

# Role played by T-helper 2 in resetting the cytokine balance in allergic patients

Mohamed Y. Attia<sup>a</sup>, Howida M. Saber<sup>b</sup>

<sup>a</sup>Ph.D in Immunology, Allergy and Immunology Unit <sup>b</sup>Ph.D. in Biochemistry, Clinical Pathology Department, Faculty of Medicine, Ain Shams University Hospitals, Cairo, Egypt

Correspondence to Mohamed Y. Attia, Abassia, Ain Shams Hospital, Cairo, Egypt  
Tel: +20 122 264 5898; fax: 0223915006  
e-mail: mohamedyousef91@yahoo.com

**Received** 27 June 2014

**Accepted** 18 August 2014

**The Egyptian Society of Internal Medicine**

2014, 26:124–129

## Background

Bronchial asthma is an allergic disorder characterized by excessive hyperactive nature of the airways, which depends on many cytokines such as interleukin-4 (IL-4) and IL-5 that are responsible for the allergic inflammatory response. One of the strategies in the management of bronchial asthma is the induction of synthesis of IL-10; it has an inhibitory effect on the synthesis of the T-helper-2 (Th2) cytokines. Th2 cells play a triggering role in the activation/recruitment of immunoglobulin E antibody-producing B cells, mast cells, and eosinophil cells. To assess regulatory changes in the immune system, in patients with allergy and asthma, we studied the cytokine profile in serum in comparison with normal healthy controls. The study was carried out in Allergy and Immunology Unit, Ain Shams University Hospitals. A total of 170 patients with various allergies and asthmatic conditions were studied, for cytokines in the serum by enzyme-linked immunosorbent assay using kits from Immune Technology, and analyzed to identify the triggering factors or main contributors toward allergy and asthma. Our study showed increase in the levels of IL-4, IL-5, and IL-6 in all groups, which was nonsignificant. However, the levels of IL-10, IL-13, and tumor necrosis factor- $\alpha$  were highly significantly increased. Besides, we found correlation of granulocyte macrophage colony-stimulating factor with IL-10. Significant positive correlation with different cytokines was observed. Most of these patients showed increase in immunoglobulin E levels. This study gives a better understanding of how cytokines are the mediators of balance of Th1 and Th2 immune responses and how immunoglobulin E synthesis is controlled by cytokines. Further studies will eventually lead to improved treatment strategies in the clinical management of immunoglobulin E-mediated allergy.

## Keywords:

allergy, asthma, cytokines, T-helper-2 cells

Egypt J Intern Med 26:124–129

© 2014 The Egyptian Society of Internal Medicine  
1110-7782

## Introduction

Inflammation plays an integral part of the clinical response in bronchial asthma. This allergic inflammatory response is a result of a complex interaction between various inflammatory cells, their mediators on the airway epithelium, and smooth muscle. Inflammation in the airways leads to airway narrowing secondary to airway remodeling performed by the T-helper-2 (Th2) cells, eosinophils, mast cells, and other leukocytes leading to airway thickening [1]. Asthmatic symptoms include wheezing, cough, and sputum production resultant from mucus hypersecretion [2]. Th2 are subsets from CD4<sup>+</sup> T cells that induce their effect mechanisms through the eosinophils and mast cells and induce B cells to produce immunoglobulin E. These cells are required for the development of airway eosinophilia and secrete interleukin-4 (IL-4), IL-5, and IL-13 [3]. Th2 cells were thought to be the major inducers of effect mechanisms that led to the development of asthma. IL-4 is responsible for initial priming of the Th2 cells, and thus necessary for the initial differentiation [4]. When natural killer cells become activated, they respond with a similar cytokine profile to that of the Th2 cells and produce IL-4 and IL-13 [5].

However, the immunological role of IL-10 was demonstrated through its inhibitory effect on cytokine secretion from allergic-specific Th2 cells [6]. IL-10 also has direct inhibitory effects on antigen-presenting cells (APCs) and T cells; it also modulates eosinophil accumulation in airways by possibly inhibiting eosinophil production in bone marrow. Those with asthma have decreased expression of IL-10 demonstrating its important role in regulating allergic diseases [3]. As asthma and other allergic diseases are a result of Th2-dominated responses, it was initially thought that resetting the cytokine balance to induce Th1 would counterbalance Th2 activity. This method is efficient in suppressing the eosinophilic airway inflammation; however, a Th1 response does not have a desired effect on reducing allergic responses [7]. Resetting the cytokine balance to regulatory T cells rather than to Th1 cells may result in a decrease in asthmatic symptoms [8,9]. It has been proposed that reduced microbial exposure in early life leads to polarization of allergen-specific T-cell memory toward the Th2 instead of the Th1 immune response [10,11]. Whether reduced microbial exposures are the only environmental stimuli influencing this immune effect is unclear; Th1 and Th2 subsets develop from the same

precursor cells, through activated APCs under the influence of naive CD4<sup>+</sup> T lymphocytes, and the pattern of differentiation is determined by environmental stimuli present early during immune responses [12].

