Soluble CD86 protein in serum samples of patients with asthma

H-Z Shi, Z-F Xie, J-M Deng, Y-Q Chen, C-Q Xiao

Background: Previous studies have reported that soluble (s) CD86 is involved in the initiation of the immune response. A study was undertaken to investigate the concentrations of sCD86 in serum samples from patients with bronchial asthma and to determine the cell origin of sCD86.

Methods: Serum sCD86 concentrations were measured in 52 asthmatic subjects and 25 non-atopic normal volunteers using an enzyme linked immunosorbent assay, and the relationship of serum sCD86 concentrations to asthma severity and to total and differential white cell counts was analysed. Each type of white blood cell was purified and cultured in vitro to determine the cell origin of serum sCD86.

Results: Serum samples from patients with an acute asthma exacerbation had much higher levels of sCD86 (585.4 (20.5) IU/ml) than those from stable asthmatics (479.6 (15.7) IU/ml, p<0.001) and healthy individuals (435.1 (13.8) IU/ml, p<0.001), and there was no difference between the latter two groups (p = 0.079). In asthmatic subjects the serum sCD86 level was inversely correlated with airway responsiveness, forced expiratory volume in 1 second, and with arterial carbon dioxide tension. In addition, the serum sCD86 level was positively correlated with numbers of lymphocytes, eosinophils, monocytes, but not neutrophils. The in vitro experiments indicated that sCD86 was produced by monocytes.

Conclusions: The serum sCD86 protein level was significantly increased in asthmatic subjects during an exacerbation and correlated with the severity of asthma. sCD86 is most probably derived from monocytes in the peripheral blood.

Methods
Subjects
The study protocol was approved by our institutional review board for human studies and informed consent was obtained from all subjects. Sixty eight asthmatic subjects were recruited consecutively from regular attenders in the pulmonary outpatient or emergency department at First Affiliated Hospital, Guangxi Medical University, PR China in the year from September 2001 to August 2002. The diagnosis and classification of the severity of asthma were based on the NHLBI/WHO Workshop on the Global Strategy for Asthma (GINA guidelines). The minimum criteria for the diagnosis of an asthma exacerbation included intense...
subjective breathlessness, audible wheezing on auscultation, and a morning peak expiratory flow <70% of the predicted value in the previous 3 months. Atopy was defined as a positive prick test to one or more common Aeroallergens. Thirteen asthmatics were excluded from the study because they had been treated with oral and/or intravenous corticosteroids in the preceding 4 weeks, and three patients refused to participate in the study. Finally, 28 patients were recruited during a moderate to severe exacerbation either on their scheduled visit to the pulmonology outpatient department or during an emergency visit, and 24 patients with stable asthma were recruited on their scheduled visit if the symptoms and peak expiratory flow were stable with no change in treatment for at least 1 month. None of the studied patients with asthma smoked, had complications of other lung diseases, or had a history suggesting intolerance to non-steroidal anti-inflammatory drugs. Twenty five non-smoking healthy volunteers were studied as controls. They had no history of atopic factors or allergic diseases, and no evidence of any lung diseases. Table 1 summarises the clinical characteristics and forced expiratory volume in 1 second (FEV₁, expressed as a percentage of the predicted value) of the patients on recruitment to the study.

Assessment of airway responsiveness
Baseline measurements of FEV₁ were followed by methacholine challenge. Methacholine inhalation tests were carried out by the method described previously¹⁴ until a provocative concentration causing a 20% decrease in FEV₁ (PC₂₀/MCh) was obtained.

Blood samples
Arterial blood samples were obtained from each subject for blood gas analysis to determine the oxygen and carbon dioxide tensions (PaO₂, PaCO₂). Venous blood samples were collected either in ethylenediamine tetraacetic acid treated tubes for total and differential blood cell counts or untreated tubes to obtain serum for determining sCD86 concentrations, and the serum samples were stored at −70°C before determination. Total cell counts were performed with a haemocytometer and differential cell counts were made from blood smears stained with Diff-Quik Staining (Sigma). Cells were classified as lymphocytes, neutrophils, eosinophils, monocytes, and basophils as standard morphological criteria and absolute numbers of each cell type were calculated.

