

Original Article

HMGB1 preferentially induces Th2 polarization and response in allergic asthma

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Abstract: Background: High Mobility Group Box 1 (HMGB1) is a common inflammatory mediator that has an increase level in asthmatic patients. HMGB1 could shift the human Th1/Th2 balance into Th2 *in vitro*. Thus, in the present study, we investigate the role of HMGB1 in the Th2-type response of allergic asthma. Methods: HMGB1, IL-4 and Th2 levels in peripheral blood of asthmatic patients were assessed. Th2 and Th1 percentages were analyzed after exogenous HMGB1 injection. The splenic CD4+ T cells from mice with allergic asthma were isolated and stimulated by IL-4 or IL-12 to differentiate into Th2 or Th1 cells, respectively. Meanwhile, both Th2 and Th1 cells were stimulated by HMGB1 in the presence of HMGB1 neutralizing antibody. Finally, the percentage of Th2 and Th1 cells, the expression levels for GATA3 and T-bet, and the levels for IL-4 and IFN- γ in the supernatant of culture media were assessed. Results: In asthmatic patients, HMGB1 level was increased, which was positively correlated with Th2 percentage and IL-4 level. Moreover, *in vivo*, HMGB1 could increase Th2 percentage but reduce Th1 in asthmatic mice. Consistently, the percentage of Th2 *in vitro* was increased and Th1 was decreased after addition of exogenous HMGB1. In addition, both GATA3 and IL-4 levels were increased after addition of HMGB1, while the expression of T-bet and IFN- γ level was decreased. All these effects could be reversed by the HMGB1 neutralizing antibody. Conclusion: HMGB1 could induce Th2 polarization and response but suppress Th1 activation in allergic asthma both *in vivo* and *in vitro*, which would provide a novel insight into immunotherapy of allergic asthma.

Keywords: HMGB1 signaling, allergic asthma, Th1/Th2 cells, immune response

Introduction

High mobility group box 1 (HMGB1) is generally a DNA binding protein relevant to many nuclear functions, but it is also involved in inflammatory responses once it is released into the extracellular milieu [1, 2]. HMGB1 level was found to be increased in the induced sputum of asthmatic patients as compared with that of healthy controls, which was related with an increased percentage of neutrophils [3]. As a result, HMGB1 level in the sputum has been proposed as a biomarker of allergic asthma in children for its relationship with asthma severity and lung function [4]. Our previous work further demonstrated in a murine model of asthma that exog-

enous HMGB1 could increase mucus secretion and eosinophil inflammation in the airway other than promoting neutrophil recruitment along with reduction of pulmonary function [5]. When blocking HMGB1 signaling, eosinophilic airway inflammation, non-specific airway hyperresponsiveness and airway remodeling can be attenuated [5-7]. Therefore, HMGB1 plays an important role in the pathogenesis of allergic asthma.

It has been accepted that the origin of atopic airway inflammation in asthma is related to T-helper 2 (Th2) type lymphocyte response and Th2-type cytokines [8]. *In vitro* study found that rhHMGB1 could decrease human Th1/Th2 ratio

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and shift the Th1/Th2 balance into Th2 [9]. However, it is still uncertain whether HMGB1 preferentially mediate Th2 response in allergic asthma. Therefore, we investigate the role of HMGB1 in the Th1/Th2 balance of allergic asthma especially in inducing Th2 response.

Materials and methods

Patients

We obtained peripheral blood samples from 10 allergic asthma patients in stable stage, 10 allergic asthma patients in exacerbation stage, and 10 controls. All controls were healthy subjects coming to the hospital for physical examination. Asthmatic patients diagnosis and asthma exacerbation judgment were defined according to the Global Initiative for Asthma (GINA, 2014 version) [10], and all patients have airway allergy history and high IgE levels. All subjects were free of infections, and none had received glucocorticoids or antibiotics within the preceding 4 weeks. The study was approved by the local ethics committee (the Affiliated Hospital of Guilin Medical University ethical committee, Guangxi, China) and written informed consent was obtained from each subject.

Animals

Female BALB/c mice (6-8 weeks old, weighing 20 ± 2 g) were provided by the Laboratory Animal Center of Guilin Medical University and housed in the SPF animal facility under a 12:12 h light/dark photoperiod. They were provided with an OVA free diet and water ad libitum. All experimental procedures were approved by the Animal Care and Use Committee of Guilin Medical University.

Induction of allergic asthma in mice

Female BALB/c mice were sensitized by intraperitoneally injection of 0.01 mg OVA, which was (Grade V; Sigma) emulsified in 2 mg of aluminum hydroxide gel, in a total volume of 200 μ L on days 1 and 13 as previously described [11]. Mice were challenged with aerosolized 5% OVA for 30 min between days 19 and 24 (PARI BOY CE, German).

Five mice in each group were intraperitoneally injected exogenous HMGB1 (0.5 μ g/g, 1 μ g/g, 2 μ g/g mouse) 30 min before each OVA aerosol challenge. For these asthmatic mice, the percentage of Th2 and Th1 in peripheral blood was

analyzed by flow cytometry to assess the effect of HMGB1 on Th2 and Th1 in asthma.

