

Increased Expression of CD86 and Reduced Production of IL-12 and IL-10 by Monocyte-Derived Dendritic Cells from Allergic Asthmatics and Their Effects on Th1- and Th2-Type Cytokine Balance

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Key Words

Allergic asthma · Costimulatory molecules · Cytokines · Dendritic cells · Naïve T cells

Abstract

Background: In allergic asthma, allergen-specific T cells have a Th2-biased phenotype, and it is thought that dendritic cells (DCs) contribute to the induction of allergic immune responses. Therefore, we hypothesized that DCs from allergic asthmatics and healthy donors differ with regard to their preference to induce Th1 or Th2 immune responses. **Objectives:** To investigate differences in DC-expressed costimulatory molecules and DC-secreted cytokines between allergic asthmatics and healthy donors, and their influence on the Th1- and Th2-type cytokine balance. **Methods:** Circulating monocytes from patients with allergic asthma and healthy donors were cultured with GM-CSF and IL-4, respectively, for 5 days and subsequently with lipopolysaccharide for 2 days to create mature DCs (mDCs). CD1a, CD83, CD40 and CD86 expression on mDCs was examined using a fluorescence-activated cell sorter. IL-12 and IL-10 secreted by mDCs were measured by ELISA. Naïve cord blood T cells were primed by mDCs from two groups, and IL-4 and IFN- γ production by polarized T-helper cells (Th) was

measured by ELISA. **Results:** (1) CD86 expression on mDCs from allergic asthmatics was higher than that from healthy donors. (2) IL-12, IL-12p40 and IL-10 production by mDCs from allergic asthmatics was significantly lower than that from healthy donors, respectively. (3) IL-4 production by Th cells primed by mDCs from allergic asthmatics was increased compared with that from healthy donors. **Conclusions:** mDCs from allergic asthmatics preferentially priming naïve T cells towards Th2-cell development might be due to increased expression of CD86 and reduced production of IL-12 and IL-10.

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Introduction

Activated T cells secreting Th2 cytokines, e.g. IL-4, IL-5 and IL-13, are believed to orchestrate the inflammatory reaction in allergic asthmatics. Activation of T cells depends on antigen presentation by antigen-presenting cells (APCs) in the context of MHC molecules. Among the APCs, dendritic cells (DCs) are regarded as professional APCs specialized for the initiation of naïve T cells [1, 2]. Many factors are important for the induction of the differentiation pathway of naïve T cells. In addition to the type and the dose of antigen, the genetic background

of the host and the route of exposure, DCs play a critical role in determining whether a Th1 or Th2 response will develop. A dense network of DCs has been identified in the airway mucosa of humans [3] and experimental animals [4, 5]. DCs form a network involved in the uptake of inhaled antigens and carry processed antigen from the lungs to the bronchial lymph nodes where they present antigens to naïve T cells and drive T-cell responses to Th1- or Th2-type responses [6]. However, the mechanisms by which DC differentially regulate Th1 and Th2 responses remain to be elucidated.

Apart from MHC molecules, DCs express an array of costimulatory and adhesion molecules, and immune response is incompletely understood. It has been proposed that differential expression of such surface molecules by DCs influences Th1/Th2 switching [7, 8]. Of the known costimulatory molecules, the B7 family (CD80/CD86) appears to be the most potent. CD86 seems to be more important than CD80 in the induction of Th2 responses [9], and in murine models of allergic airway inflammation, blockade of CD86, but not CD80, abolishes airway eosinophilia and airway hyperresponsiveness [10]. B cells from patients with allergic asthma upregulate CD86 but not CD80 [11]. Thus we aimed to determine whether costimulatory molecules expressed on DCs would differ between patients with allergic asthma and healthy donors.

