

sCD14 in Bronchoalveolar Lavage 18, 42 and 162 Hours After Segmental Allergen Provocation

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Abstract

Lipopolysaccharides (LPS) have been associated with a protective role in the development of asthma while higher levels of endotoxin have been linked with more severe asthma. LPS recruit neutrophils and eosinophils and activate macrophages via the CD14 receptor. The soluble CD 14 receptor (sCD14) has been found in bronchoalveolar lavage fluid in different diseases including allergic asthma. To elucidate the kinetics and the regulation of sCD14 concentrations in BAL in asthma, 18 patients with allergic asthma underwent segmental allergen challenge at different time points (10 min, 18, 42 and 162 h). In addition, CD14⁺ peripheral blood mononuclear cell (PBMC-CD14⁺) cultures from seven allergic and seven non-allergic subjects were stimulated with LPS, leukotrien D₄ (LTD₄), a combination of LPS and LTD₄, IL-17 and LTD₄ in presence of the leukotriene-receptor antagonist (LTRA) Montelukast for 6, 12 and 24 h. sCD14 concentrations in BAL and the supernatants were measured by ELISA. sCD14 concentrations in BAL were significantly increased 18 h after allergen challenge and peaked at 42 h. At 162 h, concentrations had returned to baseline levels. In PBMC-CD14⁺ cultures, sCD14 levels increased significantly 24 h after stimulation with LTD₄ and Montelukast was able to block LTD₄-induced stimulation. Allergen challenge leads to a significant increase in sCD14 concentrations in BAL and might modulate the allergen-induced inflammation. In addition, LTD₄ might play a role in the release of sCD14, and it could be speculated that sCD14 reduction by LTRA might contribute to the mechanisms of LTRA in the treatment of allergic asthma.

Introduction

Endotoxins have been implicated in the pathogenesis of asthma. Following the 'hygiene hypothesis', endotoxins might even have a protective role in the development of allergic asthma [1] and endotoxin exposure at home has been associated with a reduced prevalence of atopy [2]. The severity of asthma has also been shown to depend on endotoxin concentrations in the microenvironment [3], and recently, it has been suggested that this might depend on an individual's genetic background [4]. Some of the mechanisms by that endotoxin can mediate its effects include neutrophil and eosinophil recruitment as well as the activation of macrophages [3, 5, 6]. Chemically, endotoxins consist of lipopolysaccharides (LPS) that exert their effects via the CD14 receptor, a 53-kDa sur-

face glycoprotein [7] expressed on monocytes, macrophages, granulocytes and B lymphocytes [5, 6]. The molecular interactions underlying the binding of LPS have been extensively studied in recent years. Accordingly, LPS-binding protein (LBP) facilitates the binding of LPS in combination with CD14 to a receptor complex, which consists of Toll-like receptor-4 (TLR-4) and MD-2 [8–10]. The activation of the TLR induces an intracellular signalling cascade, which results in the release of cytokines such as interleukin (IL)-6, IL-8 and tumour necrosis factor (TNF)- α [6, 11] which have also been shown in elevated concentrations in asthma [12–14]. *In vitro*, CD14 is constitutively released from mononuclear cell cultures as soluble CD14 (sCD14) [15, 16]. sCD14 can be found in two isoforms, a 49- and a 55-kDa protein. The 55-kDa isoform is produced by a

shedding mechanism while the 49-kDa form is thought to derive from the interstitial space [16, 17]. The 49-kDa isoform is found in healthy subjects and is significantly elevated in patients with sepsis [18], polytrauma [19] and atopic dermatitis [20]. Shedding is increased by LPS and TNF- α *in vitro* [21] and also *in vivo* [22]. The function of sCD14 has been associated with the activation of cells which do not possess membrane-bound CD14 [8].