Cell isolation and culture
Heparinised venous blood was drawn from six healthy volunteers and six asthmatics during an acute exacerbation. Granulocytes and peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden). The neutrophil layer was collected and the remaining erythrocytes removed by hypotonic lysis. Eosinophils were purified by negative immunomagnetic selection using a magnetically activated cell separator system (Miltenyi Biotec, Auburn, California, USA) as previously described.¹⁵ By negative selection, highly purified eosinophils, depleted of neutrophils (CD16⁺ cells) and any contaminating mononuclear cells (CD3⁺/CD4⁺/CD19⁺ cells), were routinely obtained. Untouched T cells, B cells, and monocytes from PBMCs were isolated by negative immunomagnetic selection using a Pan T cell isolation kit II, B cell isolation kit II, and monocyte isolation kit II, respectively (all purchased from Miltenyi Biotec), and all magnetic separation procedures were carried out according to the protocols provided by the manufacturer. The purities of T cells, B cells, neutrophils, eosinophils, or monocytes were all >95%. One million T cells, B cells, neutrophils, eosinophils, or monocytes alone were cultured in a volume of 1 ml complete medium (RPMI 1640 supplemented with 5% fetal calf serum and antibiotics reagent). After 24 hours the cell-free supernatants were collected and stored at −70°C for determining sCD86 concentrations.

Detection of sCD86
The serum and supernatant samples previously stored at −70°C were thawed and approximately 300 µl of each sample were centrifuged for 10 minutes at 16 000 × g to remove any cell debris. The concentrations of sCD86 in serum samples and supernatants of cultured cells were measured by a sandwich enzyme-linked immunosorbent assay kit according to the manufacturer’s protocol (Diaclone, Besançon, France). The minimum detectable dose of sCD86 was 0.6 U/ml. All samples were assayed in duplicate.

Statistical analysis
Data were presented as mean (SE). Statistical analysis was done by one-way analysis of variance (ANOVA) for data conforming to a normal distribution, and by Friedman’s test for those data with a non-parametric distribution (confirmed by the Shapiro-Will W test). The Student’s t test was used for comparison of two groups, and the Pearson or Spearman rank correlation was used to analyse correlations. A p value of <0.05 was considered statistically significant.

RESULTS
Clinical characteristics
Background data for the 25 normal control subjects, 28 patients with acute asthma exacerbations, and 24 with stable asthma enrolled in the study are shown in table 1. The three groups did not differ significantly in age or sex (both p>0.05). There were no significant differences in atopic status or duration of asthma between patients with acute asthma exacerbations and those with stable asthma (both p>0.05). %FEV₁ in asthmatic subjects during an acute

| Table 1 Characteristics of patients with asthma and control subjects |
|---------------------------------|-----------------|-----------------|-----------------|
|                                  | Control subjects | Patients with asthma |
| M/F                              | 14/11           | 12/12           | 13/15           |
| Age [years]*                     | 38.1 (5.1)      | 39.2 (5.4)      | 42.2 (4.9)      |
| Asthma duration [years]*         | –               | 11.2 (2.7)      | 9.8 (2.3)       |
| Atopic/non-atopic                | 0/25            | 17/7            | 19/9            |
| FEV₁ [% predicted]*              | 92.9 (1.8)      | 54.0 (2.0)†     |                 |
| PaO₂ [kPa]*                      | 11.7 (0.1)      | 9.3 (0.3)†      |                 |
| PaCO₂ [kPa]*                     | 5.3 (0.1)       | 4.4 (0.1)†      |                 |

*Data are presented as mean (SE).
†p<0.001 compared with patients with stable asthma.
exacerbation decreased significantly compared with those with stable asthma and control subjects (p < 0.001).