In addition, the soluble factors secreted by DCs play a key role in the Th1/Th2 balance. IL-12 is a critical Th1-polarizing cytokine [12], and absence of IL-12 facilitates the development of Th2 responses [13]. A wide diversity of microbial components trigger DC to produce IL-12 in a T-cell-independent manner [14]. In patients with allergic asthma, IL-12 and IFN- γ production in whole blood cultures is significantly lower compared with that in healthy controls [15]. The major producers of IL-12 are probably monocytes and DCs [16]. Therefore, we wondered if in patients with allergic asthma Th2-cell development might be causally related to inadequate DC-derived IL-12 production. IL-10 is involved in the inhibition of Th2-driven allergic immune responses [17]. In vivo experiments with mice have shown that IL-10 inhibits delayed-type hypersensitivity [18], and animal studies indicated that lung DCs produce IL-10 and stimulate the development of regulatory T cells which mediate a state of tolerance [19]. The capacity of DCs to produce IL-10 is a crucial factor in the regulation of Th-cell development. Thereby, it was postulated that DCs from patients with allergic asthma might produce less IL-10, fail to induce T-cell tolerance and favor Th2 responses.

Table 1. Characteristics of the study participants

Characteristic	Allergic asthmatics (n = 9)	Healthy donors (n = 14)
Age, years	26.8 $\pm$ 6.1	23.0 $\pm$ 2.0
Sex, males/females	5/4	6/8
Total serum IgE, IU/ml	1,030.6 $\pm$ 954.7	102.3 $\pm$ 36.8
Eosinophils $\times 10^9/l$	2.1 $\pm$ 5.9	0.20 $\pm$ 0.16
%	17.1 $\pm$ 6.1	1.3 $\pm$ 0.6
Skin prick test	+	–
Reversibility test	+	–

+ = Positive skin prick testing to at least three allergens. Reversibility was defined as an increase in FEV₁ of at least 15% and a minimum improvement of 200 ml after inhaled albuterol.

In this study, we investigated whether monocyte-derived DCs from patients with allergic asthma show intrinsic impairments in costimulatory molecules and cytokines (IL-12 and IL-10), and whether these defects in DCs might affect the balance of Th1 and Th2 cytokines when they prime naïve Th0 cells in vitro.

Patients and Methods

Patients

Nine patients with allergic asthma, 4 females and 5 males, were recruited from the Outpatient Department of Pulmonology of the Wuhan University Renmin Hospital (table 1). Asthma was defined according to the criteria of the American Thoracic Society [20]. Selection criteria for allergic asthmatics include a history of previous recurrent episodes of wheezing, breathlessness, chest tightness and/or cough, a demonstrated reversibility defined as a 15% increase in forced expiratory volume in 1 s (FEV₁) over baseline and a minimum improvement of 200 ml in FEV₁ following 200 μ g of inhaled albuterol. All patients were atopic with positive skin prick testing to at least three allergens (pollen, *Dermatophagoides pteronyssinus* and cockroach) and a total serum IgE of more than 350 IU/ml, with eosinophil counts/percentages in the peripheral blood sample surpassing the normal range. Participants of this study had not used corticosteroids for at least 4 weeks before study entry. They inhaled only short-acting β_2 -agonists on demand as rescue medication and β_2 -agonist treatment was discontinued 12 h prior to blood sampling. The study was approved by the Ethics Committee of the Wuhan University School of Medicine, and written informed consent was given by all patients. The 14 healthy volunteers were recruited from undergraduates in the Wuhan University School of Medicine, they were nonatopic and nonasthmatic, and they had no history of respiratory diseases and a normal lung function.

Table 2. Expression of CD1a, CD83, CD40 and CD86 on mDCs from 9 allergic asthmatics and 14 healthy donors

Groups	CD1a, %	CD83, %	CD40, %	CD86, %
Allergic asthmatics	49.42 ± 8.71	42.96 ± 5.72	42.77 ± 5.48	40.75 ± 3.99
Healthy controls	49.59 ± 10.24	41.52 ± 5.96	44.03 ± 5.39	29.88 ± 1.25*

Data are presented as means ± SEM. * $p < 0.01$ vs. healthy donors.

Generation of Mature DCs

DCs were generated from lymphocyte-depleted PBMCs according to established standard procedures [21]. Blood cells were from freshly drawn heparinized blood of allergic asthmatics and healthy volunteers. PBMCs were isolated from buffy coats by centrifugation over layers of Ficoll (Haoyang Biotech, TianJing, China). These were plated (5×10^5 cells/ml per well) into 6-well culture plates in RPMI 1640 (Gibco) supplemented with 2 mM glutamine, 1% antibiotics and 10% FCS (all from Sigma, St. Louis, Mo., USA). After 2 h at 37°C in humidified 5% CO₂ in air, medium was supplemented with recombinant human GM-CSF (50 ng/ml) and IL-4 (50 ng/ml; both from PeproTech, London, UK). Every other day, these cells were fed with fresh medium, and on day 5 the maturation of DC was induced by a 2-day exposure to lipopolysaccharide (LPS; 1 µg/ml; Sigma).

