

Regulatory-T, T-Helper 1, and T-Helper 2 Cell Differentiation in Nasal Mucosa of Allergic Rhinitis with Olive Pollen Sensitivity

Ayhan Sogut^a Ozge Yilmaz^a Cengiz Kirmaz^b Kemal Ozbilgin^c Ece Onur^d
Onur Celik^e Ercan Pinar^g Seda Vatansever^c Gonul Dinc^f Hasan Yuksel^a

^aPediatric Allergy and Pulmonology Unit, ^bDivision of Allergy and Immunology, Department of Internal Medicine, Departments of ^cHistology and Embryology, ^dBiochemistry, ^eOtorhinolaryngology/Head and Neck Surgery, and ^fPublic Health, Celal Bayar University School of Medicine, Manisa, and ^gOtolaryngology and Head and Neck Department, Aaturk Training and Research Hospital, Izmir, Turkey

Key Words

Rhinitis · Pollens · Lymphocytes

Abstract

Background: Allergic rhinitis (AR) is a disease in which T-helper (Th)2 response is predominant and its pathogenic mechanism is still poorly understood. **Aim:** To evaluate the possible role of Th1, Th2 and regulatory-T (Treg) cells in the pathogenesis of AR. **Methods:** This case-control study enrolled 41 patients with seasonal AR (10–62 years old), sensitive to olive pollens, and 15 healthy controls (18–60 years old). Nasal biopsy was performed and specimens of nasal lavage fluid were obtained from all participants. The levels of interleukin (IL)-4, IL-10, interferon (IFN)- γ and transforming growth factor- β (TGF- β) were measured in nasal lavage fluid specimens. The expression of FOXP3, GATA-3 and T-bet was measured by immunohistochemical methods in the nasal biopsy specimens. **Results:** The levels of IFN- γ in the group with AR were significantly lower than those in the control group ($p = 0.008$). The levels of IL-4, IL-10 and TGF- β did not differ between the two groups. The expression of FOXP3 and T-bet in patients with AR was significantly lower than that in the control group (both $p = 0.001$). Expression of GATA-3 in the nasal mucosa was similar between the groups ($p = 0.2$). The ratios of T-bet/GATA-3 and FOXP3/GATA-3 in the AR group were significantly lower than those in the control

group ($p = 0.001$). **Conclusion:** Insufficient Treg and Th1 cells may be associated with the allergic inflammation that may be attributed to the Th2 immune response in patients suffering from AR who are sensitive to olive pollen.

Copyright © 2011 S. Karger AG, Basel

Introduction

Allergic rhinitis (AR) is a chronic inflammatory condition of the upper airway [1]. In sensitized individuals, exposure to pollen allergens causes symptoms of AR. Subgroups of T-helper (Th) cells play a pivotal role in the development of allergic diseases. The main subsets are Th1, Th2, and regulatory-T (Treg) cells [2, 3].

Th1 cells interact characteristically with exogenous pathogens (bacteria, viruses), and they secrete interleukin (IL)-2, tumor necrosis factor- α , and interferon- γ (IFN- γ) [4]. T-bet is a transcription factor for Th1 cells. It has been demonstrated that mice lacking T-bet develop airway inflammation spontaneously, which is characterized by eosinophil infiltration and airway hyperreactivity [5].

Th2 cells modulate the production of immunoglobulins by B cells and therefore regulate humoral immunity. Th2 cells secrete IL-4, IL-5, IL-9, IL-10, and IL-13. Among these cytokines, IL-4 and IL-13 facilitate switching of the

B-cell heavy chain isotype from IgG to IgE production, with subsequent release of IL-5 [4]. GATA-3 plays a major role in the development of the Th2 phenotype while inhibiting Th1 cells [6].

