

Original Article

Serum levels of soluble CD14 in allergic inflammation

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ABSTRACT

Monocytes/macrophages have recently been shown to play a significant role in the pathogenesis of allergic diseases. As the level of soluble CD14 (sCD14) in serum is considered a marker of monocyte/macrophage activation, we measured the levels of sCD14 in allergic asthma and atopic dermatitis (AD), along with acute infectious and inflammatory diseases, to see its clinical relevance. Serum samples were taken from patients with acute infectious and inflammatory diseases, allergic asthma, and atopic dermatitis. sCD14 was measured with our own ELISA system and its level in each disease was compared with normal controls as well as its disease severity. sCD14 was elevated and correlated with C-reactive protein in infectious and inflammatory diseases ($n = 26$), confirming that it reflects inflammation. sCD14 was also significantly increased both in asthma ($n = 94$) and adult chronic AD ($n = 22$). In asthmatic patients, those with higher sCD14 tended to have more severe symptoms, but there was no statistical correlation between sCD14 and severity. In adult chronic AD patients, a correlation between sCD14 and disease severity was observed. However, sCD14 was not elevated in infant AD patients ($n = 18$) irrespective of severity, suggesting differences in the degree of monocyte/macrophage involvement in the patho-

genesis between adult chronic and infant AD. The levels of sCD14 were shown to be upregulated in allergic diseases and might be useful as a marker of monocyte/macrophage involvement in allergic inflammation.

Key words: asthma, atopic dermatitis, CD14, inflammation, macrophage, monocyte.

INTRODUCTION

Atopic asthma is regarded as an inflammatory disease with secondary features that include edema, airway smooth-muscle contraction, and obstruction of airways by secretions.¹ Atopic dermatitis (AD) is also an allergic disorder with striking parallels to asthma but involving a different regional sphere of immunologic and non-immunologic influences.² Although mast cells, T lymphocytes, and eosinophils have been considered to be of particular importance,³ several studies have shown that monocytes/macrophages may also play an important role in these allergic diseases.^{2,4,5} Recently, evidence has been accumulated to suggest the importance of Fc ϵ RI-expressing antigen-presenting cells including monocytes in allergic immune response (for review, see Bieber⁶).

CD14 is a 55 kDa glycoprotein found as a glycosylphosphatidylinositol-anchored species on the surface of monocytes and macrophages.^{7,8} CD14 was originally found to bind lipopolysaccharide (LPS) and enhance immune responses of cells to LPS,⁹ and recent evidence suggests that CD14 also mediates responses to other bacterial molecules.^{10–13} CD14 is also found as an abundant (1–3 μ g/mL) soluble protein (sCD14) in human plasma.^{7,8}

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Received 20 February 1998. Accepted for publication 4 June 1998.

Although the physiological role of sCD14 has not been determined, it is known to be shed from the surface of activated monocytes and has been considered as a marker of monocyte/macrophage activation.¹⁴ In fact, a growing number of reports have shown that the level of sCD14 increases in inflammatory diseases in which activated monocytes/macrophages may play an important role, such as sepsis,^{15–18} HIV infection,¹⁹ autoimmune diseases,^{20,21} and atopic dermatitis.²² Considering the fact that allergic diseases have been regarded as inflammatory diseases and that monocytes/macrophages may have some role in the pathogenesis of allergy, we were intrigued to measure the levels of sCD14 in asthma and AD, two main allergic diseases, and to determine its clinical relevance.

METHODS

Samples

Blood samples for normal controls were taken from 20 healthy children (age range, 2 months–14 years; median \pm SD, 6.0 \pm 5.3 years) and 19 healthy adults (age range, 16–45 years; median \pm SD, 27.6 \pm 9.4 years) with no personal history or physical findings consistent with allergic diseases.

Blood samples from 26 children (age range, 6 months–14 years; median \pm SD, 3.3 \pm 3.5 years) with acute infectious and inflammatory diseases were taken both at the onset of the diseases and 1–2 weeks after the onset, when the diseases were in the recovery phase. The diseases included 10 upper respiratory tract infections due to the influenza virus, two cases of tonsillitis due to *Streptococcus pyogenes*, six lower respiratory tract infections due to bacteria such as *Hemophilus influenza* and *Streptococcus pneumonia*, four bacterial enterocolitis, and four Kawasaki disease.