Activated Th2 lymphocytes produce IL-4, IL-13, and IL-5, which are responsible for immunoglobulin E production by B cells, eosinophil activation and recruitment, and mucus production. In contrast, Th1 cells differentiate from naive CD4<sup>+</sup> cells in response to microbial activation of APCs under the influence of IL-12. Differentiated Th1 cells secrete interferon- $\gamma$ , which is important in intracellular destruction of microbes [13]. Furthermore, interferon- $\gamma$  produced by Th1 cells and IL-4 produced by Th2 counter-regulate each other.

Failure of an immune deviation from an allergen-specific Th2 response to a Th1 immune response has been proposed as the mechanism responsible for the increased allergic disease prevalence associated with reduced microbial exposure in early life. Recent evidence suggests that deregulation in the immune system involved in allergy and asthma cannot be explained simply by the Th1/Th2 dichotomy [14]. Another mechanism may involve T-regulatory/suppressor cells. It has been shown that maternal T-regulatory cells act to suppress autoimmune responses and create an immune homeostasis in the maternal relationship [15].

Hence, we undertook the study of these cytokines to identify the nature of the immune responses in our population, either Th1 type or the Th2 immune response that brings about the changes due to environmental exposure. Cytokines are categorized by their major specific function(s). IL-4 causes a switch to immunoglobulin E production by differentiating B cells [7–9,12]. Interferon- $\gamma$  can inhibit that switch and prevent the production of specific immunoglobulin E. IL-10 can actually inhibit the activity of interferon- $\gamma$ , allowing the original IL-4 to proceed in the immunoglobulin E cascade. Thus, an allergic response can be viewed as an allergen-specific production of excess IL-4 and/or IL-10, lack of adequate interferon- $\gamma$  production, or both. Eosinophilic inflammation, a major component of allergic reactions, is under control of IL-5 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). Depending on the intensity of exposure to allergen, it can influence the cytokine profile. The understanding of the allergen-specific Th1/Th2 functional rating and its importance in the pathology and physiology of allergic diseases may also have utility in clinical monitoring situations [16]. Research related to allergic inflammation and cytokines continues to move steadily from bench to bedside. The primary inflammatory lesion of asthma consists of accumulation of CD4<sup>+</sup>

Th2 lymphocytes and eosinophils in the airway mucosa [17]. Th2 cells orchestrate the asthmatic inflammation through the secretion of a series of cytokines, particularly IL-4, IL-13, IL-5, and IL-9. IL-4 is the major factor regulating immunoglobulin E production by B cells and is required for optimal Th2 differentiation. Cytokines are of particular importance for mast cell recruitment, development, and function [18].

## Patients and methods

Peripheral blood was collected in plain tubes from 170 selected patients. These patients were selected on the basis of their allergic and asthmatic conditions. The detailed history was taken and proforma was filled. Informed consent was taken from each patient. These patients were off treatment during the collection of blood. Most of the patients were treated with antihistamines, and they were informed before blood collection.

## Consents

Ethical approval was taken from the hospital management for collection of the samples and conduction of the project.

A total of 24 patients were selected from a group of healthy laboratory individuals without any ailments during the past few months.

Blood was collected in plain tubes without any anticoagulant and allowed to settle for 15 min for clotting and serum to be separated. Serum, thus separated, was then collected by centrifugation and stored in aliquots in test tubes and preserved at -70°C until assay. Enzyme-linked immunosorbent assay (ELISA) was performed for the detection of cytokines such as IL-4, IL-5, IL-6, IL-10, IL-13, TNF- $\alpha$ , and granulocyte macrophage colony-stimulating factor (GM-CSF). ELISA kits from Immune Technology (France) were used, which involved sandwich-type assay. The intensity of coloration was proportional to the concentration of the cytokine in the serum.

