

# Azithromycin Inhibits IL-5 Production of T Helper Type 2 Cells from Asthmatic Children

Syh-Jae Lin<sup>a</sup> Wan-Jung Lee<sup>b</sup> Yu-Wen Liang<sup>a</sup> Dah-Chin Yan<sup>a</sup> Po-Jen Cheng<sup>c</sup>  
Ming-Ling Kuo<sup>b</sup>

<sup>a</sup>Division of Asthma, Allergy, and Rheumatology, Department of Pediatrics, Chang Gung Memorial Hospital,

<sup>b</sup>Department of Microbiology and Immunology, Graduate Institute of Biomedical Sciences, and <sup>c</sup>Department of Obstetrics/Gynecology, Chang Gung Memorial Hospital, College of Medicine, Chang Gung University, Taoyuan, Taiwan, ROC

## Key Words

Azithromycin • Type 2 helper T cells • Asthma • Cytokine • Proliferation • Apoptosis

## Abstract

**Background:** Childhood asthma is a type 2 helper T (Th2) cell-driven inflammatory airway disease characterized by recurrent episodes of airway obstruction. Azithromycin (AZM), a macrolide antibiotic exhibiting anti-inflammatory activity aside from its antibacterial effect, may prove beneficial for asthmatic children. This study aimed to determine the effect of AZM on Th2 cells from atopic asthmatic children and non-atopic controls. **Methods:** CD4+ cells were isolated from peripheral blood mononuclear cells of 9 patients with asthma and 9 non-atopic individuals. Cells were activated as Th0 and differentiated into Th2 cells. The effect of AZM on activated CD4+ cells was evaluated with respective cell proliferation and cytokine production. **Results:** Th0 and Th2 CD4+ T cells from atopic asthmatic children produced greater interleukin (IL)-5 (Th2 cytokine) but lower interferon (IFN)- $\gamma$  (Th1 cytokine) compared to the non-atopic controls, respectively. AZM inhibited IL-5 production of Th0 and Th2 cells from atopic asthmatics in a dose-dependent fashion, without sig-

nificantly affecting their IL-13 and IFN- $\gamma$  production. A similar effect was observed in non-atopic controls except that AZM did inhibit IFN- $\gamma$  production of their Th0 cells. AZM at a higher dose decreased cell viability by inhibiting CD4+ T cell proliferation and enhanced their apoptosis, an effect similarly observed in Th0 and Th2 cells, and did not differ between asthmatic children and controls. **Conclusion:** Our finding that AZM preferentially downregulates IL-5 production suggests its therapeutic potentials in controlling childhood asthma.

Copyright © 2011 S. Karger AG, Basel

## Introduction

Asthma is a chronic inflammatory disorder of the airway characterized by bronchial hyperresponsiveness and paroxysmal attacks of wheezing. Secretions, smooth muscle contraction and airway wall thickening result in a severe narrowing of the airway calibre [1]. It is generally accepted that inflammatory cell infiltration with se-

S.-J.L. and W.-J.L. contributed equally to this paper.

## KARGER

Fax +41 61 306 12 34  
E-Mail karger@karger.ch  
www.karger.com

© 2011 S. Karger AG, Basel  
1018–2438/11/1562–0179\$38.00/0

Accessible online at:  
www.karger.com/iaa

Correspondence to: Dr. Ming-Ling Kuo  
Department of Microbiology and Immunology  
Graduate Institute of Biomedical Sciences, Chang Gung University  
259 Wen-Hwa 1st Road, Kwei-Shan, Taoyuan, Taiwan (ROC)  
Tel. +886 3 211 8800, ext. 3319, Fax +886 3 211 8293, E-Mail mingling@mail.cgu.edu.tw

cretion of pro-inflammatory cytokines plays a major role in pathogenesis of asthma [2, 3]. The major inflammatory cells that are involved in this process are type 2 helper T (Th2) cells, eosinophils and mast cells [4, 5]. Inhaled corticosteroid remains the mainstay therapy for childhood asthma as it is very effective in suppressing Th2 activation [6]. Although long-term inhaled corticosteroid treatment is generally considered safe, deteriorating side effects on bone metabolism and growth retardation may occur when used in high doses [7, 8].

Macrolides are frequently prescribed antibiotics that inhibit bacterial protein synthesis by binding to 50s ribosomal subunit of susceptible organisms [9, 10]. In addition to their anti-bacterial properties, the 14- and 15-membered ring macrolide antibiotics may have anti-inflammatory action relevant to asthma pathogenesis [10, 11]. They inhibit mucous production and adhesion molecule expression of epithelial cells, and decrease pro-inflammatory cytokine production by monocytes [12, 13]. Several clinical trials showed that macrolide antibiotics have a beneficial effect on the clinical status of asthmatic subjects by improving lung function, reducing airway concentration of IL-8 and decreasing neutrophil numbers [14–16].

Azithromycin (AZM) is a broad spectrum macrolide with the advantage of sustained tissue concentrations and excellent safety profiles [9, 17]. AZM is frequently used in children with asthma, since mycoplasma infection remains an important trigger of childhood asthma against which macrolides are active. It remains uncertain whether the anti-inflammatory action of AZM would also affect Th2 cells from asthmatic children, despite several reports showing that AZM or other macrolides ameliorate the bronchial hyperresponsiveness in asthmatic subjects [18, 19], reduced cytokine production from mixed peripheral mononuclear cell cultures [20, 21] or Th2 cells cultured from healthy subjects [22].

Most studies on the anti-inflammatory action of macrolides focused on their effect on monocytes or epithelial cells in in vitro or animal models [20, 23]. The present study addressed the immunological effect of AZM on Th1/Th2 balance in atopic asthmatic children. We investigated the effect of AZM on lymphocyte activation, proliferation and apoptosis in asthmatic children. We also sought to determine how AZM influences the Th2 polarization using magnetic bead-purified CD4<sup>+</sup> T cells from atopic asthmatic children compared to non-atopic individuals. The results suggest that AZM might benefit asthmatic children through the modulation of immunological derangement.