Blood samples for asthma were taken from 94 patients (age range, 6–20 years; median \pm SD, 12.6 \pm 3.5 years) with atopic asthma based on the criteria for the disease made by the Japanese Society of Allergology. Briefly, asthma was diagnosed by clinical symptoms (cough, chest tightness, wheezing and dyspnea), decrease and daily variability of peak expiratory flow (if available), measurements of allergic status and family history of allergic diseases. In a randomly chosen subset of these patients, eosinophil cationic protein (ECP) levels were also measured (47 patients; age range, 6–20 years; median \pm SD, 11.8 \pm 3.3 years).

For ECP samples, the methods for sample collection were standardized to avoid influences by multiple parameters. Specifically, bloods were allowed to stand at room temperature for 30 minutes to permit ECP release from eosinophils, after which serum was collected, frozen and stored until use. sCD14 levels and symptom scores were compared for 18 selected patients whose symptom scores for the past year before samples were taken had been strictly evaluated (age range, 12–18 years; median \pm SD, 14.7 \pm 1.8 years). Symptom score was determined as follows: symptoms were recorded four times per day by patients, and 1, 3, 6, and 9 points were assigned to each episode of stridor without dyspnea, mild attack, moderate attack, and severe attack, respectively. Total yearly symptom scores were determined by summing up the points for the past one year.

Serum samples for AD were taken from 22 adults (age range, 18–33 years; median \pm SD, 25 \pm 4.4 years; seven severe, nine moderate, and six mild) and 18 infants (age range, 3–15 months; median \pm SD, 7.5 \pm 2.7 months; six severe, seven moderate, and five mild) who fulfilled the criteria for AD.²³ Severity of AD was defined by the definition of Rajka and Langeland.²³ At the time of taking the samples, all the adult patients with AD were in the chronic stage, having had the condition for more than 3 years and exhibiting typical lichenified lesions, while the duration of AD for all the infants was less than a year.

Informed consent was obtained for all blood samples, either from parents or, when appropriate, from the patients themselves.

ELISA for sCD14

The ELISA for sCD14 was performed essentially as described by Detmers *et al.*²⁴ with some modification. A total of 96 well ELISA plates (Nunc, Denmark) were coated with anti-CD14 mAb, AML2–23 (Medarex, Annandale, NJ, USA), at 2.5 μ g/mL in PBS as a capture Ab, and incubated overnight at 4°C. The plates were then washed with PBS containing 0.05% Tween-20 and were blocked with blocking buffer, poly HRP diluent (Research Diagnostics Inc., NJ, USA), for 1 h at room temperature. After washing, the plates were incubated with serum samples diluted x1000 or serially diluted standard recombinant human CD14 (a gift from Dr H. Lichenstein, Amgen, CA, USA) in blocking buffer for 2 h at room temperature. Each sample and standard was run in duplicate. The plates were then washed and incubated with 0.1 μ g/mL biotinylated anti-CD14 mAb 3C10 diluted in blocking buffer for

2 h at room temperature.²⁴ The plates were washed again, then incubated with appropriately diluted poly HRP streptavidin 20 (Research Diagnostics) for 1 h at 4°C. The signal was detected using TMB (3,3',5,5'-tetramethyl benzidine) and read at A450 in an ELISA reader. The working range of the assay was 0.1–20 ng/mL with an absorbance value range of approximately 0.1–2.0 measured at 450 nm. Highly reproducible data were obtained from the same samples tested several times either in the same or in different plates (data not shown).

Measurement of serum ECP

Measurement of serum ECP was done using a radio-immunoassay kit (Kabi-Pharmacia Diagnostics, Sweden) in duplicate according to the manufacturer's instructions.

Immunohistochemistry

Skin biopsy specimens were taken from chronic active lesions of adult AD with informed consent. Immunoenzymatic staining for CD14 was performed with Dako Pap Kit (Dako Japan, Kyoto, Japan). Sections were first treated with 3% hydrogen peroxide, and treated with normal rabbit serum for 20 min. Sections were then incubated at room temperature with anti-CD14 mAb, AML2–23 (Medarex, Annandale, NJ, USA) or isotype-matched

control antibody for 30 min at a dilution of 1:20. Sections were rinsed again, incubated with IgG fraction of rabbit antiserum to mouse immunoglobulins for 30 min, rinsed, and incubated with PAP (soluble horseradish peroxidase-mouse antihorseradish peroxidase) complex for 20 min. Sections were then incubated with the substrate solution containing amino-ethylcarbazole (AEC) and hydrogen peroxide. After the rinse with distilled water, the specimens were counterstained with Mayer's hematoxylin.

