

BIOMARKERS

Elevated Interferon Regulatory Factor 4 Levels in Patients with Allergic Asthma

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Objective. Allergic asthma is a common inflammatory disease regulated by the T helper (Th) cells. Interferon regulatory factor 4 (IRF4) plays an important role in the differentiation of Th cells. This study investigated whether IRF4 is involved in the systemic immune responses in allergic asthma patients. **Methods.** Peripheral blood mononuclear cells (PBMCs) were isolated from allergic asthmatics ($n = 29$) and healthy controls ($n = 12$). The mRNA and protein levels of IRF4 in PBMCs were measured by quantitative RT-PCR and western blotting. The frequencies of Th1, Th2, and Th17 cells were analyzed by flow cytometry. The related cytokine (interferon- γ , interleukin-4 (IL-4), and IL-17) concentrations in plasma and culture supernatants were measured by ELISA. The levels of GATA binding protein 3 (GATA3), retinoic acid-related orphan receptor γ t (ROR γ t), and forkhead box P3 (FOXP3), the master transcription factors controlling Th2, Th17, and T regulatory cells differentiation, respectively, were examined by quantitative RT-PCR. **Results.** The levels of IRF4 were elevated in allergic asthmatics compared with those in healthy controls. The frequencies of Th2 and Th17 cells as well as the concentrations of Th2- and Th17-related cytokines were higher in plasma from asthma patients than those from healthy controls; similar results were observed in culture supernatants. IRF4 mRNA levels were positively correlated with GATA3, ROR γ t, and FOXP3 mRNA levels in allergic asthmatics but not in healthy controls. **Conclusions.** Our results suggested that IRF4 may play a role in the systemic inflammation of allergic asthma patients by facilitating the differentiation of Th2 and Th17 cells at least at the transcriptional level.

Keywords allergic asthma, interferon regulatory factor 4, T helper cell, transcription factor, T regulatory cell

INTRODUCTION

Allergic asthma is a common inflammatory disease of the airways, which has an increasing incidence throughout the world. CD4⁺ T cells play an important role in the initiation and maintenance of airway inflammation of allergic asthma. Cytokines secreted by T cells orchestrate the chronic inflammation of asthma by recruiting, activating, and promoting the survival of multiple inflammatory cells in the respiratory tract (1). According to their cytokine expression profile, CD4⁺ T effector cells are classified as T helper 1 (Th1), Th2, and Th17 cells. The imbalance of Th1/Th2 is currently regarded as the major mechanism of airway inflammation (2). Recently, Th17 immune abnormality has also been suggested to contribute to the pathogenesis of allergic asthma (3–5).

Interferon regulatory factor 4 (IRF4) belongs to the IRF family of transcription factors and plays a decisive role in the differentiation of Th cells (6). Naive Th cells from mice lacking IRF4 are compromised severely for the production of interleukin-4 (IL-4) and other Th2 cytokines (7). In addition, IRF4 upregulates the Th2 developmental transcription factor GATA3 and interacts with the proteins of the nuclear factor of activated T cells that are important to the regulation of IL-4 gene transcription (7, 8). These data suggest that IRF4 plays an important role in the differentiation of Th2 cells. Moreover, IRF4 is also demonstrated to be critical to the generation of Th17 cells (9–11). Study on mice showed that IRF4 depletion results in much less

expression of ROR γ t, the master transcription factor for the development of Th17 cells (9, 10).

T regulatory cells (Treg cells) are suppressing T cells that modulate the proliferation and function of CD4⁺ T cells (12, 13). The differentiation and function of Treg cells are guided by the transcription factor FOXP3. FOXP3⁺ T cells derived from Th2 memory cells could downregulate the expression of GATA3 and IRF4 (14). IRF4 is also required for the function of Treg cells during Th2 responses (15). These results suggest that IRF4 interacts with FOXP3, and this interaction may regulate the function of FOXP3 and thus plays a possible role in the differentiation of Th cells.

As mentioned above, IRF4 plays an important role in Th cell differentiation; therefore, it is possible that IRF4 contributes to the pathogenesis of allergic asthma. In fact, a previous study has shown that IRF4-deficient (IRF4^{–/–}) mice are resistant to induction of allergic airway inflammation (16). Recently, IRF4 has also been demonstrated to be involved in the pathogenesis of allergic rhinitis and regulates a large number of genes relevant to the differentiation of Th cells (17). However, the involvement of IRF4 in immune responses in allergic asthma patients has not been reported.

In this study, we investigated the expression levels of IRF4 in patients with allergic asthma and analyzed the correlation between the expression levels of IRF4 and the differentiation of Th2 and Th17 cells.