Treg cells have a pivotal role in the development of self-tolerance. They assist in the suppression of autoreactive T cells and downregulate the proinflammatory responses of both Th1 and Th2 cells. They also limit allergic airway inflammation and excessive Th2 responses to antigens [7]. FOXP3 is a specific marker expressed by Treg cells. It is a 'master switch' gene for Treg cells. The FOXP3 transcriptional regulator determines the levels and activity of CD4+CD25+ Treg cells. The expression of FOXP3 is closely related to autoimmunity and allergy [4].

In the study reported herein, we investigated the profile of Treg, Th2 and Th1 cells in the nasal mucosa of patients with AR who were sensitive to olive pollen during the pollen season.

Subjects and Methods

Subjects and Study Design

The study was conducted at the outpatient clinic of the Pediatric Allergy and Pulmonology Unit, the Division of Allergy and Immunology, Department of Internal Medicine, and the Department of Otorhinolaryngology/Head and Neck Surgery, Celal Bayar University School of Medicine, Manisa, Turkey. Participants were recruited from consecutive patients presenting to these clinics. The age and the gender of all participants were recorded.

This study consisted of 41 patients (aged 10–62 years) with seasonal allergic rhinitis (SAR) sensitized only to *Olea europaea* pollen and 15 non-allergic controls (aged 18–60 years) requiring an operation due to nasal septum deviation. In the Oleaceae family, the most allergenic pollen is produced by *O. europaea*, the olive tree, which in the Mediterranean area has been recognized as one of the most important causes of seasonal respiratory allergy. The olive pollination season lasts from May to the end of June and sometimes causes severe symptoms (ocularrhinitis and/or bronchial asthma). Olive pollinosis is quite rare in the form of mono-sensitization. Although adults are affected the most, olive sensitization can be observed in children, too [8]. This study was conducted in spring during the pollen season. The patients and control subjects were seen during the same time point of the pollen season. All patients with SAR were symptomatic. The study included patients with newly diagnosed SAR. All the controls were free of symptoms of rhinitis. Neither the patients with SAR nor the control group had previously received antihistamines, nasal corticosteroids or immunotherapy. Patients with any acute or chronic condition that might affect the immune response and those who were diagnosed with rhinosinusitis or asthma were excluded from the study.

Diagnosis of AR was based on clinical history, physical examination, as well as a positive response to skin prick test [9–11]. Symptoms of AR are rhinorrhea, nasal obstruction, nasal itching, and sneezing [9]. Allergic sensitization was demonstrated by the

skin prick test. The skin prick test was performed on all participants during the pollen season. The skin prick tests were performed according to the European Academy of Allergology and Clinical Immunology guidelines [12]. All patients with SAR had positive skin prick test reactivity to only *O. europaea* pollen extract (Allergopharma Ltd, Reinbek, Germany). These patients were not sensitized to other common environmental allergens, including *Dermatophagoides pharyngea*, *Dermatophagoides pteronyssinus*, mold mixture, cat, grass or cereal allergen mixture (*Lolium perenne*, *Festuca pratensis*, *Phleum pratense*, *Poa pratensis*, *Dactylis glomerata*, *Hordeum vulgare*, *Secale cereale*, or *Triticum sativum*), *Platanus orientalis*, *Papulus albe*, *Cotylus avellana*, *Betula verrucosa*, and *Qercus robur*.

Non-allergic control individuals were examined by an otolaryngologist. These subjects were healthy and did not have persistent or seasonal AR. Allergen skin prick tests in all non-allergic control group individuals were negative.

Nasal biopsy and nasal lavage fluid specimens were carried out from both SAR patients and control subjects during the pollen season in the spring. All patients with SAR were symptomatic in this period. After nasal biopsy and nasal lavage, fluid specimens were obtained from the patients with SAR; these patients were given antihistamines and nasal corticosteroids.

The study was approved by the Ethics Committee of the Celal Bayar University, and written informed consent was obtained from the adult participants and the parents/guardians of the children.

Nasal Lavage

For the nasal lavage procedure, we used 10 ml of physiological saline for each nasal cavity. The procedure was performed with the patient's head tipped backwards during closure of the soft palate. The fluid collected was stored at –80°C for subsequent analysis.