CD4+ T cell isolation, culture and differentiation

Asthmatic mice absence of exogenous HMGB1 was employed for isolation of CD4+ T cells. After asthma onset the mice were sacrificed to collect spleens. Native CD4+ T cell isolation and culture were carried out as previously described [12]. Briefly, single-cell suspensions were prepared by gently disrupting spleens. The cell suspension was washed twice with RPMI 1640, and mononuclear cells were then enriched from cell suspension. Cell numbers were determined by exclusion of 0.4% trypan blue using a hemocytometer. CD4+ T cells were isolated after immunization by magnetic cell sorting using CD4 positive beads (Miltenyi Biotech, Germany) followed by flow cytometry analysis.

CD4+ T cells (1×10^6 /ml) were incubated in the anti-CD3 antibody-bound plates along with 2 μ g/ml anti-mouse CD28 antibody (eBioscience, San Diego, CA) in the culture media. Some cells were further stimulated with IL-12 and anti-IL-4 antibody, or IL-4 and anti-IL-12 antibody in an atmosphere of 95% air and 5% CO₂ at 37°C for 4 days. Th2 and Th1 cells were identified by flow cytometry. Furthermore, both Th2 and Th1 cells were administrated by anti-HMGB1 antibody (Sigma Aldrich, St. Louis, MO) and HMGB1 (Sigma Aldrich, St. Louis, MO). All these cells were co-cultured with PMA, Ionomycin and Monensin for 4 hours, followed by flow cytometry analysis after washes.

Flow cytometry

Th2 cells from peripheral blood of all 30 subjects were labeled by APC-conjugated anti-human CD4 (Biolegend, San Diego, CA), and PE-conjugated anti-human IL-4 (Biolegend, San Diego, CA). Splenic CD4+ T cells from asthmatic mice were labeled by FITC-conjugated anti-mouse CD3 and PE-conjugated anti-mouse CD4 (Biolegend, San Diego, CA) to assess the cell purity. APC-conjugated anti-mouse CD4 (Biolegend, San Diego, CA), and FITC-conjugated anti-mouse IFN- γ antibodies (Biolegend, San Diego, CA) or PE-conjugated anti-mouse IL-4 (Biolegend, San Diego, CA) were used to label Th1 or Th2 surface markers according to the manufacturer's instruction.

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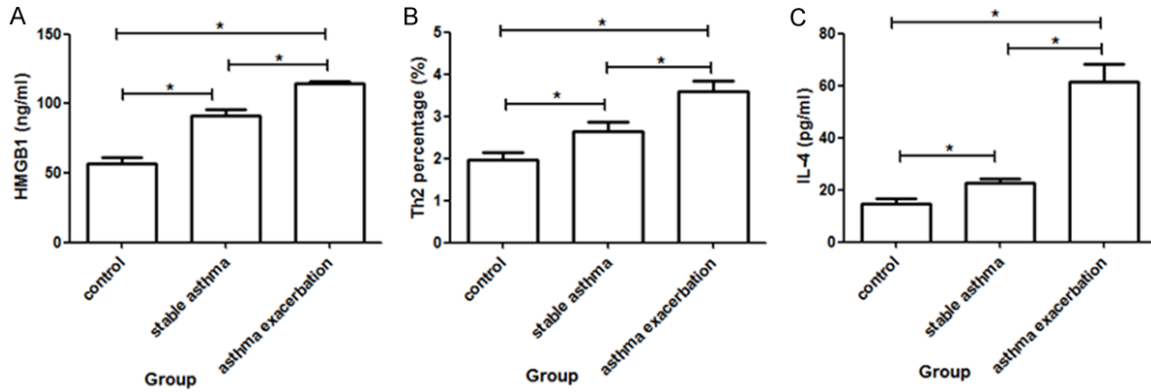


Figure 1. HMGB1, Th2 percentage and IL-4 levels in peripheral blood of asthma patients. A: HMGB1 level. B: Th2 percentage. C: IL-4 level. *: $P < 0.05$.

Western blotting

Lymphocytes were lysed by RIPA lysis buffer containing 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS, and 0.5% Sodium Deoxycholate supplemented with protease inhibitor and phosphatase inhibitor cocktails (Pierce, Thermo Scientific). The proteins were separated by electrophoresis through a 4%-12% gradient Tris-glycine SDS gel, and then transferred onto nitrocellulose membrane. The membranes were blocked with 5% milk in Tris-buffered normal saline supplemented with Tween 20 (TBST) and incubated overnight at 4°C with antibodies against T-bet (Santa Cruz Biotechnology, Santa Cruz, CA) or GATA3 (Santa Cruz Biotechnology, Santa Cruz, CA) followed by incubation with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h at room temperature. After detection of the target protein, the membrane was stripped and reprobed with anti-beta-Actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA) to assess loading equivalency.

Enzyme-linked immunosorbent assay

The concentration of IL-4 (R&D, Minneapolis, MN) and HMGB1 (Hyperheal, Shanghai, China) in peripheral blood of patients were detected by ELISA according to the manufacturer's protocols as previously described [13]. Supernatants of cultured lymphocytes from every experimental groups were collected for ELISA to measure IFN- γ (R&D, Minneapolis, MN) and IL-4 (R&D, Minneapolis, MN) production according to the protocols [13].