METHODS

Subjects

Twenty nine patients with allergic asthma, 14 females and 15 males, were recruited from the Outpatient Department

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of Zhongnan Hospital, Wuhan University. The criteria for inclusion were as follows: (1) a history of previous recurrent episodes of wheezing, breathlessness, chest tightness, and/or cough; (2) reversible airway obstruction characterized by an increase in forced expiratory volume in 1 second (FEV₁) of not less than 15% after inhalation of 200 µg of albuterol, or a provocation concentration of methacholine to produce a 20% fall in FEV₁ of not more than 8 mg/ml according to the standard of Global Initiative for Asthma (18); (3) atopic with positive skin prick testing to at least 1 of the 10 common allergens (*Dermatophagoides pteronyssinus*, *D. farinae*, mixed grass pollen, mixed tree pollen, dog hair, feather, cat fur, fluffed cotton, cockroach, and *Alternaria*); (4) no history of upper or lower airway diseases for 2 months prior to the study and no chronic heart and pulmonary diseases; (5) no treatment with systematic corticosteroids, theophylline, long-acting β₂-agonists, leukotriene modifiers, and antihistamines for at least 4 weeks before study entry, but allowing to receive inhaled steroids. Asthma severity was evaluated on the basis of the Global Initiative for Asthma criteria (18). Twelve healthy volunteers were recruited from undergraduates of Wuhan University. They were nonatopic, nonasthmatic, and had a normal lung function and no history of respiratory diseases. Informed consent was obtained from all subjects, and the study was approved by Medical Ethics Committee of Wuhan University. The subjects' characteristics were shown in Table 1.

Isolations of Peripheral Blood Mononuclear Cells

Fifteen milliliters of peripheral blood was obtained from every subject and kept in heparinized tubes. The blood was diluted by the addition of equal volume of phosphate-buffered saline (PBS) and layered over Lymphoprep ($d = 1.077$ mg/ml; Nycomed, Oslo, Norway) with the ratio of 2:1, then centrifuged at 800g for 20 min at room temperature. Peripheral blood mononuclear cells (PBMCs) were collected from the medium interface and washed with PBS twice. Cell viabilities were more than 95% as examined by trypan blue exclusion assays. Plasma samples were collected and preserved at -20°C for the measurement of cytokines by ELISA.

Cell Culture and Treatment

PBMCs were seeded at a density of 1×10^6 cells/ml in RPMI 1640 (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO, USA) in six-well plates and cultured at 37°C in 5%

CO_2 atmosphere. Cells were incubated with ionomycin (500 ng/mL; Sigma, St. Louis, MO, USA) plus phorbol 12-myristate 13-acetate (PMA; 100 ng/ml; Sigma, St. Louis, MO, USA) for 2 h and were then stimulated for additional 4 h in the presence of 2 µM monensin (eBioscience, San Diego, CA, USA). After treatment, cells were harvested and used for flow cytometry, real-time quantitative PCR analysis, and protein expression assay. The culture supernatants were collected and preserved at -20°C for ELISA.

Flow Cytometry

All the antibodies and solutions for flow cytometry were purchased from eBioscience. Single-cell suspensions were stained with phycoerythrin (PE)-cy5-labeled anti-human CD3 and fluorescein isothiocyanate (FITC)-labeled anti-human CD8 for 30 min at 4°C in the dark. CD3⁺CD8⁻ cells, but not CD3⁺CD4⁺ cells, were defined as Th cells, due to the downregulation of surface CD4 molecules after stimulation with PMA and ionomycin. After washing with staining buffer, cells were fixed with fixation buffer and incubated for 20 min at room temperature in the dark. Cells were then washed twice with permeabilization buffer and incubated with PE-labeled anti-human cytokine antibodies (IL-4, IL-17, and interferon-γ (IFN-γ)) for 20 min at room temperature in the dark. After washing with staining buffer, cells were resuspended in staining buffer in an appropriate volume and subjected to fluorescence-activated cell sorting (FACS) analysis (Epics Altra II, Beckman). Data were analyzed with software Expo32 v1.2 (Applied Cytometry Systems, Sacramento, CA). PE-cy5-conjugated mouse IgG1, FITC-conjugated mouse IgG1, and PE-conjugated rat IgG2a were used as isotype controls.

Quantitative RT-PCR Analysis

Total RNA was isolated with TRIzol (Invitrogen, Carlsbad, CA, USA), and reverse transcription was performed with ReverTra Ace (Toyobo, Osaka, Japan) according to the manufacturer's instructions. Expression of target genes was determined by real-time PCR using SYBR Premix Ex Tap™ (Takara, Dalian, China). Amplification was conducted under the following condition: initial denaturation at 94°C for 30 s, then 40 cycles at 94°C for 10 s, at 61.8°C for 15 s, and at 72°C for 10 s. All measurements were performed three times. Glyceraldehyde phosphate dehydrogenase (GAPDH) was used as house-keeping gene. Data were analyzed by comparative delta-delta Ct method with

TABLE 1.— Characteristics of the subjects.

	Healthy controls ($n = 12$)	Allergic asthmatics ($n = 29$)		
		Mild ($n = 10$)	Moderate ($n = 10$)	Severe ($n = 9$)
Age (years)	29.5 ± 3.4	36.8 ± 14.8	38.0 ± 12.1	48.2 ± 13.9
Sex (male/female)	5/7	6/4	4/6	5/4
Inhaled glucocorticoid	0	0	2	3
Plasma IgE (ng/ml)	161.6 ± 39.1	$939.6 \pm 158.0^*$	$1552.9 \pm 255.7^*$	$1798.1 \pm 457.6^{**}$
FEV ₁ pred. %	109.1 ± 5.8	$89.3 \pm 9.6^*$	$71.5 \pm 10.4^{**}$	$49.3 \pm 9.3^{***}$

Data are expressed as means $\pm$ SD. FEV₁ pred. % = Predicted percentage of forced expiratory volume in the first second. * $p < .05$ versus healthy controls; ** $p < .05$ versus mild asthma; *** $p < .05$ versus all other groups.

software Rotor-Gene 6. The sequences for the primers used were as follows: IRF4-forward, 5-TGG ACA TCT CAG ACC CGT ACA AAG-3 and reverse, 5-ATG GAC ATC TGC GGG TCC TC-3; GATA3-forward, 5-GAA CCG GCC CCT CAT TAA G-3 and reverse, 5-ATT TTT CGG TTT CTG GTC TGG AT-3; ROR γ t-forward, 5-ACC TCA CCG AGG CCA TTC AG-3 and reverse, 5-TAGG CCC GGC ACA TCC TAA C-3; Foxp3-forward, 5-ATC TAC CAC TGG TTC ACA CGC AT-3 and reverse, 5-CTC CAC CCG CAC AAA GCA C-3; GAPDH-forward, 5-GGT GTG AAC CAT GAG AAG TAT GAC A-3 and reverse, 5-GTC CTT CCA CGA TAC CAA AGT TGT-3.

