

Considerations for Use of Acupuncture as Supplemental Therapy for Patients with Allergic Asthma

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Abstract This study examines the clinical and immunomodulatory effects of acupuncture in the treatment of patients with allergic asthma. The acupuncture points GV14, BL12, and BL13 were selected based on the theory of traditional Chinese medicine in treating asthma. Manual acupuncture was performed once every other day (three times per week) for 5 weeks. The needles were twisted approximately 360° evenly at the rate of 60 times/min for 20 s, manipulated every 10 min and withdrawn after 30 min. Concentrations of sIgA and total IgA in secretions were determined by the combination of sucrose density gradient ultracentrifugation and RIA. Levels of cortisol in the plasma were measured by RIA. Total IgE in the sera was examined by ELISA. Flow cytometry was used to detect the numbers of CD3+, CD4+, CD8+, and IL-2R + T lymphocytes in the peripheral blood. The absolute and differential numbers of eosinophils in peripheral blood were counted with eosin staining. The total efficacy of the acupuncture treatment in patients with allergic asthma at the end of one course of treatment was 85 %. After treatment, the concentrations of sIgA and total IgA in the saliva ($P<0.01$, $P<0.02$) and nasal secretions ($P<0.02$, $P<0.02$) were significantly decreased in patients with allergic asthma. The levels of total IgE in sera ($P<0.001$), the counts of IL-2R + T lymphocytes ($P<0.001$), and the absolute and differential numbers of eosinophils ($P<0.01$, $P<0.01$) in the peripheral blood were also significantly decreased. The numbers of CD3+, CD4+, and CD8+ T lymphocytes in the peripheral blood were significantly increased in the allergic asthmatics treated by

acupuncture ($P<0.001$, $P<0.01$, and $P<0.001$, respectively). The concentration of cortisol in the plasma of asthmatic patients did not change significantly after the acupuncture treatment ($P>0.05$). Acupuncture has regulatory effects on mucosal and cellular immunity in patients with allergic asthma and may be an adjunctive therapy for allergic asthma.

Keywords Acupuncture · Allergic asthma · Mucosal immunity · Cellular immunity

Introduction

Mucosal immunity is an important arm of the immune system, operating in various mucous membranes, including saliva, tears, secretions from the nose and bronchi, intestinal juice, colostrums, etc., which are involved in everyday defense against infections as well as in tolerance against innocuous environmental and dietary antigens [1, 2]. Secretory IgA (sIgA) is the first line of defense within the effector component of mucosal immunity [1]. This isotype constitutes >80 % of all antibodies produced in mucosa-associated tissues and is induced, transported, and regulated by mechanisms that are remarkably distinct from those involved in systemic antibody responses [3]. In considering the allergic asthma induced by inhaled antigens and the serum IgA deficiency that is often associated with atopy [4], researchers surmised that the allergic asthma could possibly be related to mucosal immunodeficiency [5, 6]. However, until now, no studies have been conducted to determine if asthmatics may have a selective IgA deficiency, even though the latter is associated with an increased risk of respiratory tract infection, often a trigger for asthma exacerbations [7–9]. The concentration of serum IgA in the sputum of patients with asthma has been studied through

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immunoelectrophoretic and agar diffusion analyses, yet clear results have not been obtained [10, 11].

Analysis of the concentrations of exocrine IgA (sputum, saliva, nasal secretions) suggests that there is no significant difference between allergic asthmatics and normal controls [12–14], though there were increasing [15–17] and decreasing [13] trends noted in these studies. Furthermore, histological examination of the IgA-secreting cells in the lungs of bronchial asthmatics showed that there is no marked difference compared with normal controls, but the distribution of IgA secretory cells are unusual in some of the patients [18, 19]. The determination of IgA in the exocrine fluid of other allergic diseases (such as atopic rhinitis, hay fever, etc.) indicates that the concentration of IgA may increase in allergic asthma [20, 21]. Recent research reported that children on corticosteroid therapy showed lower levels of sIgA compared with asthmatic children on antihistamine therapy [22], and that low levels of total sIgA and high levels of allergen-specific sIgA indicate a local pathogenic factor in the tears of patients with allergic conjunctivitis [23]. Additional research reported that high levels of sIgA antibodies in sensitized infants were associated with significantly less late-onset wheezing [24]. Until now, the data regarding the levels of mucosal sIgA in patients with allergic asthma have been conflicting [24], and limited research has been conducted related to identifying treatments for the regulation of sIgA.