Naïve T Cell Preparation

Cord blood mononuclear cells (CBMCs) were obtained by centrifugation over Ficoll (Haoyang Biotech), and CD4⁺ T cells were isolated from CBMCs by an immunomagnetic method according to the manufacturer's instructions. In brief, CBMCs were incubated at 4°C for 30 min with mouse anti-human CD4 antibody (PeproTech), and then cells were washed and incubated at 4°C for another 15 min with human anti-mouse IgG-coated magnetic beads (Dyna, Oslo, Norway). Positive cells were separated in a magnetic particle concentrator (Dyna) and washed three times with RPMI 1640/1% FCS. Naïve T cells (CD4⁺CD45RA⁺ T cells) were obtained by secondly positive selection using mouse anti-human CD45RA antibody (PeproTech) and separated as described above. After three washes with RPMI 1640/1% FCS, the purity of the naïve T cells was verified by FACS Calibur (Becton Dickinson, Franklin Lakes, N.J., USA) as over 98%.

Priming of Naïve T Cells

24-well tissue culture plates were seeded with 5×10^4 mature DCs (mDCs) and 5×10^5 purified naïve T cells. The cultures with a final volume of 1 ml of RPMI 1640/10% FCS were further cultured with IL-2 (200 U/ml, PeproTech). After 3 days, the cultures were restimulated with PMA and ionomycin (both gifts from Jing-Mei Biotech) at the concentrations indicated by the manufacturer for another 2 days.

Flow-Cytometric Cell Surface Marker Analysis

5×10^5 DCs/ml were plated into 24-well multiwell tissue culture plates and were incubated for 45 min at 4°C with FITC-conjugated mouse-anti human CD1a, CD83, CD40, CD86 and rabbit-anti mouse IgG (all from Ancell, Bayport, Minn., USA). After two washes with 10% PBS, the cells were resuspended in 1 ml PBS and subjected to FACS Calibur analysis.

Cytokine Detection

After DC stimulation with LPS for 24 h, the supernatant was harvested, and cytokines were measured by enzyme-linked immunosorbent assay (ELISA) using antibodies specific for IL-12, IL-12p40 and IL-10 (from R&D, Minneapolis, Minn., USA). After priming of naïve T cells for 5 days, cytokines were measured in the supernatant by ELISA using antibodies specific for IFN-γ and IL-4 (R&D, Minneapolis, Minn., USA).

Statistical Analysis

Data are expressed as means ± SEM or median values with a range from the minimal to the maximal values. When the variables showed normal distribution, comparison was performed using Student's unpaired t test. When the variables did not show normal distribution, they were compared by Mann-Whitney U test (two tailed). Statistical significance was set at a level of $p < 0.05$.

Results

Increased Expression of CD86 on mDCs from Allergic Asthmatics

Monocyte-derived DCs from patients with allergic asthma and healthy donors were stimulated with LPS. CD1a, CD83, CD40 and CD86 expression on LPS-pulsed DCs were measured. Increased CD86 expression on mDCs from patients was observed compared with healthy donors ($p < 0.01$); CD1a, CD83 and CD40 expression did not differ between patients and healthy donors (table 2). One representative experiment out of 9 is shown in figure 1.

mDCs from Allergic Asthmatics Produce Less IL-12, IL-12p40 and IL-10

mDCs from patients with allergic asthma and healthy donors were compared regarding their ability to secrete IL-12, IL-12p40 and IL-10. The production levels of IL-12 and IL-12p40 secreted by mDCs from patients with allergic asthma were both significantly lower than those from healthy donors ($p = 0$ for IL-12 and $p = 0.017$ for IL-12p40). mDCs from patients with allergic asthma secreted significantly less IL-10 than those from healthy donors ($p = 0.029$; table 3).