Elevated levels of sCD14 have been found in bronchoalveolar lavage in several diseases such as tuberculosis, sarcoidosis, allergic alveolitis and idiopathic pulmonary fibrosis [6, 23–27]. sCD14 also seems to play a role in allergic asthma. Dubin *et al.* [28] showed an increase in sCD14 in bronchoalveolar lavage fluid (BALF) 24 h after allergen provocation which was confirmed by others [29]. Increased concentrations were also found in children with status asthmaticus [30]. In addition, CD14 expression has been correlated to the influx of neutrophils into the airways [22]. It has been suggested that this might be related to a remodelling processes in the airways as has been shown in an animal model with endotoxin-sensitive mice [31]. Moreover, distinct gene-polymorphisms of the C14 gene have been associated with an increased risk to develop an atopic phenotype [32]. It can therefore be hypothesized that an elevated expression of the LPS receptor might be involved in the activation of the inflammatory cascade in asthma which could lead to chronic inflammation, remodelling of the airways and subsequently an accelerated loss in FEV1. Alternatively, it has been argued that sCD14 could bind to and inactivate LPS in a protective manner [33].

Our study was undertaken to establish the kinetics of sCD14 concentrations in BALF in patients with allergic asthma following segmental allergen challenge at different time points (10 min, 18, 42 and 162 h). Moreover, the study attempted to establish stimuli involved in sCD14 production and/or shedding, respectively, such as LPS, which has been shown to increase sCD14 levels *in vitro* [21] and *in vivo* [22] and leukotriene D₄ (LTD₄), which has been implicated in allergic inflammation and the pathogenesis of airway hyperresponsiveness [34]. Furthermore, LTD₄ has been shown to induce TNF- α release from macrophages [35] that was inhibited by the leukotriene-receptor antagonist (LTRA) Verlukast [35].

IL-17 has been associated with an increase in IL-8 and GM-CSF production from bronchial epithelial cells [36], the regulation of ICAM-1 expression [37] and the selective recruitment of neutrophils [38]. Moreover, it plays a role in the LPS-induced chemotaxis of neutrophils into the airways after LPS inhalation [39] and increases after organic dust inhalation in healthy subjects [40]. We therefore investigated whether IL-17 might influence sCD14 production in cell cultures.

Materials and methods

Patients

Eighteen patients with allergic asthma (nine men and nine women), mean age 26.3 ± 5.4 years with a duration of asthma for more than 2 years (mean duration 11.7 ± 5.2 years), were studied. Bronchial asthma had previously been diagnosed by an independent physician. Each patient had a positive skin prick test to either birch pollen ($n = 8$), rye pollen ($n = 3$), or house dust mite allergen ($n = 7$) extracts (Allergopharma, Reinbek, Germany), and all had either elevated total IgE levels (293.6 ± 233.1 kU/l) and / or elevated specific IgE levels (32.2 ± 49.1 kU/l) (Kabi Pharmacia CAP System, Uppsala, Sweden) to their respective allergen as well as a history of reversible bronchoconstriction after inhalation of these particular allergens. There was no history or clinical evidence in any of the patients, suggesting a respiratory tract infection prior to or at the time of segmental allergen challenge. All patients were non-smokers. Baseline FEV₁ (forced expiratory volume in 1 s) was 3.8 ± 0.96 l ($94.9 \pm 13.1\%$ of predicted, normal values [41]). All patients received inhaled β_2 -agonist therapy on an as needed basis except for three patients who did not require any medication. In addition, five patients had inhaled corticosteroids and three had inhaled cromoglycate. Both were withheld at least 10 days prior to entry into the study. All patients gave their written informed consent. The study protocol was approved by the local Ethics Committee.

Inhaled allergen provocation

Prior to the segmental allergen provocation, patients underwent an inhaled allergen challenge as previously described [29, 42] to establish dual bronchial reactions to the inhaled allergen and to determine the individual PD_{20FEV1} for the respective allergen. All allergen preparations tested negative for LPS in the Limulus amoebocyte lysate assay [43]. The individual PD_{20FEV1} $\times 10$ was then used for the subsequent Segmental Allergen Provocation (SAP). Inhaled and segmental allergen challenges were separated by at least 4 weeks.