## Subjects and Methods

### Study Subjects

The study subjects included 9 atopic asthmatics aged 8–12 years (mean  $9.6 \pm 0.6$  years) and 9 non-atopic controls with written informed consent with the pre-approval of the study by Medical Ethics and Human Clinical Trial Committee of the Chang Gung Memorial Hospital. The diagnosis of atopic asthma was confirmed by a history of recurrent episodes of dyspnoea with wheeze, reversible bronchoconstriction, and a positive skin prick test or a positive specific IgE test to the common aeroallergens: house dust mites *Dermatophagoides pteronyssinus* or *Dermatophagoides farinae* [24]. Patients with predominant symptoms of allergic rhinitis and atopic dermatitis were excluded in this study. Non-atopic controls showed a negative skin prick test or specific IgE test. All 9 asthmatic patients were asymptomatic with normal pulmonary function as determined by percent predicted forced expiratory volume in 1 s (% FEV<sub>1</sub>) using spirometry (Vitalograph, Buckingham, UK) at the time of investigation. None of these children had received oral prednisolone or inhaled corticosteroids within 1 month before this study.

### Purification of CD4<sup>+</sup> T Cells

Peripheral blood samples were collected in sterile heparinized tubes and processed within 24 h. Mononuclear cells were obtained using Ficoll-Hypaque density gradient centrifugation (Amersham Pharmacia Biotech, Pittsburgh, Pa., USA). CD4<sup>+</sup> T cells were enriched by depleting the CD4<sup>−</sup> cells in mononuclear cells with an isolation kit containing a cocktail of anti-human monoclonal antibodies against CD8, CD14, CD16, CD19, CD36, CD56, CD123, TCR $\gamma/\delta$  and glycophorin A (Milenyi Biotec, Auburn, Calif., USA). The separation was performed with LS<sup>+</sup> columns (Milenyi Biotec), according to the manufacturer's instruction. The purity of CD4<sup>+</sup> T cells (checked by anti-TCR- $\alpha\beta$ -FITC and anti-CD4-PE) were confirmed by FACS to be greater than 90%.

### Cell Cultures and the Induction of Th2 Cells

The enriched CD4<sup>+</sup> T cells were resuspended in RPMI with 10% foetal calf serum at a concentration of  $1 \times 10^6$  cells/ml and placed in a 24-well plate (TPP®, Trasadingen, Switzerland) pre-coated with anti-CD3 monoclonal antibodies at 1  $\mu$ g/ml (BD Biosciences, San Diego, Calif., USA) and soluble anti-CD28 (100 ng/ml; BD Biosciences) and incubated at 37°C in a 5% CO<sub>2</sub>/95% air atmosphere for 5 days (Th0). For the induction of Th2 cells, IL-4 (10 ng/ml; Pepro Tech EC, London, UK) and anti-IFN- $\gamma$  (20 ng/ml; R&D Systems, Minneapolis, Minn., USA) were added at the beginning of culture. AZM (Ben Venue Laboratories Inc., Bedford, Ohio, USA; kindly provided by Pfizer, Amboise, France) was added at a final concentration of 10 or 50  $\mu$ g/ml to each well ( $1 \times 10^6$  cells) on day 0 of cell culture. The viability of CD4<sup>+</sup> T cell count was determined by trypan blue (Sigma, St. Louis, Mo., USA) exclusion.

### Monitoring Cell Proliferation by Carboxyfluorescein Diacetate Succinimidyl Ester Staining

To follow CD4<sup>+</sup> T cell division, purified cells were pulsed with 1  $\mu$ M fluorescent dye carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Montlucon, France) in free RPMI-1640 medium (Invitrogen-Gibco, Grand Island, N.Y., USA) and

incubated at 37°C for 10 min. RPMI-1640 with 10% heat-inactivated foetal bovine serum (Biological Industries, Haemek, Israel), 1% penicillin and streptomycin (Invitrogen-Gibco), and 25  $\mu$ M  $\beta$ -mercaptoethanol (complete RPMI, also cRPMI; Sigma) was then immediately added to block the reaction. The CFSE-labelled cells were washed 3 times with cRPMI and the cell pellet was resuspended in cRPMI to the cell density of  $1 \times 10^6$  cells/ml at 37°C for 5 days. We analyzed CFSE staining (FL1) gated on viable CD4+ T cells by a FACSCalibur (BD Biosciences).

#### Assessment of Cell Death

Cell viability was determined by flow cytometry according to their forward and side scatter characteristics. Also, the percentage of dead cells was determined by cellular staining with the FITC-annexin-V/propidium iodide (PI) apoptosis detection kit (BD Biosciences). Cultured cells were harvested and washed with PBS. The pellet was resuspended in 200  $\mu$ l binding buffer and stained for 10 min at room temperature with 5  $\mu$ l FITC-annexin-V. Cells were then resuspended in 200  $\mu$ l binding buffer and added 5  $\mu$ l of PI (1  $\mu$ g/ml). The fluorescent staining was analyzed on a FACS Calibur (BD Biosciences) flow cytometer. The activated cells were gated as R1 and the annexin-V+ cells represented as apoptotic cell populations.

#### Cytokine Analysis by ELISA

The concentration of human IL-5, IL-13 and IFN- $\gamma$  were assessed using DuoSet® ELISA Development System (R&D Systems). The lowest detection limits for IL-5, IL-13 and IFN- $\gamma$  were 23.4, 93.7 and 15.6 pg/ml, respectively.

#### Statistical Analysis

All statistical analyses were performed using the Wilcoxon signed-rank test for paired data or the Mann-Whitney test for two independent data. The data are presented as medians  $\pm$  SEM, and  $p < 0.05$  is considered to be significant.

## Results

#### Effect of AZM on CD4+ T Cell Viability and Recovery

The potential toxic effect of AZM on CD4+ T cells was firstly determined. The effect of AZM on the cell viability of unpolarized CD4+ T (Th0) cells was shown in figure 1. Culturing with AZM had a dose-dependent inhibitory effect on cell viability in asthmatic children and controls alike. The CD4+ T cell viability in asthmatic children slightly decreased in the presence of AZM at 10  $\mu$ g/ml ( $0.84 \pm 0.07$ ,  $p = 0.086$ ). AZM at 50  $\mu$ g/ml, however, significantly decreased the viable CD4+ T cell ratio in asthmatics ( $0.72 \pm 0.07$ ,  $p = 0.021$ ), similar to that observed in corresponding controls ( $0.80 \pm 0.07$ ,  $p = 0.028$ ).