Statistical analysis

A software package (Statview, CA, USA) was used for statistical analysis on a Macintosh computer. Differences between groups were analyzed by the Mann–Whitney U-test.

RESULTS

Normal levels of sCD14 in adults and children

We first evaluated the normal levels of sCD14 both in adults and children in our assay. The mean ± SD of sCD14 from children aged 1 month to 15 years was 0.89 ± 0.26 µg/mL, which was almost the same as that in normal adults (0.73 ± 0.24 µg/mL) (Fig. 1). There

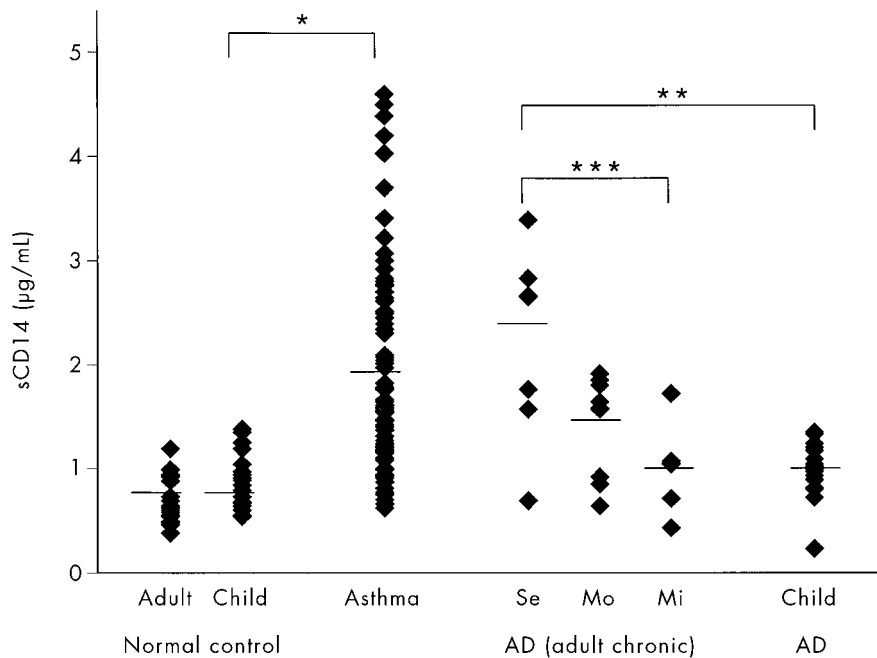


Fig. 1 Serum levels of sCD14 in normal controls and in patients with allergic diseases. Se, severe; Mo, moderate; Mi, mild; AD, atopic dermatitis; *, $P < 0.01$; **, $P < 0.01$; ***, $P < 0.05$.

was no age difference in the level of sCD14 in childhood (data not shown).

Increase of sCD14 in infectious and inflammatory diseases

We measured the sera of 26 cases with various infectious and inflammatory diseases. sCD14 was significantly elevated at the onset and then decreased in the recovery phase, 1–2 weeks after the onset (Fig. 2). Levels of sCD14 were correlated with C-reactive protein (CRP) ($r = 0.58$, $P < 0.001$), the most common marker of inflammation (Fig. 3). These results confirm the validity of sCD14 as a marker of inflammation. Levels of sCD14 were not correlated with white blood cell counts or monocyte counts in peripheral blood (data not shown), indicating that it reflects activation, not the cell number, of monocytes. Correlation of sCD14 with CRP was more prominent in influenza virus infection ($n = 10$, $r = 0.846$, $P < 0.001$) than in bacterial infection ($n = 12$, $r = 0.41$, $P = 0.046$) or Kawasaki disease ($n = 4$, $r = 0.56$, $P = 0.154$).