Protein Expression Assay

Protein expression of IRF4 was determined by western blotting. Briefly, 5×10^6 PBMCs were lysed on ice with radioimmunoprecipitation assay (RIPA) lysis buffer in the presence of henylmethyl sulfonylfluoride (PMSF) (both from Beyotime Biotechnology, Shanghai, China). Proteins (30 μ g) were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane (Sigma, St. Louis, MO, USA). The membrane was blocked with 5% skim milk in Tris-Buffered Saline with Tween-20 (TBST) for 1 h at room temperature and then incubated with antibodies against IRF4 or β -actin (both diluted 1:200; Santa Cruz Biotechnology, CA, USA) overnight at 4°C. The membrane was washed and incubated with horse radish peroxidase (HRP)-conjugated secondary antibodies (1:10,000; Santa Cruz Biotechnology, CA, USA) at room temperature for 1 h. The protein bands were visualized by enhanced chemiluminescence detection reagents (ECL; Applygen Technologies Inc., Shanghai, China) and analyzed using Fusion Fx7 image acquisition system (Vilber, France).

Detection of Cytokines

The levels of cytokines in plasma and culture supernatants from activated PBMCs were measured by ELISA according to the manufacturer's protocols (IgE, IFN- γ , IL-4 ELISA kits, R&D Systems, Minneapolis, MN, USA; IL-17 ELISA kits, eBioscience). The minimal detectable concentrations were 8 ng/ml for IgE, 8 pg/ml for IFN- γ , and IL-4, 0.5 pg/ml for IL-17. All samples were measured in duplicate.

Statistics

Statistical analysis was performed with SPSS 11.5 software. All data were presented as means $\pm$ standard error (SE). Unpaired Student's *t*-test was used for comparison between allergic asthmatics and healthy controls. One-way ANOVA was used for comparison within three groups. Correlations were analyzed by Pearson test. $p < .05$ was considered as significant.

RESULTS

Characteristics of the Subjects

Twelve healthy volunteers and 29 allergic asthmatics were recruited in this study, characteristics of these participants

were shown in Table 1. The IgE levels in plasma were significantly higher in all the three groups of allergic asthmatics of different severities than those in healthy controls ($p < .05$), and the IgE levels of severe asthma were significantly higher than those of mild asthmatics ($p < .05$). The predicted percentage FEV₁ was significantly lower in allergic asthmatics than that in healthy controls ($p < .05$), and the predicted percentage FEV₁ of allergic asthmatics declined significantly with the severity of the disease ($p < .05$).

Elevated Expression of IRF4 in Allergic Asthmatics

We first assessed the expression levels of IRF4 in PBMCs from healthy controls and allergic asthma patients. Our real-time quantitative PCR analysis showed that the mRNA expression of IRF4 was higher in PBMCs from asthma patients ($p < .05$; Figure 1a). Similar results were obtained by protein expression assay ($p < .05$; Figure 1b and c). We then checked the expression of IRF4 in asthma patients with different severities. As shown in Figure 1d and e, both the mRNA and protein levels of IRF4 were significantly higher in all three groups of patients of different severities than those in healthy controls ($p < .05$). IRF4 mRNA levels were similar in the three groups, although there was a trend that IRF4 mRNA levels of mild asthma was higher than that of moderate and severe asthma ($p > .05$).

Frequencies of Th1, Th2, and Th17 Cells in PBMCs

As mentioned above, IRF4 plays an important role in the differentiation of Th2 and Th17 cells in mice (7–11). We then checked whether IRF4 also influenced the frequencies of Th1, Th2, and Th17 cells in PBMCs from allergic asthma patients. The expression of surface antigens and intracellular cytokines was measured by flow cytometry. Representative cytometric profiles of cytokine-positive Th cells from a healthy control and an allergic asthma patient were shown in Figure 2a. Percentages of IL-4- and IL-17-positive CD3⁺CD8[−] T cells in PBMCs were elevated in allergic asthmatics compared with those in healthy controls ($p < .05$; Figure 2b). The percentage of IFN- γ -positive CD3⁺CD8[−] T cells in PBMCs did not differ significantly between the two groups ($p > .05$; Figure 2b). The ratio of Th1/Th2 (IFN- γ -positive CD3⁺CD8[−] T cells/IL-4-positive CD3⁺CD8[−] T cells) was lower in asthmatics than that in healthy controls ($p < .05$; data not shown).