#### Effect of AZM on Proliferation and Apoptosis of Th0 and Th2 Cells

A representative profile of CD4+ T cells activated by anti-CD3/anti-CD28 under the influence of AZM is

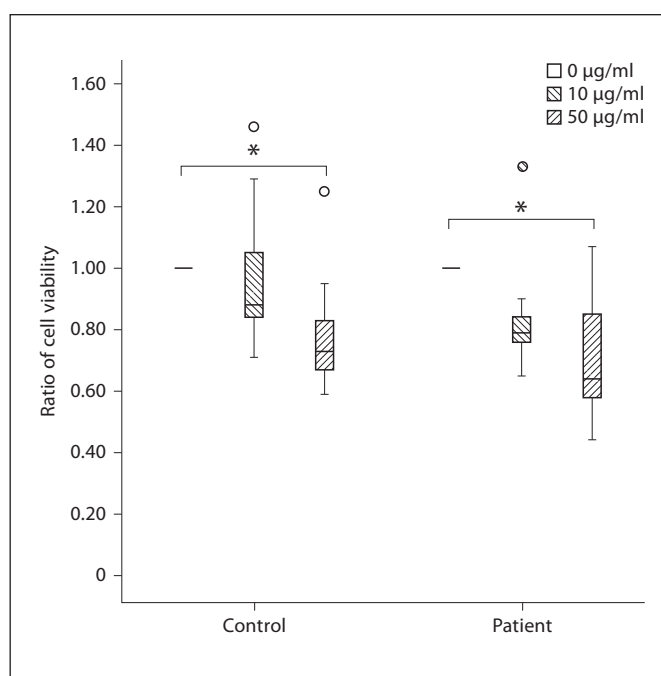


**Fig. 1.** AZM reduced the percentages of viable cells in activated-CD4+ T cell cultures. CD4+ T cells were stimulated with anti-CD3 (1  $\mu$ g/ml, plate coated) and anti-CD28 (100 ng/ml, soluble) for 5 days without or with AZM at 10 and 50  $\mu$ g/ml. The ratio of inhibition by AZM equals viable cell count in the presence of AZM on day 5 divided by viable cell count without AZM count on day 5, as determined by trypan blue exclusion. Data were obtained from 9 asthmatics and 9 controls. ° = Outlier of each group. \*  $p < 0.05$ , compared with no AZM group.

shown in figure 2. CD4+ T cells undergoing multiple divisions showed progressively decreased fluorescent intensity of CFSE (fig. 2a). AZM exerted a dose-dependent inhibition on CD4+ T cell division. Although AZM at 10  $\mu$ g/ml did not affect Th0 and Th2 proliferation, AZM at 50  $\mu$ g/ml resulted in significant suppression of TCR-activated CD4+ T cell division for Th0 ( $61.6 \pm 3.3\%$  vs.  $71.3 \pm 1.9\%$ ,  $p = 0.028$ ) and Th2 cells ( $59.9 \pm 5.0\%$  vs.  $72.4 \pm 1.1\%$ ,  $p = 0.043$ ).

Annexin-V was used to detect viable CD4+ T cells committed to apoptosis. The effect of AZM on the percentages of viable CD4+ T cells that expressed annexin-V (early apoptosis, % annexin-V+/PI-) is shown in figure 3. AZM at 50  $\mu$ g/ml resulted in significantly increased apoptosis for Th0 ( $22.7 \pm 2.8\%$  vs.  $14.1 \pm 2.4\%$ ,  $p = 0.046$ ) and Th2 cells ( $28.2 \pm 9.6\%$  vs.  $16.5 \pm 2.0\%$ ,  $p = 0.028$ ).

