

Increase in Concentration of Soluble
CD86 After Segmental Allergen
Challenge in Patients With Allergic
Asthma*

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Study objective: To investigate the effects of segmental allergen challenge on the concentration of soluble CD86 (sCD86) in BAL fluids in patients with allergic asthma.

Methods: BAL fluid and peripheral blood were collected at baseline, 24 h after segmental saline solution or allergen challenge by fiberoptic bronchoscopy and venepuncture, respectively, from 10 patients with allergic asthma. Total and differential cell counts in BAL fluid were performed, and sCD86 levels in both BAL fluid and serum were measured by enzyme-linked immunosorbent assay.

Results: In allergic asthmatics, there was no significant increase in BAL sCD86 concentrations after saline solution challenge (median, 2.0 IU/L; 25th to 75th percentiles, 0 to 3.4) compared with baseline control subjects (median, 1.2 IU/L; 25th to 75th percentiles, 0 to 3.6 IU/mL; $p = 0.735$); however, sCD86 concentrations were significantly elevated after allergen challenge (median, 8.1 IU/L; 25th to 75th percentiles, 4.4 to 17.0 IU/mL; $p < 0.001$). The concentrations of sCD86 in BAL fluid after allergen challenge exceeded levels that could be accounted for passive transudation from the circulation, based on the magnitude of increases in BAL albumin concentrations.

Conclusions: These data indicate that allergen challenge results in a significant local accumulation of sCD86 within the airways, and that the local release of sCD86 may play a role in allergen-induced inflammatory processes in the asthmatic airways.

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Key words: airway; allergy; asthma; bronchoscopy

Abbreviations: AHR = airway hyperresponsiveness; APC = antigen-presenting cell; CTLA-4 = cytotoxic T-lymphocyte-associated antigen 4; HDM = house dust mite; IL = interleukin; PC_{20} = provocative concentration required to reduce FEV_1 by 20%; sCD86 = soluble CD86; Th2 = T-helper type 2

Bronchial asthma is prevalent worldwide, but especially in developed countries its prevalence is increasing to epidemic proportions.¹ Patients with asthma have acute bronchoconstriction and mucus formation directly after inhalation of the allergen.

Chronic symptoms include airway hyperresponsiveness (AHR) to bronchospasmogenic stimuli, inflammation, and airway remodeling. Central to the process of airway inflammation is the T-lymphocyte.²

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Thus, T-cell recruitment and differentiation are critical elements in the evolution of the asthmatic state. The factors that regulate these aspects of T-cell function are incompletely understood but may include engagement of specific accessory molecules on the T-cell.³ Because B7 molecules B7-1 (CD80) and B7-2 (CD86) and related B7 homologs are especially significant on antigen-presenting cells (APCs) for delivering requisite costimulatory signals to lymphocytes, the expressions of B7 molecules on clinical samples from patients with asthma have been well studied.⁴ A soluble form of CD86 might play an important role in immune regulation by binding with the CD28 molecules, thus interfering with the binding of CD28 or/and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4).⁵ We have shown⁶ that sera from patients with acute asthma exacerbation had much higher levels of soluble CD86 (sCD86) than sera from stable asthmatics and healthy individuals, and there was no difference between the two latter groups. In asthmatics, the serum sCD86 level was positively correlated with AHR, and was inversely correlated with FEV₁ as well as arterial PCO₂.⁶ More recently, our data further demonstrated that serum sCD86 concentration increased after allergen inhalation in allergic asthmatic subjects, and that serum sCD86 concentrations were downregulated by prednisolone therapy.⁷ The aim of the present study was to evaluate the local production of sCD86 at the site of allergic airway inflammation in patients with bronchial asthma.

