

## Acupuncture Research

# Acupuncture Regulates the Balance of CD4<sup>+</sup> T Cell Subtypes in Experimental Asthma Mice\*

DONG Ming<sup>1</sup>, WANG Wen-qian<sup>2,3</sup>, CHEN Juan<sup>4</sup>, LI Mi-hui<sup>2,3</sup>, XU Fei<sup>2,3</sup>,  
 CUI Jie<sup>2,3</sup>, DONG Jing-cheng<sup>2,3</sup>, and WEI Ying<sup>2,3</sup>

**ABSTRACT** **Objective:** To evaluate the involvement of different CD4<sup>+</sup> T cell subtypes in the anti-asthmatic effects of acupuncture in asthmatic mice. **Methods:** BALB/c mice were challenged by ovalbumin (OVA) for the establishment of experimental asthma model. Mice were divided into 4 groups by a random number table including the normal control, asthma model, acupuncture and sham acupuncture groups (14 per group). Acupoints Dazhui (GV 14), bilateral Fengmen (BL 12) and Feishu (BL 13) were selected for manual acupuncture treatment every other day for 4 weeks and Huantiao (GB 30) was selected for sham acupuncture. Airway hyperresponsiveness was examined by Buxco Pulmonary System. Pulmonary histopathology analysis was performed for inflammatory cell infiltration and mucus hypersecretion by haematoxylin eosin staining and periodic acid-Schiff staining. Inflammatory mediators assays of serum were investigated by enzyme-linked immunosorbent assay and Bio-Plex. CD4<sup>+</sup> T cell subpopulations including the expression levels of important factors in T lymphocyte polarization in lung tissue were examined by flow cytometric and Western blot analyses. Related pathways were detected by Western blot assay. **Results:** Compared with the OVA-induced asthma model group, acupuncture could attenuate airway hyperresponsiveness, inhibit inflammatory cell infiltration and mucus hypersecretion ( $P < 0.05$  or  $P < 0.01$ ). Furthermore, acupuncture increased the expressions of T-bet and Foxp3<sup>+</sup>, the cell numbers of CD4<sup>+</sup> interferon gamma (IFN- $\gamma$ )<sup>+</sup> and CD4<sup>+</sup> Foxp3<sup>+</sup> in lung tissue and the level of Treg type cytokine interleukin (IL)-10 in serum ( $P < 0.05$  or  $P < 0.01$ ). Meanwhile, acupuncture reduced the RAR-related orphan receptor gamma t (ROR  $\gamma$  t) level, the cell numbers of CD4<sup>+</sup> IL-17A<sup>+</sup> as well as the levels of IL-5, IL-13 and IL-17A in serum ( $P < 0.05$  or  $P < 0.01$ ). In addition, both acupuncture and sham acupuncture could inhibit the phosphorylation of p38 and p44/42 ( $P < 0.01$ ). **Conclusion:** Acupuncture could alleviate allergic airway inflammation by strengthening the activities of Th1 and Treg, thus regulating the balance of CD4<sup>+</sup> T cell subtypes in experimental asthmatic mice.

**KEYWORDS** acupuncture, asthma, Th2, Th17, Treg

Asthma is a common disease with various causes and complicated pathophysiology.<sup>(1)</sup> A common belief is that many immune cells, especially the activated T cells subtypes, release cytokines and act together to cause cardinal symptoms of asthma.<sup>(2)</sup> Th2 cells and related cytokines, which induce eosinophilic airway inflammation and IgE synthesis, participate in the pathogenesis of asthma, and this pathological progress could be inhibited by Th1 cells and interferon gamma (IFN- $\gamma$ ). Th17 cells and related cytokines cause neutrophilic inflammation while Treg can release Foxp3 and inhibit RAR-related orphan receptor gamma t (ROR  $\gamma$  t) to abort the differentiation of Th17 cells.<sup>(3)</sup> These considerable evidences indicate that asthma features are suppressed through Th1, Treg cells and their cytokines, while they are aggravated through Th2, Th17 cells and their cytokines,<sup>(4)</sup> suggesting that the activities of these cells are associated with the

pathology and therapeutic effect of asthma.<sup>(5)</sup>

As important parts of mitogen-activated protein kinases families, p38 and p44/42 not only play important roles in inflammation and stress, but also participate in the process of cell survival, differentiation

©The Chinese Journal of Integrated Traditional and Western Medicine Press and Springer-Verlag GmbH Germany, part of Springer Nature 2018

\*Supported by the National Natural Science Foundation of China (No. 81403476)

1. Gumei Community Health Center of Minhang District, Shanghai (201102), China; 2. Department of Integrative Medicine, Huashan Hospital, Fudan University, Shanghai (200040), China; 3. Institutes of Integrative Medicine of Fudan University, Shanghai (200040), China; 4. Department of Pediatric Neurological Rehabilitation, Maternal and Child Health Hospital of Dengfeng, Dengfeng (452470), Henan Province, China

Correspondence to: Associate Prof. WEI Ying, Tel: 86-21-52888263, E-mail: [weiyong\\_acup@126.com](mailto:weiyong_acup@126.com)

DOI: <https://doi.org/10.1007/s11655-018-3055-6>

and apoptosis.<sup>(6)</sup> Previous reports observed that p38 and p42/44 were activated by responding to inflammatory signals in asthma.<sup>(7)</sup> p38 and p42/44 were also involved in eosinophil differentiation and cytokine production,<sup>(8)</sup> suggesting that they may participate in asthmatic T cell polarization.