Briefly, 100  $\mu$ l of the calibrator or the sample was added to the wells of a 96-well ELISA plate coated with the respective monoclonal antibody to the cytokine of interest and incubated for 2 h at 18–25°C with shaking. After 3 $\times$  washes, 50  $\mu$ l of antibody and 100  $\mu$ l of conjugate were added at appropriate dilution and incubated for 30 min at 18–25°C with constant shaking. After 3 $\times$  washes, 100  $\mu$ l of substrate was added to the test wells and further incubated for 20 min at 18–25°C with constant shaking. 50  $\mu$ l of stop solution was added to end the reaction. The color

reaction was then read at 450 nm using ELISA reader. Depending on the assay, the conjugate was diluted according to the kit insert. The assay was performed according to the protocol given in the kit insert.

### Statistical analysis

The results were analyzed using Statistical Package of the Social Science (SPSS version 15) and the Student *t*-test was used for evaluation of statistical significance. Spearman's coefficient correlation was used for comparison with different parameters and one-way analysis of variance test for significant correlation between groups.

## Results

From our studies, we observed that most of our patients were in the age group of 25–45 years. Age differences in different groups were not seen. There was also no significant difference as far as the duration of the disease was concerned. Comparison of total white blood cell count did not show any significant changes in percentage. Significant or increased eosinophil percentage was seen when compared with controls. Significant increase in total immunoglobulin E was observed in different allergic groups when compared with normal ( $P < 0.001$ ) (Table 1).

To assess the regulatory changes in the immune system, in patients with allergy and asthma, we studied the cytokine levels in the serum and compared the levels with the normal controls as well as with different allergic groups. Cytokines derived from Th1 and Th2 type cells, which included IL-4, IL-5, IL-6, IL-10, IL-13, TNF- $\alpha$ , and GM-CSF, were studied using ELISA kits from Immune Technology. Levels of cytokines in the allergic patients were compared with that of healthy controls as shown in Table 2.

From Table 2, it is observed that, in the group of patients showing allergic rhinitis, IL-4 and IL-13 cytokines are significantly increased as compared with normal, whereas only IL-13 is significantly increased in patients showing urticaria, dermatitis, and those showing symptoms with respiratory as well as skin problems.

From our studies, we can see that functional analysis of the role of cytokines seems to be the main factor in determining the degree of the airway inflammation. It would thus appear that cytokine production rather than influx of eosinophils or the production of immunoglobulin E is the cause of allergic conditions.

From Table 3, analysis of our normal data shows that there should be an association of IL-4 cytokine with IL-5, a negative correlation of IL-5 with IL-10, and a positive correlation of TNF- $\alpha$  with IL-6 and IL-13.

Studies conducted on allergic rhinitis showed that there is an association of IL-4 with significant increase in IL-13 and TNF- $\alpha$ . Similarly, IL-5 is significantly associated with IL-4, IL-10, and IL-13.

In asthma, we found a positive association of IL-4 with IL-5 and significant association with IL-13, TNF- $\alpha$  as well as GM-CSF, whereas IL-5 was positively associated with IL-4, IL-10, and GM-CSF. There was strong association of IL-13 with TNF- $\alpha$  and GM-CSF.

In dermatitis, there was positive correlation of IL-4 with total immunoglobulin E and TNF- $\alpha$  only. In patients showing urticaria, there was a positive correlation of IL-4 with IL-5 and that of IL-5 with IL-10. Similarly, there was association of TNF- $\alpha$  with IL-6 and IL-13. In patients with both types of allergy, that is, skin and rhinitis, there was a positive correlation of IL-5 with IL-10 and that of IL-6 with TNF- $\alpha$ .

Table 4 depicts the significant levels of cytokines compared between different groups as analyzed by analysis of variance test. From Table 4, we observed significant levels of IL-13 between groups. Similarly, total immunoglobulin E was also found to be significantly correlated within the groups with *P*-value less than 0.01. Thus, our studies show significant correlation of IL-13 as the main cytokine orchestrating the total immunoglobulin E production.