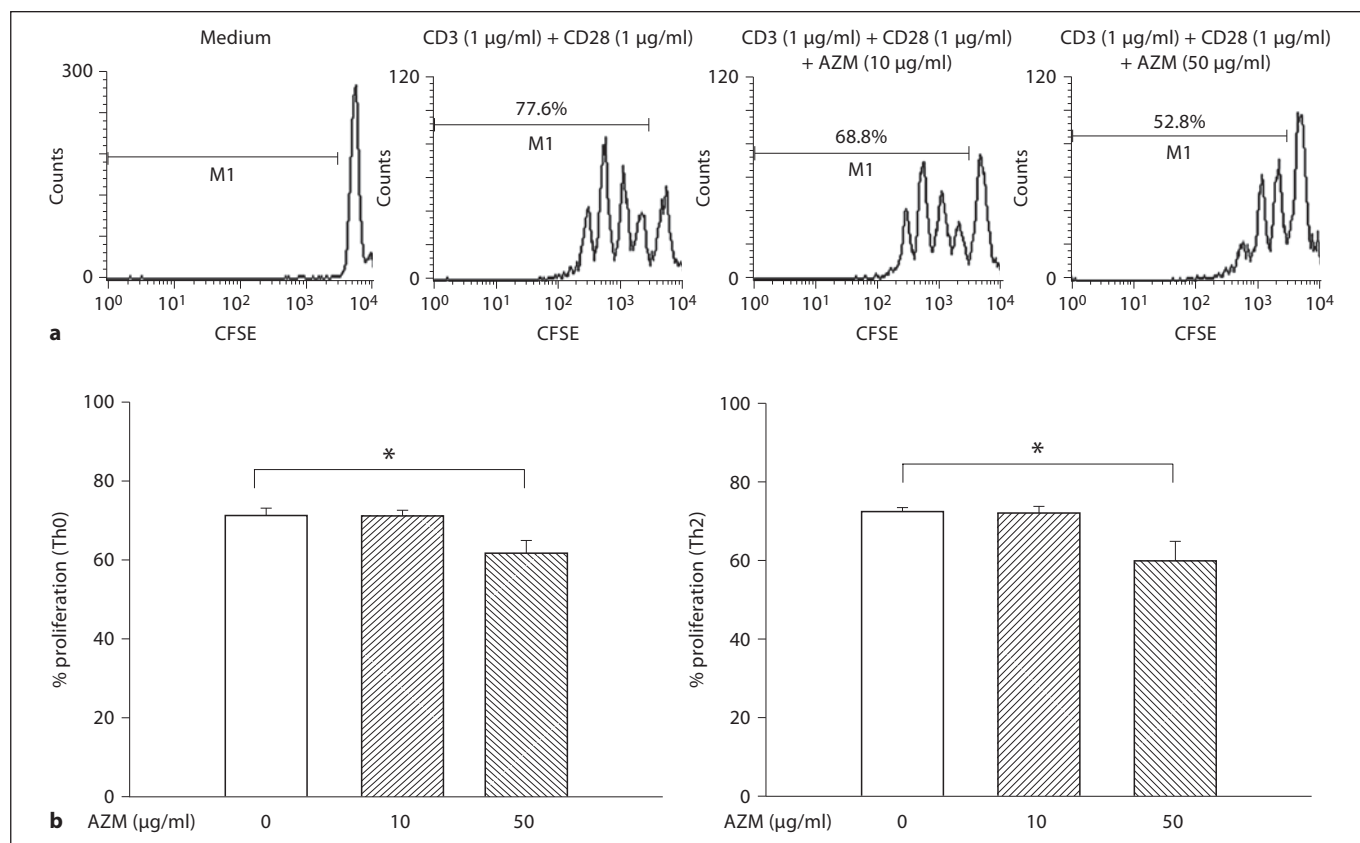


**Fig. 2.** AZM suppressed the proliferation of Th0 and Th2 cells. CFSE-labelled Th0 and Th2 cells were cultured alone or in the presence of 10 and 50 µg/ml AZM. **a** Representative profiles of histograms showing the effect of increasing dose of AZM on proliferation. The percentage of CD4<sup>+</sup> T cells that underwent cell division are determined by cell number in the M1 area divided by total CD4<sup>+</sup> cells. **b** Collective data from Th0 and Th2 cells are presented as mean  $\pm$  SEM from 6 Th0 and 5 Th2 experiments. \*  $p < 0.05$ , compared with no AZM culture.

#### Effect of AZM on IL-5, IL-13 and IFN- $\gamma$ Production of Th0 and Th2 Cells from Asthmatic Children

The IL-5 and IFN- $\gamma$  production of Th0 CD4<sup>+</sup> T cells from asthmatic children and normal controls is shown in figure 4. Increased IL-5 production was detected from anti-CD3/anti-CD28-stimulated CD4<sup>+</sup> T cells of the atopic asthmatics ( $846 \pm 247$  pg/ml) compared to the controls ( $313 \pm 43$  pg/ml,  $p = 0.136$ ). In contrast, a significantly lower level of IFN- $\gamma$  was obtained from activated CD4<sup>+</sup> T cells of the asthmatic children ( $523 \pm 138$  pg/ml vs.  $1,947 \pm 557$  pg/ml,  $p = 0.014$ ).

AZM at 10 µg/ml significantly suppressed IL-5 production from Th0 cells of asthmatic children ( $698 \pm 210$  pg/ml,  $p = 0.008$ ), as well as from controls ( $241 \pm 41$  pg/ml,  $p = 0.008$ ). AZM at 50 µg/ml further decreased IL-5 production from asthmatic children ( $526 \pm 182$  pg/ml,  $p = 0.008$ ; fig. 5a). Both concentrations of AZM had no

significant effect on IL-13 production from Th0 cells (fig. 5b). Although AZM had little effect on IFN- $\gamma$  production (fig. 5c) of Th0 cells from asthmatics at 10 µg/ml ( $670 \pm 173$  pg/ml,  $p = 0.066$ ), and at 50 µg/ml ( $551 \pm 181$  pg/ml,  $p = 0.889$ ), AZM at 10 µg/ml significantly decreased IFN- $\gamma$  production of Th0 cells from controls ( $1,588 \pm 529$  pg/ml,  $p = 0.021$ ).

For Th2 cells, AZM exerted a dose-dependent suppression on IL-5 production of Th2 cells from asthmatics (fig. 6a). AZM inhibited IL-5 production of Th2 cells from asthmatics at 10 µg/ml ( $897 \pm 213$  pg/ml,  $p = 0.015$ ), and further at 50 µg/ml ( $439 \pm 157$  pg/ml,  $p = 0.008$ ). Similarly, AZM inhibited IL-5 production of Th2 cells from controls at 10 µg/ml ( $399 \pm 57$  pg/ml,  $p = 0.021$ ), and further at 50 µg/ml ( $244 \pm 22$  pg/ml,  $p = 0.008$ ). AZM, however, had no effect on IL-13 (fig. 6b) and IFN- $\gamma$  (fig. 6c) production of Th2 cells from asthmatics and controls.