As a traditional therapy supplement to glucocorticoid, acupuncture has been applied in treating asthma symptoms for centuries.<sup>(9)</sup> Many controlled trials suggest that acupuncture is effective in treating asthma,<sup>(10)</sup> like alleviating asthmatic symptoms,<sup>(11,12)</sup> improving lung function and decreasing medication dosage.<sup>(13)</sup> Furthermore, previous studies have identified that acupuncture can elevate Th1 and Treg cytokines and decrease Th2 and Th17 cytokines in bronchoalveolar lavage fluid (BALF) of asthmatic model.<sup>(14,15)</sup> But the regulation role of acupuncture on these 4 CD4<sup>+</sup>T cell subtypes in asthma has not been explicitly explained. Aiming to clarify the influence of acupuncture in balancing these CD4<sup>+</sup>T cell subtypes, we created experimental allergic asthma mice and detected the related cytokines, transcription factors, cell polarization and pathway, in which we may enhance the understanding of asthma as well as the development of acupuncture research.

## METHODS

### Reagents

Ovalbumin (OVA, grade V) and methacholine (Mch) were purchased from Sigma (St. Louis, MO, USA). Mouse premixed multi-analyte kit was obtained from R&D (Minneapolis, MN, USA). Sterile acupuncture needles (0.25 mm × 13 mm) were purchased from Shenlong Medical Apparatus Co., Ltd. (Wujiang, China). Mouse interleukin 5 (IL-5), IL-10, IL-13 Bio-Plex kits were custom-made from Bio-Rad Corp. (Hercules, CA, USA). Mouse IL-17A enzyme-linked immunosorbent assay (ELISA) kit was purchased from Anogen Biopharmaceutical Company (Mississauga, Ontario, Canada). Cell stimulation cocktail (500X) was obtained from eBioscience (San Diego, USA). FITC-labeled CD4, phycoerythrin/cyanine 7 (PE/Cy7)-labeled IFN- $\gamma$ , allophycocyanin (APC)-labeled IL-4, brilliant violet (BV) 421-labeled IL-17A and phycoerythrin (PE)-labeled Foxp3 were obtained from Biolegend (anti-mouse, San Diego, USA). Anti-mouse IgG of GAPDH and horseradish peroxidase (HRP)-conjugated secondary antibodies were obtained from Kangchen (Shanghai, China).

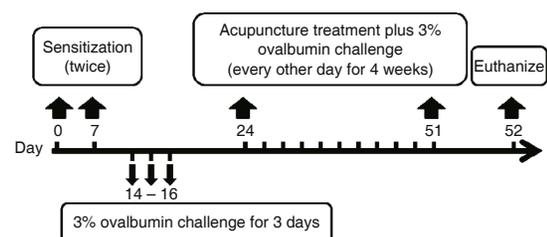
Anti-mouse T-bet, GATA binding protein 3 (GATA3), ROR  $\gamma$ t, Foxp3, p38, p-p38, p44/42, and p-p44/42 antibodies were obtained from CST (Danvers, MA, USA) and Abcam (Cambridge, MA, USA).

### Animals and Grouping

Healthy female BALB/c mice (certificate No. 0003425), weighing 12–15 g, were provided by Shanghai Laboratory Animal Co., Ltd. The mice were reared in a pathogen-free rodent facility according to approved procedures and housed in separate stainless steel cages in a temperature-controlled environment (20–24 °C) with a 12-h light/dark cycle. Fifty-six mice were feeding in pathogen-free animal facilities with free access to diet in a 12-h light/dark cycle and randomly divided to 4 groups (14 per group) according to the random number table: the normal control group (NC), the OVA-induced asthma model group (model), the acupuncture group, and the sham acupuncture group. The protocol of this study was following the guidelines and approval of the Animal Care and Use Committee on the Ethics of Animal Experiments of Fudan University.

### Establishment of Experimental Asthma Mice

On Day 0 and 7, mice were sensitized with 0.2 mL normal saline containing 50  $\mu$ g of OVA and 1 mg of alum. From Day 14 to 16, mice were put into plexiglas chambers and challenged with 3% OVA for 30 min every day using ultrasonic nebulizers (402AI, Yuyue Medical Equipment Company, Jiangsu, China). After a week of non-treatment period, all mice were challenged every other day for 4 weeks (Day 24–51). The NC mice were managed with normal saline with the same method (Figure 1).