Acupuncture treatment in patients with allergic asthma and chronic bronchitis has been traditionally used in China for over a thousand years. The World Health Organization listed the diseases treatable by acupuncture in 1979 [25] and then again classified the diseases as treatable by acupuncture in 2002 [26]. The National Institutes of Health has accepted the validity of acupuncture treatment [27] and recommends acupuncture as an adjunctive treatment in comprehensive management programs for indications including asthma [28]. Whether acupuncture had a regulative role in the mucosal immunity of allergic asthma is under further experimentation.

In this study, the sIgA in secretions, which were determined by the combination of sucrose density gradient ultracentrifugation and radioimmunoassay established by our laboratory [29] and related cellular and humoral immune responses were studied in patients with allergic asthma treated with acupuncture.

Subjects and Methods

Subjects

In this study, 100 patients with allergic asthma (average age 36.41, age range 7–67) and 74 patients with chronic bronchitis (average age 49.24, age range 19–76) were included. Allergic asthma and chronic bronchitis were diagnosed under the guiding

principle of clinical research on new drugs of the People's Republic of China [30]. All patients were non-smokers. None of the patients were steroid-dependent. None had taken any drugs during the week prior to sampling. Samples were collected at 8–9 o'clock in the morning on the day before acupuncture treatment and the day after acupuncture treatment. The study was conducted with the approval of the Ethics Committee of the Shanghai University of Traditional Chinese Medicine.

Acupuncture Treatment

The acupuncture points Dazhui (GV 14, on the posterior median line in the depression below the spinous process of the seventh cervical vertebra), bilateral Fengmen (BL12, 1.5 cun lateral to the lower border of the spinous process of the second thoracic vertebra), and bilateral Feishu (BL13, 1.5 cun lateral to the lower border of the spinous process of the third thoracic vertebra) were selected based on the theory of TCM in treating asthma [31]. The stainless needles (0.30×25 mm, Suzhou Medical Appliance Factory, Suzhou, China) were manually inserted into the skin about 18 mm deep at the BL13 and BL12 points. Point GV14 was manually stimulated with 0.30×40 mm stainless needle, and the depth of the insertion was about 32 mm. The needles were twisted approximately 360° evenly at the rate of 60 times/min for 20 s, manipulated every 10 min and withdrawn after 30 min. Manual acupuncture was performed three times a week by the same experienced acupuncture doctor. Fifteen treatments were taken within five consecutive weeks during the summer.

Sample Collection

Unstimulated whole saliva was collected by aspirating the saliva from the mouth with a glass capillary dropper after breakfast [32]. Saliva was then placed into a 1.5-ml stopped plastic tube, followed by centrifugation at 3000×g at 4 °C for 5 min (SCR 20 BA, Hitachi, Japan), after which the insoluble components and debris were disposed. Supernatant samples were diluted in 1:20 with normal saline and were stored at −4 °C.

Nasal secretions were collected using the modified filter paper method [33]. Briefly, two pieces of filter paper (10×50 mm) were folded into 5×5 mm. The two paper pieces were weighed together with a small plastic tube and a stopper. After filling the tube with 0.8 ml of a solution containing normal saline, a new weight was measured. The paper pieces were placed in each nasal cavity by means of a pair of pincers. When the nose started dripping, or after 15 min, the paper was removed and placed in the plastic tube with saline and then weighed again. The weight of the secretion collected was then calculated. Finally, the secretions in saline were placed in a sterile tube and stored at −40 °C until analysis.