Levels of sCD14 in atopic asthma

We then tried to see the clinical relevance of sCD14 in allergic inflammation. We first evaluated the levels of sCD14 in sera from 94 patients with atopic asthma. As shown in Fig. 1, the levels of sCD14 in asthmatic patients were significantly higher than those in normal controls (1.86 ± 0.95 , $P < 0.01$). These samples were taken when there was no asthma attack or symptoms of infection, and CRP was not elevated in any samples tested, indicating that sCD14 can be elevated in allergic inflammation without the concomitant elevation of CRP. We also compared sCD14 levels with ECP in 47 randomly chosen samples and found that those two parameters were not correlated (data not shown). We then selected 18 asthmatic patients whose symptom scores for the past one year had been strictly evaluated, and their sCD14 levels and symptom scores were compared. There was no statistical correlation between these parameters (Fig. 4). However, those with higher sCD14 levels had the tendency to have higher symptom scores.

Levels of sCD14 in atopic dermatitis

We measured sCD14 levels in sera from 22 adults and 18 infants with AD (Fig. 1). Compared with normal controls, the levels of sCD14 in adult chronic AD were significantly elevated ($P < 0.01$). Moreover, sCD14 levels were

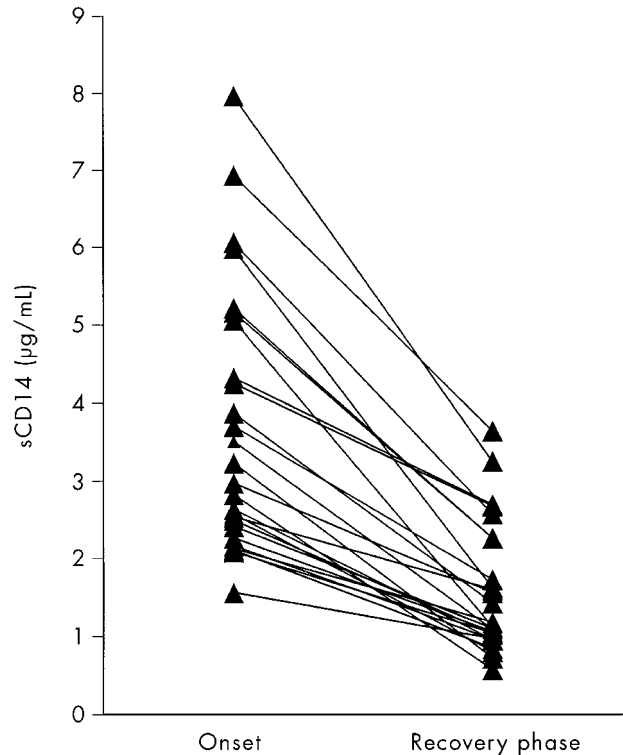


Fig. 2 Serum levels of sCD14 in patients with acute infectious and inflammatory diseases.

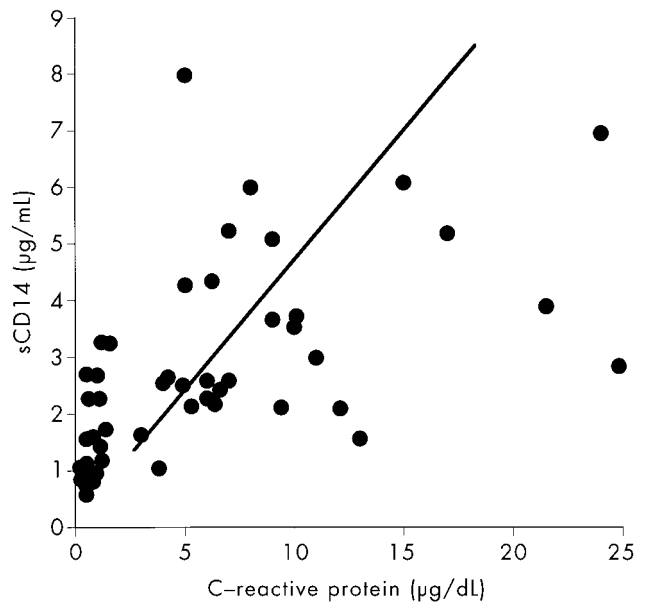


Fig. 3 Correlation between sCD14 and C-reactive protein in acute infectious and inflammatory diseases. $r = 0.58$; $P < 0.001$.