**Figure 1. Time Line of Establishment of Mouse Model and Acupuncture Treatment**

### Acupuncture Treatment

To perform the acupuncture treatment, the acupoints of Dazhui (GV 14), bilateral Fengmen (BL 12) and Feishu (BL 13) were selected according to the theory of acupuncture.<sup>(16)</sup> For mice in the sham

acupuncture group, the irrelevant distal acupoints of bilateral Huantiao (GB 30) were selected for sham acupuncture by operator blinded to experimental group allocation. The acupuncturist was not involved in asthma establishment and the following measurements. From Day 24, both acupuncture and sham acupuncture procedures were performed 1 h before each challenge with a fixation device in an awakened state. Sterile needles were inserted approximately 3 mm into the acupoints and withdrawn after 30 min. During the needling time, no manual manipulations were performed. Mice were sacrificed by cervical dislocation after the airway hyper-reactivity (AHR) measurement.

### AHR Measurement

The measurements of airway responsiveness were performed after the last challenge. Mice were inhaled with gradient doses of Mch (3.125, 6.25, and 12.5 mg/mL) under anaesthesia with pentobarbital sodium using Buxco Pulmonary System (Buxco Electronics, Troy, NY, USA) to assess pulmonary resistance (RL) and lung dynamic compliance (Cdyn). Results were expressed as the percentage change in baseline following Mch challenge.

### Histopathological Assessment

After fixed in 4% paraformaldehyde, the lung was embedded in paraffin and then cut into 4- $\mu$ m-thick sections. The sections were stained with hematoxylin eosin (HE) and periodic acid-Schiff (PAS) to assess the severity of lung pathology, leukocyte infiltration and mucous/goblet cells proliferation. The inflammatory changes were observed in the perspective of 10 times with an optical microscope (Eclipse 80i, Nikon Corporation, Tokyo, Japan).

### Cytokines Determination in Serum

The blood was collected after the AHR detection by ophthalmia and thereafter centrifuged for serum collection. Levels of cytokines including IL-5, IL-10, IL-13 and IL-17A in serum were detected by Bio-Plex and ELISA assays.

### Flow Cytometric Analysis

The single-cell suspension of lung tissue was dissociated by gentleMACS™ Dissociator (Miltenyi, Germany) for further flow cytometric analysis. Lung tissue cells were cultured with 1X cell stimulation cocktail for 4 h and then stained with CD4 antibody for 30 min. After incubation with the fixation/permeabilization

solution for 1 h, the lung tissue cells were incubated with antibodies for 40 min, followed by detection with a FACS Calibur Instrument (BD, San Jose, CA, USA).

### Western Blot Analysis

The extraction of lung total proteins in each group was finished according to the instructions. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 20  $\mu$ g protein, and then the targeted proteins including T-bet, GATA3, Foxp3, ROR  $\gamma$ t, p38, p-p38, p44/42 and p-p44/42 were transferred to polyvinylidene fluoride membranes and blocked. Thereafter, targeted proteins were blotted at 1:10000 dilution using specific antibodies and HRP-conjugated secondary antibodies. The results were quantified by Bio-Rad Image Lab software 3.0 (USA).

### Statistical Analysis

Data was expressed as mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ). Statistical analysis was performed using one-way analysis of variance followed by post-hoc test of least significant difference or Games-Howell tests (depending on the data and on the hypothesis tested) by SPSS 18.0. A *P* value < 0.05 indicated a statistically significant difference.

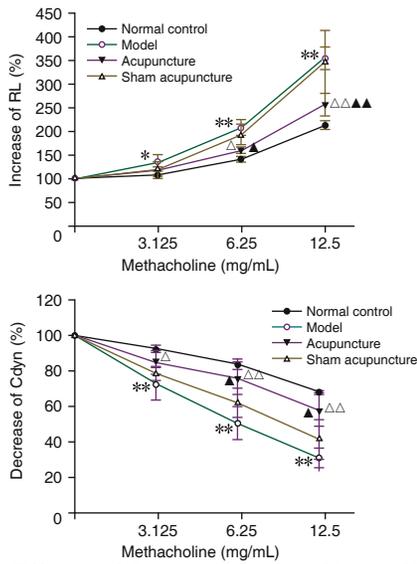
## RESULTS

### Effects of Acupuncture on AHR in Asthma Mice

With the increasing dose of Mch administration, mice in the model group showed an obvious increase of RL and a significantly decrease of Cdyn compared with the NC group (*P* < 0.05 or *P* < 0.01). After acupuncture treatment, RL was decreased and Cdyn was increased significantly compared with the model mice (*P* < 0.05 or *P* < 0.01). However, changes in RL and Cdyn of mice in the sham acupuncture group were not obviously (*P* > 0.05, Figure 2).