Measurement of Cytokines

The levels of IL-4, IL-10, transforming growth factor- β (TGF- β) and IFN- γ in nasal lavage fluid samples were measured by using the enzyme-linked immunosorbent assay (ELISA) method. The appropriate RayBio Human IL-4, IL-10, TGF- β and IFN- γ immunoassay kits (Ray Biotech, Inc., Norcross, Ga., USA) were used to measure the levels of IL-4, IL-10, TGF- β and IFN- γ . The intra- and interassay coefficients of variation for all the kits were <10 and <12%, respectively. The sensitivity of detection of IL-4, IL-10, TGF- β and IFN- γ was 5, 1, 80 and 15 pg/ml, respectively.

Nasal Biopsy Specimens

Inferior nasal turbinate biopsy specimens of patients with SAR were obtained with a cup forceps device from the patient under local anesthesia. The same procedure was performed on the control group during septoplasty by the otolaryngologist.

Immunohistochemical Staining

Nasal biopsy specimens were first fixed with 10% formalin in phosphate-buffered solution (PBS) for a maximum duration of 24 h and then washed with distilled water for 12 h. They were then dehydrated through a series of ethanol solutions for 30 min each. Then specimens were kept in xylene alcohol for 30 min, in xylene twice for 45 min and in xylene-paraffin for 30 min. They were then kept in dissolved paraffin for 1 h in the sterilizer at 60°C, in paraffin I for 1 h and in paraffin II for 1 h. Sections taken from the sterilizer were blocked and embedded in paraffin. Then,

5- μ m-sized sections in series were obtained by using microtome from paraffin blocks.

The samples were deparaffinized for a night in the sterilizer at 60°C before the staining process began. The samples were first kept in xylene twice for 30 min and then rehydrated through a series of ethanol solutions for 2 min each. The sections were incubated in the citrate buffer (50 ml citrate + 450 ml distilled water) at the microwave stove for 6 min. Then they were washed with distilled water for 5 min. The tissues were then washed with PBS 3 times for 5 min and incubated in 3% hydrogen peroxidase for 5 min. The tissues were washed with PBS 3 times for 5 min. They were then washed with blocking solution and kept for 1 h. Nasal tissue specimens were stained with antibodies against GATA-3, FOXP3, and T-bet (GATA-3 [sc-268], FOXP3 [sc-80792], and T-bet [sc-21003], all from Santa Cruz Biotechnology Inc., Santa Cruz, Calif., USA) using the immunohistochemical technique and kept overnight. The tissues were then washed in PBS 3 times for 5 min. The secondary antibody with biotin was applied for 30 min and washed in PBS 3 times for 5 min. The streptavidin-peroxidase complex was added for 30 min and washed in PBS 3 times. Sections were then stained with diaminobenzidine (Dako, Glostrup, Denmark) to detect immunoreactivity and then counterstained with Mayer's hematoxylin. Sections were then applied a series of ethanol solutions for 2 min each and then kept in xylene for 30 min.

The samples were covered with Entellan and observed under an Olympus BX-40 light microscope (Olympus, Center Valley, Pa., USA). The analyses were performed in a blinded fashion. All sections were scored in a semiquantitative fashion, considering both the intensity and percentage of cell staining at each intensity. Intensities were classified as 0 (no staining), +1 (weak staining), +2 (distinct staining), and +3 (very strong staining). The expression of T-bet, FOXP3 and GATA-3 was determined as an immunohistochemical score (HSCORE) from the specimens.

For each slide, a value designated the histochemical score (HSCORE) was obtained by application of the following algorithm: $HSCORE = \sum Pi (i + 1)$, where i is the intensity of staining with a value of 1, 2, or 3 (weak, moderate, or strong, respectively) and Pi is the percentage of stained cells for each intensity, varying from 0 to 100%. The HSCORE has been used in previous studies in patients with SAR [13].