Segmental allergen challenge

Bronchoscopy was performed as previously described [29, 42]. A volume of 2.5 ml of 0.9% saline was instilled into the anterior basal segment of the left lower lobe (B8 left) and one of the segments of the lingula (B4 or B5 left). Allergen diluted in 2.5 ml of saline was instilled into the anterior basal segment of the right lower lobe (B8 right) and the medial or lateral segment of the right

middle lobe (B4 or B5 right). After 10 min, bronchoalveolar lavage was performed in the anterior basal segments of the right and left lower lobes. Patients were re-bronchoscope at different time points: In the first group, the second lavage was performed after 18 h in segments B4 or B5 right and left. Some of these patients also participated in the second part of the trial. This second group was lavaged 10 min and 42 h after segmental allergen challenge (Table 1). In the third arm of the trial, four patients were lavaged 10 min and 162 h after allergen challenge. In patients who participated repeatedly the segmental allergen challenges were separated by at least six months. In all patients, peripheral blood was taken before bronchoscopy.

PBMC-CD14⁺ cultures

From seven healthy subjects and seven patients with allergic asthma, 250-ml whole blood was drawn and mixed well with heparin. Cell subtypes were separated by

Ficoll centrifugation. PBMC-CD14⁺ were harvested, and after washing and counting monocytes were separated via immunomagnetic separation by AutoMACS system (Miltenyi Biotec GmbH, Germany) after labelling with CD14 antibody (Miltenyi Biotec GmbH). CD14⁺ monocytes were washed; purification was controlled by flow-cytometry (94–98% purified monocytes, contamination with lymphocytes was <2%), and 5×10^5 cells per well were cultured in 1 ml RPMI 1640 medium (GIBCO, Paisley, Scotland, UK) + 10% FCS (Seromed, Berlin, Germany) + 1% penicillin/streptomycin (Biochrom AG, Berlin, Germany) at 37 °C and 5% CO₂. Cells differentiated to macrophages in about 5 days. Medium was exchanged every 2 days.

Cellular stimulation

The above-mentioned PBMC-CD14⁺ cells (5×10^5 cells in 1 ml) were stimulated with either human IL-17 (50 ng/ml), LPS (10 ng/ml), leukotriene D₄ (LTD₄)

Table 1 Patient characteristics.

No	Age	Gender	Asthma duration	FEV ₁ (l)	FEV _{1%} pred.	Allergen	Total IgE (kU/l)	Spec. IgE	Allergen dose (AU)	Medication	2nd BAL (h after SAP)
1	33	F	18	3.08	87	Birch	71.8	6.19	160	β ₂	18
2	27	F	17	2.92	84	Birch	199	7.8	2.5	ICS, β ₂	18
4	29	M	21	5.1	107	Birch	229	5.7	10	ICS, β ₂	18
5	26	M	19	3.68	87	Birch	314	4.8	526	β ₂	18
6	27	M	11	5.72	116	Rye	986	>100	100	β ₂	18
7	23	M	10	6.24	115	D. pter.	234	26	4700	CR, β ₂	18
8	25	F	10	3.08	86.9	D. pter.	483	>100	1	ICS, β ₂	18
1	33	F	18	3.08	87	Birch	95.9	71.8	160	β ₂	18
9	26	M	8	3.54	75	Birch	204	21.1	2220	CR	18
10	18	F	6	3.2	110	D. pter.	247	>100	650	ICS, β ₂	18
11	24	F	14	3.3	106	D. pter.	551	>100	2650	ICS, β ₂	18
8	26	F	11	2.8	79.1	D. pter.	483	>100	35	ICS, β ₂	18
12	25	M	11	3.72	86.3	Rye	282	171	1.8	CR, β ₂	18
13	43	F	10	2.96	102.8	Birch	65.1	64.4	775	β ₂	18
1	34	F	19	3.08	87	Birch	95.9	32.3	160	β ₂	42
4	30	M	22	5.1	107	Birch	229	5.7	10	ICS, β ₂	42
6	28	M	12	5.72	116	Rye	986	>100	100	β ₂	42
11	24	F	14	3.3	106	D. pter.	715	>100	265	ICS, β ₂	42
9	27	M	9	3.54	75	Birch	204	21.1	2250	CR	42
10	18	F	6	3.2	110	D. pter.	247	>100	6500	ICS, β ₂	42
14	20	M	8	3.97	92	Birch	-	-	6950	β ₂	42
11	25	F	15	3.2	103	D. pter.	746	>100	2650	ICS, β ₂	42
15	25	F	5	3.4	107	Birch	221	18.4	12.9	No	42
13	44	F	11	2.96	102	Birch	139	64.4	775	β ₂	42
4	30	M	22	4.48	96.5	Birch	242	24.1	112.5	ICS, β ₂	42
16	23	F	2	3.09	100	D. pter.	264	>100	190	No	42
10	19	F	7	3.16	110	D. pter.	114	>100	65	ICS, β ₂	42
17	29	M	14	3.43	78	D. pter.	64.6	19.7	1000	No	42
1	34	F	19	3.12	90	Birch	90.8	16.8	160	β ₂	42
18	25	F	16	4.14	84	Rye	512	>100	127	β ₂	162
4	32	M	24	4.48	96.5	Birch	264	24.1	112.5	ICS, β ₂	162
16	24	F	3	3.09	100	D. pter.	264	>100	190	No	162
20	25	M	10	3.6	83.5	D. pter.	64.2	9.85	1000	β ₂	162