### Effect of Acupuncture on Inflammation in Asthma Mice

The HE results showed that there were lots of inflammatory cells infiltrating around the airway in asthma mice. Acupuncture treatment revealed obvious decrease in the infiltration of inflammatory cells. Sham acupuncture group also showed a trend of improving in lung inflammation, but the improvement is limited. The PAS staining results denoted that there were a large number of mucous/goblet cells proliferating and producing mucin/mucous around the



**Figure 2. Effects of Acupuncture on Airway Hyper-Reactivity to Mch in OVA Inhalation Mice (n=14)**

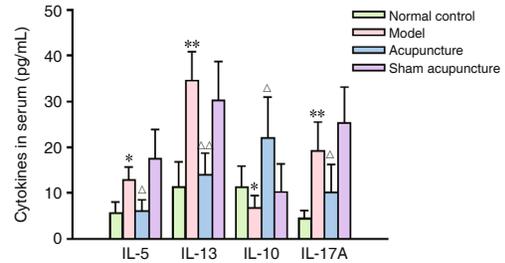
Notes: RL: pulmonary resistance; Cdyn: lung dynamic compliance; OVA: ovalbumin. Data was expressed as percentage change of the baseline value. \* $P < 0.05$ , \*\* $P < 0.01$  vs. normal control group;  $\Delta P < 0.05$ ,  $\Delta\Delta P < 0.01$  vs. model group;  $\blacktriangle P < 0.05$ ,  $\blacktriangle\blacktriangle P < 0.01$  vs. sham acupuncture group

airway in asthma mice. In contrast, the lung tissues of acupuncture group were protected from mucin/mucous hyper-secretion by airway epithelial cells and sham acupuncture also did manifest improvements (Figure 3).

**Effect of Acupuncture on Cytokine Levels of Different CD4<sup>+</sup> T Cell Subtypes in Serum of Asthma Mice**

Th2 cytokines (IL-5 and IL-13) and Th17 cytokine (IL-17A) elevated obviously in the model group compared with the NC group ( $P < 0.05$  or  $P < 0.01$ ), while acupuncture presented an inhibiting effect on these cytokine levels ( $P < 0.05$  or  $P < 0.01$ ). The level

of IL-10 was decreased in the model group compared with the NC group ( $P < 0.05$ ), while it was obviously increased in the acupuncture group ( $P < 0.05$ ). However, sham acupuncture did not have any effect on the production of IL-5, IL-13, IL-17A and IL-10 compared with the asthma model mice ( $P > 0.05$ , Figure 4).

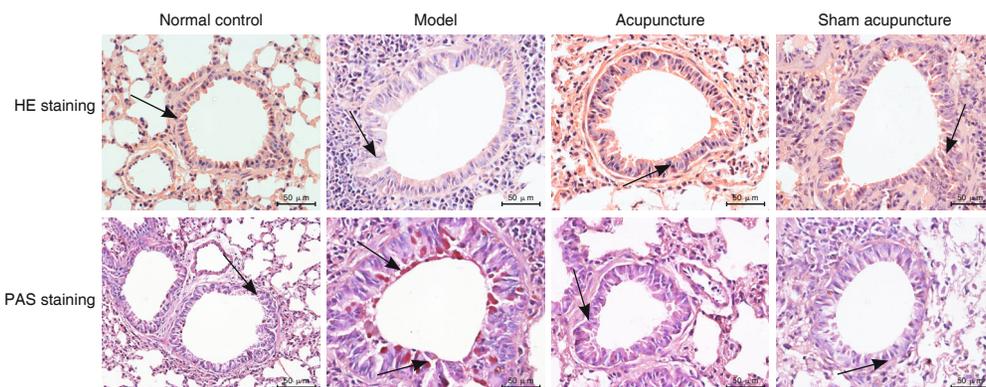


**Figure 4. Acupuncture Regulated Cytokine Levels of Different CD4<sup>+</sup> T Cell Subtypes in Serum of Asthma Mice ( $\bar{x} \pm s$ , n=14)**

Notes: IL: interleukin. \* $P < 0.05$ , \*\* $P < 0.01$  vs. normal control group;  $\Delta P < 0.05$ ,  $\Delta\Delta P < 0.01$  vs. model group