MATERIALS AND METHODS

Subjects

The study protocol was approved by the Ethics Committee of Guangxi Medical University, People's Republic of China, and all subjects provided written consent. A total of 10 nonsmoking patients who met the criteria for a diagnosis of asthma as defined by the National Heart, Lung, and Blood Institute/World Health Organization workshop on the Global Strategy for Asthma

(Global Initiative for Asthma guidelines)⁸ were enrolled in this study (Table 1). All 10 subjects demonstrated a stable baseline FEV₁ of at least 70% of the predicted value, with $\geq 15\%$ reversibility to inhaled β_2 -agonist or a $> 20\%$ decline in FEV₁ on exposure to < 8 mg/mL of nebulized methacholine. All of these subjects used only short-acting β_2 -agonists for control of asthma symptoms; none of the subjects were taking inhaled steroids, methylxanthines, or leukotriene modifiers. Each subject with asthma had an abnormal allergen skin-prick test result, defined as a wheal > 5 mm (averaged orthogonal diameters) in response to house dust mite (HDM) or/and other aeroallergens. Following skin-prick testing, quantitative skin testing was performed with HDM solution (Allergon AB; Ångelholm, Sweden). The weakest dilution that produced a 3-mm wheal 15 min after administration was determined. At a second visit, when lung function was determined to be stable, with an FEV₁ of at least 70% of the predicted value, HDM solutions of increasing concentration were administered by nebulization according to the method of Cockcroft and coworkers,⁹ and the provocative concentration required to reduce the FEV₁ by 20% (PC₂₀) was determined. This concentration was used in segmental allergen challenge as detailed below.

Fiberoptic Bronchoscopy and Segmental Allergen Challenge

The procedure for bronchoscopy, BAL, and segmental allergen provocation was as previously described.^{10,11} Subjects were premedicated with 0.5 mg IV atropine, and a blood sample was obtained by venipuncture to provide a baseline serum sample. Following inhalation of nebulized 4% lidocaine, a fiberoptic bronchoscope (Olympus BF-20; Olympus; Tokyo, Japan) was inserted into the lower airways. Local anesthesia was supplemented with 2% lidocaine. Initially BAL was performed in a subsegment of the right upper lobe with five 20-mL aliquots of prewarmed normal saline solution to serve as baseline control. Subsequently, a sham challenge was performed by instilling 5 mL of normal saline solution into a subsegment of the lingula or right middle lobe. An allergen challenge was performed by instilling HDM in 5 mL of saline solution into a subsegment of the opposite lung (middle lobe or lingula). HDM solution was prepared just before the procedure, with 1:10 volume/volume dilution of the HDM concentration that caused a 20% reduction in FEV₁, that is, 10% of PC₂₀ value.¹² No lavage was performed at the sites of saline solution or allergen challenge during the initial bronchoscopy. Twenty-four hours later, a second bronchoscopy was performed. Each of the previously challenged subsegments was lavaged with five 20-mL aliquots of prewarmed normal saline solution, and fluids recovered from each site were separated pooled.

Table 1—Characteristics of the Asthmatic Subjects

Subject No.	Age, yr	Sex	Atopy	IgE, U/mL	FEV ₁ , % Predicted	Methacholine PC ₂₀ , mg/mL
1	65	Male	+	298	98	0.08
2	28	Female	+	720	88	1.42
3	45	Male	+	280	102	0.18
4	19	Female	+	462	92	0.78
5	24	Female	+	228	98	2.54
6	18	Male	+	378	78	3.61
7	24	Female	+	510	104	3.66
8	34	Male	+	604	92	0.26
9	29	Female	+	440	80	0.24
10	25	Female	+	202	86	0.64

The volume of fluid recovered from each 100-mL lavage was recorded, and the fluid was filtered through two layers of gauze. Cytospins were prepared from resuspended cells, and BAL supernatants were divided into 0.5-mL aliquots and stored at -80°C until use. A differential cell count was performed on cytopins of BAL cells using May-Grünwald-Giemsa stain. A total of 500 cells were enumerated for differential cell counts, which identified macrophages, lymphocytes, neutrophils, eosinophils, and bronchial cells. Blood was centrifuged at 3,000 revolutions per minute for 10 min, and then serum was removed and divided into 0.5-mL aliquots and stored at -80°C until use.