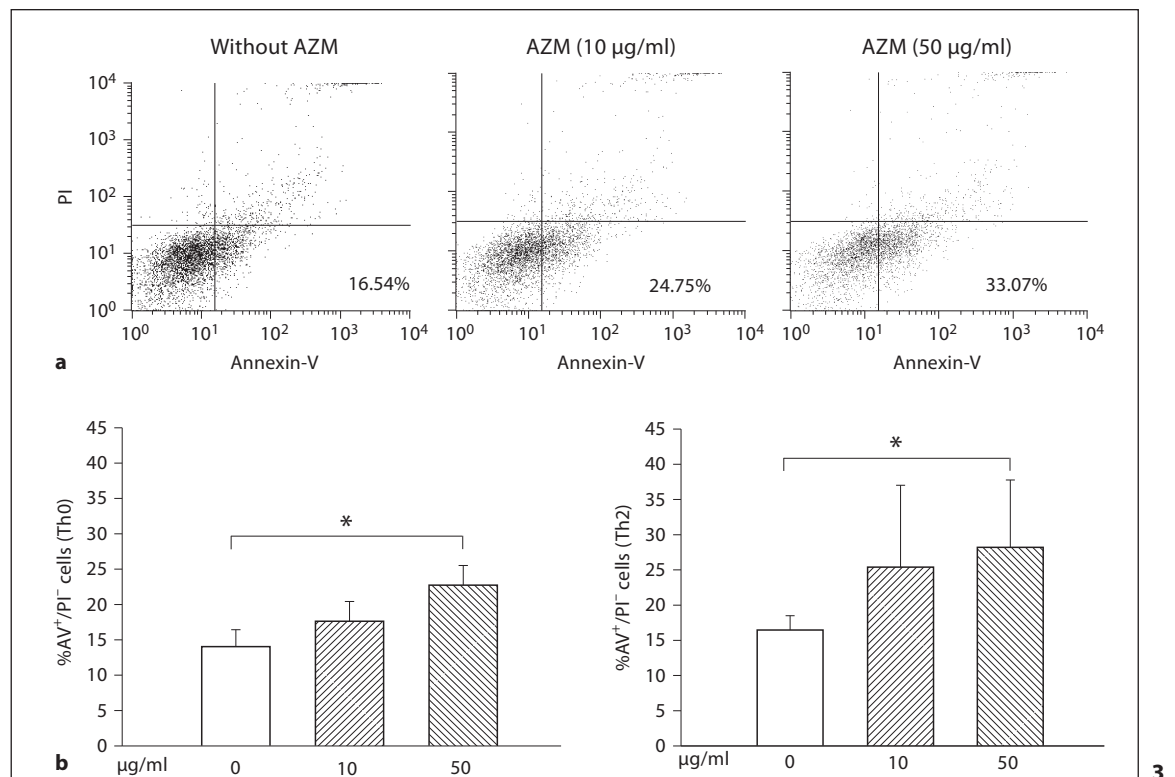
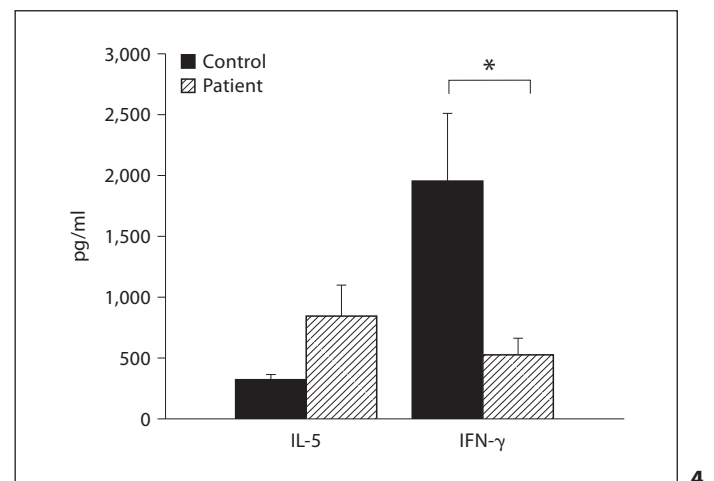


**Fig. 3.** The induction of early apoptosis by AZM on Th0 and Th2 cells. Th0 and Th2 cells cultured with or without AZM were stained with annexin-V (AV) and PI to assess apoptosis. **a** Representative profiles of dot plots showing the effect of increasing dose of AZM on AV+PI<sup>-</sup> expression. The number in the dot plot indicates the percentage of AV+PI<sup>-</sup> cells in each population. **b** Data are presented as means ± SEM from 6 Th0 and 6 Th2 experiments. \*  $p < 0.05$ , compared to media alone.

**Fig. 4.** Higher IL-5 and lesser IFN- $\gamma$  secretion of activated CD4<sup>+</sup> T cells of atopic individuals. CD4<sup>+</sup> T cells were stimulated with anti-CD3 (1 µg/ml, plate coated) in conjunction with soluble anti-CD28 (100 ng/ml) for 5 days. The IL-5 and IFN- $\gamma$  levels in culture supernatants were assessed by ELISA. The data are shown as means ± SEM from 9 atopic asthmatic patients and 9 non-atopic controls. \*  $p < 0.05$ .



## Discussion

The present study specifically examined the effect of AZM on survival, proliferation and cytokine production of Th2 cells, a key player in allergic inflammation, from atopic asthmatic children and non-atopic controls. To our knowledge, this is the first study demonstrating that AZM at 50 µg/ml, a concentration that could be achieved in polymorphonuclear cells or sputum [25, 26], decreased

viable CD4<sup>+</sup> T cell recovery and reduced IL-5 production from activated Th0 and Th2 cells cultured from atopic asthmatic children.

We found that AZM dose-dependently suppressed CD4<sup>+</sup> T cell proliferation upon TCR stimulation, in contrast to Asano et al. [27] who showed that roxithromycin did not affect T cell proliferation. We also observed that AZM at 50 µg/ml enhanced apoptosis of TCR-activated CD4<sup>+</sup> T cells, confirming previous studies using higher

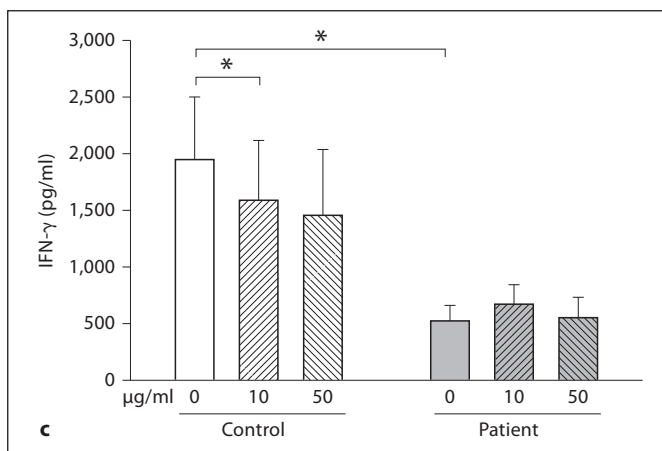
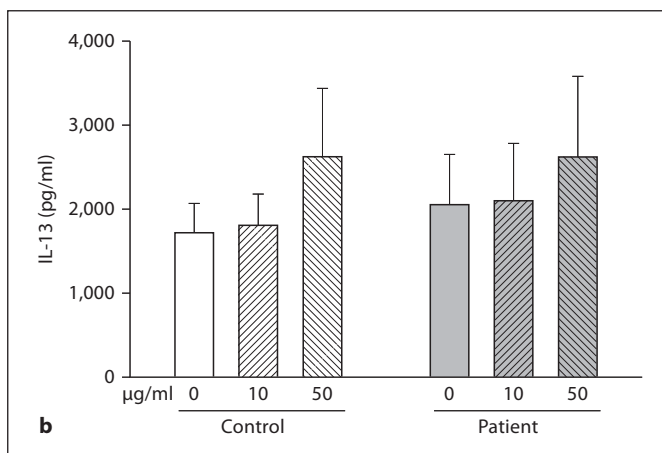
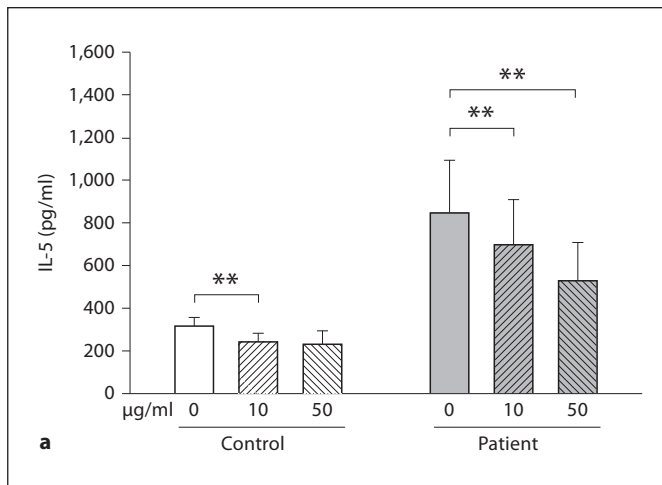