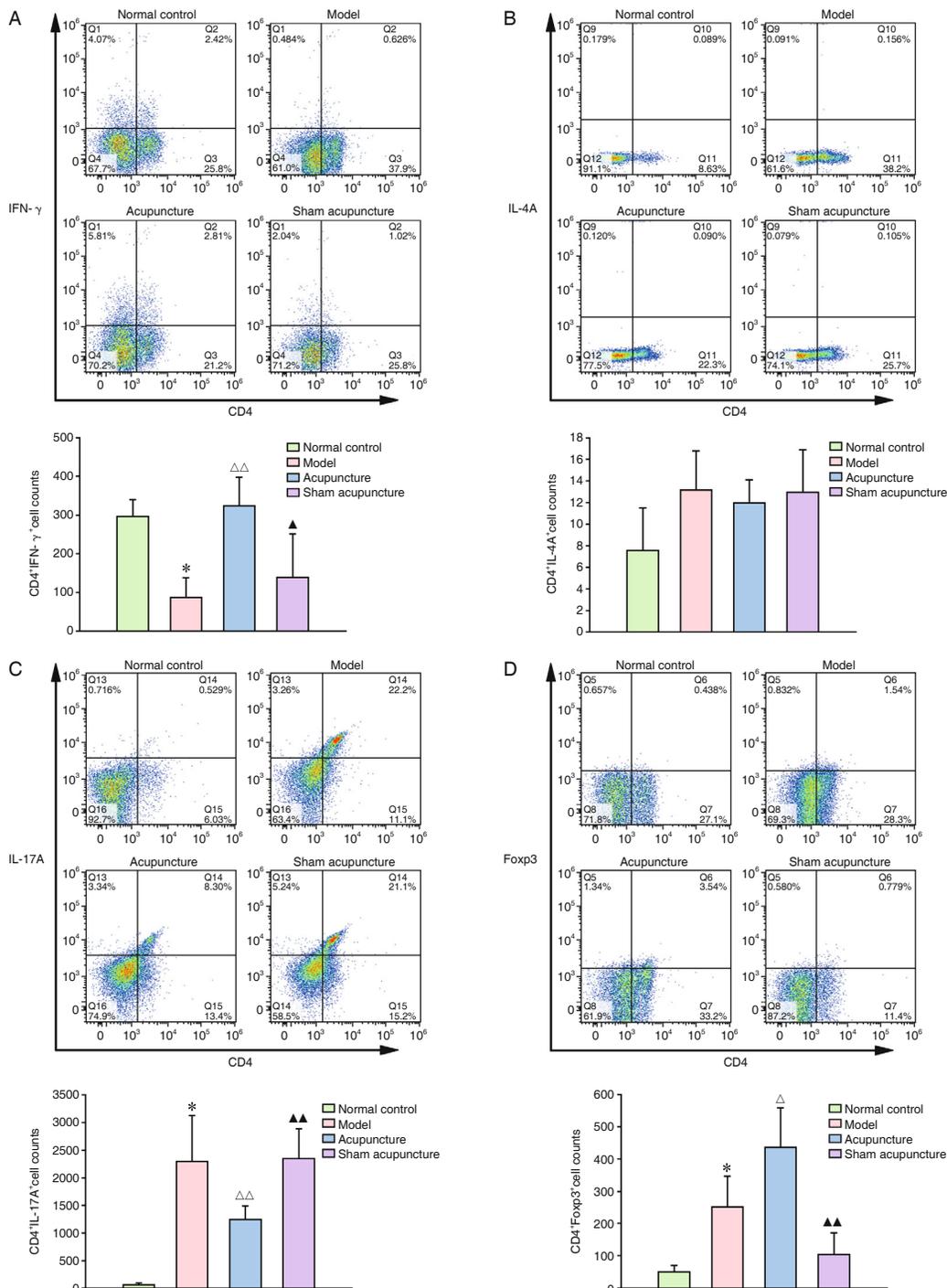
**Effect of Acupuncture on CD4<sup>+</sup> T Cell Subtypes in Lung Tissues of Asthma Mice**

The model group had significantly lower CD4<sup>+</sup> IFN- $\gamma$ <sup>+</sup> cells in lung tissue compared with the NC group ( $P < 0.01$ ). After acupuncture treatment, the mice had a prominent increase in CD4<sup>+</sup> IFN- $\gamma$ <sup>+</sup> cells ( $P < 0.01$ ), and there was no decrease in CD4<sup>+</sup> IL-4A<sup>+</sup> cells compared with the model group ( $P > 0.05$ ). Mice in the model group had significantly higher CD4<sup>+</sup> IL-17A<sup>+</sup> cells and CD4<sup>+</sup> Foxp3<sup>+</sup> cells compared with the NC group ( $P < 0.01$ ). The mice in the acupuncture group had an obvious reduction in CD4<sup>+</sup> IL-17A<sup>+</sup> cells and a prominent increase in CD4<sup>+</sup> Foxp3<sup>+</sup> cells in lung tissue compared with the model group ( $P < 0.05$  or  $P < 0.01$ ). There were no obvious changes in these cell populations in the sham acupuncture group compared with the asthma mice ( $P > 0.05$ , Figure 5).



**Figure 3. Changes of Inflammatory Cells Infiltration and Mucous/Goblet Cells Proliferating around the Airway of Asthma Mice by HE and PAS Stainings ( $\times 20$ )**

Notes: HE: hematoxylin eosin; PAS: periodic acid-Schiff. The arrows indicate the inflammatory changes and mucus secretion around the airways.