Sputum samples were collected in sterile jars during forced coughing in the early morning [34]. Then, the samples were transferred into polypropylene centrifuge tubes and centrifuged at $4500\times g$ for 2 h in 4 °C (HIMAC, Hitachi, Japan). Finally, the sol-phase of sputum was diluted into 1:50 (v/v) with normal saline and was put in another sterile tube for storing at -70 °C. Ten milliliters of peripheral whole blood was collected from the left median cubital vein of the patients for cell culture and preparation of serum and plasma.

Measurement of sIgA in Saliva, Nasal Secretion, and Sputum

A method was established by our laboratory for the separation and determination of sIgA and IgA in secretions [29]. Briefly, 4.5-ml linear sucrose gradients were formed with 5–20 % (wt/vol) sucrose in PBS containing 0.1 g/L NaN_3 . Salivary samples (0.3 ml) were applied to the individual sucrose density gradient immediately before ultracentrifugation. The sucrose density gradients were centrifuged at $160,000\times g$ for 15 h at 4 °C using an RPS-40 T rotor (70P-72, Hitachi, Japan) and were fractionated from the base of the tube with the aid of a Fraction Recovery System (DGF-U, Hitachi, Japan). Six drops (approximately 0.2 ml) were collected in each fraction, resulting in an average of 25 fractions per gradient. The concentrations of sIgA and IgA in the sucrose density gradient fractions were determined by radioimmunoassay using sIgA and IgA RIA Kits (Shanghai Institute of Radioimmunological Technology, Shanghai, China), respectively. The peak position was at 5.61 ± 0.46 tubes for sIgA and at 12.18 ± 0.28 tubes for IgA.

Flow Cytometry of T Lymphocytes in Peripheral Blood

CD3+, CD4+, CD8+, and IL-2R + T lymphocytes in the peripheral blood were analyzed by flow cytometry (FACStar PLUS, Becton-Dickinson, USA) with the use of a 488-nm Innova 90-5 W argon laser. Mouse anti-human CD3, CD4, and CD8 monoclonal antibodies were purchased from the Department of Immunology, Peking Medical University, and mouse anti-human IL-2R monoclonal antibody was purchased from the Wuhan Biological Products Factory.

Isolation of Human Peripheral Blood Mononuclear Cells and Culture Conditions

Human peripheral blood mononuclear cells (PBMC) were isolated from the patients by centrifugation on a Ficoll-Hypaque (Sigma) density gradient. The cells were resuspended at a concentration of $5\times 10^5/\text{ml}$ in RPMI 1640 medium (GIBCO) supplemented with 10 % heat-inactivated fetal calf serum (GIBCO), penicillin (100 U/ml), streptomycin (100 $\mu\text{g}/\text{ml}$), and 2 mM L-glutamine (GIBCO). The PBMC were stimulated

with pokeweed mitogen (PWM, GIBCO) at a concentration 10 $\mu\text{l}/\text{ml}$ and cultured at 37 °C in a moist atmosphere containing 5 % CO_2 . After 7 days of culture, the cell supernatants were centrifuged and frozen at -20 °C. IgA concentrations in the supernatants were detected by ELISA [35, 36].

Determination of IgE and IgA in Serum and Cortisol in Plasma

Concentrations of total IgE in the serum were determined by ELISA (Basic Medicine Institute, Chinese Academe of Medical Science, Peking). Concentrations of IgA in the serum were determined by turbidimetry (ICS Analyzer II, Beckman, USA). The albumin in the serum and nasal secretions was measured by spectrophotometry with bromocresol green. Levels of cortisol in the plasma were determined by radioimmunoassay (Shanghai Institute of Biological Products, Shanghai, China).

Counts of Eosinophils in Peripheral Blood

The absolute and differential numbers of eosinophils in the peripheral blood were counted with an eosin stain.