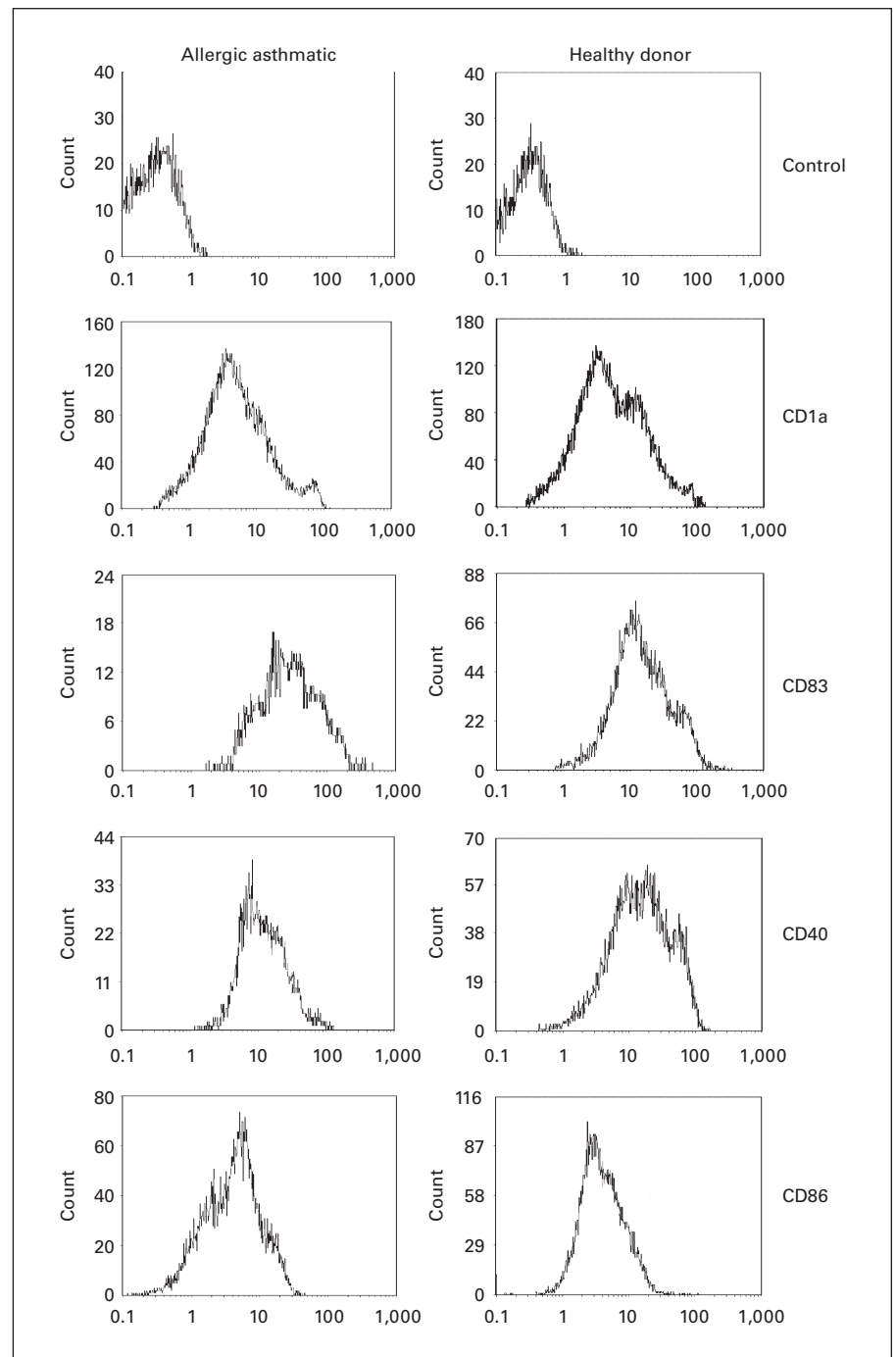


Fig. 1. Expression of costimulatory molecules on mDCs from allergic asthmatics and healthy controls. Monocyte-derived DCs from allergic asthmatics (left panels) and from healthy controls (right panels) were matured by treatment with LPS (1 μ g/ml) for 24 h. The cells were then collected and analyzed by flow cytometry for CD1a, CD83, CD40 and CD86 expression.