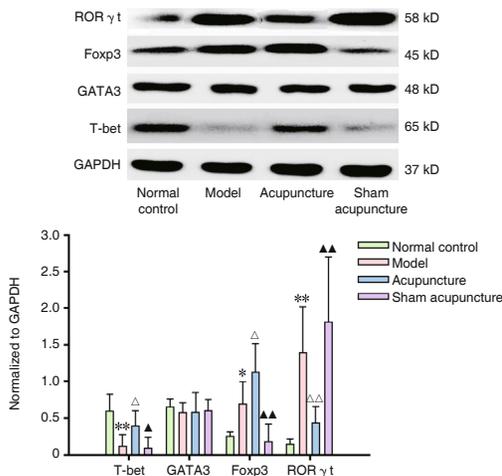
**Figure 5. Acupuncture Regulated Proportion and Counts of Different CD4<sup>+</sup>T Cell Subtypes in Lung Tissues of Asthma Mice ( $\bar{x} \pm s, n=14$ )**

Notes: IL: interleukin; IFN- $\gamma$ : interferon gamma. \* $P<0.01$  vs. normal control group;  $\Delta P<0.05$ ,  $\Delta\Delta P<0.01$  vs. model group;  $\Delta P<0.05$ ,  $\Delta\Delta P<0.01$  vs. acupuncture group

### Effect of Acupuncture on Expressions of CD4<sup>+</sup> T Cell Subtypes Associated Factors in Asthma Mice

The level of T-bet was reduced obviously in the asthma mice compared with the NC group ( $P<0.01$ ), while it was increased significantly in the acupuncture group ( $P<0.05$ ). However, there were no marked changes in Th2 transcription factor GATA3 expression

in each group ( $P>0.05$ ). Compared with the NC group, Foxp3<sup>+</sup> and ROR  $\gamma$  t were elevated significantly in the model group ( $P<0.05$  or  $P<0.01$ ). Acupuncture resulted in a significant increase of Foxp3<sup>+</sup> and a significant reduction of ROR  $\gamma$  t ( $P<0.05$  or  $P<0.01$ ). However, sham acupuncture has no effect on the expressions mentioned above ( $P>0.05$ , Figure 6).

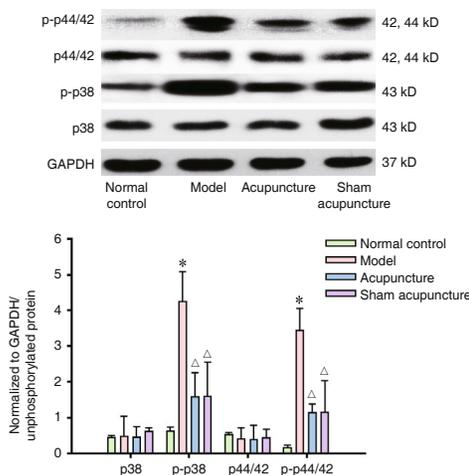


**Figure 6. Acupuncture Regulated Expressions of CD4<sup>+</sup> T Cell Subtypes Associated Factors in Lung Tissue of Asthma Mice ( $\bar{x} \pm s, n=14$ )**

Notes: ROR  $\gamma$  t: RAR-related orphan receptor gamma t; GATA3: GATA binding protein 3. \* $P < 0.05$ , \*\* $P < 0.01$  vs. normal control group;  $\Delta$   $P < 0.05$ ,  $\Delta\Delta$   $P < 0.01$  vs. model group;  $\blacktriangle$   $P < 0.05$ ,  $\blacktriangle\blacktriangle$   $P < 0.01$  vs. acupuncture group

**Effect of Acupuncture on Phosphorylation of p38 and p44/42 in Lung Tissue of Asthma Mice**

OVA inhalation induced a sharp up-regulation of p-p38, p-p44/42 expressions ( $P < 0.01$ ). And obvious declines were observed in the acupuncture group and the sham acupuncture group compared with the asthma mice ( $P < 0.01$ ). However, there was no marked change in p38 and p44/42 expressions in each group ( $P > 0.05$ , Figure 7).



**Figure 7. Acupuncture Suppresses Phosphorylation of p38 and p44/42 Expression in Lung Tissue of Asthma Mice ( $\bar{x} \pm s, n=14$ )**

Notes: \* $P < 0.01$  vs. normal control group;  $\Delta$   $P < 0.01$  vs. model group

**DISCUSSION**

It is widely accepted that the disorder of CD4<sup>+</sup> T cell subtypes was an important mechanism to induce

allergic asthma.<sup>(17)</sup> Such as Th2 cells and their cytokines are participated in eosinophil chemotaxis, goblet cell hyperplasia and mucus hypersecretion, AHR development and lung remodeling.<sup>(18)</sup> On the other hand, Th1 cell is protecting us from intracellular pathogens. Th17 cells and related cytokines can aggravate allergic inflammation,<sup>(19)</sup> as well as the accumulation of neutrophil and eosinophil.<sup>(20)</sup> Treg cells and IL-10, are increasingly believed to play key roles in asthma peripheral tolerance.<sup>(21)</sup> Furthermore, evidences have suggested that Th2-mediated pathological changes in asthma could be further synergized by specific Th17 cells.<sup>(22)</sup> Therefore, the discord of CD4<sup>+</sup> T cell subtypes may be a key role for asthma mechanism, and Th1 and Treg are critical in suppressing asthma features.

In this experiment, OVA challenge caused obvious AHR, inflammatory cells infiltration, mucus hypersecretion and increased cytokines of Th2 and Th17 cells. An obvious weakness in Th1 response as well as the enhancement of Th17 response represented the characteristics of allergic asthma. Meanwhile, a remarkable up-regulation of Treg response was also observed, but the up-regulation of Treg response was weaker than that of Th17 response. All the results leading to a Th2 and Th17 dominated inflammation in experimental asthma mice.