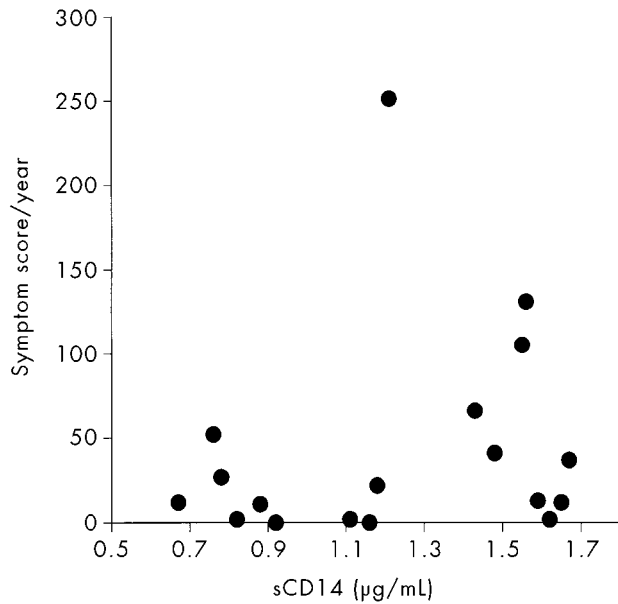


Fig. 4 Relationship between sCD14 and symptom score/year in patients with allergic asthma. $r = 0.20$.

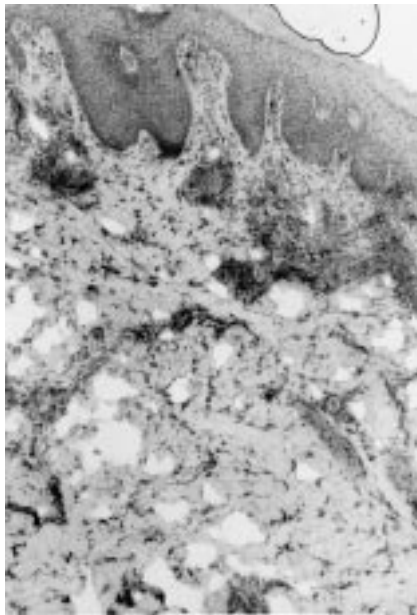


Fig. 5 Immunohistochemical staining of adult chronic atopic dermatitis lesion. Immunohistochemical staining for CD14 was performed as described in Methods.

higher as the severity of AD became stronger. In contrast to the levels in adults, sCD14 in infants with AD were not elevated, irrespective of severity. Again, CRP was not elevated in any samples with AD.

Immunohistochemistry of CD14 in chronic AD lesions

Immuno-enzymatic staining for CD14 was performed to the skin biopsy specimens from adult chronic AD lesions. A typical example is shown in Figure 5. Marked accumulation of CD14 was observed around the vessels in dermis, suggesting that the CD14 molecule in the lesional skin came from monocytes infiltrating into the dermis through the vessels. No staining was observed when control antibody was used instead of anti-CD14 (data not shown). In this assay, it was difficult to determine whether the stained CD14 is on the cell surface or solubilized.

DISCUSSION

In this paper, we confirmed that sCD14 correlates with CRP in acute infectious and inflammatory diseases, and thus reflects inflammation. We further showed that sCD14 is significantly elevated in allergic diseases such as asthma and AD, although CRP is not elevated in these diseases. As sCD14 has been considered a marker of monocyte/macrophage activation, these data suggest the significant contribution of monocyte/macrophage in the pathogenesis of allergic inflammation. The mechanisms for the elevation of sCD14 without the concomitant elevation of CRP is yet to be evaluated. However, sCD14 in bronchoalveolar fluid has been shown to be elevated after transbronchial allergen challenge, indicating that sCD14 release can occur in a mechanism different from that in infectious diseases.^{25,26}

The role of mononuclear phagocytes in allergy has not been fully described. However, several studies have shown that monocyte/macrophages from asthmatic patients are in an activated state and may orchestrate immune reactions in asthma.^{27,28} Recently, Maurer *et al.* showed elevated FcεRI expression on monocytes of atopic individuals,²⁹ and that FcεRI on atopic monocytes mediates IgE-dependent allergen presentation.³⁰ Several other reports have also shown that antigen presenting cells including monocytes/macrophages may play a role through FcεR I expressed on these cell.⁶ These FcεR-dependent mechanisms may be one way of monocyte/macrophage involvement in allergic inflammation. Evidence of monocyte/macrophage involvement in atopic dermatitis had come from studies showing that monocytes from these patients exhibit: (i) priming for superoxide generation;^{31,32} (ii) elevated phosphodiesterase

activity which is linked to elevated secretion of IL-10 and prostaglandin E₂,^{33,34} and (iii) non-responsiveness to IL-4-induced apoptosis which may contribute to the chronic inflammation.³⁵ Our data are consistent with these findings, as sCD14 is considered a marker of monocyte/macrophage activation.