**Serum sCD86 levels in patients with asthma**

Figure 1 shows combined data of serum sCD86 concentrations from normal healthy volunteers, patients with acute asthma exacerbations, and those with stable asthma. sCD86 was detectable in all subjects studied. There was no obvious relationship between levels of sCD86 and the sex or age of the subjects. Serum samples from patients with acute asthma exacerbations had much higher levels of sCD86 (585.4 (20.5) U/ml (95% confidence interval (CI) 543.4 to 627.4)) than serum from stable asthmatics (479.6 (15.7) U/ml (95% CI 447.1 to 512.1) p < 0.001) and healthy individuals (435.1 (13.8) U/ml (95% CI 406.6 to 463.6) p < 0.001), and there was no difference in serum sCD86 concentration between stable asthmatics and healthy individuals (p = 0.079). In addition, the serum sCD86 concentration in 36 patients with atopic asthma (531.8 (17.7) U/ml (95% CI 495.8 to 567.8) did not differ from that in 16 patients with non-atopic asthma (547.2 (28.8) U/ml (95% CI 485.9 to 608.3), t = 0.469, p = 0.641).

**Correlation between serum sCD86 levels and various clinical parameters**

Of 52 asthmatic patients, %FEV1 was more than 60% in 38 and airway responsiveness was measured in 25 of these (20 atopic and five non-atopic asthmatics). The geometric mean (SE) PC20MCh was 0.89 (1.07) mg/ml. Details of %FEV1, PaO2 and PaCO2 in the study subjects are shown in table 1. The serum sCD86 level was inversely correlated with PC20-MCh (r = -0.498, p = 0.011), % FEV1 (r = -0.664, p < 0.001), and with PaCO2 (r = -0.659, p < 0.001, fig 2), but did not correlate with PaO2 (r = -0.321, p = 0.555). Total and differential blood cell counts in healthy controls and asthmatic subjects are shown in table 2. In 52 patients with asthma the serum sCD86 level was positively correlated with numbers of lymphocytes (r = 0.628, p < 0.001), eosinophils (r = 0.659, p < 0.001), or monocytes (r = 0.729, p < 0.001) but not neutrophils (r = -0.135, p = 0.339; fig 3).

**Cell origin of sCD86**

To reveal the cell origin of sCD86, untouched T cells, B cells, neutrophils, eosinophils, and monocytes from peripheral blood were purified and cultured to determine the presence of sCD86 in the supernatants. No sCD86 could be detected in the supernatants of cultured T cells, B cells, neutrophils, or eosinophils from either asthmatic subjects or healthy volunteers. The most important finding was that monocytes
were the only cell type which released sCD86 into the culture supernatants, and that monocytes from asthmatic subjects produced more sCD86 than those from healthy volunteers (fig 4).

**DISCUSSION**

We have shown that sCD86 is detected in the serum of healthy controls and both atopic and non-atopic asthmatic subjects, and that sCD86 levels are raised in the serum of patients with acute asthma exacerbations. The concentration of serum sCD86 in asthmatic patients was inversely correlated with PC20-MCh, %FEV1, and PaCO2. These findings suggest a potentially important role for sCD86 in asthma. To our knowledge, this study is the first to suggest the possible clinical significance of sCD86 in human asthma.

Co-stimulation through the B7/CD28 pathway plays a critical role in the establishment of antigen driven immune responses. Hofer and coworkers found that atopic patients with asthma who were exposed to allergens had significantly higher levels of CD86 expression on B cells than atopic asthmatic subjects not exposed to allergen in vivo or non-atopic controls. When PBMCs from asthmatic patients or normal control subjects were stimulated with IL-4 or IL-13, the expression of CD86 (but not CD80) was significantly increased on B cells. It has been shown that blocking CD86 but not CD80 co-stimulation

![Figure 3](image-url) **Figure 3** Correlation between concentrations of serum sCD86 and numbers of (A) lymphocytes, (B) neutrophils, (C) eosinophils, and (D) monocytes in peripheral blood of 52 patients with asthma.