**Fig. 5.** Effect of AZM on IL-5, IL-13 and IFN- $\gamma$  production of Th0 cells from asthmatics and controls. CD4<sup>+</sup> T cells were stimulated with anti-CD3 (1  $\mu$ g/ml, plate coated) in conjunction with soluble anti-CD28 (100 ng/ml) for 5 days alone or in the presence of AZM at 10 and 50  $\mu$ g/ml. The IL-5 (a), IL-13 (b) and IFN- $\gamma$  (c) levels in culture supernatants were assessed by ELISA. The data were presented as means  $\pm$  SEM from 9 atopic asthmatic patients and 9 non-atopic controls. \*  $p < 0.05$ ; \*\*  $p < 0.01$ .

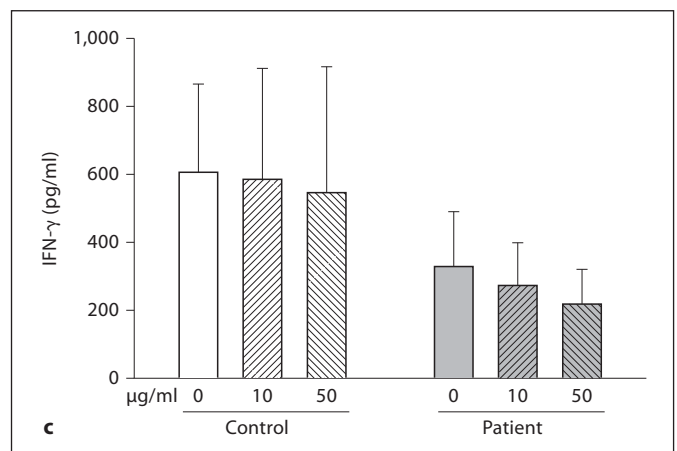
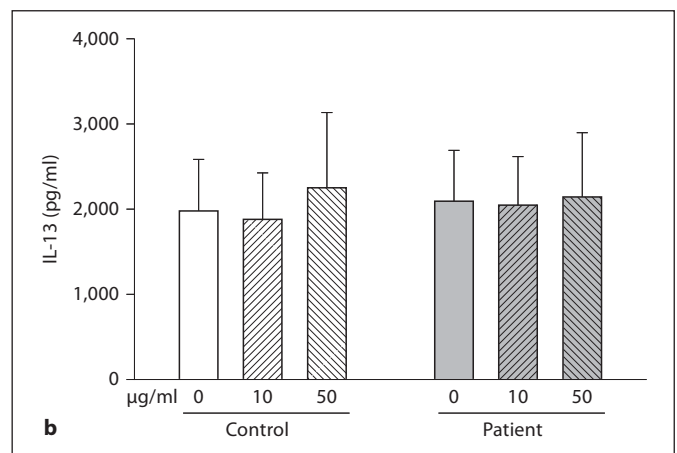
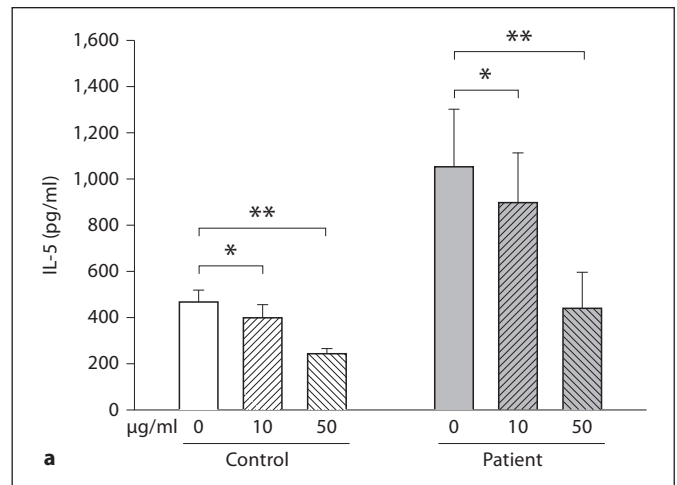


**Fig. 6.** Effect of AZM on IL-5, IL-13, and IFN- $\gamma$  production of Th2 cells from asthmatics and controls. Enriched CD4<sup>+</sup> T cells were stimulated with anti-CD3 (1  $\mu$ g/ml, plate coated) in conjunction with soluble anti-CD28 (100 ng/ml), together with IL-4 (10 ng/ml) and anti-IFN- $\gamma$  (20 ng/ml) for 5 days without or with AZM at 10 and 50  $\mu$ g/ml. The IL-5 (a), IL-13 (b) and IFN- $\gamma$  (c) levels in culture supernatants were assessed by ELISA. The data were presented as means  $\pm$  SEM from 9 atopic asthmatic patients and 9 non-atopic controls. \*  $p < 0.05$ ; \*\*  $p < 0.01$ .

concentrations of AZM (>100 µg/ml) [20, 28]. The apoptosis-inducing capacity of macrolides has also been described in Jurkat cells [29] and neutrophils [30], and is therefore not specific to CD4+ T cells. The dual effect of AZM in suppressing CD4+ T cell proliferation and accelerating apoptosis may account for the decrease in the numbers of both Th0 and Th2 cells of asthmatic children and controls observed in this study.

