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.

N
ative T lymphocytes require two distinct signals from antigen-presenting cells (APC) to be functionally activated.1-2 The first signal, which confers specificity, is provided by the interaction of the T cell receptor with major histocompatibility complex II complexes on APCs. A second co-stimulatory signal can be provided by APC borne ligands for the CD28 and cytotoxic lymphocyte associated antigen-4 (CTLA-4) receptors on T cells. CD28 is constitutively expressed by T cells and interacts with the B7 molecules B7-1 (CD80) and B7-2 (CD86).3,4 CD80 and CD86 are capable of forming homodimers, allowing for interactions with homodimers of either CD28 or CTLA-4. The interaction of CD80 with its ligands is weaker than the interaction with CTLA-4. Human CD80 binds to human CTLA-4 and CD28 with Kd values of 0.42 and 4 μM, respectively, for the monomeric interactions.5 The CD86:CTLA-4 interaction is of an affinity similar to CD80:CD28 and the CD82:CD86 interaction is of even lower affinity.6 CD80 and CD86 are type 1 membrane glycoproteins belonging to the immunoglobulin (Ig) supergene family. Despite having the same ligands, CD80 and CD86 appear to be involved in different mechanisms; CD80 can be more potent than CD86 in inducing an antitumoral response while CD86 preferentially induces the production of a helper (Th) 2 response.7 Due to its constitutive expression on human APCs, it has been suggested that CD86 may be involved in the initiation of the immune response.

Allergic asthma is a disease characterised by airway hyperresponsiveness, pulmonary inflammation, and raised serum IgE levels. Production of Th2 cytokines such as interleukin (IL)-4, IL-5 and IL-13 in allergic asthma is at least partly responsible for eliciting the cardinal pathogenic changes of the asthmatic phenotype.8 The factors in asthma that govern the production of Th2 cytokines over Th1 cytokines, such as interferon-γ, are slowly being revealed. One likely factor is the cytokine profile of T cells which is influenced by APCs, possibly through co-stimulatory signals. Such signals may be provided by ligation of CD28 or CTLA-4 with CD80 or CD86.8 Jeannin and coworkers have reported that soluble (s) CD86 results from an alternatively spliced transcript characterised by the deletion of the transmembrane domain; and that sCD86 provides a co-stimulatory signal to memory human T cells.9 Circulating sCD86 has been reported to be detected in the plasma of normal individuals and patients with leukaemia.10 Reverse transcriptase polymerase chain reaction analysis has shown that the transcript for sCD86 is expressed in normal monocytes, dendritic cells, as well as some leukaemic cells, but not in normal T, B and NK cells.11 Monocytes in peripheral blood might therefore be able to produce sCD86. We speculated that a reasonable starting point in the search for sCD86 expression would be in patients with asthma. To that end, we determined the concentrations of sCD86 in serum samples from patients with asthma and the correlations between sCD86 levels and clinical indices as well as the cell origin of sCD86.

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).12 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 pulmonary 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 (FEV<sub>1</sub>, expressed as a percentage of the predicted value) of any lung diseases. Table 1

### Characteristics of patients with asthma and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Control subjects</th>
<th>Patients with asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 25)</td>
<td>Stable (n = 24)</td>
</tr>
<tr>
<td>M/F</td>
<td>14/11</td>
<td>12/12</td>
</tr>
<tr>
<td>Age [years]</td>
<td>38.1 (5.1)</td>
<td>39.2 (5.4)</td>
</tr>
<tr>
<td>Asthma duration [years]</td>
<td>–</td>
<td>11.2 (2.7)</td>
</tr>
<tr>
<td>Atopic/non-atopic</td>
<td>0/25</td>
<td>17/7</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; [% predicted]</td>
<td>–</td>
<td>92.9 (5.8)</td>
</tr>
<tr>
<td>PaCO&lt;sub&gt;2&lt;/sub&gt; [kPa]</td>
<td>–</td>
<td>11.7 (0.1)</td>
</tr>
<tr>
<td>PaCO&lt;sub&gt;2&lt;/sub&gt; [kPa]</td>
<td>–</td>
<td>5.3 (0.1)</td>
</tr>
</tbody>
</table>

*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 PC20MCh (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. In contrast, there were no differences in CD80 expression among the three study groups. 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** 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.

**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>Total cell count (&lt;10^9/ml)</td>
<td>Stable (n = 24)</td>
</tr>
<tr>
<td>Lymphocytes (&lt;10^9/ml)</td>
<td>6.08 (0.28)</td>
<td>6.82 (0.22)*</td>
</tr>
<tr>
<td>Neutrophils (&lt;10^9/ml)</td>
<td>1.57 (0.11)</td>
<td>1.87 (0.06)*</td>
</tr>
<tr>
<td>Eosinophils (&lt;10^9/ml)</td>
<td>3.94 (0.22)</td>
<td>4.08 (0.21)</td>
</tr>
<tr>
<td>Monocytes (&lt;10^9/ml)</td>
<td>0.21 (0.03)</td>
<td>0.48 (0.04)*</td>
</tr>
<tr>
<td></td>
<td>0.30 (0.02)</td>
<td>0.29 (0.02)</td>
</tr>
</tbody>
</table>

*p < 0.05 compared with control subjects.
M1 peptide. sCD86 molecule therefore appears to be a specific CD8+ eosinophilia, Ig isotype switching, or Th2 cytokine secretion. Prevention of CD86 in CD28-deficient mice restored lymphocytes after stimulation with anti-CD3 monoclonal antibody. CD86 is an important co-stimulatory molecule in the regulation of the immune system. Tsuyuki and coworkers have also reported that CD86 inhibition is effective in inhibiting allergic responses and that these results show that the serum sCD86 level is significantly increased in asthmatic patients during an exacerbation and that this level correlates with the severity of asthma. Our results also suggest that sCD86 is probably derived from monocytes in the peripheral blood. Further elucidation of the roles and functions of sCD86 in bronchial asthma will require longitudinal studies and detailed molecular research, comparing the immunopathological characteristics of asthma in diverse clinical settings using samples of induced sputum, bronchoalveolar lavage fluid, or bronchial biopsy specimens.

ACKNOWLEDGEMENTS

The authors thank Miss Jian Li for her skilful technical assistance, and Feng Gao for helpful discussions and critical review of the manuscript.

Authors’ affiliations
H-Z Shi, Z-F Xie, J-M Deng, Y-Q Chen, C-Q Xiao, Departments of Respiratory and Critical Care Medicine, First Affiliated Hospital, Guangxi Medical University, Nanning 530021, Guangxi, People’s Republic of China

This study was supported in part by research grant no 30060079 from the National Natural Science Foundation of China, in part by research grant no 200260 from the Ministry of Education, People’s Republic of China, and in part by research grant no 200203 from the Bureau of Health, Guangxi Zhuang Autonomous Zone, People’s Republic of China.

REFERENCES


**LUNG ALERT**

---

**Sensitivity of pulmonary function tests in assessing whether bronchodilatation is different in healthy subjects and asthmatics**


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 (FEV₁ >80% predicted), and 12 with moderate asthma (FEV₁ <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.

M Menon
Specialist Registrar, Department of Respiratory Medicine, Homerton University Hospital, London, UK; mkmnenor@doctors.org.uk

---

www.thoraxjnl.com
Soluble CD86 protein in serum samples of patients with asthma

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

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

Updated information and services can be found at:
http://thorax.bmj.com/content/59/10/870

These include:

References
This article cites 28 articles, 8 of which you can access for free at:
http://thorax.bmj.com/content/59/10/870#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

Asthma (1782)
Airway biology (1100)
Lung function (773)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/