In asthmatic patients we could not find a strict correlation of sCD14 with disease severity, as defined by symptom score, although there was a tendency for those with higher sCD14 to have higher symptom scores. Larger-scaled prospective studies would be necessary to further evaluate the meaning of sCD14 in asthma. In the meantime, sCD14 was not correlated with ECP. As it is known that serum ECP reflects activation of eosinophils,^{36,37} this lack of correlation suggests that sCD14 levels have no direct relation to the status of eosinophils.

A clear relationship of sCD14 with disease severity was found in adult chronic AD. This is consistent with Wüthrich *et al.* and suggests significant involvement of monocytes/macrophages in the pathogenesis of AD.²² In contrast to the levels in adults, sCD14 in infants with AD were not elevated, irrespective of severity. The reason for this difference is not clear, but several possibilities exist. Disease duration may contribute since histologic features of AD depend on the acuity, and macrophages have been shown to dominate in the dermal mononuclear cell infiltrate in chronic lichenified lesions that are frequently seen in adult patients.² Consistent with these observations, it has been shown that *in situ* expression of interferon- γ , the main activator of monocytes/macrophages, is linked to the clinical course of AD,³⁸ and that interferon- γ expression has major pathogenic relevance for the chronic phase of AD.³⁹ Alternatively, differences in the allergens that exacerbate the disease may contribute to the difference between adults and infants. Some studies have shown that food allergens can exacerbate skin rashes in at least a subset of patients with AD, especially children, while the majority of patients, particularly adults, do not have food allergy and alternatively, inhalation or contact with aero-allergen may play a role.² In fact, all AD infants in our study had a positive reaction to egg white and no reaction to aero-allergens, while most adult AD patients had a positive reaction to house dust mite and no reaction to food allergens, as determined by radio-allergosorbent assay (data not shown). These differences may cause different degree of involvement of monocyte/macrophage in the pathogenesis of AD.

In a separate experiment, we confirmed the existence of CD14 molecule at the dermis of skin biopsy specimens

with AD by immunohistochemical staining with anti-CD14. We have recently showed the existence of amphiphilic molecules from *Staphylococcus aureus* (*S. aureus*) that bind CD14 and stimulate inflammatory cells.^{10,11} As *S. aureus* is found in over 90% of AD skin lesions,² CD14 in lesional skin may contribute to exacerbation of the disease by interacting with such *S. aureus* molecules and activating inflammatory cells migrating to the lesion.

There are many questions unanswered regarding sCD14. First, it remains to be evaluated how the level of sCD14 is regulated in serum, although several studies have shown that IL-4 and granulocyte macrophage-colony stimulating factor (GM-CSF) both downregulate CD14 expression and release.^{40,41} CRP, the commonest and most useful marker of inflammation, is produced as an acute phase protein from hepatocytes stimulated with inflammatory cytokines such as IL-6.⁴² It may be of interest to determine whether the same cytokines can upregulate sCD14 as well. Second, it is yet to be defined which types of cells (other than CD14-positive cells) can produce sCD14. Our unpublished data suggests that human hepatocytes also express and secrete CD14 and studies in mice show many tissues may express CD14 upon stimulation.⁴³ Finally, the physiological role of sCD14 has not been determined. In this respect, a role of sCD14 as a lipid transfer protein has been recently proposed by Yu *et al.*⁴⁴ Evaluation of regulatory mechanisms and physiological function of sCD14 will shed more light on the importance and the meaning of the level of sCD14.

Our data strongly suggest the involvement of monocytes/macrophages in the pathogenesis of allergic diseases. Further evaluation of the role of monocytes/macrophages and sCD14 in allergic diseases may be a fruitful area of study.

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