Statistical Analysis

Statistical analysis was performed with SPSS software, version 10.0 for Windows (SPSS Inc., Chicago, Ill., USA). The Mann-Whitney U test was used to compare subject-based means of cytokine levels in patients with AR and controls. Student's t test was used to compare field-based means of the HSCOREs. $p < 0.05$ was considered significant.

Results

Demographic Features

The mean age of the 41 patients with AR was 30.8 ± 15.2 years (range 10–62) and the mean age of the 15 controls was 29.6 ± 10.1 years (range 18–60) ($p > 0.05$). Males constituted 31.7 and 40% of the AR and control groups, respectively.

Cytokine Analysis

The levels of IFN- γ in the AR group were significantly lower than those in the control group (52.96 ± 22.04 vs. 78.47 ± 40.00 , $p = 0.008$). However, there was no significant difference in the levels of IL-4, IL-10 and TGF- β in nasal lavage fluid between the cases and controls ($p = 0.4$, $p = 0.07$, $p = 0.3$, respectively).

Expression of GATA-3, T-bet, FOXP3

The HSCOREs for FOXP3 in nasal tissue specimens from patients with AR and controls were 23.75 ± 4.49 and 57.39 ± 10.25 , respectively. The HSCOREs for FOXP3 in patients with AR were significantly lower than those of the control group ($p = 0.001$). The HSCOREs for T-bet determined in nasal tissue specimens from patients with AR and controls were 23.91 ± 6.31 and 57.20 ± 11.96 , respectively. HSCOREs of T-bet in patients with the AR were significantly lower than that in the control group ($p = 0.001$). The HSCOREs for GATA-3 determined in nasal tissue specimens from patients with AR and controls were 30.50 ± 7.65 and 32.25 ± 6.71 , respectively. There was no significant difference between the groups ($p = 0.2$). The ratios of cells staining for FOXP3/GATA-3 (0.81 ± 0.22 , 1.84 ± 0.46) and T-bet/GATA-3 (0.82 ± 0.27 , 1.83 ± 0.51) in the AR group were significantly lower than those in the control group (both $p = 0.001$).

Discussion

In this study, we demonstrated that the expression of FOXP3⁺ and T-bet⁺ cells in nasal biopsy specimens of patients with AR is significantly lower than that in non-allergic subjects. In addition, we identified significantly lower ratios of T-bet⁺/GATA-3⁺ and FOXP3⁺/GATA-3⁺ cells in patients with AR. We also detected significantly lower levels of the IFN- γ cytokine in nasal lavage fluid from allergic subjects in comparison with controls.

The development of AR has been thought to be caused by an imbalance between allergen-specific Th2 and Th1 cells. The perceived importance of Treg cells suggests that allergy is more likely to be due to an imbalance between Treg cells and Th2 cells [14]. FOXP3 is a specific marker expressed by the Treg cells [4]. Xu et al. [15] compared nasal tissue and peripheral blood mononuclear cells between adult patients with AR and healthy controls. They reported that the patients had significantly fewer FOXP3⁺ lymphocytes and decreased expression of FOXP3 mRNA. Recently, Lee et al. [16] suggested that either reduced ex-

pression of the FOXP3 gene or defective FOXP3 function could be one of the mechanisms in the development of allergic disease in humans.

In patients with AR, Malmh  ll et al. [17] demonstrated a higher number of FOXP3⁺ and GATA-3⁺ cells, but not T-bet⁺ cells, in the nasal mucosa compared with that of healthy subjects before the start of the pollen season. Although, the number of FOXP3⁺ cells in the nasal mucosa remained the same in AR patients after the pollen season, it decreased significantly following treatment with fluticasone propionate. The pollen season caused a substantial increase in the number of GATA-3⁺ cells, which was inhibited by fluticasone propionate. The number of T-bet⁺ cells was not affected by pollen or by treatment with fluticasone propionate.