Increased IL-4 Production by T Cells Primed with mDCs from Allergic Asthmatics

To evaluate the potential effect of DCs from allergic asthmatics or healthy donors on the Th1/Th2 response, the production of IFN- γ and IL-4 by naïve T-cell-primed mDCs was analyzed. T cells coincubated with mDCs

from allergic asthmatics demonstrated increased IL-4 production, which was significantly higher than that from healthy controls ($p = 0$). However, IFN- γ production by T cells primed by mDCs from allergic asthmatics was low and did not significantly differ from healthy donors ($p = 0.339$; table 4).

Table 3. Cytokine levels produced by mDCs from 9 allergic asthmatics and 14 healthy donors

Patient groups	IL-12, pg/ml	IL-12p40, pg/ml	IL-10, pg/ml
Allergic asthmatics	217.44 (76.41–349.68)**	2,134.16 (421.68–3,878.07)*	282.71 (69.31–634.71)*
Healthy donors	904.33 (378.43–1,481.46)	5,264.25 (2,447.73–8,874.32)	917.49 (350.26–3,029.87)

Data are presented as medians (ranges). * $p < 0.05$; ** $p < 0.01$, vs. healthy donors.

Table 4. Cytokine levels released by T cells primed with mDCs from 9 allergic asthmatics and 14 healthy donors

Patient groups	IFN- γ , pg/ml	IL-4, pg/ml
Allergic asthmatics	455.98 (90.23–554.08)	436.40 (238.15–606.82)*
Healthy donors	562.07 (267.52–949.25)	55.25 (20.58–74.17)

Data are presented as medians (ranges). * $p < 0.01$ vs. healthy donors.

Discussion

In allergic asthma, allergen-specific T cells have a Th2-biased phenotype [22]. It is thought that DCs contribute to the induction of allergic immune responses, leading to the assumption that DCs from patients with allergic asthma and healthy donors differ with regard to their preference to induce Th1 or Th2 immune responses. In an attempt to determine whether intrinsic defects exist in DCs in allergic asthmatics, we cultured circulating monocytes from patients with allergic asthma and healthy donors with GM-CSF and IL-4, followed by LPS to create mDCs, and then the amount of cytokines released by mDCs and the expression of costimulatory molecules on mDCs were analyzed. The results showed that CD86 expression on mDCs from patients with allergic asthma is increased compared to healthy donors. IL-10 and IL-12 production by mDCs from patients with allergic asthma is significantly less than that from healthy donors, and the reduced production could be due to intrinsic defects in DCs from allergic asthmatics, since monocytes were cultured in vitro and extrinsic factors influencing IL-12 and IL-10 production were excluded.

To test whether these defects in mDCs from patients with allergic asthma which present the antigen to naïve T cells could influence its commitment to a Th1 or Th2 lineage (Th1/Th2 balance), we cultured mDCs from patients and healthy donors with naïve T cells isolated from umbilical cord blood. Our results showed that the production of the type-2 cytokine IL-4 by T cells primed by mDCs was significantly higher in the patient group than

that in the healthy donors, which demonstrates that mDCs from the patient group drive naïve T cells towards a polarized predominant Th2 phenotype. In our experiments, we expected a decreased IFN- γ production by T cells primed by mDCs from the patient group. However, the results presented here showed that IFN- γ production was low and did not significantly differ between patients and healthy controls. It seems that our study needs further confirmation in additional samples.

Effective antigen presentation to naïve T cells by mDCs needs three signals, i.e. coordinate regulation of MHC, costimulatory molecules and a wide range of cytokines. Upon activation by LPS, immature DCs undergo maturation and upregulate costimulatory molecules (CD86 and CD80), DC-specific markers (CD83) and adhesion molecules [23]. Some reports suggest that CD86 costimulation, but not CD80 costimulation, can preferentially activate IL-4 expression and Th2 development [24]. A study in asthmatic children has suggested that CD86 expressed by DCs remarkably increased in the asthmatic children compared with normal control subjects [25]. Our data showed that CD86 expression on mDCs from adult patients with allergic asthma was higher than that on mDCs from healthy donors, and naïve T cells primed by mDCs from these patients produced more IL-4. It was inferred that high expression of CD86 on mDCs from allergic asthmatics might contribute to the induction of naïve T cells to become IL-4 producer, Th2-type cells. CD1a and CD83 are maturation markers of DC derived from PBMC [26]. CD1a and CD83 expression on mDCs did not differ between patients and healthy

controls. We had anticipated that there might be significant changes in CD40 expression on mDCs from allergic asthmatics as compared to healthy donors, given that the interaction between CD40 on DCs and CD40 ligand (CD40L) expressed on T cells is reported to induce IL-12 production [27]. However, since the expression of CD40 on mDCs from patients with allergic asthma and healthy donors showed no difference, CD40 costimulation might not be involved in the occurrence of asthma.