Our studies show the importance of IL-4 and IL-5 cytokines in response to allergy. We observed an increase

**Table 1 Data showing general characteristics of patients with total immunoglobulin E**

Groups/ variables	Age (mean) (years)	Sex (male/ female)	Duration (mean) (months)	Total leukocytic count/ Per Cubic millilitre (mm <sup>3</sup> ) (mean)	Eosinophils count (mean) (%)	Total immunoglobulin E (mean) (IU/ml)	Peak Expiratory Flow Rate (PEFR) (mean) (%)	Basophils count (mean) (%)
Normal (24)	Normal (34)	13/11	—	8700	1.2	26.3	96	0
Asthma (20)	Asthma (29.5)	9/11	15	10900	6.1	211.5	55	2
Rhinitis (57)	Rhinitis (36)	24/33	20	9600	5.4	177.8	78	1
Urticaria (58)	Urticaria (32)	27/31	8	8300	4.5	283.6	89	2
Dermatitis (35)	Dermatitis (23.5)	16/19	5	7700	6.4	291.9	90	2

in the IL-4 as well as IL-5 cytokine production, which clearly shows the association of both the cytokines in allergic conditions and their important role with respect to hypersensitivity reactions. As the individuals are from Mumbai-based population, they are exposed to environmental pollution, which is very high in this urban area, and hence there is an increase in allergic immune response with consequent increase in total immunoglobulin E. This increase is higher than the normal level observed in the western population. Hence, our study could reveal the role of Th2 cytokines in hypersensitivity reactions.

## Discussion

We found that there is a strong association of IL-4 cytokines with IL-5 in most cases of allergic rhinitis,

**Table 2 Quantity of cytokines by enzyme-linked immunosorbent assay in different allergic groups**

Variables/groups	Normal (24)	Asthma (20)	Rhinitis (57)	Urticaria (58)	Dermatitis (35)
Total immunoglobulin E	26.3	211.5	177.8	283.6	291.9
IL-4	3.1	6.8	21.7	5.9	10.8
IL-5	2.4	3.7	4.4	4.1	6.1
IL-6	10.3	12.1	11.6	14.8	13.2
IL-10	11.6	15.2	17.2	16.3	14.9
IL-13	2.9	3.7	35.8	28.5	23.9
TNF- $\alpha$	2.7	5.5	9.3	7.4	10.5
TG-CSF	8.5	10.2	9.18	12.7	15.1

IL, interleukin; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

asthma, urticaria as well in patients showing skin allergy and rhinitis. This shows that IL-4 is the main cytokine that brings about the increase in immunoglobulin E synthesis. IL-10 is also associated with IL-5 in urticaria as well as in those showing skin allergy and rhinitis. The association of IL-13 with TNF- $\alpha$  was seen only in urticaria patients. However, GM-CSF shows its presence in urticaria as well as in allergic rhinitis along with IL-13. These studies confirm the importance of IL-5 in eosinophilic inflammation in man but question the role of eosinophils in asthma. IL-13 has many actions similar to those of IL-4 and also regulates total immunoglobulin E production, but, unlike IL-4, it does not regulate T-cell differentiation to Th2 cells and T lymphocytes do not respond to IL-13.

Cytokines are important in the chronic inflammation of asthma and play a critical role in orchestrating the allergic inflammatory response. Of particular importance to allergic disease is the recent recognition of the regulation of helper immune function by two lineages of T-helper cells, that is, Th1 and Th2, by these cytokines [19].

The Th2 hypothesis of allergy considers atopy as a Th2-driven hypersensitivity reaction to allergens of complex genetic and environmental origins, in which the Th1 lineage, normally driven by IL-2, TNF- $\alpha$ , and interferon- $\gamma$ , is deficient and in which a predominant Th2 response is seen, which is driven by IL-4, IL-13, IL-5, and IL-10. This knowledge is finding application in both the diagnosis and therapy of allergic diseases,

**Table 3 Association of individual cytokines in the presence of other cytokines; analysis by Spearman's correlation**

Normal (N = 24)	Total immunoglobulin E	IL-4	IL-5	IL-6	IL-10	IL-13	TNF- $\alpha$	TG-CSF
Total immunoglobulin E	1	-0.092	-0.050		0.006	-0.380	-0.001	-0.2
IL-4	0.092	1	0.006***	-0.066	-0.362	0.102	0.052	0.1
IL-5	0.054	0.005***	1	0.035	0.046***	-0.029	-0.104	0.2
IL-6	0.195	-0.006	0.025	1	-0.040	0.142	0.476*	-0.1
IL-10	0.006	-0.382	0.045**	-0.040	1	0.074	0.062	-0.1

IL, interleukin; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

**Table 4 Significant levels of cytokines compared between groups as analyzed by analysis of variance**

Variables/statistics	Sum of squares	Difference	Mean of squares	F	Significance
<b>IL-4</b>					
Between groups	147.849	5	29.570	1.406	0.224
Within groups	3954.089	189	21.032		
Total	4101.938	193			
<b>IL-5</b>					
Between groups	28.564	5	5.713	0.725	0.605
Within groups	1480.812	188	7.877		
Total	1509.372	192			
<b>IL-13</b>					
Between groups	12 415.313	5	2523.663	2.764	0.020*
Within groups	170 674.405	187	922.697		
Total	1977.89	104			

IL, interleukin.



through the measurement or use of cytokines, which may replace deficient quantities, or the use of anticytokines, which may neutralize elevated quantities of cytokines, events that collectively contribute to the immunologic imbalance characteristic of the allergic state [20,21].