F = Female, M = Male, specific IgE (kU/l), Allergen dose in Allergen Units (AU), BAL after 10 min each and 18, 42 and 162 h, respectively; ICS = inhaled corticosteroids, β₂ = inhaled beta-2-agonist, CR = chromoglycate, No = no medication.

(10^{-11} M) or a combination of LPS and LTD₄ for a duration of 6, 12 and 24 h.

Cells were also stimulated with LTD₄ in the presence of the leukotriene antagonist Montelukast. LTD₄ was added to cell cultures 30 min after stimulation with Montelukast (10^{-11} M), and cultures were incubated for 6, 12 and 24 h.

Cell culture supernatants were stored at -20°C until sCD14 measurement with an ELISA kit (IBL Hamburg, Germany) according to the manufacturer's instructions.

Statistical analysis

Data were analysed by SPSS software package. Results are reported as median (range) or as single values and median (Figs. 2–5). Differences between groups were analysed using the Mann–Whitney *U*-test or the Wilcoxon signed ranks test. *P*-values ≤ 0.05 were considered statistically significant. Potential correlations were analysed by Spearman's rank correlation coefficient.

Results

sCD14 in BALF 10 min, 18, 42 and 162 h after SAP

sCD14-concentrations in BAL fluid were significantly elevated 18 and 42 h after allergen challenge, compared to the control segment at 10 min, 18 and 42 h, respectively, as well as to the segment 10 min after allergen provocation (Fig. 1). sCD14 levels reached baseline levels 162 h after allergen provocation.

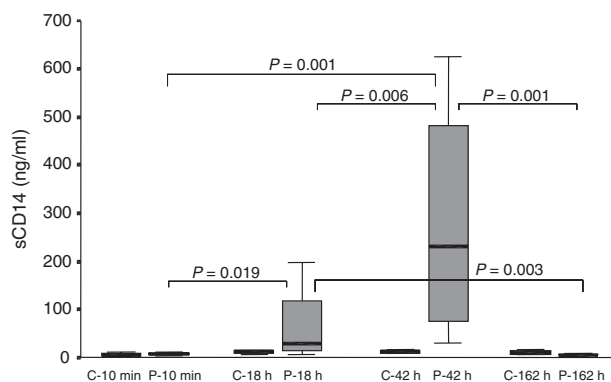


Figure 1 sCD14 levels in BAL 10 min, 18, 42 and 162 h after allergen provocation. sCD14 was measured in BAL by ELISA 10 min ($n = 14$), 18 h ($n = 14$), 42 h ($n = 15$) and 162 h ($n = 4$) after allergen provocation. Data are represented non-parametric in box plots showing the median (line within the box), interquartile range (edges of the box) and the range of values less distant than 1.5 interquartile ranges from the upper or lower quartile (vertical lines). C = control segment stimulated with NaCl; P = allergen provocation; different *P*-values are given in the figure (Mann–Whitney *U*-test).