Statistical Analysis

All data are expressed as the mean $\pm$ SE. A paired *t* test was used to compare the variances before and after acupuncture treatment. *P* values lower than 0.05 were considered significant.

Results

Clinical Efficacy of the Acupuncture Treatment

The overall efficacy of acupuncture on patients with allergic asthma and chronic bronchitis at the end of one course of treatment was 85 % (85/100) and 85.14 % (63/74), respectively. Twenty seven patients with allergic asthma and 25 with chronic bronchitis were followed-up. For patients with allergic asthma and chronic bronchitis, the efficacy was 37.04 and 28 % after 1 year of treatment, respectively; after 3 years of treatment, the clinical control rates were 22.22 and 12 %, respectively.

Concentrations of sIgA in the Saliva and Nasal Secretions of Patients Treated with Acupuncture

After acupuncture treatment, the concentrations of sIgA and total IgA in the saliva ($P<0.01$ and $P<0.02$, respectively) and nasal secretions ($P<0.02$ and $P<0.02$, respectively) were significantly decreased in patients with allergic asthma, and there were no significant

changes in the concentration of sIgA in the sputum (Table 1). There was no significant alteration in the sIgA concentrations in the saliva and nasal secretions of patients with chronic bronchitis, but the concentrations of sIgA, IgA, total IgA, and sIgA/T were significantly increased, and IgA/T concentrations were markedly decreased in the sputum of patients treated with acupuncture (Table 1).

IgA Relative Coefficient of Excretion in the Nasal Secretions of Patients Treated with Acupuncture

In order to determine the contribution of serum versus local origin of the proteins, an IgA relative coefficient of excretion (RCE) was calculated as the secretion-to-serum concentration ratio secretion/(protein in serum)/[(albumin in secretion)/(albumin in serum)] [37]. There were no significant changes in the RCE, serum IgA, or albumin in the serum and nasal secretions of patients with allergic asthma and those with chronic bronchitis (Table 2). The results indicated that any changes in IgA in the secretions were not from exudation of the protein from the serum.

T Lymphocyte Subsets and Eosinophil Counts in the Peripheral Blood of Patients Treated with Acupuncture

The CD3⁺, CD4⁺, and CD8⁺ T lymphocytes in the peripheral blood were significantly increased after acupuncture treatment in patients with allergic asthma and those with chronic bronchitis, but IL-2R⁺ T lymphocytes in the peripheral blood were significantly decreased after acupuncture in the two groups (Table 3). There were no significant changes in the ratio of CD4⁺/CD8⁺ cells in the two groups before and after acupuncture. The absolute and differential counts of eosinophils in the peripheral blood were significantly decreased after acupuncture treatment in patients with allergic asthma (Table 4). In patients with chronic bronchitis, there was a significant reduction in the differential counts of eosinophils in the peripheral blood after acupuncture treatment.

Concentrations of IgA in the PBMC Supernatants, Total IgE in the Serum, and Cortisol in the Plasma of Patients Treated with Acupuncture

There were no significant changes in the concentration of IgA in the supernatants and cortisol in the plasma of patients with allergic asthma and those with chronic bronchitis after

Table 1 Concentrations of sIgA in the saliva, nasal secretions and sputum of patients treated with acupuncture