TGF-   is important in the induction of Treg cells and participates directly in the suppression of effector T cells. It also mediates the remodeling of airway tissues [18]. In humans, IL-10 is released by Treg cells as well as by monocytes, B lymphocytes and dendritic cells. Treg may increase the capacity of these cells to produce IL-10 [19]. IL-10 inhibits the production of proinflammatory cytokines and the activation of both Th1 and Th2 cells. Sun et al. [20] showed that ragweed stimulation significantly increased the production of IL-10 in patients with AR, whereas the production of TGF-   was increased only in normal individuals. Conflicting results have been reported regarding the role of TGF-   in AR. Benson et al. [21] reported that low expression of TGF-  -inducible transcripts may indicate a decreased TGF-   activity in AR. However, Ouyang et al. [22] suggested that TGF-   signaling is activated in epithelial cells in the nasal mucosa in AR and may contribute to the development of goblet cell hyperplasia. Ciprandi et al. [23] found that TGF-   serum levels were significantly lower in patients with AR evaluated outside the pollen season in comparison with during the pollen season and after one pre-seasonal sublingual immunotherapy course.

We found that the number of FOXP3⁺ cells and the FOXP3⁺/GATA-3⁺ cell ratio were reduced in patients with AR compared with healthy controls. In addition, we demonstrated that the levels of TGF-   and IL-10 are similar to those in healthy controls. These results may indicate that the suppressive function of Treg cells is reduced in patients with AR patients.

T-bet is required for Th1 cell differentiation and the production of IFN-   [24, 25]. It has been shown that mice lacking T-bet develop airway inflammation and airway hyperreactivity [5]. In addition, polymorphisms of the T-bet gene have been found to be associated with asthma

and airway hyperreactivity [26]. These findings suggest that T-bet may be a negative regulator in allergic airway inflammation and airway hyperreactivity in both human beings and mice. Malmh  ll et al. [17] showed that similar levels of T-bet are present in the nasal mucosa in patients with AR and healthy controls, before and during the pollen season. However, a significant decrease in the T-bet⁺/GATA-3⁺ ratio was determined during the pollen season. Liu et al. [27] showed that the expression of T-bet mRNA was not significantly different in persistent AR, intermittent AR or control patients.

The main Th1 effector cytokine, IFN-  , is important for the resolution of allergic inflammation. In studies of asthma in mice, IFN-   has prevented the development of antigen-induced airway eosinophilia and hyperreactivity [28]. In another study, mice that were deficient in the IFN-   receptor exhibited prolonged airway eosinophilia in response to the inhalation of allergen [29].

In the study reported herein, we showed that both the number of T-bet⁺ cells and the level of the IFN-   cytokine in the nasal mucosa of patients with AR were significantly lower than those of controls during the pollen season. In addition, we found that the ratio of T-bet⁺/GATA-3⁺ cells in patients with AR was significantly lower than those of controls during the pollen season. These results indicate that the Th1 immune response in the nasal mucosa is reduced in patients with AR during the pollen season.

GATA-3 plays a major role in the differentiation of Th2 cells and is required for the release of IL-4, IL-5 and IL-13 from Th2 cells [30]. Several studies have demonstrated that the expression of GATA-3 is increased significantly in patients with AR compared with healthy controls [17, 31]. Liu et al. [27] showed that the expression of GATA-3 mRNA is increased significantly in persistent AR patients compared with those with intermittent AR. GATA-3 is also increased in patients with AR following natural exposure during the pollen season. In the present study, we found a similar number of GATA-3⁺ cells and similar levels of the IL-4 cytokine in AR patients and controls. In addition, we found that the ratios of FOXP3⁺/GATA-3⁺ and T-bet⁺/GATA-3⁺ cells in the AR group were significantly lower than those in the controls.

In conclusion, our data demonstrate that the expression of FOXP3 and T-bet cells, as well as cytokine production by Th1 cells, are lower in the nasal mucosa of patients with AR during the pollen season than in controls. Moreover, the ratios of Th1/Th2 and Treg/Th2 are reduced in patients with AR. These results suggest that the Th1 immune response and the suppression of Treg cells are de-

creased in patients with AR. As a result, insufficient Treg cell and Th1 cell activity may be associated with allergic inflammation caused by the Th2 immune response during the pollen season in patients with AR who have sensitivity to olive pollen.