sCD14 in peripheral blood before and 18, 42 and 162 h after SAP

Peripheral blood samples were drawn as in Fig. 1. Median sCD14 concentrations in peripheral blood were 6709 ng/ml (range 9528) before SAP ($n = 33$) and 6985 ng/ml (range 15862) after SAP ($n = 32$). There was no statistically significant difference between sCD14 values in peripheral blood before and 18, 42 or 162 h after allergen challenge (data not shown).

sCD14 levels in PBMC-CD14⁺ cell cultures 6, 12 and 24 h after stimulation with LTD₄, LPS, the combination of LPS ± LTD₄ and IL-17

PBMC-CD14⁺ of healthy subjects ($n = 7$) and patients with allergic asthma ($n = 7$) were stimulated with LPS (10 ng/ml), LTD₄ (10^{-11} M) or a combination of LPS + LTD₄ (10 ng/ml + 10^{-11} M), for 6, 12 and 24 h, and sCD14 levels in the supernatant were measured at the different time points.

sCD14 levels increased significantly 24 h after stimulation with LTD₄ in comparison to control, 6 and 12 h after stimulation ($P < 0.02$, Wilcoxon signed ranks test – Fig. 2). PBMC-CD14⁺ cells from healthy volunteers and patients with allergic asthma reacted similarly.

Stimulation of PBMC-CD14⁺ cells with LPS leads to increased sCD14 levels but failed to reach statistical significance in comparison to control (Fig. 3). Similar results were seen when cells were stimulated with the combination of LPS and LTD₄.

Similarly, PBMC-CD14⁺ cells were stimulated with IL-17 (50 ng/ml), and supernatants were measured for sCD14 6, 12 and 24 h after stimulation (Fig. 4). However, sCD14 levels were not different to control.

Co-stimulation of LTD₄ with Montelukast

PBMC-CD14⁺ cells of two healthy volunteers and four patients with allergic asthma were stimulated with LTD₄ (Fig. 5) which lead to a significant increase in sCD14 levels (median 57.1 ng/ml, range 92.4) compared to control (median 43.2 ng/ml, range 73.0; $P = 0.028$, Wilcoxon signed ranks test). Addition of Montelukast to LTD₄ stimulation resulted in reduced sCD14 levels after 24 h compared to LTD₄ stimulation (median 38.1 ng/ml, range 93.5; $P = 0.028$, Wilcoxon signed ranks test).

Discussion

The soluble LPS ligand sCD14 has been shown in increased concentrations at 18 and 24 h after segmental allergen challenge in patients with allergic asthma [28, 29]. In this study, we were able to expand this with kinetic data showing a further, approximately, 10-fold

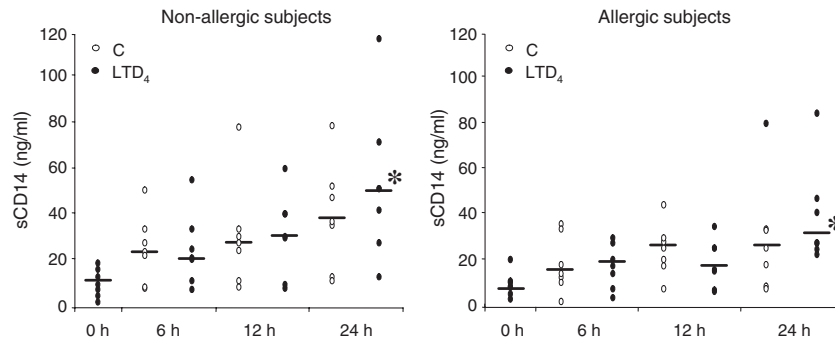


Figure 2 sCD14 levels in PBMC-CD14⁺ cultures 0, 6, 12 and 24 h after stimulation with LTD₄. sCD14 levels were measured in PBMC-CD14⁺ cultures before (0 h), 6, 12 and 24 h after stimulation with LTD₄ in seven allergic and seven non-allergic subjects. 0 h: before stimulation. 24 h after stimulation with LTD₄, sCD14 concentrations were significantly elevated in allergic and non-allergic subjects compared to 6 and 12 h. * $P < 0.03$ for LTD₄ 24 h compared to C 6 h, C 12 h, C 24 h as well as LTD₄ 6 h and LTD₄ 12 h in allergic and non-allergic subjects (Wilcoxon signed ranks test). Open dots represent unstimulated cells (control), filled dots represent LTD₄-stimulated cells. Lines represent the median.