Statistical analysis

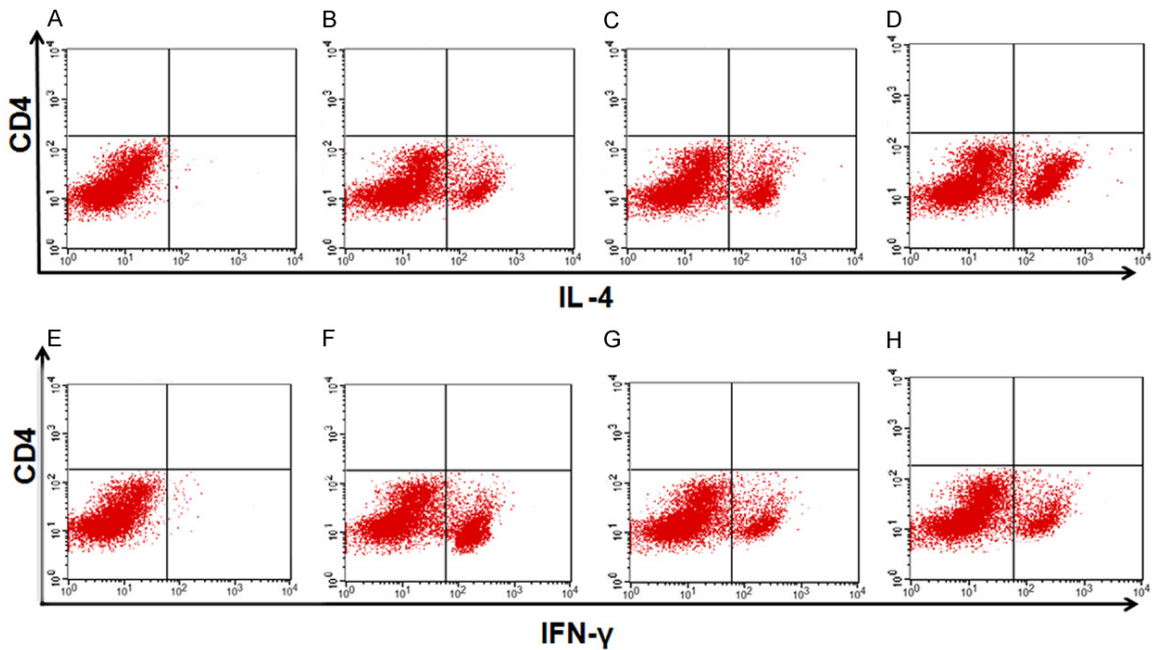
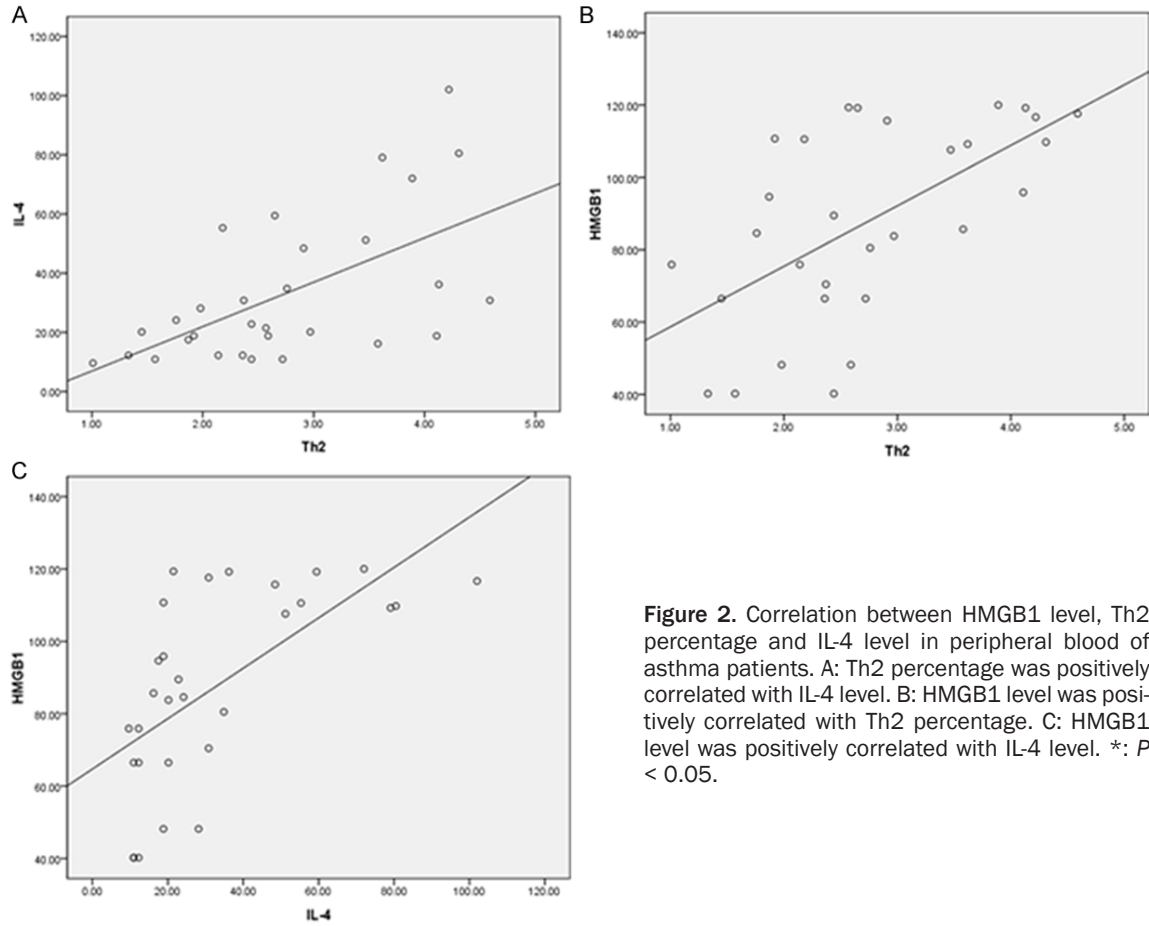
All experiments were performed at least twice for each group per experiment. Group data are expressed as the mean \pm standard deviation (SD). Significant differences were evaluated by Student's t-test (two-tailed) or one-way ANOVA test. Correlation coefficients were calculated using Spearman's rank method. P values ≤ 0.05 were considered statistically significant. The statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA).