		sIgA (μg/ml)	IgA (μg/ml)	T ^a (μg/ml)	sIgA/T (%)	IgA/T (%)
Saliva						
Allergic asthma (n=14)	Before acupuncture	144.11±21.15	9.41±1.87	153.35±22.16	93.06±1.32	6.94±1.31
	After acupuncture	111.09±17.62	9.13±2.10	120.22±19.07	92.03±1.87	7.97±1.87
	<i>P</i> value	<0.01	>0.50	<0.02	>0.20	>0.20
Chronic bronchitis (n=14)	Before acupuncture	86.09±10.97	10.69±1.67	96.78±11.78	88.20±1.73	11.80±1.73
	After acupuncture	91.91±11.48	11.42±1.54	103.33±11.91	87.92±1.53	12.08±1.53
	<i>P</i> value	>0.20	>0.50	>0.20	>0.50	>0.50
Nasal secretion						
Allergic asthma (n=24)	Before acupuncture	905.23±190.28	123.74±26.71	1,028.98±210.11	85.92±2.00	14.08±2.00
	After acupuncture	411.35±49.49	70.80±12.27	482.15±54.58	84.90±1.97	15.10±1.97
	<i>P</i> value	<0.02	>0.05	<0.02	>0.50	>0.50
Chronic bronchitis (n=10)	Before acupuncture	1,673.69±602.56	229.03±135.97	1,902.72±732.34	90.72±1.28	9.28±1.28
	After acupuncture	2,756.77±942.54	451.70±245.02	3,208.47±1,169.76	87.93±2.26	12.07±2.26
	<i>P</i> value	>0.10	>0.05	>0.20	>0.20	>0.20
Sputum						
Allergic asthma (n=5)	Before acupuncture	103.97±18.25	36.41±16.76	140.38±28.76	77.14±6.16	22.86±6.16
	After acupuncture	457.33±151.10	54.76±14.14	512.50±164.52	87.74±2.32	12.26±2.32
	<i>P</i> value	>0.05	>0.10	>0.05	>0.20	>0.20
Chronic bronchitis (n=7)	Before acupuncture	159.15±57.53	45.22±14.51	204.38±70.86	77.15±4.04	22.85±4.04
	After acupuncture	1,173.88±347.65	83.09±12.81	1,256.95±355.91	91.62±1.64	8.38±1.64
	<i>P</i> value	<0.05	<0.05	<0.05	<0.02	<0.02

Data are shown as mean ± SE. *P* values represent the statistical probability between the values before and after acupuncture by the paired-samples *t* test

^a T is total IgA, the sum of sIgA and IgA

Table 2 IgA relative coefficient of excretion (RCE) in nasal secretions of the patients treated with acupuncture

	IgA in serum g/L	Albumin in serum	Albumin in nasal secretion	RCE
Allergic asthma				
Before acupuncture	2.38±0.11 (<i>n</i> =37)	50.21±0.81 (<i>n</i> =18)	1.654±0.66 (<i>n</i> =12)	3.300±1.21 (<i>n</i> =12)
After acupuncture	2.39±0.10 (<i>n</i> =37)	49.20±1.54 (<i>n</i> =18)	0.699±0.17 (<i>n</i> =12)	2.803±0.78 (<i>n</i> =12)
<i>P</i> value	>0.50	>0.50	>0.20	>0.50
Chronic bronchitis				
Before acupuncture	2.26±0.13 (<i>n</i> =35)	46.63±1.33 (<i>n</i> =10)	40.976±0.49 (<i>n</i> =6)	1.955±0.41 (<i>n</i> =6)
After acupuncture	2.28±0.09 (<i>n</i> =35)	43.29±0.92 (<i>n</i> =10)	1.449±0.48 (<i>n</i> =6)	2.164±0.41 (<i>n</i> =6)
<i>P</i> value	>0.50	>0.50	>0.50	>0.50

Data are shown as mean ± SE. *P* values represent the statistical probability between the values before and after acupuncture by the paired-samples *t* test

acupuncture treatment. Total IgE concentrations in the serum of patients with allergic asthma were significantly decreased after acupuncture treatment (Table 5).

Discussion

Acupuncture is a complex intervention for diseases, and many studies have demonstrated that acupuncture can cause multiple biological responses and regulate many cellular and physiological processes [28]. In an animal model, our study indicated that the gene expression profile at the transcriptional level during the early airway response phase of asthma could be effectively and specifically regulated by acupuncture, which suggested that the gene expression of the immune response may play an important role in the treatment [38]. The results of this study revealed that acupuncture treatment in patients with allergic asthma and chronic bronchitis had similar total efficacies: 85 and 85.15 %, respectively, which was in accordance with other reports that used the same acupuncture method [31]. The level of total IgE was significantly decreased in patients with allergic asthma, further supporting the efficacy of acupuncture in allergic asthma.