Th1-, Th2-, and Th17-Related Cytokine Production in Plasma and Culture Supernatants

The concentrations of IFN- γ , IL-4, and IL-17 in plasma and culture supernatants from activated PBMCs were measured by ELISA. As shown in Figure 3a–b, IL-4 and IL-17 levels in plasma were significantly increased in asthma patients compared with those in healthy controls ($p < .05$). The expression of IFN- γ in plasma does not differ between the two groups ($p > .05$; Figure 3c). Similar results were observed in culture supernatants (Figure 3d–f). The ratio of IFN- γ /IL-4 both in plasma (Figure 3g) and in culture supernatants (Figure 3h) was lower in allergic asthmatics than that in healthy controls ($p < .05$).

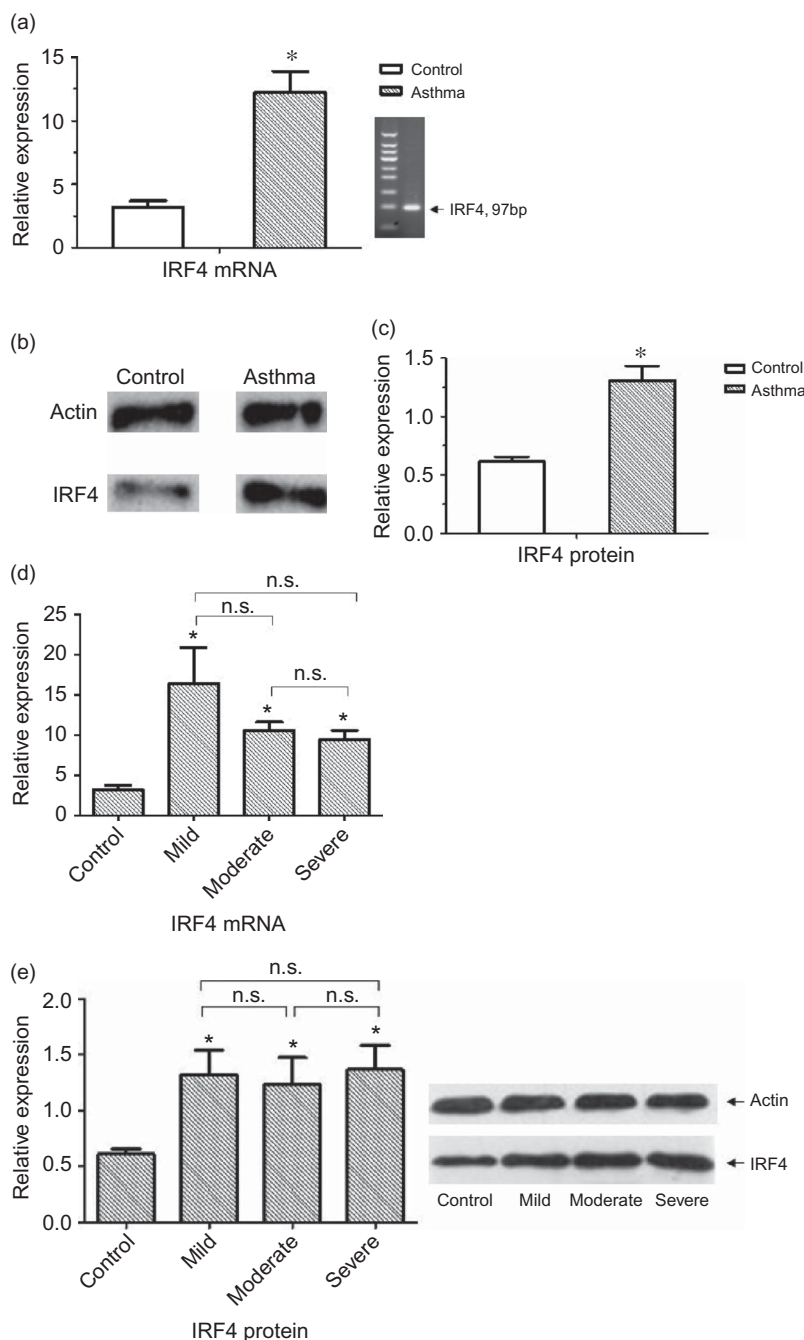


FIGURE 1.—Expression of IRF4 in PBMCs from allergic asthmatics and healthy controls. (a) IRF4 mRNA levels were detected by quantitative RT-PCR and data were analyzed by comparative delta-delta Ct method with software Rotor-Gene 6. Mean $\pm$ SE are shown. (b, c) Western blot analysis of IRF4 protein expression was performed in whole cell lysates and images were analyzed by software ImageJ. Representative measurement of IRF4 protein expression from a healthy control and an allergic asthma patient (b). Mean $\pm$ SE are shown (c). (d, e) IRF4 mRNA (d) and protein (e) expression in asthma patients with different severities. Note: * $p < .05$ versus healthy controls. n.s., not significant.

Expression of GATA3, ROR γ t, and FOXP3 in PBMCs

GATA3, ROR γ t, and FOXP3 are key transcription factors that control the differentiation of Th2, Th17, and Treg cells, respectively. We then examined the expression levels of these transcription factors in asthmatics and healthy controls. Our PCR results showed that the mRNA levels of GATA3 and ROR γ t were significantly increased in activated PBMCs from allergic asthmatics compared with those in healthy controls ($p < .05$;

Figure 4a–b). There was no significant difference in the mRNA expression of FOXP3 between the two groups ($p > .05$; Figure 4c).