Results

HMGB1 and Th2 response in peripheral blood of asthmatic patients

There were no significant differences between three groups of subjects in terms of age (control group: 40 \pm 3 yrs, stable asthma group: 40 \pm 7 yrs, and asthma exacerbation group: 46 \pm 9 yrs). The level of HMGB1 in peripheral blood of asthma exacerbation group was the highest (114.57 \pm 4.76 ng/ml), followed by the stable asthma group (91.51 \pm 14.47 ng/ml). HMGB1 level was the lowest in control group (56.82 \pm 14.85 ng/ml) (P values < 0.05) (**Figure 1A**). The percentage of Th2 and IL-4 level in peripheral blood of patients were consistent with HMGB1 level. Th2 percentage in asthma exacerbation group was 3.60 \pm 0.79%, stable asthma group was 2.64 \pm 0.76%, and control group was 1.96 \pm 0.59% (P values < 0.05) (**Figure 1B**). Similarly, IL-4 level in asthma exacerbation group (61.51 \pm 21.51 pg/ml) was higher than the stable asthma group 22.57 \pm 5.99 pg/ml and the control group (14.64 \pm 5.90 pg/ml) (**Figure 1C**).

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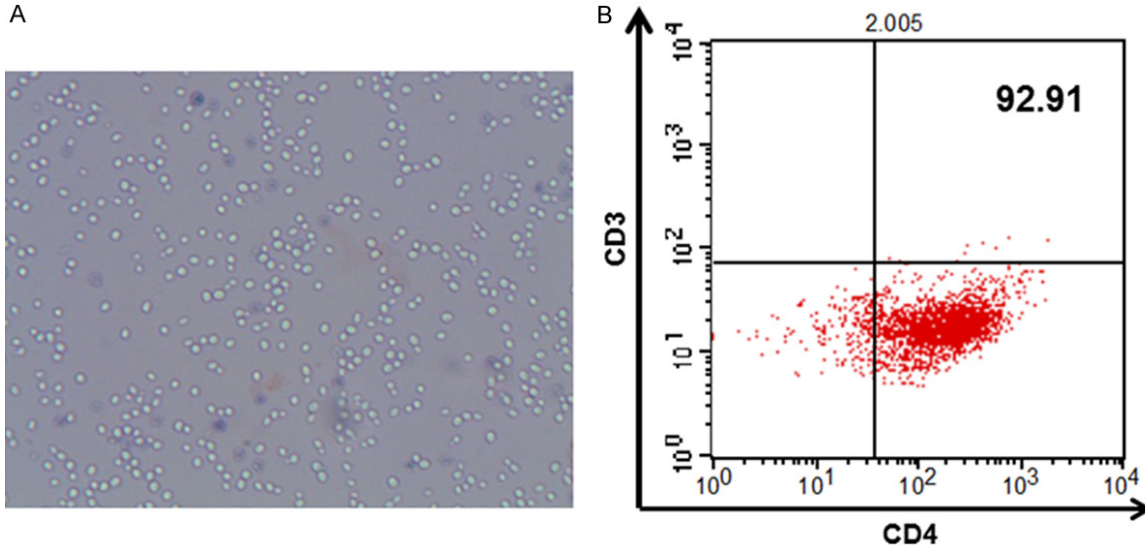


Figure 4. Splenic CD4+ T cells were determined by exclusion of 0.4% trypan blue. The number of isolated splenic CD4+ T cell was more than 90%.

Spearman's rank method was used to assess the correlation coefficients. The percentage of Th2 was positively correlated with IL-4 level ($r=0.590$, $P=0.001$) (**Figure 2A**). Consistently, the HMGB1 level was positively correlated with Th2 percentage ($r=0.589$, $P=0.001$, **Figure 2B**) and IL-4 level ($r=0.691$, $P < 0.001$, **Figure 2C**), respectively.

HMGB1 induces Th2 and inhibits Th1 differentiation in vivo

Exogenous HMGB1 were intraperitoneally injected into asthmatic mice. The percentage of Th2 in blood was increasingly raised after HMGB1 injection with an increasing concentration (**Figure 3B-D**), whereas Th1 percentage was decreased (**Figure 3F-H**). Thus, exogenous HMGB1 could induce Th2 polarization of asthmatic mice, but inhibit Th1 in a dose-dependent manner.

