The levels of CD4+CD25+ regulatory T cells in patients with allergic rhinitis

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Key words
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The levels of CD4+CD25+ regulatory T cells in patients with allergic rhinitis

Background: The involvement of CD4+CD25+ regulatory T cells (CD4+CD25+ TRegs) in allergic diseases was reported previously. However, it remains unclear whether CD4+CD25+ TRegs are involved in allergic rhinitis (AR).

Methods: Fresh whole blood from 20 patients with AR and 16 healthy donors was used to investigate the frequency of CD4+CD25+ and CD4+CD25hi Treg cells using flow cytometry. In addition, serum total IgE (IU/mL) levels were determined using enzyme-linked immunosorbent assays.

Results: Patients with AR had fewer CD4+CD25+ Treg cells (2.80 ± 1.36% vs. 3.94 ± 0.97%, P < 0.01) and CD4+CD25hi TRegs (1.53 ± 0.62% vs. 2.00 ± 0.52%, P < 0.05) than control subjects. The number of CD4+CD25+ and CD4+CD25hi TRegs was correlated negatively with total immunoglobulin E levels (r = −0.79, P < 0.01 and r = −0.61, P < 0.01, respectively).

Conclusion: Deficient regulatory T cells might play a role in the development of AR.

Introduction

Allergic rhinitis (AR) is an example of a persistent inflammatory disease of the nasal mucosa. It is caused by the secretion of interleukin (IL)-4, IL-5, and IL-13 by CD4+ Th2 effector cells in response to harmless environmental antigens [1]. The T cells that infiltrate the nasal mucosa are predominantly Th2 in nature, and they release cytokines that promote immunoglobulin E (IgE) production by plasma cells. In turn, IgE production triggers the release of mediators, such as histamine and leukotrienes, which are responsible for arteriolar dilation, increased vascular permeability, itching, rhinorrhea, mucous secretion, and smooth muscle contraction [2].

Recently, regulatory T cells (TRegs) were identified as being essential for immune tolerance. In addition, effector Th lymphocytes and TRegs play important roles in AR [3].

Approximately 15% of human peripheral blood CD4 T cells can express CD25, which is the IL-2 growth factor receptor α-chain. However, the CD25hi cells represent only 1 – 2% of the total CD4+ T cell population, while the CD25low cells can represent up to 16% of CD4+ T cells [4]. Thus, CD4+CD25hi T cells can mainly represent CD4+CD25+ Tregs. CD4+CD25+ TRegs account for 5 – 10% of CD4+ T cells in healthy humans and play a critical role in preventing organ-specific autoimmunity and allograft rejection as well as maintaining self-tolerance by preventing the activation and proliferation of autoreactive T cells that have escaped thymic deletion [5, 6]. The transition from the early activation stage to the differentiated Th2 state might be blocked in patients by CD4+CD25+ TRegs specifically, which limits airway allergic inflammation and prevents the inappropriate Th2 responses to environmental allergens [7, 8]. Several independent lines of evidence suggest that the number or function of TRegs is impaired or altered in patients with allergies compared with healthy individuals [9, 10].

Some studies suggest that the number and function of CD4+CD25+ TRegs is not impaired in patients with allergies, whereas other reports indicate that a decreased number of CD4+CD25+ Tregs might be related to allergic disease [11, 12, 13, 14]. However, the role of TRegs in the pathogenesis of allergic disease was not defined until recently, and data concerning the role of TRegs in the pathogenesis of AR are rare.
The aim of the current study was to investigate the role of TRegs in the pathogenesis of AR by exploring the CD4+CD25+ Treg population during AR, and to determine whether the number of TRegs was associated with disease severity in patients with AR.

Materials and Methods

Study participants

We selected 20 patients with persistent AR and 16 age-matched healthy control subjects without allergies. Table 1 shows the subjects’ clinical characteristics. AR was diagnosed if an IgE-mediated response, such as nasal itching, sneezing, watery rhinorrhea and/or nasal stuffiness, was induced after allergen sensitization. Patients in the AR group had symptom scores of at least 6 as assessed by the Total 5 Symptom Score (T5SS, including rhinorrhea, sneezing, nasal congestion, and nasal and ocular pruritus) [15]. Serum total IgE (IU/mL) levels were determined by an enzyme-linked immunosorbent assay method. Subjects in the non-allergic group had no history of allergies, a negative skin-prick test using a panel of common aeroallergens (mixed grass pollen, tree pollen, weeds, house-dust mites, cat hair, dog hair, and mold), and were negative for IgE. Patients with other complications, such as asthma or sinusitis, were also excluded. The study was approved by the Institutional Review Board at the Tongji Hospital, Tongji University, Shanghai City, China.