In future, the application of cytokines will continue to find clinical application in allergic disease, and it behoves the clinical allergist — immunologist to keep abreast of the exciting new developments that are occurring in this field.

*In vitro*, IL-4 is necessary for differentiation of the naive CD<sup>+</sup> T cells within the Th2 subpopulation secreting IL-4, IL-5, IL-6, IL-10, and IL-13. Although IL-4 induces total immunoglobulin E synthesis and enables the immediate type of hypersensitivity reaction, there is certain evidence suggesting in-vitro and in-vivo anti-inflammatory effects of IL-4 [22]. IL-4 is critical in switching B lymphocytes to produce total immunoglobulin E, for expression of VCAM-1 on endothelial cells and for inducing the differentiation of Th2 cells and IL-5, which is essential for the differentiation of eosinophils. IL-4 is of critical importance in the differentiation of Th2 cells, and is therefore an 'upstream' cytokine that is an attractive therapeutic target in the treatment of atopic diseases [23]. Excessive IL-4 production by Th2 cells has been associated with elevated total immunoglobulin E production and allergy.

The critical role of IL-5 in eosinophilia has been confirmed by the use of an anti-IL-5 antibody in asthmatic patients, which almost depletes circulating eosinophils and prevents eosinophil recruitment into the airway after allergen [24]. IL-5 is a cytokine that is not encountered at high levels in healthy individuals. The control of IL-5 protein production takes place at the level of transcription.

IL-10 is a potent anti-inflammatory cytokine that inhibits the synthesis of many inflammatory proteins, including cytokines (TNF- $\alpha$ , GM-CSF, IL-5, chemokines) and inflammatory enzymes (inducible nitric oxide synthase) that are overexpressed in asthma [25,26].

In addition, IL-10 inhibits antigen presentation and sensitization. IL-13 signals through the IL-4 receptor  $\alpha$ -chain but may also activate different intracellular pathway [27].

In contrast, Th2 cells secrete IL-4, IL-5, IL-9, IL-10, and IL-13, which are involved in the same type switching of B cells as well as proliferation and

differentiation into antibody-secreting plasma cells. In particular, IL-4 and IL-13 are involved in the same type switch from immunoglobulin M to immunoglobulin E, the antibody responsible for classic allergy and implicated in the pathology and physiology of allergic asthma. IL-4 and IL-10 are also regulatory cytokines, antagonizing the activities of Th1 cytokines. Thus, the nature, intensity, and duration of a specific immune response depend on the delicate balance between Th1 and Th2 numbers or activities (or both).

## Conclusion

This study gives a better understanding of how cytokines are the mediators of balance of Th1 and Th2 immune responses and how immunoglobulin E synthesis is controlled by cytokines. Further studies will eventually lead to improved treatment strategies in the clinical management of asthma and immunoglobulin E-mediated allergy.

## Acknowledgements

The authors thank Professor Dr. Maged Refaat for his help in allergic diagnosis of diseases and clinical part.

## Conflicts of interest

There are no conflicts of interest.