Correlations of the mRNA Levels of IRF4 and the Differentiation of Th2 and Th17 Cells

IRF4 has been shown to be involved in the airway inflammation in the mice model of allergic asthma (16). To further assess the possible role of IRF4 in human allergic

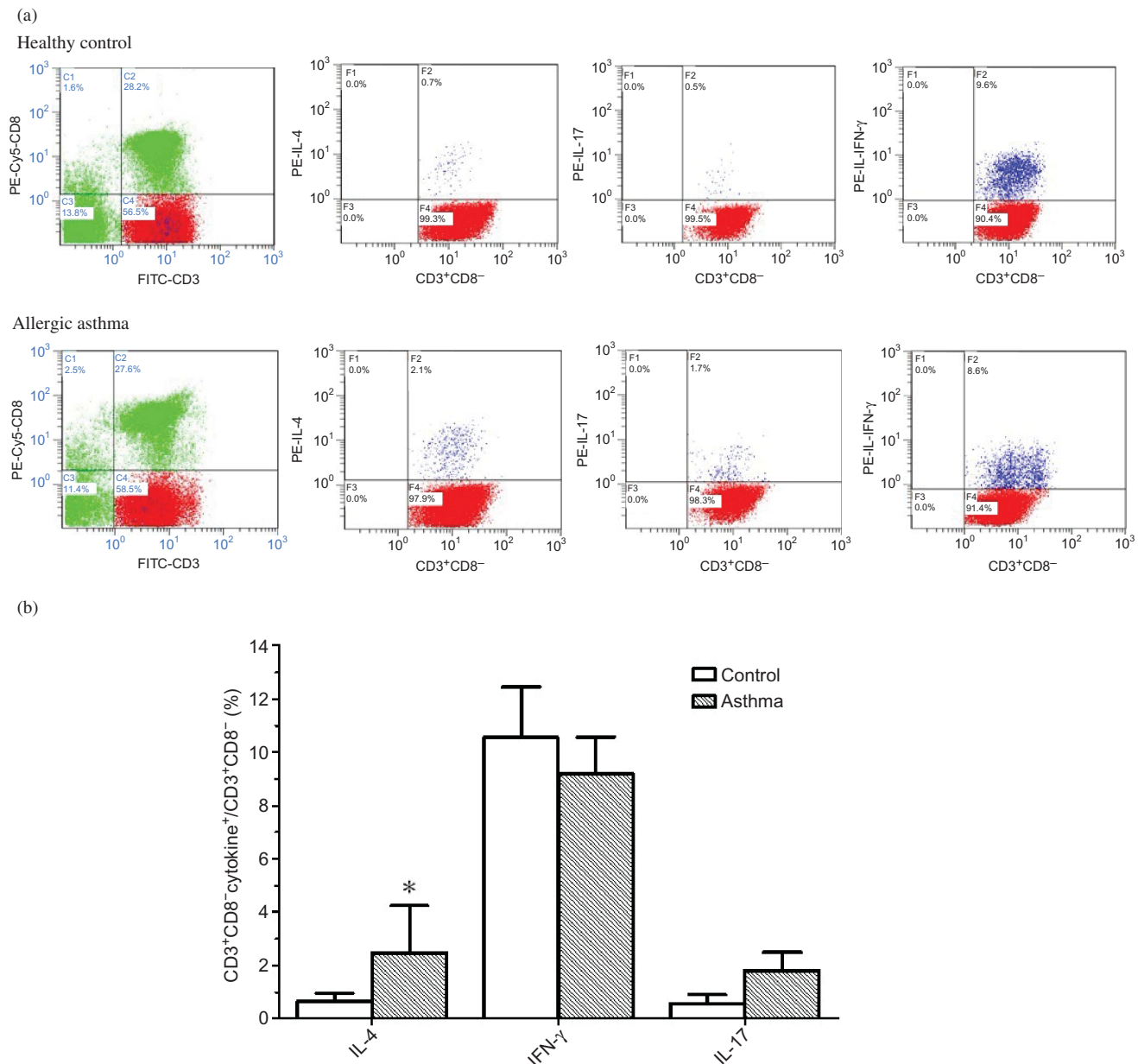


FIGURE 2.—The respective frequencies of IL-4-, IL-17-, and IFN- γ -expressing cells in PBMCs from allergic asthmatics and healthy controls. (a) Representative flow-cytometric profiles of the IL-4-, IL-17-, and IFN- γ -producing CD3⁺CD8⁻ T cells from a healthy control and an allergic asthma patient. Percentages of the indicated cells are shown in F2 quadrants. (b) Percentages of CD3⁺CD8⁻ T cells with positive intracellular staining for IL-4, IL-17, and IFN- γ in PBMCs from allergic asthmatics and healthy controls. Columns represent mean $\pm$ SE.

Note: * $p < .05$ versus healthy controls.

asthma, we analyzed the correlation of expression levels of IRF4 and that of GATA3, ROR γ t, and FOXP3. The mRNA levels of IRF4 were positively correlated with those of GATA3, ROR γ t, and FOXP3 in allergic asthmatics ($p < .05$; Figure 5a–c) but not in healthy controls ($p > .05$; Figure 5d–f). We did not find correlations of IRF4 mRNA levels and the frequencies of Th2 and Th17 cells in PBMCs from asthma patients and healthy controls (data not shown). We also analyzed the correlation of IRF4 mRNA levels and the productions of Th2- and Th17-related cytokines in plasma and in culture supernatants. There were no correlations between IRF4 mRNA levels

and IL-4 or IL-17 concentrations in plasma as well as in culture supernatants in allergic asthmatics; similar results were observed in healthy controls (data not shown).