The airway mucosa, which is continuously exposed to inhaled antigens and biotoxins, represents a major challenge for the immune system [39]. The first line of defense mechanisms of the upper (nose, pharynx, and larynx) and lower (trachea, cartilaginous bronchi, and membranous bronchioles) conducting airways rely mainly on sIgA. However, it remains unclear how and to what extent the mucosal IgA response plays a role in allergic asthma [40]. Our previous research indicated that the local secretion rate of sIgA in the saliva and the concentration of sIgA in the nasal secretions were significantly increased in patients with allergic asthma when compared with those in normal controls [41], which was supported by others [17, 42]. After acupuncture treatment, the concentrations of sIgA and total IgA in the saliva and nasal secretions were significantly decreased in patients with allergic asthma. The role of eosinophil activation in asthma has been well documented [43]. Several studies showed that sIgA is a potent stimulus for eosinophils and represents the main trigger of eosinophil degranulation [44, 45]. The increase of sIgA in the saliva and nasal secretions may be an inducer of eosinophil degranulation. After acupuncture treatment, the reduction of sIgA in the secretions and the decrease in the numbers of absolute and differential eosinophils in the peripheral blood may be associated with the amelioration of eosinophilic inflammation in patients with allergic asthma.

Table 3 T lymphocyte subsets in the peripheral blood of patients treated with acupuncture

	CD3 %	CD4	CD8	IL-2R	CD4/CD8
Allergic asthma (<i>n</i>=17)					
Before acupuncture	67.45±1.64	45.44±1.67	18.72±1.23	10.27±0.49	2.62±0.21
After acupuncture	76.19±1.58	49.24±1.43	24.54±1.77	7.62±0.38	2.27±0.24
<i>P</i> value	<0.001	<0.01	<0.001	<0.001	>0.10
Chronic bronchitis (<i>n</i>=12)					
Before acupuncture	60.27±2.94	38.12±1.70	19.54±5.75	9.77±1.66	2.11±0.21
After acupuncture	71.06±2.49	43.41±1.40	25.72±5.74	7.09±0.53	1.78±0.14
<i>P</i> value	<0.001	<0.001	<0.001	<0.001	>0.05

Data are shown as mean ± SE. *P* values represent the statistical probability between the values before and after acupuncture by the paired-samples *t* test

Table 4 Counts of eosinophils in the peripheral blood of patients treated with acupuncture

	Absolute eosinophils ($\times 10^8/L$)	Differential eosinophils (%)
Allergic asthma ($n=20$)		
Before acupuncture	303.60 $\pm$ 50.71	7.08 $\pm$ 1.28
After acupuncture	192.50 $\pm$ 38.70	4.05 $\pm$ 0.59
<i>P</i> value	<0.012	<0.01
Chronic bronchitis ($n=12$)		
Before acupuncture	100.83 $\pm$ 18.65	3.00 $\pm$ 0.44
After acupuncture	88.92 $\pm$ 15.46	2.21 $\pm$ 0.35
<i>P</i> value	>0.10	<0.01

Data are shown as mean $\pm$ SE. *P* values represent the statistical probability between the values before and after acupuncture by the paired-samples *t* test

Although there were no significant changes in sIgA concentrations in the saliva and nasal secretions of patients with chronic bronchitis treated with acupuncture, there were some increasing trends as indicated in Table 1. The concentrations of sIgA, IgA, total IgA, and sIgA/T were significantly increased in the sputum of patients with chronic bronchitis who were treated with acupuncture. Whether the results were related with the enhancement of “anti-infection” in the patients should be further researched.