Detection of Albumin and sCD86

The BAL fluid and serum samples previously stored at -80°C were thawed, and were centrifuged for 10 min at 16,000g to remove any cell debris. Both BAL fluid and serum samples were assayed for albumin concentrations using a sensitive sandwich enzyme-linked immunosorbent assay method in which rabbit antihuman albumin polyclonal antibody and peroxidase-labeled rabbit anti-human albumin antibody (both purchased from Dako Corporation; Carpinteria, CA) were used. The concentrations of sCD86 in BAL fluid and serum samples were measured by a sandwich enzyme-linked immunosorbent assay kit according to the protocol of the manufacturer (Diacclone; Besançon, France). The minimum detectable dose of sCD86 was 0.6 IU/mL. All samples were assayed in duplicate.

Calculation of the Expected Increase in Concentrations of sCD86 in BAL Fluid

In the subjects who demonstrated increases in sCD86 in BAL fluid from allergen-challenged segment compared with that from their saline-challenged counterparts, we calculated the expected increase in each subject using the method described by Takahashi et al.¹³ This value was then compared with that of the actual increase measured by assay. Making the assumption that the measured increase in albumin in the BAL fluid should be accompanied by the same proportional amount of sCD86 associated with it in the serum, the expected values for each sCD86 concentration were calculated as follows:

Expected release

$$= (\text{albumin concentration in allergen-challenged BAL} \\ - \text{albumin concentration in saline solution-} \\ \text{challenged BAL}) \\ \times (\text{sCD86 concentration in allergen-challenged serum} / \\ \text{albumin concentration in allergen-challenged serum})$$

Statistical Analysis

Data are presented as medians (25th to 75th percentiles). For comparison of paired data (baseline, saline solution, and allergen), significant variability was first established using the Friedman nonparametric test. The Wilcoxon signed-ranks test was then used for individual comparisons. The difference between the expected and actual concentrations of sCD86 from allergen-challenged segments was also compared using Wilcoxon signed-ranks test. Correlations were determined by Spearman rank analysis; $p < 0.05$ was considered significant.

RESULTS

Effect of Segmental Allergen Challenge on BAL Cytology

BAL recovery did not differ between baseline control BAL (median, 43.9%; 25th to 75th percentile, 38.9 to 50.7%), saline solution-challenged BAL (median, 43.4%; 40.7 to 46.8%), and allergen-challenged BAL (median, 47.3%; 41.7 to 49.1%) [all $p > 0.05$]. There was an increased total cell count after allergen challenge in patients with allergic asthma (Table 2). The percentage and absolute numbers of neutrophils were increased after saline solution and allergen challenge, whereas the percentage and numbers of eosinophils were elevated only after allergen challenge in patients with asthma.

Table 2—BAL Fluid Recovery and Cell Enumeration Before and 24 h After Segmental Saline Solution or Allergen Challenge*

Variables	Total Cells, /10 ⁶	Macrophages		Neutrophils		Lymphocytes		Eosinophils	
		%	/10 ⁶	%	/10 ⁶	%	/10 ⁶	%	/10 ⁶
Baseline control									
Median	11.8	87.6	10.1	2.6	0.3	8.8	0.9	0.8	0.1
25th to 75th percentiles	6.2–16.5	74.3–91.2	5.4–12.5	1.1–5.3	0.1–0.9	5.3–18.3	0.5–3.3	0–1.7	0–0.2
Saline solution challenge									
Median	14.5	75.5	7.6	8.0†	1.1†	10.7	1.6	0.8	0.1
25th to 75th percentiles	8.4–18.7	60.9–88.9	6.7–16.4	4.9–17.4	0.6–2.4	4.3–22.9	0.8–3.4	0.4–1.7	0.1–0.2
Allergen challenge									
Median	54.9*	23.7*	21.6	13.3†	7.5†	9.7	5.5†	42.6*	22.3*
25th to 75th percentiles	22.7–122.8	14.6–45.7	7.1–29.1	7.8–27.3	5.4–9.2	7.8–16.6	2.1–17.2	24.0–58.7	5.0–60.4

* $p < 0.01$ compared with baseline control or saline solution challenge by Wilcoxon signed-ranks test.