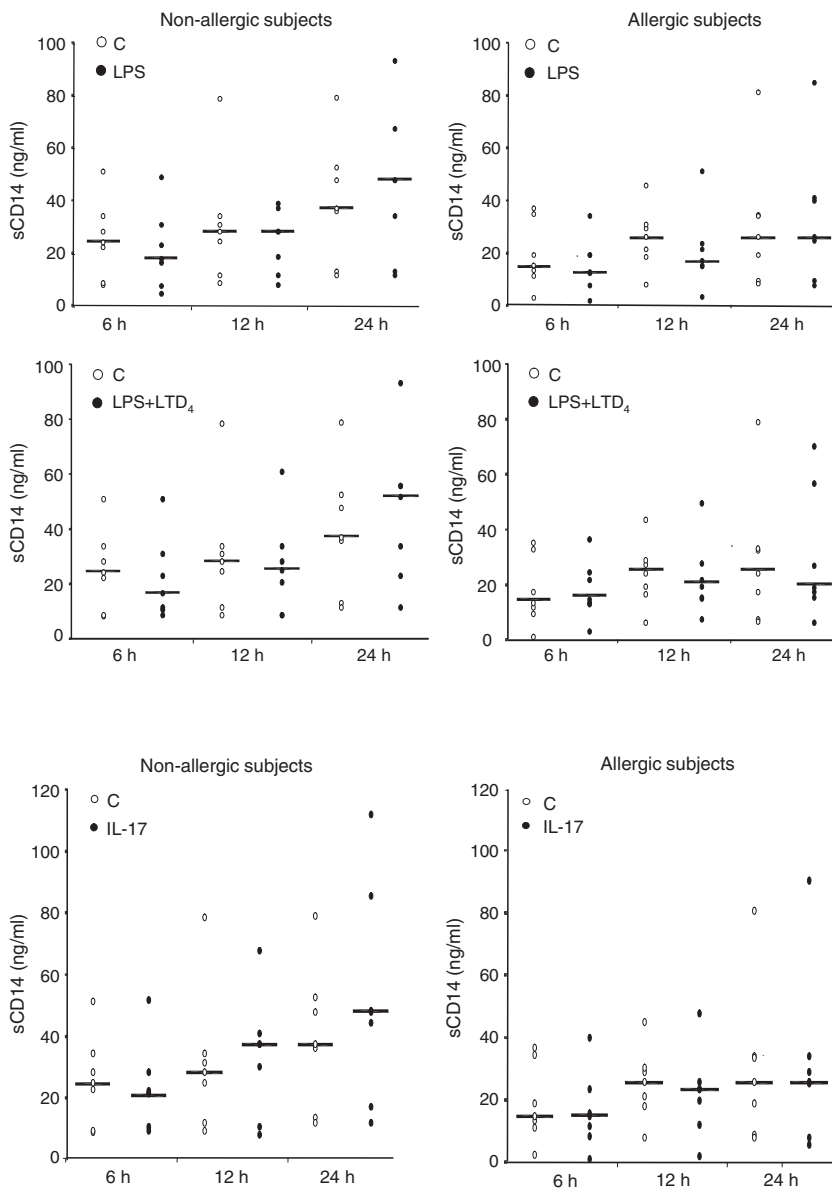


Figure 3 sCD14 levels in PBMC-CD14⁺ cultures 6, 12 and 24 h after stimulation with LPS and LPS + LTD₄. sCD14 levels were measured in PBMC-CD14⁺ cultures 6, 12 and 24 h after stimulation with LPS and the combination of LPS and LTD₄ in seven allergic and seven non-allergic subjects. $P > 0.05$. Open dots represent unstimulated cells (control); filled dots represent LPS- or LPS + LTD₄-stimulated cells. Lines represent the median.