## References

- 1 Lambrecht BN. An unexpected role for the anaphylax toxin C5a receptor in allergic sensitization. *J Clin Invest* 2006; 116:628–632.
- 2 Spahn JD, Leung DY, Szefer SJ. New insights into the pathogenesis and management of steroid resistant asthma. *J Asthma* 2007; 34:177–194.
- 3 Janeway CA, Travers P, Walport M, Shiomchik MJ. *Immunobiology*. New York: Garland Science; 2005.
- 4 Herrick CA, Bottonly K. To respond or not to respond: T cells in allergic asthma. *Nat Rev* 2003; 3:405–412.
- 5 Meyer EH, Goya S, Akbari O, Berry GJ, Savage PB, Kronenberg M, *et al*. Glycolipid activation of invariant T cell receptor NK T cells is sufficient to induce airway hyper reactivity independent of conventional CD4<sup>+</sup> T cells. *PNAS* 2006; 103:2782–2787.
- 6 Lee CG, Kumar S, Griswold DE, Underwood DC. Transgenic over expression of interleukin 10 in the lung causes mucus metaplasia, tissue inflammation, and airway remodeling via IL-13 dependent-independent pathways. *J Biol Chem* 2002; 277:35466–35474.
- 7 Tournoy KG, Hove CV, Grooten J, Moerloose K, Brusselle GG, Joos GF. Animal models of allergen induced tolerance in asthma: are T-regulatory-1 cells, the solution for T-helper-2 cells in asthma?. *Clin Exp Allergy* 2006; 36:8–20.
- 8 Akbari O, Stock P, DeKruyff RH, Umetsu DT. Role of regulatory T cells in allergy and asthma. *Curr Opin Immunol* 2003; 15:627–633.
- 9 Chikanza IC. Mechanisms of corticosteroid resistance in rheumatoid arthritis: a putative role for the corticosteroid receptor beta isoform. *Ann N Y Acad Sci* 2002; 966:39–48.
- 10 Mishan-Eisenberg G, Borovsky Z, Weber MC, Gazit R, Tykocinski ML, Rachmilewitz J. Differential regulation of Th1/Th2 cytokine responses by placental protein 14. *J Immunol* 2004; 173:5524–5530.
- 11 Zhou Y, McLane M, Levitt RC. Th2 cytokines and asthma – interleukin-9 as a therapeutic target for asthma. *Respir Res* 2001; 2:80–84.
- 12 Ngoc LP, Gold DR, Tzianabos AO, Weiss ST, Celedon JC. Cytokines, allergy and asthma. *Curr Opin Allergy Clin Immunol* 2005; 5:161–166.

- 13 Malviya AM. Cytokine network and its manipulation in rheumatoid arthritis. *J Assoc Physicians India* 2006; 54:15–18.
- 14 Olsson A, Cagnoni F, Dignetti P, Melioli G, Canonica GW. Low concentration of cytokines produced by allergen-stimulated peripheral blood mononuclear cells have potent effects on nasal polyp-derived fibroblasts. *Clin Exp Immunol* 2003; 132:254–260.
- 15 Bischoff SC, Sellge G. Mast cell hyperplasia: role of cytokines. *Int Arch Allergy Immunol* 2002; 127:118–122.
- 16 Will-Karp 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.
- 17 Chung F. Anti-inflammatory cytokines in asthma and allergy: interleukin-10, interleukin-12, interferon-gamma. *Mediators Inflamm* 2001; 10:51–59.
- 18 Frieri M. Advances in the understanding of allergic asthma. *Allergy Asthma Proc* 2007; 28:614–619.
- 19 Aziz N, Nishanian P, Fahey JL. Levels of cytokines and immune activations markers in plasma in human immunodeficiency virus infection, quality control procedures. *J Infect Dis* 1998; 5:755–761.
- 20 Boyton RJ, Altamann DM. Asthma: new developments in cytokine regulation. *Clin Exp Immunol* 2004; 136:13–14.
- 21 Barnes PJ. Th2 cytokines and asthma: an introduction. *Respir Res* 2001; 2:64–65.
- 22 Hawrylowicz CM, O'Garra A, Richards D. Potential role of IL-10 secreting regulation T cells in allergy and asthma. *Nat Rev Immunol* 2005; 5:271–283.
- 23 Iruen E, Matthews JG, Takahashi A, Barnes PJ, Chung KF, Adcock IM. Mitogen activated protein kinase induced glucocorticoid receptor phosphorylation reduces its activity: role in steroid insensitive asthma. *J Allergy Clin Immunol* 2002; 109:649–657.
- 24 Hawrylowicz CM, Richards D, Corrigan C, Lee T. A defect in corticosteroid induced interleukin 10 production in T lymphocytes from corticosteroid resistant asthmatic patients. *J Allergy Clin Immunol* 2002; 9:369–370.
- 25 Xystrakis E, Kusumakar S, Boswell S, Peek E, Urry Z, Richards DF, *et al.* Reversing the defective induction of IL-10 secreting regulatory T cells in glucocorticoid resistant asthma patients. *J Clin Invest*, 2006; 116:146–155.
- 26 Centers for Disease Control. Forecasted state specific estimates of self reported asthma prevalence – United States, 2006. *MMWR* 2006; 47:1022–1025.
- 27 Franchimont, D Davenport MG, Garg R. Tumor neurosis factor alpha decreases and interleukin-10 increases, the sensitivity of human monocytes to dexamethasone: potential regulation of glucocorticoid receptor. *J Clin Endocrinol Metab*, 2001; 84:2834–2839.