Antibodies

The following monoclonal antibodies against human cell-surface molecules were purchased from Becton-Dickinson Immunocytometry Systems, San Jose, CA, USA: fluorescein isothiocyanate-conjugated mouse anti-human CD4 (CD4-FITC), phycoerythrin-conjugated mouse anti-human CD25 (CD25-PE), FITC-conjugated mouse immunoglobulin G1 isotype control (IgG1-FITC), and PE-conjugated mouse immunoglobulin G1 isotype control (IgG1-PE).

Flow cytometric analysis

All antibodies were used at concentrations that were determined to be optimal for staining in antibody titrations. Briefly, a sample of whole blood was incubated with 5 μL CD4-FITC and 5 μL CD25-PE or isotype control (IgG1-FITC and IgG1-PE) in the dark for 30 min at room temperature. They were then washed with phosphate-buffered saline twice, and fixed with 1% paraformaldehyde at room temperature. Then, two-color immunofluorescence analysis was performed using MPL FC 500 (Beckman Coulter, Brea, CA, USA). Data analysis was performed using CXP software. An appropriate gate was drawn around the lymphocyte population, as defined by forward scatter and side scatter characteristics. The gated cells were then analyzed for CD4 and CD25 expression, and CD25+ cells were distinguished from those with a distinct (> 7-fold) brighter fluorescence signal as CD25hi.

Statistical analysis

Standard two-tailed t-tests or nonparametric tests for two independent samples were used for the statistical analyses. Data are presented as the means. Spearman correlations were used to analyze relationships between variants. p values of < 0.05 were considered to be significant. All analyses were performed with statistical software SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

Results

Characteristics and IgE values of study participants

The clinical characteristics of the study cohort are shown in Table 1. The subjects in
each group were matched for age and gender. Patients with persistent AR had significantly higher serum total IgE values than did healthy individuals (312.8 ± 165.8 IU/mL vs. 70.1 ± 14.6 IU/mL, p < 0.01).

As shown in Figures 1 and 2, patients with AR had significantly decreased numbers of CD4+CD25+ T lymphocytes compared with control subjects (2.80 ± 1.36% vs. 3.94 ± 0.97%, p < 0.01) (Figure 2a). The numbers of CD4+CD25hi TRegs were also decreased significantly in AR patients compared with control subjects (1.53 ± 0.62% vs. 2.00 ± 0.52%, p < 0.05) (Figure 2b).

Serum total IgE levels were measured in all patients to investigate the association between AR and the number of TRegs. Serum total IgE levels were increased significantly in AR patients compared with control subjects (312.8 ± 165.8 vs. 70.1 ± 14.6, p < 0.01) (Figure 2c). The number of TRegs and CD4+CD25hi TRegs were negatively correlated with total serum IgE levels (r = −0.79, p < 0.01; r = −0.61, p < 0.01, respectively) (Figure 3a and b).

Discussion

The activation of Th2 cells is thought to play an important role in allergic reactions by mediating IgE synthesis and eosinophilic inflammation via IL-4, IL-5, and IL-13 [16,
Allergen-induced T cell proliferation can be detected in primary cultures of peripheral blood mononuclear cells (PBMCs) from individuals both with and without allergies. However, PBMCs from allergy patients produce increased levels of Th2-type cytokines including IL-4, IL-5, and IL-13 [18, 19]. However, it is unclear why subjects with allergies elicit a Th2-type T-cell response whereas other individuals do not. One hypothesis is that Treg-based mechanisms prevent the IgE responses to allergens in normal individuals, including the suppression of Th2 responses by regulatory T lymphocytes.