In addition to costimulatory molecules, induction and activation of T lymphocytes require cytokines secreted by DCs. Bioactive IL-12 is a heterodimeric cytokine composed of p40 and p35, which strongly promotes the differentiation of naïve T cells to the Th1 phenotype and suppresses the synthesis of Th2-type cytokine [28]. In patients with allergic asthma, IL-12 and IFN- γ production in whole blood cultures is significantly lower compared with healthy donors [15]. Therefore, we aimed to determine if DCs from patients with allergic asthma affect IL-12 production. Reider et al. [29] have shown that upon activation by CD40L, monocyte-derived DCs from allergic patients produce less IL-12. Here, we specifically tested IL-12 production by DCs in response to LPS, a stimulus that mimics DC maturation in vivo, in which immature DCs are fed exogenous allergen and migrate from the peripheral to the lymphoid tissue. Importantly, in response to LPS, DCs from allergic asthmatics produce less IL-12, which is in line with the data of Reider et al. [29]. Because IL-12 is a key cytokine for the induction of the Th1 response [12], a reduced capacity to produce this cytokine by DCs could lead to aberrant Th2 development. In HIV-infected individuals, a reduced production of IL-12 corresponds with the reported shift from Th1 to Th2 or Th0 cytokine production [30]. Our study has demonstrated that upon contact with mDCs from allergic asthmatics, naïve T cells differentiate into the Th2 type, which is characterized by increased IL-4 production. The explanation for this result might be that less IL-12 is secreted by mDCs from patients with allergic asthma.

IL-10 is produced by DCs in response to different antigens such as bacteria and the products thereof [31]. IL-10 is a kind of anti-inflammatory cytokine which inhibits the development of airway inflammation in a murine model of airway hyperreactivity [32, 33]. In addition, IL-10 production is critical for the induction of tolerance. Under normal conditions, soluble nonreplicating proteins passing the mucosal surface do not provoke strong immune reactions, but instead induce a state of hyporesponsiveness. IL-10 or IL-10-treated DC induce a long-term antigen-specific anergic state in human CD4+ and

CD8+ T cells [34, 35] and the differentiation of a regulatory T cell (Tr cells) subset which is characterized by increased IL-10 secretion and strong suppressive properties [36]. In the absence of IL-10, e.g. when antigen is presented by DCs from IL-10 $^{-/-}$ mice or from mice with allergic asthma [19], Tr cells fail to develop. The studies have reported that DCs from the asthmatic children secrete less IL-10 [25, 37]. Our findings showed that mDCs from adult patients with allergic asthma release less IL-10 compared with healthy donors, and these mDCs preferentially prime naïve T cells towards the differentiation into Th2 cells with increased IL-4 levels, possibly suggesting that these mDCs producing inadequate IL-10 upon contact with naïve T cells are not capable of inducing T-cell unresponsiveness, which protects against the development of Th2-biased responses and allergen-induced airway hyperreactivity, a cardinal feature of asthma. We have shown that the capacity to produce IL-10 by DCs from allergic asthmatics is reduced in vitro. If this situation is confirmed in studies in vivo, the administration of IL-10 to induce T-cell tolerance in allergic asthmatics would deserve close examination.

In conclusion, monocyte-derived mDCs from patients with allergic asthma are intrinsically aberrant. A high expression of CD86 and reduced IL-12 production by mDCs from allergic asthmatics might contribute to the differentiation into the Th2-biased immune response. Diminished IL-10 might not favor the induction of T-cell unresponsiveness. These data support the idea that DCs could be exploited as targets for therapy in allergic asthma.

Acknowledgments

The authors thank Wei Guo and Li Zhang for their excellent technical assistance, and the personnel of the Division of Respiratory Medicine at the Wuhan University Renmin Hospital for the selection of patients and blood collection. This work was supported by a grant from the China National Natural Science Fund (project No. 30370619 to J.Y.).

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