DISCUSSION

Our previous studies showed that abnormal Th17 immunity and an impaired increment of Treg cells in response to allergen challenge contribute to the airway inflammation of allergic asthma (4, 19). Here, we demonstrated that IRF4 levels were elevated in allergic asthmatics, and the frequencies of Th2 and Th17 cells as well as the

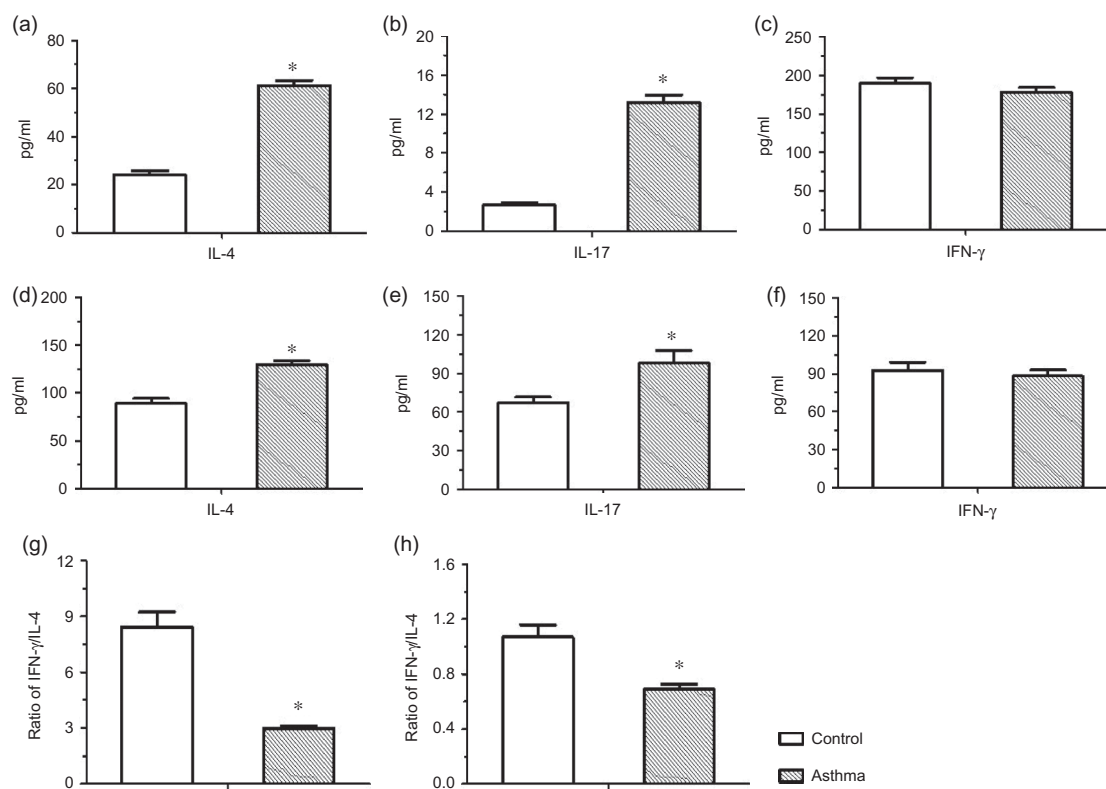


FIGURE 3.—IL-4 and IL-17 are significantly increased in plasma and culture supernatants from PBMCs of allergic asthmatic patients. (a–c) Concentrations of IL-4, IL-17, and IFN- γ in plasma from allergic asthmatics and healthy controls. (d–f) Concentrations of IL-4, IL-17, and IFN- γ in culture supernatants of activated PBMCs from allergic asthmatics and healthy controls. (g–h) The ratio of IFN- γ /IL-4 concentration in plasma (g) and in culture supernatants (h). Mean $\pm$ SE are shown.

Note: * $p < .05$ versus healthy controls.