Splenic CD4+ T cells identification from mice

Splenic CD4+ T cells viability from mice were determined by exclusion of 0.4% trypan blue. The number of splenic CD4+ positive T cell after isolated by magnetic cell sorting was more than 90%. As shown in **Figure 4**, the purity of CD4+ T cell was high enough.

HMGB1 induces Th2 and inhibits Th1 differentiation in vitro

Splenic CD4+ T cells were stimulated by IL-12 in the presence of anti-IL-4 antibody, or IL-4 and in

the presence of anti-IL-12 antibody, followed by flow cytometry analysis of the proportion of Th1 and Th2 cells. As shown in **Figure 5**, the percentage of Th2 increased from 22.57% to 34.30% after administrated of HMGB1, while the Th1 percentage decreased from 34.40% to 24.29%. Of note, the effect of HMGB1 on differentiation of CD4+ T cells was reversed by the HMGB1 neutralizing antibody (Th2: 22.19%; Th1: 32.43%) (all P values were < 0.01). These results suggest that HMGB1 induces Th2 but inhibits Th1 differentiation.

We further assessed the effect of HMGB1 on Th2 and Th1 by analyzing the expression of their nuclear transcription factors, GATA3 and T-bet, by western blotting, respectively. Consistently, the Th2 transcription factor GATA3 was increased after stimulated by HMGB1 (from 0.167 to 0.489), while the Th1 transcription factor T-bet was decreased (from 0.559 to 0.121). However, administration of HMGB1 neutralizing antibody restored the expressions of GATA3 (0.139) and T-bet (0.347) (**Figure 6**, all P values were < 0.01). Together, these data support that HMGB1 regulates an imbalance of Th2/Th1 ratio.

HMGB1 promotes IL-4 and represses IFN- γ production in vitro

Th2 and Th1 were first stimulated by HMGB1 and then administrated with an HMGB1 neutralizing antibody, and the levels for Th2-type

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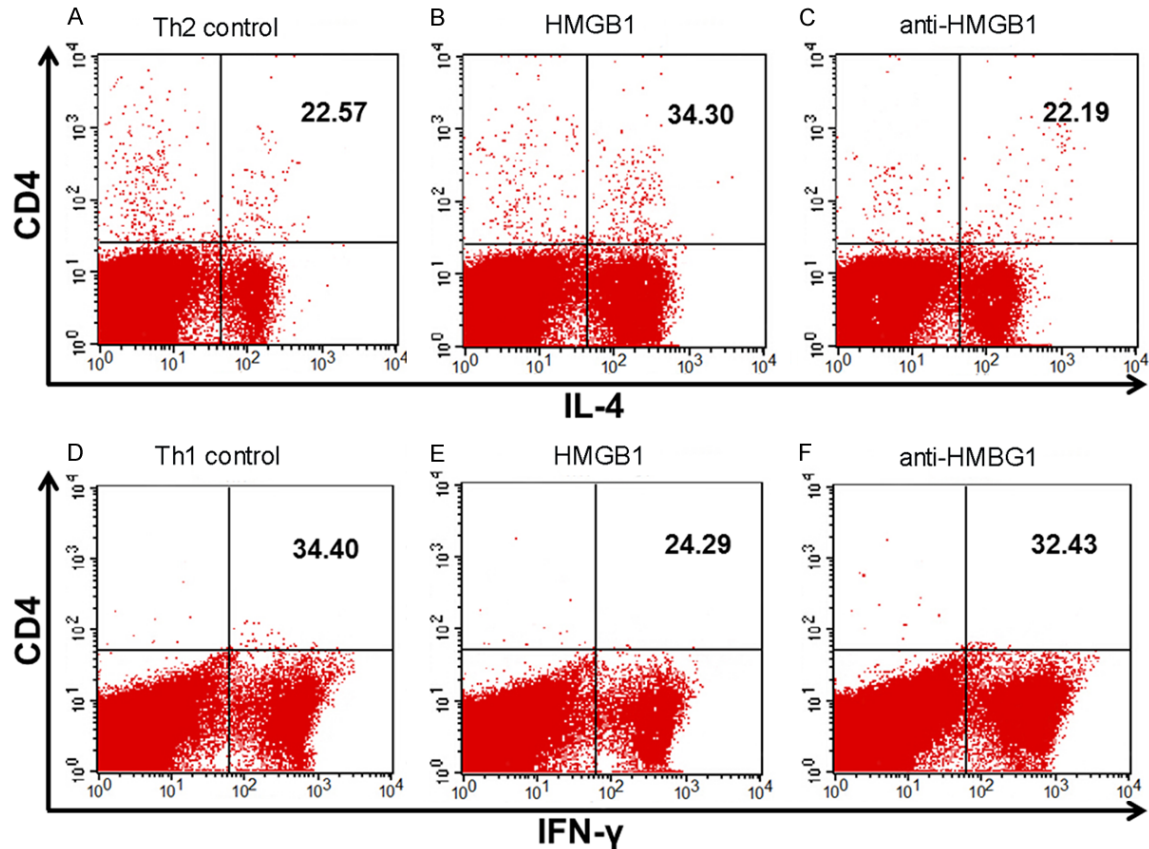