† $p < 0.05$ compared with baseline control by Wilcoxon signed-ranks test.

‡ $p < 0.05$ compared with baseline control or saline solution challenge by Wilcoxon signed-ranks test.

Effect of Segmental Allergen Challenge on Concentrations of sCD86 in BAL Fluids

In patients with asthma, sCD86 protein was detectable in unconcentrated BAL fluid samples from six asthmatics at baseline control (median, 1.2 IU/mL; 0 to 3.6 IU/mL) and seven patients after saline solution challenge (median, 2.0 IU/mL; 0 to 3.4 IU/mL) without significant differences ($p = 0.735$) [Fig 1, Table 3]. After allergen challenge, there was a significant increase in all 10 patients with asthma (median, 8.1 IU/mL; 4.4 to 17.0 IU/mL; both $p < 0.001$). We also noted that the serum sCD86 concentrations in allergic asthmatics increased from baseline control subjects (median, 416.2 IU/mL; 364.5 to 479.4 IU/mL) to 439.9 IU/mL (414.0 to 527.8 IU/mL) 24 h after allergen challenge ($p = 0.017$).

Albumin was used as index of serum transudation into the airways under baseline conditions, and it was noted that the ratio of sCD86 in BAL fluid to that in serum before challenge was fourfold higher than the ratio of albumin in these two compartments (Table 3), suggesting that the movement of circulating sCD86 into airway luminal spaces was greatly facilitated compared with that of albumin, or that sCD86 in luminal spaces derived from an additional source. In the seven subjects who demonstrated an increase

Table 3—Effects of Allergen Challenge on Concentrations of sCD86 and Albumin in BAL Fluids and Sera

Segmental Challenge	Baseline Control	Saline Solution Challenge	Allergen Challenge
BAL sCD86, IU/mL			
Median	1.2	2.0	8.1*
25th to 75th percentiles	0–3.6	0–3.4	4.4–17.0
Serum sCD86, IU/mL			
Median	416.2		439.9†
25th to 75th percentiles	364.5–479.4		414.0–527.8
BAL albumin, μ g/mL			
Median	43.5	53.1	433.1*
25th to 75th percentiles	39.1–51.0	43.4–60.3	299.9–626.3
Serum albumin, mg/mL			
Median	38.8		39.5
25th to 75th percentiles	36.1–42.8		34.9–43.9

* $p < 0.01$ compared with baseline control or saline solution challenge by Wilcoxon signed-ranks test.

† $p < 0.05$ compared with baseline control by Wilcoxon signed-ranks test.