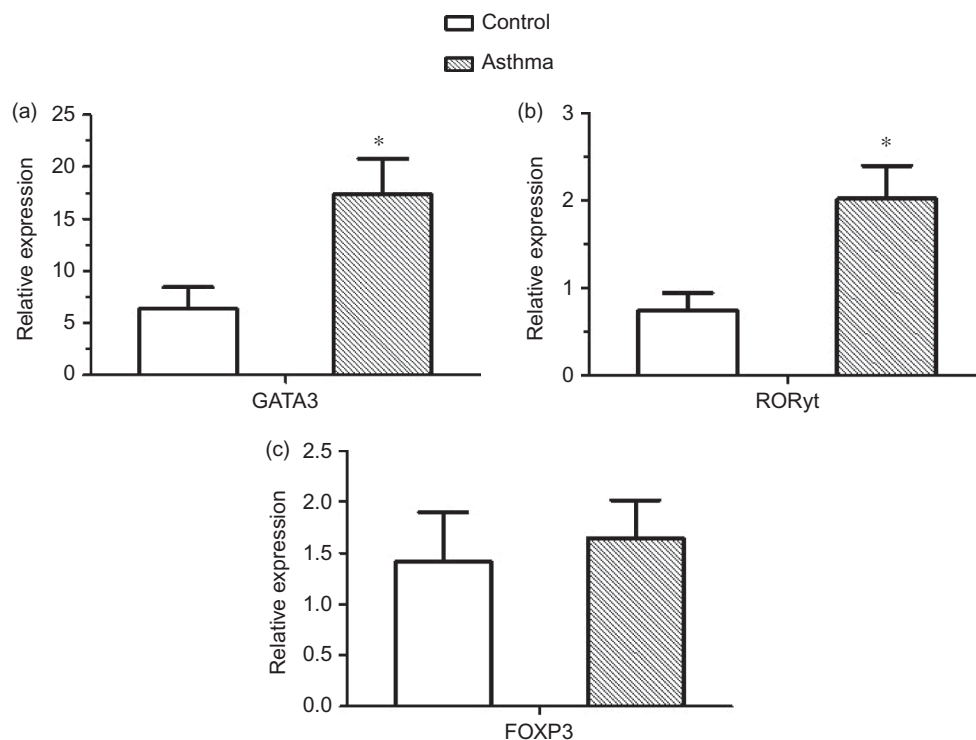


FIGURE 4.—The mRNA expression of GATA3, ROR γ t, and FOXP3 in PBMCs from allergic asthmatics and healthy controls. The mRNA levels were detected by quantitative RT-PCR, and data were analyzed by comparative delta-delta Ct method with software Rotor-Gene 6. Mean $\pm$ SE are shown.

Note: * $p < .05$ versus healthy controls.

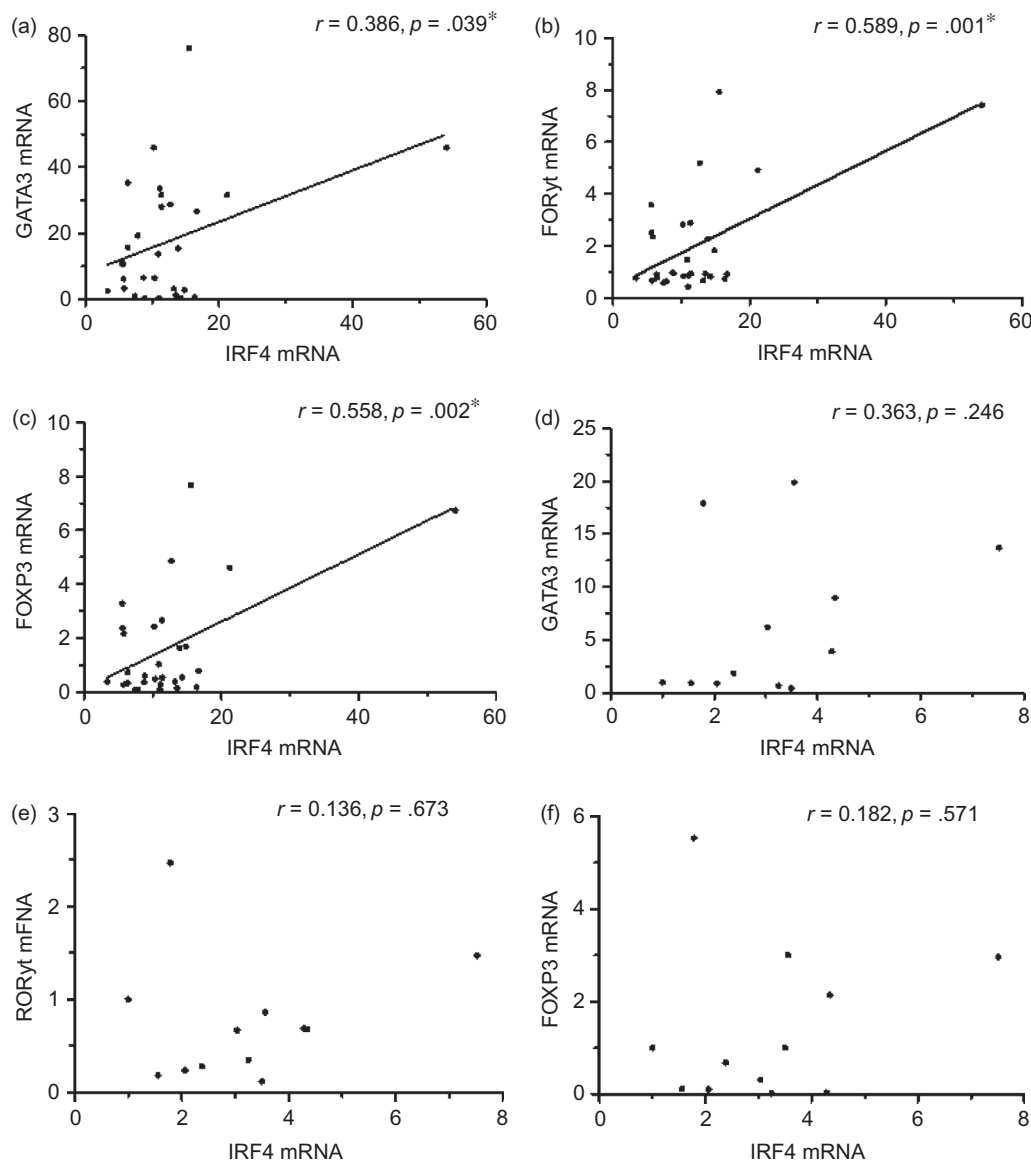


FIGURE 5.—Correlations of IRF4 mRNA levels with GATA3, RORγt, and FOXP3 mRNA levels in allergic asthmatics (a–c) and in healthy controls (d–f). Data were analyzed by Pearson test.