Figure 5. Effect of HMGB1 on the percentage of Th2 and Th1. The percentage of Th2 and Th1 were assessed by flow cytometry. The percentage of Th2 was higher after administrated by HMGB1 (B) than before (A), whereas the Th1 percentage was decreased (D, E). After inhibited by anti-HMGB1 antibody, the percentage of Th2 (C) was reduced but Th1 increased (F).

cytokine IL-4 and Th1-type cytokine IFN- γ in culture media were assessed by ELISA. IL-4 level was increased after HMGB1 administrated (from 2.57 ng/ml to 3.46 ng/ml), while IFN- γ level was decreased (from 4.19 ng/ml to 2.46 ng/ml). However, the production of IL-4 and IFN- γ were restored after administrated by anti-HMGB1 antibody (IL-4: 2.41 ng/ml; IFN- γ : 3.46 ng/ml) (**Figure 7**).

Discussion

In this study, we found that HMGB1 level in peripheral blood was remarkably increased in asthmatic patients, particularly in asthmatic patients at the exacerbation stage, which was co-related with the increase of Th2 percentage and IL-4 level in peripheral blood. In asthmatic mouse model, we injected exogenous HMGB1, and then confirmed that HMGB1 could increase Th2 percentage but decrease Th1 in peripheral

blood. Furthermore, we investigated the role of HMGB1 in Th1 and Th2 differentiation in asthmatic mice through co-culturing Th1 or Th2 with HMGB1 in vitro. We showed that HMGB1 could promote Th2 development but inhibit Th1 in asthmatic mice, which could be reversed by blocking HMGB1. The levels of IL-4 and IFN- γ in the culture media were consistent with cell percentages and expression of GATA3 and T-bet. All of these results suggest that HMGB1 signaling could promote the shift of Th1/Th2 balance into Th2-type response in allergic asthma.

Our study also found an increased level of HMGB1 in peripheral blood of asthmatic patients in an asthma severity-dependent manner, which was positively correlated with Th2 percentage and IL-4 level. These findings confirmed the role of HMGB1 in asthma and its relationship with asthma severity, which was consistent with previously reported studies [3,

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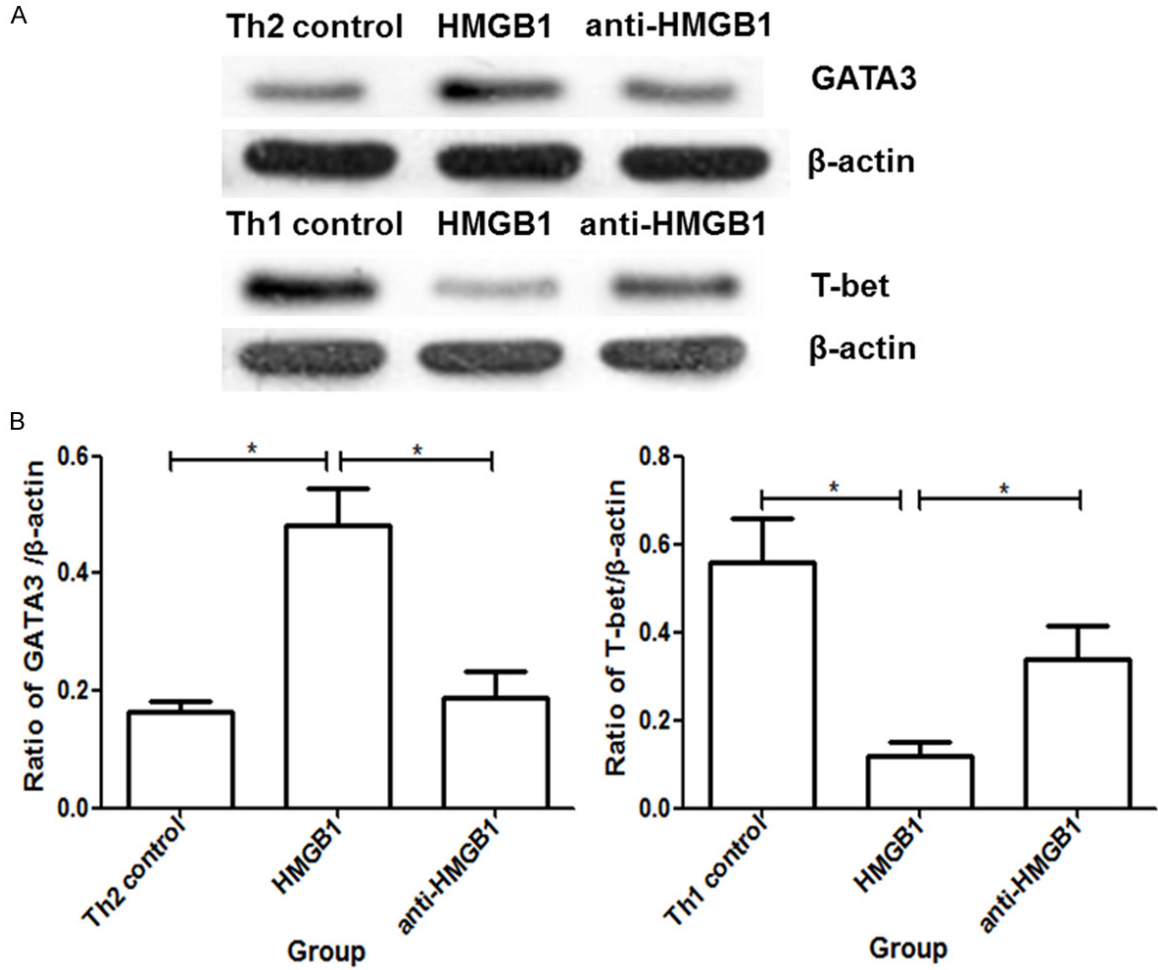


