol* 1987; 63: 1147–1155.
 34. Maciejewski JP, Young NS, Yu M, Anderson SM, Sloand EM. Analysis of the expression of glycosylphosphatidylinositol anchored proteins on platelets from patients with paroxysmal nocturnal hemoglobinuria. *Thrombosis Research* 1996; 83: 433–447.
 35. Hunt LW, Gleich GJ, Ohnishi T, *et al.* Endotoxin contamination causes neutrophilia following pulmonary allergen challenge. *Am J Respir Crit Care Med* 1994; 149: 1471–1475.
 36. Robinson DS, Hamid Q, Ying S, *et al.* Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992; 326: 298–304.
 37. Michie HR, Manogue KR, Spriggs DR, *et al.* Detection of circulating tumor necrosis factor after endotoxin administration. *N Engl J Med* 1988; 318: 1481–1486.