Acupuncture is now a popular comprehensive and alternative therapy of asthma in Western countries.<sup>(9)</sup> In patients with allergic asthma, acupuncture can increase T lymphocytes and attenuate symptoms.<sup>(16)</sup> So the disorder of CD4<sup>+</sup> T cells is considered to be involved in the anti-asthma effect of acupuncture. In this experiment, as compared with OVA-challenged mice, acupuncture could attenuate AHR by increased RL and decreased Cdyn, airway inflammation, mucus hypersecretion in experimental asthma model. Furthermore, acupuncture treatment simultaneously promoted Th1 and Treg activities, including the higher levels of IL-10 secretion, the high expressions of T-bet and Foxp3 protein, as well as the increase of Th1 and Treg. Our data was in accordance with previous research in which Treg cells can suppress asthma features and drive peripheral tolerance of asthma through the increase of IL-10.<sup>(23)</sup> In the meantime, acupuncture treatment markedly decreased the productions of IL-5 and IL-13, which are correlating with airway eosinophilia

degree, mounting AHR and goblet cell metaplasia.<sup>(24)</sup> The remodeling of airway and counteract of Treg was also weakened by the decrease of Th17 cells and IL-17A<sup>(25,26)</sup> after acupuncture treatment. These findings revealed that acupuncture could switch asthma model from a predominant Th2 and Th17 response to Th1 and Treg response, which reasonably attenuated asthma symptoms. However, no obvious effect of sham acupuncture treatment on airway inflammation was observed in OVA inhalation mice except the pulmonary histopathology results, indicating acupuncture effect is not equal to the placebo effect, and the different effects between acupuncture and sham acupuncture may depend on acupoint specificity.

Considered as a transfer station of many signal transduction pathways, p38 and p44/42 are responsive to multiple extracellular stimuli, involved in eosinophil differentiation and cytokine production<sup>(8)</sup> and activated in response to inflammatory signals in asthma.<sup>(7,27)</sup> Previous reports observed that when asthma progression was inhibited, the p38, p42/44 activation were suppressed,<sup>(7,28)</sup> which indicating a relationship between asthma and the activation of p38 and p42/44. The current results showed that OVA challenged induced a sharp increase of p-p38 and p-p44/42 expressions in experimental asthma group. Furthermore, we observed that both acupuncture and sham acupuncture could reduce the level of p-p38 and p-p44/42, indicating that both specific and non-specific effects contributed to the inhibition of the p38 and p44/42 activities, and might further affect the downstream signal transduction. Unfortunately, our results did not clearly establish whether the inhibition of p38 and p44/42 activities directly contribute to the anti-asthmatic effects of up-regulation of Th1, Treg activities and down-regulation of Th2, Th17 activities. In our study, specific acupuncture could simultaneously promote the activities of Th1 and Treg. But non-specific effects of sham acupuncture could not regulate the differentiation of CD4<sup>+</sup> T cells. Except cell differentiation activated by inflammatory molecules, the p38 and p44/42 are also involved in signaling pathways to stress stimuli, like oxidative stress.<sup>(6,29)</sup> So the reduction of phosphorylation expressions in both acupuncture and sham acupuncture treatment might be more inclined to oxidative stress, which needs further investigation. In spite of this limitation, this study indicated that acupuncture could attenuate asthma symptoms in experimental asthma mice by

strengthening Th1 and Treg activities and regulating the balance of CD4<sup>+</sup> T cell subtypes.

### Conflict of Interest

All the authors have declared that they had no competing interests.

### Author Contributions

Wei Y and Dong JC conceived and supervised the study. Dong M and Wang WQ performed the experiment and wrote the manuscript. Chen J, Li MH, Xu F and Cui J analysed output data and gave technical support. All authors read and approved the final manuscript.

## REFERENCES

1. Erle DJ, Sheppard D. The cell biology of asthma. *J Cell Biol* 2014;205:621-631.
2. Lambrecht BN, Hammad H. The immunology of asthma. *Nat Immunol* 2015;16:45-56.
3. Moldaver DM, Larche M, Rudulier CD. An update on lymphocyte subtypes in asthma and airway disease. *Chest* 2017;151:1122-1130.
4. Lambrecht BN, Hamida H. Lung dendritic cells in respiratory viral infection and asthma: from protection to immunopathology. *Immunology* 2012;30:243-270.
5. Shi YH, Shi GC, Wan HY, Jiang LH, Ai XY, Zhu HX, et al. Coexistence of Th1/Th2 and Th17/Treg imbalances in patients with allergic asthma. *Chin Med J* 2011;124:1951-1956.
6. Mielke K, Herdegen T. JNK and p38 stresskinases—degenerative effectors of signal-transduction-cascades in the nervous system. *Prog Neurobiol* 2000;61:45-60.
7. Subhashini, Chauhan PS, Dash D, Paul BN, Singh R. Intranasal curcumin ameliorates airway inflammation and obstruction by regulating MAPKinase activation (p38, Erk and JNK) and prostaglandin D2 release in murine model of asthma. *Int Immunopharmacol* 2016;31:200-206.
8. Chialda L, Zhang M, Brune K, Pahl A. Inhibitors of mitogen-activated protein kinases differentially regulate costimulated T cell cytokine production and mouse airway eosinophilia. *Respir Res* 2005;6:36.
9. Acupuncture. NIH consensus statement. Available at <https://consensus.nih.gov/1997/1997acupuncture107html.htm>
10. Fung KP, Chow OK, So SY. Attenuation of exercise-induced asthma by acupuncture. *Lancet* 1986;328:1419-1422.
11. World Health Organization. Acupuncture: review and analysis of reports on controlled clinical trials. Available at <http://apps.who.int/bookorders/anglais/detart1.jsp?sesslan=1&codlan=1&codcol=93&codcch=196>
12. Han J. Observation on effect of acupuncture at Yuji (LU 10) on the pulmonary function of patients with bronchial asthma