Figure 6. Effect of HMGB1 on the expression of GATA3 and T-bet. Western blotting (A) and quantitation of protein bands (B) showing differentially expressed GATA3 and T-bet in cells. The expression of GATA3 in Th2 was increased after administrated by HMGB1, whereas the T-bet expression in Th1 was decreased. After inhibited by anti-HMGB1 antibody, the GATA3 expression was reduced but T-bet expression increased. *: $P < 0.05$.

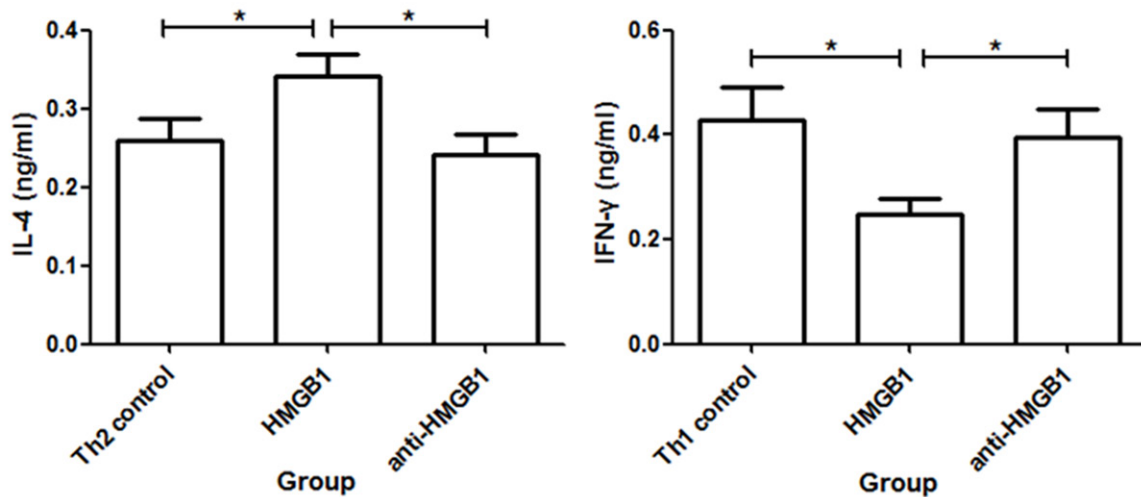


Figure 7. HMGB1 induces IL-4 and inhibits IFN- γ secretion. The levels of Th2 cytokine IL-4 and Th1 cytokine IFN- γ in culture media were assessed by ELISA. IL-4 level was increased after HMGB1 administrated, whereas IFN- γ level was decreased. Their levels were restored after administrated by anti-HMGB1 antibody. *: $P < 0.05$.

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4]. Based on these findings, we further validated the role of HMGB1 in asthmatic mouse model. Our results showed that exogenous HMGB1 could increase Th2 percentage but reduce Th1 in a dose-dependent manner *in vivo*. Together, our data demonstrated the role of HMGB1 in promoting Th2-type immune response in allergic asthma.

To further explore the effect of HMGB1 on Th2 and Th1 differentiation and their function, we cultured isolated CD4⁺ T cells in the presence of exogenous HMGB1. It was noted that the percentage of Th2 from asthmatic mice was increased after HMGB1 administration, whereas the Th1 percentage was decreased. Similarly, the effect of exogenous HMGB1 could be reversed by HMGB1 neutralizing antibody. Since the expression of HMGB1 was increased in asthmatic patients [4, 6], our study demonstrated that HMGB1 may act as an upstream regulator involved in asthma pathogenesis via promoting Th2 response.

We also investigated the expression of transcription factors for Th2 (GATA3) and Th1 (T-bet) in response to exogenous HMGB1 in allergic asthma, and obtained consistent results correlated with the percentages of Th2 and Th1. Given that GATA3 plays a crucial role in Th2 differentiation and can inhibit Th1 differentiation [14], while T-bet is essential in Th1 differentiation [15], those data relevant to the expression of GATA3 and T-bet further indicated the role of HMGB1 in Th2 polarization in allergic asthma.

Finally, we measured Th2-type cytokine IL-4 levels after HMGB1 stimulation and noted a significant increase upon HMGB1 administration, but Th1-type cytokine IFN- γ level was decreased. Similarly, the effect of HMGB1 on IL-4 and IFN- γ production could be reversed by the addition of HMGB1 neutralizing antibody. Given that IL-4 and IFN- γ are important in Th2 and Th1 development and function, respectively [16, 17], our results suggest that HMGB1 not only regulates the ratio of Th2 and Th1 cells, but also promotes the functionality of Th2 cells.