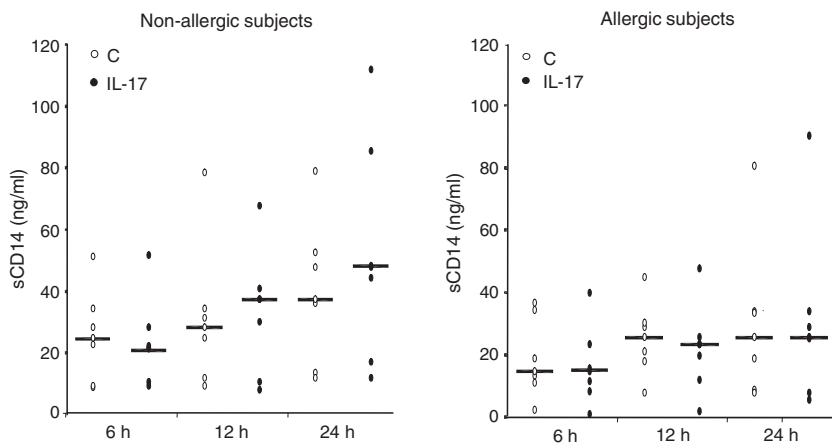


Figure 4 sCD14 levels in PBMC-CD14⁺ cultures 6, 12 and 24 h after stimulation with IL-17. sCD14 levels were measured in PBMC-CD14⁺ cultures 6, 12 and 24 h after stimulation with IL-17 in seven allergic and seven non-allergic subjects. $P > 0.05$. Open dots represent unstimulated cells (control); filled dots represent IL-17-stimulated cells. Lines represent the median.

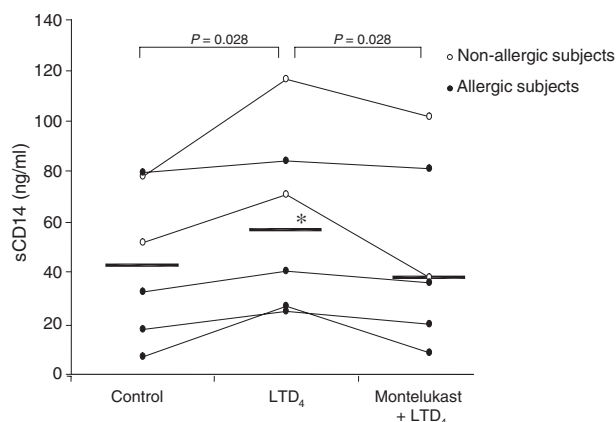


Figure 5 sCD14 levels in PBMC-CD14⁺ cultures 24 h after stimulation with LTD₄ and LTD₄ in the presence of Montelukast. sCD14 levels were measured in PBMC-CD14⁺ cultures 24 h after stimulation with LTD₄ and LTD₄ in the presence of Montelukast in four allergic (closed dots) and two non-allergic subjects (open dots). $P = 0.028$ for LTD₄ compared to control and to LTD₄ + Montelukast (Wilcoxon signed ranks test). Lines represent the median.

increase in sCD14 concentrations 42 h after allergen challenge compared to control segments. Interestingly, sCD14 levels returned to baseline within 7 days after allergen challenge. Nevertheless, the exact time point at which sCD14 concentration in BALF reach their maximum remains unknown as the maximal concentration might be before or after the 42 h at which BAL was performed following allergen challenge. *In vitro* experiments support the hypothesis that the maximum influx of sCD14 might be around that time, because Landmann *et al.* have also shown a significant increase in sCD14 production in cell cultures 44 h after stimulation with LPS [44].