- and immediate efficacy of relieving asthma. *China Med Abstr (Int Med, Chin)* 2012;32:891-894.
13. Lewith GT, Watkins AD. Unconventional therapies in asthma: an overview. *Allergy* 1996;51:761-769.
  14. Carneiro ER, Xavier RA, De Castro MA, Do Nascimento CM, Silveira VL. Electroacupuncture promotes a decrease in inflammatory response associated with Th1/Th2 cytokines, nitric oxide and leukotriene B4 modulation in experimental asthma. *Cytokine* 2010;50:335-340.
  15. Dong M, Xie SY, Li FC, Lu N, Wei XP. Is acupuncture better than sham acupuncture for attenuated airway inflammation and regulated cytokines produced by diverse Th subtypes in chronic OVA inhalation in asthma induced mice. *Eur J Integr Med* 2015;7:485-491.
  16. Yang YQ, Chen HP, Wang Y, Yin LM, Xu YD, Ran J. Considerations for use of acupuncture as supplemental therapy for patients with allergic asthma. *Clin Rev Allergy Immunol* 2013;44:254-261.
  17. Wei B, Zhang H, Li L, Li M, Shang Y. T helper 17 cells and regulatory T-cell imbalance in paediatric patients with asthma. *J Int Med Res* 2011;39:1293-1305.
  18. Wills-Karp M. Interleukin-13 in asthma pathogenesis. *Immunol Rev* 2004;202:175-190.
  19. Bettelli E, Korn T, Oukka M, Kuchroo VK. Induction and effector functions of T(H)17 cells. *Nature* 2008;453:1051-1057.
  20. McKinley L, Alcorn JF, Peterson A, Dupont RB, Kapadia S, Logar A, et al. TH17 cells mediate steroid-resistant airway inflammation and airway hyperresponsiveness in mice. *J Immunol* 2008;181:4089-4097.
  21. Holt PG, Strickland DH, Wikstrom ME, Jahnsen FL. Regulation of immunological homeostasis in the respiratory tract. *Nat Rev Immunol* 2008;8:142-152.
  22. Ji X, Li J, Xu L, Wang W, Luo M, Luo S, et al. IL4 and IL-17A provide a Th2/Th17-polarized inflammatory milieu in favor of TGF-beta1 to induce bronchial epithelial-mesenchymal transition (EMT). *Int J Clin Exp Pathol* 2013;6:1481-1492.
  23. Huang MT, Dai YS, Chou YB, Juan YH, Wang CC, Chiang BL. Regulatory T cells negatively regulate neovasculature of airway remodeling via DLL4-Notch signaling. *J Immunol* 2009;183:4745-4754.
  24. Wills-Kar M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998;282:2258-2261.
  25. Bellini A, Marini MA, Bianchetti L, Barczyk M, Schmidt M, Mattoli S. Interleukin (IL)-4, IL-13, and IL-17A differentially affect the profibrotic and proinflammatory functions of fibrocytes from asthmatic patients. *Mucos Immunol* 2012;5:140-149.
  26. Zhao J, Lloyd CM, Noble A. Th17 responses in chronic allergic airway inflammation abrogate regulatory T-cell-mediated tolerance and contribute to airway remodeling. *Mucos Immunol* 2013;6:335-346.
  27. Su X, Pan J, Bai F, Yuan H, Dong N, Li D, et al. IL-27 attenuates airway inflammation in a mouse asthma model via the STAT1 and GADD45gamma/p38 MAPK pathways. *J Transl Med* 2016;14:283.
  28. Bhavsar P, Hew M, Khorasani N, Torrego A, Barnes PJ, Adcock I, et al. Relative corticosteroid insensitivity of alveolar macrophages in severe asthma compared with non-severe asthma. *Thorax* 2008;63:784-790.
  29. Ono K, Han J. The p38 signal transduction pathway: activation and function. *Cell Signal* 2000;12:1-13.

(Accepted June 26, 2017; First Online December 5, 2018)

Edited by YU Ming-zhu