![Figure 4](image-url) **Figure 4** sCD86 production by non-activated monocytes in vitro. One million monocytes from six normal control subjects and six asthmatic subjects with an exacerbation were purified and cultured in a volume of 1 ml for 24 hours and the supernatants were collected for determination of sCD86 concentrations.

**Table 2** Mean (SE) white blood cell counts and differential counts in asthmatic and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Control subjects (n = 25)</th>
<th>Patients with asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stable (n = 24)</td>
<td>Exacerbation (n = 28)</td>
</tr>
<tr>
<td>Total cell count (x10^9/ml)</td>
<td>6.08 (0.28)</td>
<td>6.82 (0.22)*</td>
</tr>
<tr>
<td>Lymphocytes (x10^9/ml)</td>
<td>1.57 (0.11)</td>
<td>1.87 (0.06)*</td>
</tr>
<tr>
<td>Neutrophils (x10^9/ml)</td>
<td>3.94 (0.22)</td>
<td>4.08 (0.01)</td>
</tr>
<tr>
<td>Eosinophils (x10^9/ml)</td>
<td>0.21 (0.03)</td>
<td>0.48 (0.04)*</td>
</tr>
<tr>
<td>Monocytes (x10^9/ml)</td>
<td>0.30 (0.02)</td>
<td>0.29 (0.02)</td>
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*p < 0.05 compared with control subjects.
is effective in inhibiting allergic responses and that these effects are associated with reduced Th2 cytokine production. Tsuyuki and coworkers have also reported that airway allergen challenge upregulates the expression of CD86 on B cells from the lung and airway administration of anti-CD86 monoclonal antibody inhibits IgE production in a mouse model of asthma. In addition, blockade of costimulation with CTLA4-Ig (a fusion protein known to prevent costimulation by blocking CD28/B7 interactions) inhibits airway hyperresponsiveness, inflammatory cell infiltration, and allergen-specific responsiveness of thoracic T cells in a murine model of asthma. CD28-deficient mice did not manifest airway inflammation in response to allergen challenge, despite evidence of systemic sensitisation, and this was due to a failure in T cell recruitment and defective Th2 cell development. Prevention of CTLA-4 engagement by blockade of CD86 in CD28-deficient mice restored lymphocyte recruitment to the airway but did not result in tissue eosinophilia, Ig isotype switching, or Th2 cytokine secretion. The eosinophilia, Ig isotype switching, or Th2 cytokine secretion.

**REFERENCES**

The ability of different pulmonary function tests to detect dose-response effects depends on both the variability and sensitivity of the methods used. In clinical trials bronchodilatation is usually assessed by spirometry. Body plethysmography and impulse oscillation (IOS) are alternative techniques. The advantage of IOS is that it is simpler to perform and requires minimal effort from the subject. It can also measure different components of respiratory impedance, including both central and peripheral airways resistance. The authors of this paper compared the sensitivity and variability of the above pulmonary function methods to measure the dose-response effects of salbutamol in both healthy subjects and asthmatics. The study involved 12 healthy subjects, 12 with mild asthma (FEV$_1$ >80% predicted), and 12 with moderate asthma (FEV$_1$ <80% predicted). Pulmonary function was measured by all three methods on day 1 and repeated after 30 minutes to assess variability. One week later the measurements were repeated at the same time of day, after which increasing doses of nebulised salbutamol were administered and pulmonary function was recorded after each dose. The most sensitive measurements were spirometry in healthy individuals and plethysmography in those with mild asthma. All three pulmonary function methods showed similar sensitivity in individuals with moderate asthma. However, spirometry showed the least variability of the tests. The sensitivities of the tests also varied with the degree of airflow obstruction.

This study suggests that the airways of healthy subjects respond differently from those of asthmatic subjects. In clinical trials the results of dose-response curves in healthy individuals are not necessarily applicable to those with asthma. This needs to be taken into account when choosing the most appropriate test to measure pulmonary function.

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Thorax 2004 59: 870-875
doi: 10.1136/thx.2004.021840

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