Disclosure Statement

The authors have no conflicts of interest to disclose.

References

- Bousquet J, Jacquot W, Vignola M, Bachert C, Van Cauwenberge P: Allergic rhinitis: a disease remodeling the upper airways? *J Allergy Clin Immunol* 2004;113:43–49.
- LaRosa DE, Orange JS: Lymphocytes. *J Allergy Clin Immunol* 2007;121(suppl 2):S364–S369.
- Buckner JH, Ziegler SF: Regulating the immune system: the induction of regulatory T cells in the periphery. *Arthritis Res Ther* 2004;6:215–222.
- Paik Y, Dahl M, Fang D, Calhoun K: Update: the role of FOXP3 in allergic disease. *Curr Opin Otolaryngol Head Neck Surg* 2008;16:275–279.
- Finotto S, Neurath MF, Glickman JN, Qin S, Lehr HA, Green FH, Ackerman K, Haley K, Galle PR, Szabo SJ, Drazen JM, De Sanctis GT, Glimcher LH: Development of spontaneous airway changes consistent with human asthma in mice lacking T-bet. *Science* 2002;295:336–338.
- Ouyang W, Ranganath SH, Weindel K, Bhattacharya D, Murphy TL, Sha WC, Murphy KM: Inhibition of Th1 development mediated by GATA-3 through an IL-4-independent mechanism. *Immunity* 1998;9:745–755.
- Freitas A, Wildin RS: IPEX and FOXP3; clinical and research perspectives. *J Autoimmun* 2005;25:56–62.
- Liccardi G, D'Amato M, D'Amato G: Oleaceae pollinosis: a review. *Int Arch Allergy Immunol* 1996;111:210–217.
- Bousquet J, Khaltaev N, Cruz AA, et al: World Health Organization (WHO), GA(2) LEN and AllerGen: Allergic Rhinitis and Its Impact on Asthma (ARIA) 2008 Update; in collaboration with the WHO, GA(2)LEN and AllerGen. *Allergy* 2008;63(suppl 86):8–160.
- Ng ML, Warlow RS, Chrishanthan N, Ellis C, Walls RS: Preliminary criteria for the definition of allergic rhinitis: a systematic evaluation of clinical parameters in a disease cohort (II). *Clin Exp Allergy* 2000;30:1417–1422.
- Van Rijswijk JB, Blom HM, Fokkens WJ: Idiopathic rhinitis, the ongoing quest. *Allergy* 2005;60:1471–1481.
- Dreborg S, Backman A, Basomba A: Skin tests used in type I allergy testing. Position paper. *Allergy* 1989;44(suppl 10):S1–S59.
- Yuksel H, Kirmaz C, Yilmaz O, Pinar E, Vatansever S, Degirmenci PB, Ozbilgin K: Nasal mucosal expression of nitric oxide synthases in patients with allergic rhinitis and its relation to asthma. *Ann Allergy Asthma Immunol* 2008;100:12–16.
- Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Cramer R, Thunberg S, Deniz G, Valenta R, Fiebig H, Kegel C, Disch R, Schmidt-Weber SB, Blaser K, Akdis CA: Immune responses in healthy and allergic individuals are characterized by a fine balance between allergic-specific T-regulatory 1 and T-helper 2 cells. *J Exp Med* 2004;199:1567–1575.
- Xu G, Mou Z, Jiang H, Cheng L, Shi J, Xu R, Oh Y, Li H: A possible role of CD4+CD25+ T cells as well as transcription factor Foxp3 in the dysregulation of allergic rhinitis. *Laryngoscope* 2007;117:876–880.
- Lee SM, Gao B, Dahl M, Calhoun K, Fang D: Decreased Foxp3 gene expression in the nasal secretions from patients with allergic rhinitis. *Otolaryngology Head Neck Surg* 2009;140:197–201.