It is well known that the induction of sIgA in external secretions is not accompanied by the appearance of corresponding serum antibodies [2]. Secretory and circulatory IgA systems are independent. After antigen ingestion, PWM-stimulated PBMC produced antibodies predominantly of the IgA isotype [46]. Our results demonstrated that, before and after acupuncture treatment, there were no significant changes in the RCE, serum IgA and albumin in the serum, and nasal secretions in patients with allergic asthma and those with chronic bronchitis, indicating that any changes in IgA in the secretions and in the PWM-stimulated PBMC supernatants were not the result of exudation of the protein from the serum. However, after acupuncture treatment, there were no significant changes in IgA in the PWM-stimulated PBMC in the two groups of patients. Further research would provide more detailed explanations for these observations.

Our research showed that the peripheral blood CD3+, CD4+, and CD8+ T lymphocytes were significantly

increased after acupuncture treatment in patients with allergic asthma and those with chronic bronchitis and IL-2R + T lymphocytes (activated CD4+ T cells) were significantly decreased in the two groups. Research has indicated that T lymphocytes, particularly Th2-like cells, play a central role in the late inflammation of allergic asthmatics. Activated T lymphocytes in the peripheral blood, bronchoalveolar lavage fluid, and bronchial mucosa of allergic asthmatics have characteristics of Th2-like cells [47], and these activated Th2-like cells mainly secrete IL-4 and IL-5. IL-4 is known to play a key role in IgE synthesis, while IL-5 is important in the secretion of sIgA in the mucosa [48] and in the development, maturation, chemo-taxis, and activation of eosinophils [49], which result in the increase of blood serum IgE and the excessive secretion of mucosal sIgA in allergic asthmatics. The results showed that the reductions of total IgE in the serum and sIgA in secretions were closely associated with the decrease in IL-2R + T lymphocytes and eosinophils in the peripheral blood, which may play roles in the acupuncture treatment of allergic asthma.

In summary, the results suggested that acupuncture is efficacious as an adjunctive therapy in the treatment of allergic asthma. The regulatory effects of acupuncture in patients with allergic asthma included decreases in the concentrations of sIgA and total IgA in the saliva and nasal secretions and total IgE in the serum, as well as reduction in the numbers of IL-2R + T lymphocytes and eosinophils in the peripheral blood.

Table 5 Concentrations of IgA in PBMC supernatants, total IgE in serum and cortisol in plasma of the patients treated with acupuncture

	IgA in supernatants (ng/ml)	Total IgE in serum (IU/ml)	Cortisol in plasma (μ g/100 ml)
Allergic asthma			
Before acupuncture	74.21 $\pm$ 9.77 ($n=15$)	1,966.29 $\pm$ 378.99 ($n=35$)	21.23 $\pm$ 1.89 ($n=20$)
After acupuncture	82.15 $\pm$ 10.02 ($n=15$)	984.85 $\pm$ 201.90 ($n=35$)	20.03 $\pm$ 1.57 ($n=20$)
<i>P</i> value	>0.50	<0.001	>0.50
Chronic bronchitis			
Before acupuncture	59.26 $\pm$ 8.28 ($n=16$)	712.99 $\pm$ 123.73 ($n=28$)	24.33 $\pm$ 2.87 ($n=12$)
After acupuncture	75.22 $\pm$ 10.26 ($n=16$)	614.46 $\pm$ 91.64 ($n=28$)	21.96 $\pm$ 2.71 ($n=12$)
<i>P</i> value	>0.20	>0.20	>0.50

Data are shown as mean $\pm$ SE. *P* values represent the statistical probability between the values before and after acupuncture by the paired-samples *t* test

Acupuncture has also been studied in many other allergic and immune disease states [50, 51]. While there have similarly been promising observations in those studies as well as other asthma studies [52, 53], the efficacy, safety, and pathogenic mechanism of its action in these diseases remain unclear, and further well-conducted clinical and laboratory studies are needed to clarify the role of acupuncture in allergy and immunology.

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