Conclusions

Our study suggested that HMGB1 could induce Th2/Th1 polarization and promote Th2-type response, but inhibit Th1 activation in allergic asthma both *in vivo* and *in vitro*. Those results would provide a novel insight into the immuno-

therapy of allergic asthma. In future, *in vivo* study would be necessary to further identify the effect of HMGB1 on allergic asthma and to characterize the related underlying mechanisms.

Acknowledgements

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Disclosure of conflict of interest

None.

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References

- [1] Fiuza C, Bustin M, Talwar S, Tropea M, Gerstenberger E, Shelhamer JH, Suffredini AF. Inflammation-promoting activity of HMGB1 on human microvascular endothelial cells. *Blood* 2003; 101: 2652-2660.
- [2] Hreggvidsdottir HS, Ostberg T, Wahamaa H, Schierbeck H, Aveberger AC, Klevenvall L, Palmblad K, Ottosson L, Andersson U, Harris HE. The alarmin HMGB1 acts in synergy with endogenous and exogenous danger signals to promote inflammation. *J Leukocyte Biol* 2009; 86: 655-662.
- [3] Zhou Y, Jiang YQ, Wang WX, Zhou ZX, Wang YG, Yang L, Ji YL. HMGB1 and RAGE levels in induced sputum correlate with asthma severity and neutrophil percentage. *Hum Immunol* 2012; 73: 1171-1174.
- [4] Cuppari C, Manti S, Chirico V, Caruso R, Salpietro V, Giacchi V, Laganà F, Arrigo T, Salpietro C, Leonardi S. Sputum high mobility group box-1 in asthmatic children: a noninvasive sensitive biomarker reflecting disease status. *Ann Allergy Asthma Immunol* 2015; 115: 103-107.
- [5] Ma L, Zeng J, Mo B, Wang C, Huang J, Sun Y, Yu Y, Liu S. High mobility group box 1: a novel mediator of Th2-type response induced airway inflammation of acute allergic asthma. *J Thorac Dis* 2015; 7: 1732-1741.
- [6] Shim EJ, Chun E, Lee HS, Bang BR, Kim TW, Cho SH, Min KU, Park HW. The role of high-mobility group box-1 (HMGB1) in the pathogenesis of asthma. *Clin Exp Allergy* 2012; 42: 958-965.

HMGB1 induces Th2 response in allergic asthma

- [7] Lee CC, Lai YT, Chang HT, Liao JW, Shyu WC, Li CY, Wang CN. Inhibition of high-mobility group box 1 in lung reduced airway inflammation and remodeling in a mouse model of chronic asthma. *Biochem Pharmacol* 2013; 86: 940-949.
- [8] Kay AB. TH2-type cytokines in asthma. *Ann N Y Acad Sci* 1996; 796: 1-8.
- [9] Huang L, Yao Y, Meng HD, Zhao XD, Dong N, Yu Y, Sheng ZY. The effect of high mobility group box-1 protein on immune function of human T lymphocytes in vitro. *Zhongguo Wei Zhong Bing Ji Jiu Yi Xue* 2008; 20: 7-13.
- [10] Global Initiative for Asthma Management and Prevention (2014). <http://www.ginasthma.org/>.
- [11] Mcmillan SJ, Lloyd CM. Prolonged allergen challenge in mice leads to persistent airway remodelling. *Clin Exp Allergy* 2004; 34: 497-507.
- [12] Ma L, Li J, Wang G, Gong S, Zhang L, Li K, Ji X, Liu Y, Chen P, Xiang X. Atrial natriuretic peptide suppresses Th17 development through regulation of cGMP-dependent protein kinase and PI3K-Akt signaling pathways. *Regul Peptides* 2013; 181: 9-16.
- [13] Ma L, Zeng J, Mo B, Wang C, Sun Y, Zhang M, Liu S, Xiang X, Wang CY. ANP/NPRA signaling preferentially mediates Th2 responses in favor of pathological processes during the course of acute allergic asthma. *Int J Clin Exp Med* 2015; 8: 5121-5128.
- [14] Nawijn MC, Dingjan GM, Ferreira R, Lambrecht BN, Karis A, Grosveld F, Savelkoul H, Hendriks RW. Enforced expression of GATA-3 in transgenic mice inhibits Th1 differentiation and induces the formation of a T1/ST2-expressing Th2-committed T cell compartment in vivo. *J Immunol* 2001; 167: 724-732.
- [15] Yates A, Callard R, Stark J. Combining cytokine signalling with T-bet and GATA-3 regulation in Th1 and Th2 differentiation: a model for cellular decision-making. *J Theor Biol* 2004; 231: 181-196.
- [16] Fields PE, Kim ST, Flavell RA. Cutting edge: changes in histone acetylation at the IL-4 and IFN-gamma loci accompany Th1/Th2 differentiation. *J Immunol* 2002; 169: 647-650.
- [17] Zhu J, Guo L, Min B, Watson CJ, Hu-Li J, Young HA, Tschlis PN, Paul WE. Growth factor independent-1 induced by IL-4 regulates Th2 cell proliferation. *Immunity* 2002; 16: 733-744.