We demonstrated a higher IL-5 and lower IFN-γ production following polyclonal activation of CD4+ T cells from the atopic asthmatic patients compared to non-atopic controls, confirming previous studies showing that asthma is a Th2-biased disease [31, 32]. There was an additional increase in IL-5 production from CD4+ T cells from asthmatics and controls in the presence of IL-4 (Th2 cells). We found that AZM inhibited IL-5 production of both Th0 and Th2 cells from asthmatic patients without affecting IFN-γ production.

As IL-4 was present in our Th2-polarized culture, we chose to measure another Th2 cytokine, IL-13, in view of similar intron and exon structure and sequence homology between IL-4 and IL-13 genes [33, 34]. In contrast to its effect on IL-5, AZM did not affect the IL-13 production from Th0 or Th2 cells of both asthmatics and controls. The discrepant effect of AZM on IL-5 and IL-13 levels observed in the present study may be explained in part by the differential transcription regulation of IL-5 and IL-13 gene expression [35]. The production of IL-13 was also more ubiquitous than IL-4 or IL-5 [33, 34]. Alternatively, IL-13 may participate in asthma pathogenesis using pathways independent of IL-5 [36]. Higher levels of serum IL-5, but not IL-13, are associated with severe airway obstruction in asthmatics [37].

Whether AZM would downregulate Th2 response in asthma remained unclear. Our finding, however, adds to

the list of the multifaceted immune-modulating effects of macrolides. A recent animal study lends support to our results by showing that AZM attenuated the ovalbumin-dependent airway inflammation by decreasing the eosinophil counts and IL-5 expression in bronchoalveolar lavage [38]. As IL-5 is crucial for eosinophil recruitment in asthma, our findings also help explain why macrolides suppress bronchial hyperresponsiveness associated with eosinophilic inflammation in patients with asthma [39–41]. AZM targeting IL-5-producing CD4+ T cells may be particularly beneficial for asthmatic subjects with severe eosinophilic inflammation refractory to conventional glucocorticosteroid treatment [42]. However, sustained use of AZM may not be justified considering the risk of emergence of resistant strains.

In summary, our findings suggest that AZM preferentially downregulated IL-5 production from activated Th0 and polarized Th2 cells as a hitherto reported benefit of AZM on asthma pathogenesis, further supporting its therapeutic roles in childhood asthma. Larger randomized, controlled clinical trials are needed to confirm whether our findings are of clinical relevance in application to asthma.

## Acknowledgements

This study was supported partially by grants from the National Science Council of the Republic of China (NSC98-2314-B-182-003-MY3 and NSC97-2314-B-182-017) and grants from the Chang Gung Memorial Hospital (CMRPD160332, CMRPG460082 and CMRPG460083). The authors would like to thank Hsiu-Shan Hsiao and Ya-Fen Lu for their technical support with flow cytometry. AZM was generously provided by Pfizer.

## References

- 1 Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM: Asthma. From bronchoconstriction to airways inflammation and remodeling. *Am J Respir Crit Care Med* 2000; 161:1720–1745.
- 2 Nakajima H, Takatsu K: Role of cytokines in allergic airway inflammation. *Int Arch Allergy Immunol* 2007;142:265–273.
- 3 Yang KD: Childhood asthma: aspects of global environment, genetics and management. *Chang Gung Med J* 2000;23:641–661.
- 4 Bjerner L: Time for a paradigm shift in asthma treatment: from relieving bronchospasm to controlling systemic inflammation. *J Allergy Clin Immunol* 2007;120:1269–1275.
- 5 Lovett CJ, Whitehead BF, Gibson PG: Eosinophilic airway inflammation and the prognosis of childhood asthma. *Clin Exp Allergy* 2007;37:1594–1601.
- 6 Allen DB, Bielory L, Derendorf H, Dluhy R, Colice GL, Szefer SJ: Inhaled corticosteroids: past lessons and future issues. *J Allergy Clin Immunol* 2003;112:S1–S40.
- 7 Barnes PJ, Pedersen S, Busse WW: Efficacy and safety of inhaled corticosteroids. New developments. *Am J Respir Crit Care Med* 1998;157:S1–S53.
- 8 Skoner DP, Maspero J, Banerji DP: Assessment of the long-term safety of inhaled ciclesonide on growth in children with asthma. *Pediatrics* 2008;121:e1–e14.
- 9 Shinkai M, Rubin BK: Macrolides and airway inflammation in children. *Paediatr Respir Rev* 2005;6:227–235.