As expected, sCD14 concentrations differed markedly interindividually. While some subjects reacted with a large increase in sCD14 into the bronchoalveolar space at 18 and 42 h after allergen challenge, others had only minor increases. Whether this is owing to interindividual CD14 polymorphisms that have been shown to influence serum levels [45] or whether this merely reflects interindividual variability remains unclear. There was no correlation between sCD14 concentrations and lung function or the type or dose of allergen used for challenge. Similarly, no correlation was found regarding sCD14 levels in BAL and IgE levels in blood (data not shown) as has been demonstrated by others [46]. sCD14 levels in BAL and in PBMC-CD14⁺ cultures were lower than sCD14 measured in peripheral blood. This could be because of a methodical influence of the BAL procedure where 100 ml of normal saline are used which dilute bronchoalveolar lining fluids, and the measured concentrations of sCD14 might therefore also be diluted. sCD14 levels in peripheral blood were in the range of sCD14 measured in other

studies [47] but tended to be higher than those measured in cord and peripheral blood from allergic and non-allergic asthmatic children [30, 48]. Lundell *et al.* also found a trend for lower sCD14 levels in peripheral blood of children developing allergies compared to healthy controls [48]. In our study, we also observed a slight trend towards lower sCD14 levels in allergic subjects compared to non-allergic controls (Figs. 2–4) but this did not reach statistical significance.

The predominant isoform of sCD14 in BALF is the 49-kDa isoform, which is produced intracellularly in mononuclear cells and secreted [28]. Therefore, sCD14 is produced locally in the bronchi.

Recently, sCD14 has been discussed as an acute-phase protein, and sCD14 levels were correlated to CRP levels in patients with bacterial and non-bacterial inflammation [49]. Also, it is known that CRP and lipopolysaccharide-binding protein are elevated in peripheral blood serum after allergen challenge [28]. Therefore, it could be speculated that endobronchial sCD14 is also a parameter for asthmatic inflammation.

To elucidate potential mechanisms that might contribute to sCD14 increase in allergic asthma, PBMC-CD14⁺ cultures were stimulated with LPS, LPS + LTD₄, LTD₄ and IL-17. IL-17 is a cytokine that mediates the LPS-induced accumulation of neutrophils in the respiratory tract [39], and incubation of PBMC-CD14⁺ cultures with IL-17 results in an increase in IL-6, IL-10, IL-12 and TNF- α production [50]. In our study, stimulation with IL-17 *in vitro*, however, had no effect on sCD14 levels in PBMC-CD14⁺ cultures.

On the other hand, incubation with LTD₄, a proinflammatory mediator which has been implicated in the pathophysiology of the asthmatic response, leads to a significant increase in sCD14 production from PBMC-CD14⁺ cultures after 24 h. LTD₄ is known to prime alveolar macrophages to produce mediators such as MIP-1 α , TNF and NO when stimulated with LPS [35]. In our study, sCD14 production in PBMC-CD14⁺ cultures was blocked by the co-incubation of LTD₄ with the LTRA Montelukast. This observation supports the hypothesis that LTRAs could exert some of their anti-inflammatory effects by inhibiting LPS-induced augmentation of the asthmatic inflammation. This is further supported by previous reports where the LTRA pranlukast was able to suppress NF- κ activation, an intracellular signalling pathway which is also activated by LPS in human monocytes/macrophages as well as by Tcells [51].

In our study, there was a trend towards an increase in sCD14 production in PBMC-CD14⁺ cultures following stimulation with LPS and the combination of LPS and LTD₄ that, however, failed to reach statistical significance, possibly as a result of a relatively short stimulation interval, as *in vivo* the maximal sCD14 concentrations were measured 42–44 h after allergen and

LPS stimulation [44], respectively. Therefore, we cannot rule out that a more prolonged stimulation of PBMC-CD14⁺ cultures might have resulted in a significant increase in sCD14 production.

In conclusion, kinetic analysis of the local endobronchial sCD14 production suggests that sCD14 concentrations reach their maximum around 42 h after segmental allergen challenge. We provide evidence that LTD₄ stimulates sCD14 production in PBMC-CD14⁺ cultures which could contribute to the proinflammatory potential of this mediator. The leukotriene-receptor antagonist Montelukast is able to block this effect, suggesting that this is indeed a CysLTR-1 mediated effect.

As LPS seem to have a protective role in the development of asthma on the one hand [1], possibly related to LPS dose and genetic constellation [2, 4], it can aggravate existing asthma on the other hand [3]. Based on our *in vitro* findings, it could be speculated that leukotriene-receptor antagonists might be able to block the effects of LPS-induced aggravation of allergic asthma *in vivo*.

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