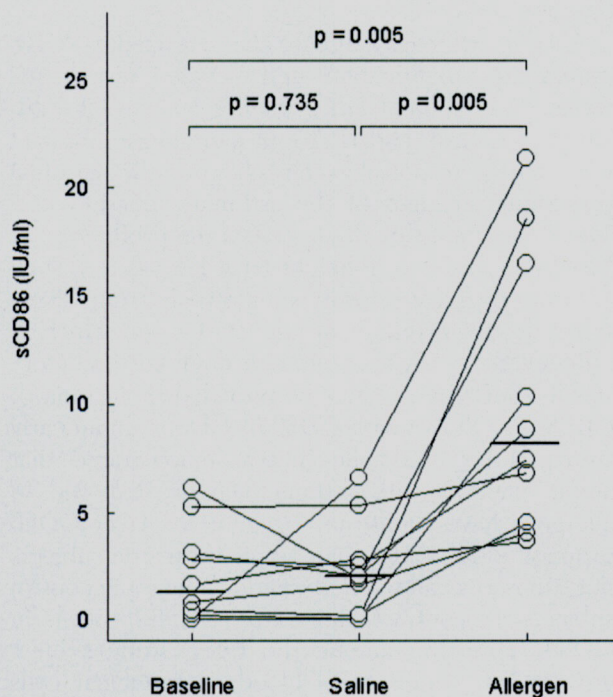


FIGURE 1. Comparison of concentrations of sCD86 in BAL fluid from segments unchallenged or challenged with saline solution or allergen. Bars represent medians; statistical analysis was done using Wilcoxon signed-ranks test.

in sCD86 concentration in allergen-challenged vs saline solution-challenged segments, we calculated an expected sCD86 value based on assayed concentrations of albumin and compared each with the actual sCD86 concentrations observed after allergen. It was found that the actual increases in sCD86 levels in BAL fluid from allergen-challenged vs saline solution-challenged segments (median, 7.4 IU/mL; 4.0 to 16.5 IU/mL) was significantly greater than expected values (median, 5.6 IU/mL; 3.3 to 6.6 IU/mL; Wilcoxon signed-ranks test, $p = 0.001$). These results suggest that significantly more sCD86 was recovered from the airways following allergen challenge than could be accounted for by passive transudation with serum.

Correlation Between sCD86 Levels and Cytologic Parameters

Using lavage data obtained from allergen-challenged segments, sCD86 levels correlated significantly with the numbers of total white cells ($r = 0.760$, $p = 0.011$), macrophages ($r = 0.749$, $p = 0.013$), lymphocytes ($r = 0.688$, $p = 0.028$), and eosinophils ($r = 0.670$, $p = 0.034$) but not with the numbers of neutrophil ($r = -0.048$, $p = 0.895$) [Fig 2]. No relationship was observed between sCD86 levels and any cell counts in BAL fluid recovered from control or saline solution-challenged segments.

DISCUSSION

Bronchoscopy with segmental bronchoprovocation was used to determine the effects of allergen on