- 10 Amsden GW: Anti-inflammatory effects of macrolides – an underappreciated benefit in the treatment of community-acquired respiratory tract infections and chronic inflammatory pulmonary conditions? *J Antimicrob Chemother* 2005;55:10–21.
- 11 Mitsuyama T, Hidaka K, Furuno T, Hara N: Neutrophil-induced endothelial cell damage: inhibition by a 14-membered ring macrolide through the action of nitric oxide. *Int Arch Allergy Immunol* 1997;114:111–115.
- 12 Tamaoki J: The effects of macrolides on inflammatory cells. *Chest* 2004;125:41S–51S.
- 13 Shinkai M, Mo H, Rubin BK: Macrolide antibiotics as immunomodulatory medications: proposed mechanisms of action. *Pharmacol Ther* 2008;17:393–405.
- 14 Gotfried MH: Macrolides for the treatment of chronic sinusitis, asthma, and COPD. *Chest* 2004;125:52S–60S.
- 15 Kostadima E, Tsiodras S, Alexopoulos EI, Kaditis AG, Mavrou I, Georgatou N, Pampichalopoulos A: Clarithromycin reduces the severity of bronchial hyperresponsiveness in patients with asthma. *Eur Respir J* 2004;23:714–717.
- 16 Simpson JL, Powell H, Boyle MJ, Scott RJ, Gibson PG: Clarithromycin targets neutrophilic airway inflammation in refractory asthma. *Am J Respir Crit Care Med* 2008;177:148–155.
- 17 Amsden GW: Advanced-generation macrolides: tissue-directed antibiotics. *Int J Antimicrob Agents* 2001;18:S11–S15.
- 18 Ekici A, Ekici M, Erdemoglu AK: Effect of azithromycin on the severity of bronchial hyperresponsiveness in patients with mild asthma. *J Asthma* 2002;39:181–185.
- 19 Piacentini GL, Peroni DG, Bodini A, Pigozzi R, Costella S, Loiacono A, Boner AL: Azithromycin reduces bronchial hyperresponsiveness and neutrophilic airway inflammation in asthmatic children: a preliminary report. *Allergy Asthma Proc* 2007;28:194–198.
- 20 Mizunoe S, Kadota J, Tokimatsu I, Kishi K, Nagai H, Nasu M: Clarithromycin and azithromycin induce apoptosis of activated lymphocytes via down-regulation of Bcl-xL. *Int Immunopharmacol* 2004;4:1201–1207.
- 21 Williams AC, Galley HF, Watt AM, Webster NR: Differential effects of three antibiotics on T helper cell cytokine expression. *J Antimicrob Chemother* 2005;56:502–506.
- 22 Ito T, Ito N, Hashizume H, Takigawa M: Roxithromycin inhibits chemokine-induced chemotaxis of Th1 and Th2 cells but regulatory T cells. *J Dermatol Sci* 2009;54:185–191.
- 23 Lin CC, Liaw SF, Wu KM, Lin CH: Effect of erythromycin on bronchial hyperreactivity and inflammation in ovalbumin-sensitized brown Norway rats. *Respir Physiol Neurobiol* 2008;161:267–272.
- 24 Johansson SG, Hourihane JO, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T, Kowalski ML, Mygind N, Ring J, van Cauwenberge P, van Hage-Hamsten M, Wuthrich B: A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001;56:813–824.
- 25 Liu P, Allaudeen H, Chandra R, Phillips K, Jungnik A, Breen JD, Sharma A: Comparative pharmacokinetics of azithromycin in serum and white blood cells of healthy subjects receiving a single-dose extended-release regimen versus a 3-day immediate-release regimen. *Antimicrob Agents Chemother* 2007;51:103–109.
- 26 Wilms EB, Touw DJ, Heijerman HGM: Pharmacokinetics of azithromycin in plasma, blood, polymorphonuclear neutrophils and sputum during long-term therapy in patients with cystic fibrosis. *Therapeut Drug Monitor* 2006;28:219–225.
- 27 Asano K, Kamakazu K, Hisamitsu T, Suzuki H: Modulation of Th2 type cytokine production from human peripheral blood leukocytes by a macrolide antibiotic, roxithromycin, in vitro. *Int Immunopharmacol* 2001;1:1913–1921.
- 28 Ishimatsu Y, Kadota J, Iwashita T, Nagata T, Ishii H, Shikuwa C, Kaida H, Mukae H, Kohno S: Macrolide antibiotics induce apoptosis of human peripheral lymphocytes in vitro. *Int J Antimicrob Agents* 2004;24:247–253.
- 29 Jun YT, Kim HJ, Song MJ, Lim JH, Lee DG, Han KJ, Choi SM, Yoo JH, Shin WS, Choi JH: In vitro effects of ciprofloxacin and roxithromycin on apoptosis of Jurkat T lymphocytes. *Antimicrob Agents Chemother* 2003;47:1161–1164.
- 30 Aoshiba K, Nagai A, Konno K: Erythromycin shortens neutrophil survival by accelerating apoptosis. *Antimicrob Agents Chemother* 1995;39:872–877.
- 31 Huang JL, Chen LC, Yeh KW, Lin SJ, Hsieh KH, Kuo ML: Th1 and Th2 cytokine production among asthmatic children after immunotherapy. *J Asthma* 2003;40:273–279.
- 32 Hirose H, Matsuse H, Tsuchida T, Fukahori S, Fukushima C, Mizuta Y, Kohno S: Cytokine production from peripheral blood mononuclear cells of mite allergen-sensitized atopic adults stimulated with respiratory syncytial virus and mite allergen. *Int Arch Allergy Immunol* 2008;146:149–155.
- 33 Minty A, Asselin S, Bensussan A, Shire D, Vita N, Vyakarnam A, Wijdenes J, Ferrara P, Caput D: The related cytokines interleukin-13 and interleukin-4 are distinguished by differential production and differential effects on T lymphocytes. *Eur Cytokine Network* 1997;8:203–213.
- 34 Jiang H, Harris MB, Rothman P: IL-4/IL-13 signaling beyond JAK/STAT. *J Allergy Clin Immunol* 2000;105:1063–1070.
- 35 Georas SN, Guo J, De Fanis U, Casolaro V: T-helper cell type-2 regulation in allergic disease. *Eur Respir J* 2005;26:1119–1137.
- 36 Eum SY, Maghni K, Tolloczko B, Eidelman DH, Martin JG: IL-13 may mediate allergen-induced hyperresponsiveness independently of IL-5 or eotaxin by effects on airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2005;288:576–584.
- 37 Lee YC, Lee KH, Lee HB, Rhee YK: Serum levels of interleukins (IL)-4, IL-5, IL-13, and interferon-gamma in acute asthma. *J Asthma* 2001;38:665–671.
- 38 Beigelman A, Gunsten S, Milols CL, Vidaskey I, Cannon CL, Brody SL, Walter MJ: Azithromycin attenuates airway inflammation in a noninfectious mouse model of allergic asthma. *Chest* 2009;36:498–506.
- 39 Garey KW, Rubinstein I, Gotfried MH, Khan JJ, Varma S, Danziger LH: Long-term clarithromycin decreases prednisone requirements in elderly patients with prednisone-dependent asthma. *Chest* 2000;118:1826–1827.
- 40 Amayasu H, Yoshida S, Ebana S, Yamamoto Y, Nishikawa T, Shoji T, Nakagawa H, Hasegawa H, Nakabayashi M, Ishizaki Y: Clarithromycin suppress bronchial hyperresponsiveness associated with eosinophilic inflammation in patients with asthma. *Ann Allergy Asthma Immunol* 2000;84:594–598.
- 41 Johnston SL, Blasi F, Black PN, Martin RJ, Farrell DJ, Nieman RB: TELICAST Investigators: The effect of telithromycin in acute exacerbations of asthma. *N Engl J Med* 2006;354:1589–1600.
- 42 Tagaya E, Tamaoki J, Kondo M, Nagai A: Effect of a short course of clarithromycin therapy on sputum production in patients with chronic airway hypersecretion. *Chest* 2002;122:213–218.