- Malmhäll C, Bossios A, Pullerits T, Lötvall J: Effects of pollen and nasal glucocorticoid on FOXP3+, GATA-3+ and T-bet+ cells in allergic rhinitis. *Allergy* 2007;62:1007–1013.
- Schmidt-Weber CB, Blaser K: The role of TGF- β in allergic inflammation. *Immunol Allergy Clin North Am* 2006;26:233–244.
- Akdis CA, Blesken T, Akdis M, Wüthrich B, Blaser K: Role of IL-10 in specific immunotherapy. *J Clin Invest* 1998;102:98–106.
- Sun J, Wong B, Cundall M, Goncharova S, Conway M, Dalrymple A, Coyle AJ, Wasserman S, Jordana M: Immunoreactivity profile of peripheral blood mononuclear cells from patients with ragweed-induced allergic rhinitis. *Clin Exp Allergy* 2007;37:901–918.
- Benson M, Carlsson B, Carlsson LM, Mostad P, Svensson PA, Cardell LO: DNA microarray analysis of transforming growth factor- β and related transcripts in nasal biopsies from patients with allergic rhinitis. *Cytokine* 2002;18:20–25.
- Ouyang Y, Miyata M, Hatsushika K, Ohnuma Y, Katoh R, Ogawa H, Okumura K, Masuyama K, Nakao A: TGF- β signaling may play a role in the development of goblet cell hyperplasia in a mouse model of allergic rhinitis. *Allergol Int* 2010;59:313–319.
- Ciprandi G, De Amici M, Tosca M, Marseglia G: Serum transforming growth factor- β levels depend on allergen exposure in allergic rhinitis. *Int Arch Allergy Immunol* 2010;152:66–70.
- Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH: A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 2000;100:655–669.
- Szabo SJ, Sullivan BM, Stemmann C, Satoskar AR, Sleckman BP, Glimcher LH: Distinct effects of T-bet in TH1 lineage commitment and IFN- γ production in CD4 and CD8 T cells. *Science* 2002;295:338–342.
- Raby BA, Hwang ES, Van Steen K, Tantisira K, Peng S, Litonjua A, Lazarus R, Giallourakis C, Rioux JD, Sparrow D, Silverman EK, Glimcher LH, Weiss ST: T-bet polymorphisms are associated with asthma and airway hyperresponsiveness. *Am J Respir Crit Care Med* 2006;173:64–70.
- Liu F, Zhang J, Liu Y, Zhang N, Holtappels G, Lin P, Liu S, Bachert C: Inflammatory profiles in nasal mucosa of patients with persistent vs. intermittent allergic rhinitis. *Allergy* 2010;65:1149–1157.
- Iwamoto I, Nakajima H, Endo H, Yoshida S: Interferon- γ regulates antigen-induced eosinophil recruitment into the mouse airways by inhibiting the infiltration of CD4+ T cells. *J Exp Med* 1993;177:573–576.
- Coyle AJ, Tsuyuki S, Bertrand C, Huang S, Aguet M, Alkan SS, Anderson GP: Mice lacking the IFN- γ receptor have impaired ability to resolve a lung eosinophilic inflammatory response associated with a prolonged capacity of T cells to exhibit a Th2 cytokine profile. *J Immunol* 1996;156:2680–2685.
- Maneechotesuwan K, Xin Y, Ito K, Jazrawi E, Lee KY, Usmani OS, Barnes PJ, Adcock IM: Regulation of Th2 cytokine genes by p38 MAPK-mediated phosphorylation of GATA-3. *J Immunol* 2007;178:2491–2498.
- Nakamura Y, Christodoulopoulos P, Cameron L, Wright E, Lavigne F, Toda M, Muro S, Ray A, Eidelman DH, Minshall E, Hamid Q: Upregulation of the transcription factor GATA-3 in upper airway mucosa after in vivo and in vitro allergen challenge. *J Allergy Clin Immunol* 2000;105:1146–1152.