Note: r = Pearson correlation. $*p < .05$.

concentrations of Th2- and Th17-related cytokines were higher in asthma patients than those in healthy controls. The IRF4 mRNA levels were positively correlated with mRNA levels of GATA3, RORγt, and FOXP3, the master transcription factors controlling the development of Th2, Th17, and Treg cells, respectively. Our results suggest that over-expression of IRF4 might be an important mechanism involved in the pathogenesis of human allergic asthma by facilitating the differentiation of Th2 and Th17 cells at the transcriptional level.

Th2 cells have a predominant role in allergic asthma pathogenesis by inducing the survival and recruitment of eosinophils and mast cells, by inducing goblet-cell hyperplasia, and by causing bronchial hyper-reactivity. Several studies have demonstrated that the development of Th2 responses is critically dependent on the presence of IRF4

(7, 20, 21). A previous study showed that IRF4^{−/−} mice are resistant to induction of allergic airway inflammation (16). Accordingly, we further assessed the role of IRF4 in the systemic immune responses in allergic asthmatic patients. IRF4 has been demonstrated to be involved in the pathogenesis of autoimmune diseases such as inflammatory arthritis and chronic intestinal inflammation (22, 23). In addition, IRF4 was shown to be elevated in patients of inflammatory bowel disease and allergic rhinitis (17, 24). Similarly, we found increased expression of IRF4 mRNA and protein in human allergic asthma. We did not find significant difference of IRF4 mRNA levels among asthma patients with different severities. An *in vitro* study in CD4⁺ T cells demonstrated that glucocorticoid significantly decreased IRF4 expression levels in these cells (17). There were five patients of moderate and severe

asthma receiving inhaled glucocorticoid in our study. To rule out the possible influence of inhaled glucocorticoid on IRF4 expression levels in this study, we then checked IRF4 expression levels in these five patients. However, we did not find significant difference of IRF4 mRNA levels between these five patients and those patients without inhaled glucocorticoids treatment (Figure S1 available online at <http://informahealthcare.com/doi/suppl/10.3109/02770903>)). Similar results were obtained by protein expression assay. Further investigations including more allergic asthmatics receiving inhaled glucocorticoid are needed to elucidate the exact role of inhaled glucocorticoid in the regulation of IRF4 expression.

IRF4 plays a predominate role in the regulation of Th2 responses by binding to and transactivating the IL-4 promoter (7). IRF4 also controls the expression of GATA3, the master transcription factors of Th2 cells, and thus further amplify the commitment of Th cells toward the Th2 lineage (8, 21). Consistent with these studies, we found IRF4 mRNA levels were positively correlated with GATA3 mRNA levels in allergic asthmatics but not in healthy controls. This result further indicates that IRF4 may play an essential role in the pathogenesis of human allergic asthma by regulating the activities of master transcription factors of Th2 cells.

Besides the imbalance of Th1/Th2, Th17 immune abnormality was also shown to be involved in the systemic immune responses of allergic asthma. Our previous study demonstrated that the frequencies of Th2 and Th17 cells as well as the concentrations of Th2- and Th17-related cytokines were higher in asthma patients than those in healthy controls (4); in this study, we also observed this imbalance of Th cells in allergic asthma. In addition to being a major regulator of Th2 responses, IRF4 has also been shown to play a fundamental role in Th17 differentiation (9–11). In this study, we found a correlation between mRNA levels of IRF4 and ROR γ t in allergic asthmatics but not in healthy controls. Consistent with our results, a study highlights IRF4 as a decisive factor during the IL-21-mediated steps of Th17 development by influencing the balance of several transcription factors (10). Th17 is considered to be associated with neutrophil inflammation that is more commonly seen in severe asthma (25, 26). As IRF4 regulates the differentiation and cytokine production of Th17 cells, it is necessary to study the relationship of IRF4 and neutrophil airway inflammation, especially its role in severe asthma.

Peripheral immune tolerance and homeostasis are dynamically maintained by Treg cells (12, 13). Treg cells can modulate the Th2 responses in allergy (13). In addition, FOXP3 has also been shown to interact with ROR γ t and then regulate the differentiation of Th17 cells (27). Interestingly, we observed a correlation between the mRNA expression of IRF4 and FOXP3 in allergic asthmatics. Whether IRF4 regulates Th2 and Th17 responses of allergic asthma by modulating the function of Treg cells is still not elucidated. Similar to our result, a recent study demonstrated that IRF4 and transcription factor Blimp-1 jointly control the differentiation and function of effector

Treg cells (28). The exact role of IRF4 in regulating the activity of Treg cells in asthma is still unknown.

We did not find significant correlation of IRF4 expression with the frequencies and the productions of characteristic cytokines of Th2 and Th17 cells, although IRF4 was reported to regulate the production of these cytokines (29, 30). The cause of this discrepancy is not known, but a previous study on allergic rhinitis demonstrated that Th-cytokines were not the direct targets of IRF4, suggesting complex regulation of the production of these cytokines (17). Further studies are needed to elucidate the regulating mechanisms of IRF4 on the expression of these cytokines in asthma.

CONCLUSIONS

We found increased levels of IRF4 in asthmatic patients and a close correlation of the expression of IRF4 with Th2-, Th17-, and Treg-related transcription factors. Our results suggested that elevated IRF4 levels may play a role in the systemic inflammation of allergic asthma patients possibly by facilitating the differentiation of Th2 and Th17 cells at least at the transcriptional level.

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DECLARATION OF INTEREST

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