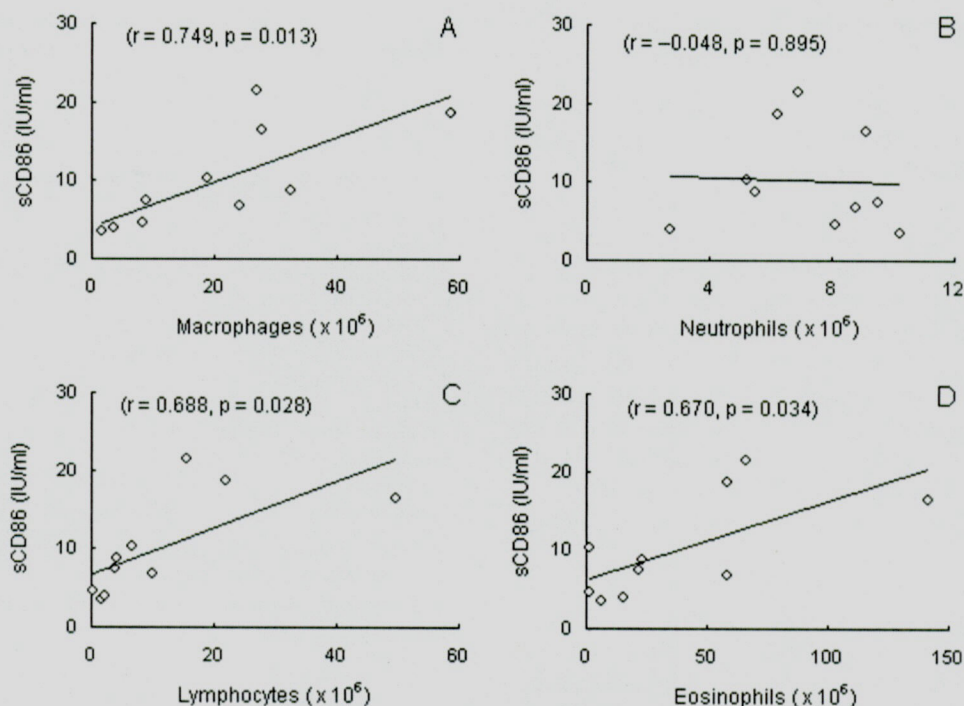


FIGURE 2. The concentration of sCD86 correlated with the numbers of macrophages (top left, A), lymphocytes (bottom left, C), or eosinophils (bottom right, D), but did not correlate with the number of neutrophils (top right, B) in BAL fluid. Correlations were determined by Spearman rank correlation coefficients.

changes in concentrations of sCD86 in BAL fluid. We found that instillation of allergen in the airway resulted in the local release of sCD86 into the BAL fluid and influx of eosinophils. Furthermore, after allergen challenge, we also found that sCD86 concentrations in BAL fluid in the allergen-challenged segments correlated with the total cell counts and numbers of macrophages, lymphocytes, and eosinophils, but not of neutrophils.

It is well known that naïve T-lymphocytes require two distinct signals from APCs to be functionally activated.^{3,14} The first signal is provided by the interaction of the T-cell receptor with major histocompatibility complex II complexes on APCs. A second costimulatory signal can be provided by APC-borne ligands for the CD28 and CTLA-4 receptors on T-cells. CD28 is constitutively expressed by T-cells and interacts with the B7 molecules CD80 and CD86.^{15,16} CD80 and CD86 are type 1 membrane glycoproteins belonging to the Ig supergene family, and they are capable of forming homodimers, allowing for interactions with homodimers of either CD28 or CTLA-4. 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 T-helper type 2 (Th2) response.^{17,18} Due to its constitutive

expression on human APCs, CD86 has been suggested of being involved in the initiation of the immune response.

Allergic asthma is a disease characterized by AHR, pulmonary inflammation, and elevated 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.¹⁹ The factors in asthma that govern the production of Th2 cytokines over T-helper type 1 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 costimulatory signals. Such signals may be provided by ligation of CD28 or CTLA-4 with CD80 or CD86.⁴ In an early study, Hofer and colleagues²⁰ demonstrated that atopic patients with asthma who are exposed to allergens have significantly higher levels of CD86 expression on B-cells than atopic asthmatic subjects not exposed to allergen *in vivo* or nonatopic control subjects. In contrast, there were no differences in CD80 expression among the three study subject groups. When peripheral blood mononuclear cells 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. Cytoflowmetric analysis revealed that

alveolar macrophages from asthmatics, unlike those from normal subjects or patients with pulmonary sarcoidosis or extrinsic allergic alveolitis, overexpressed CD86, and to a lesser extent, CD80 surface molecules.²¹ In addition, in asthmatic subjects, the level of CD80 expression on alveolar macrophages did not change following allergen challenge; in contrast, CD86 membrane expression was up-regulated following allergen challenge.²² Like human asthmatic patients, allergen exposure up-regulated expression of CD86, but not CD80, on B-cells from murine lungs within 24 h.²³ Moreover, airway administration of an anti-CD86 monoclonal antibody inhibited eosinophil infiltration, IgE production, and Th2 cytokine secretion. In addition, the anti-CD86, but not anti-CD80, monoclonal antibody also inhibited allergen-induced AHR *in vivo*.²³ We and the studies of other authors^{24–26} suggest that the CD86 costimulatory ligand plays a major role in the development of allergic inflammation and AHR in allergen-challenged mice, and that T-cell/B-cell interactions during allergic sensitization are amenable to therapeutic manipulation and that selective blockade of accessory signals can be an effective means for modulating distinct T-cell functions.²⁶

Jeannin and coworkers²⁷ have reported that sCD86 results from an alternatively spliced transcript characterized by the deletion of the transmembrane domain, and that sCD86 provides a costimulatory signal to memory human T-cells. We have shown⁶ that sera from patients with acute asthma exacerbation had much higher levels of sCD86 than sera from stable asthmatics and healthy individuals, and there was no difference between the two latter groups. In asthmatics, the serum sCD86 level was correlated with AHR, percentage of FEV₁, and with PaCO₂. More recently, we have extended the above findings and demonstrated that the sCD86 concentrations in the dual-responder group of allergic asthmatics increased significantly after HDM inhalation.⁷ In the present study, our finding that the concentrations of sCD86 in BAL fluid is strikingly increased after segmental allergen challenge has important mechanistic implications: it demonstrates that, in humans, allergic exposure is associated with the synthesis of sCD86 protein and implies that airway sCD86 is involved in the allergen-induced asthmatic response in patients with allergic asthma. In addition, we also noted that there was a small but statistically significant increase in sCD86 concentration segmental allergen challenge. The small magnitude of the increase may be related to the relative area of lung involved in the allergic response.

We did not identify the cell origins of BAL sCD86 in the present study. It is known that membrane CD86 is expressed predominantly on monocytes,

dendritic cells, lymphocytes, eosinophils, and neutrophils.⁴ Reverse transcriptase polymerase chain reaction analysis demonstrated that the transcript for sCD86 is expressed in normal monocytes, dendritic cells, as well as some leukemic cells, but not in normal T-cells, B-cells and natural killer cells.²⁸ However, our previous *in vitro* experimental results showed that cultured T-cells, B-cells, neutrophils, or eosinophils from either asthmatics or healthy volunteers did not produce detectable sCD86. In contrast, without any stimulation, monocytes were capable of releasing sCD86 into the culture supernatants. We also noted that monocytes from asthmatics produced more sCD86 than did those from healthy volunteers.⁶ Therefore, we inferred that serum sCD86 derives from monocytes in peripheral blood, and that monocytes were further responsible for the elevation of serum sCD86 in asthmatics with acute exacerbation. In the present study, we also noted that the concentrations of sCD86 in BAL fluid after allergen provocation exceeded those predicted to result from passive transudation from the circulation, based on the magnitude of the increases in BAL albumin concentrations. Although the possibility of active transport cannot be excluded, the results suggest that sCD86 appearing in BAL fluid following segmental airway challenge came from a source other than the circulation. Our present results showed that the BAL sCD86 level from allergen-challenged segments was positive correlated with numbers of macrophages, lymphocytes, and eosinophils, but not neutrophils. These results suggested that sCD86 detected in asthmatic airway after allergen challenge might derive from macrophages, lymphocytes, or/and eosinophils. In the present study, cell counts in BAL fluid after allergen challenge demonstrated a very high proportion of eosinophils: the percentage is changed from 0.8 to 42.6%; the absolute number of eosinophils is enhanced by a factor of 220. It is known that activated human eosinophils are able to express CD86^{29,30}; actually, our previous studies^{31–34} have shown that eosinophils within the lumina of airways can process inhaled antigen function *in vitro* and *in vivo* as APCs to promote expansion of Th2 cells. Therefore, airway eosinophils not only act as terminal effector cells but also modulate immune responses in asthma by presenting inhaled allergen.

In summary, the concentrations of sCD86 increased after allergen challenge, and these values correlated with increased inflammatory cell presence in the airway. This finding suggests that allergen challenge resulted in local production of sCD86 by airway cells. It should be acknowledged that the sCD86 may be a downstream by product of airway inflammation, or alternatively it is central to driving the asthmatic response in response to allergen. Fur-

ther elucidation of sCD86-producing cell types and the roles and functions of sCD86 in asthma should be considered in future research.

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