TRegs control the development of autoimmune disease and transplant rejection, and also play a major role in regulating allergic reactions including AR and asthma. A distinct type of TRegs was identified in mammalian and humans, CD4+ T cells, which are characterized by the expression of the surface marker CD25 and account for 5 – 10% of the normal CD4+ T cell population [5, 20]. Autoreactive T cells are also present in the peripheral blood of healthy individuals without any evidence of autoimmune disease, suggesting that some mechanisms regulate the autoreactive T cells to prevent autoimmune disease. CD4+CD25+ Treg cells play a critical role in the induction and maintenance of peripheral self-tolerance. Specifically, they prevent the activation and proliferation of the potentially autoreactive T cells that have escaped thymic deletion [21, 22, 23, 24]. CD4+CD25+ Treg cells play a key role in modulating Th2-mediated pulmonary inflammation by suppressing the

Figure 2. Comparison of the numbers of CD4+CD25+ (a) and CD4+CD25hi (b) Tregs and serum total IgE (c) levels between control subjects and AR patients. The number of CD4+CD25+ and CD4+CD25hi Tregs was decreased significantly, whereas serum total IgE levels were increased in AR patients.

Figure 3. Correlation between the numbers of CD4+CD25+ Tregs (a) and CD4+CD25hi Tregs (b) and serum total IgE levels. The numbers of CD4+CD25+ Treg cells and CD4+CD25hi Treg cells were both negatively correlated with total IgE levels (r = −0.66, p < 0.01 and r = −0.61, p < 0.01, respectively).
development of the Th2 phenotype, which effectively promotes airway eosinophilia in vivo [8]. The specific mechanisms by which CD4+CD25+ Treg cells function and their specific characteristics are still being investigated.

The current study suggested that a lower frequency of CD4+CD25+ Treg cells is associated with the pathogenesis of AR. In addition, the number of T_{RegS} was correlated with the inflammation status, as determined by measuring serum total IgE levels, in subjects with AR, but not in healthy subjects. These observations are consistent with previous observations in patients with allergic asthma. Xue and Shi investigated the number and functional role of CD4+ CD25+ T_{RegS} in the PBMCs of patients with allergic asthma [25, 26]. They used flow cytometry to reveal a decrease in the CD4+CD25+ Treg ratio of CD4+ T cells and function in the PBMCs of patients with asthma. The authors concluded that CD4+CD25+ T_{RegS} are critical for maintaining self-tolerance, and are associated with moderate to severe asthma. Consistent with this, Ling et al. [27] reported that the number of allergen-specific Th2 cells in allergic patients was enhanced significantly by the depletion of CD4+CD25+ Treg cells. Therefore, CD4+CD25+ T_{RegS} play a crucial role in preventing inappropriate Th2 responses in allergic diseases.

However, other studies contradict the above findings. For example, Han et al. showed that the number and function of CD4+CD25+ T_{RegS} from patients with allergies was not impaired [28]. In addition, Hoffmann et al. studied a cohort of asthmatic patients and showed that the number of CD4+ CD25+ T_{RegS} was similar in allergic patients and control subjects [29]. Additional studies reported that the numbers of T_{RegS} increased during the exacerbation of asthma [30, 31]. These findings are not consistent with the current results. These differences might be related to the varying ages and disease statuses of the patients in the different studies. In addition, several reports have suggested that the suppressive activity of CD4+CD25+ T_{RegS} is affected by various factors including the type of allergen, allergen exposure, and individual allergic status [31]. Some studies also suggested that high levels of CD25 and CD25^{hi} T_{RegS} in patients might represent the generation of induced or adaptive T_{RegS} during the exacerbation of allergic inflammation as a consequence of the immune response [32].

In conclusion, AR patients with airway allergies had decreased numbers of CD4+CD25+ and CD4+CD25^{hi} T_{RegS}, and the number of Treg cells was correlated negatively with total serum IgE levels. This suggests that lower numbers of regulatory T cells might be associated with inflammation of AR.

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Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this manuscript.

Literatur

[1] Greiner AN, Hellings PW, Rotiroti G, Scadding GK. Allergic rhinitis. Lancet. 2011; 378: 2112-2122. CrossRef PubMed

[2] Akdis CA, Akdis M. Mechanisms and treatment of allergic disease in the big picture of regulatory T cells. J Allergy Clin Immunol. 2009; 123: 735-746., quiz 747-748. CrossRef PubMed

[3] Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune
CD4+CD25+ TReg in allergic rhinitis

[4] Baecher-Allan C, Wolf E, Haftter DA. Functional analysis of highly defined, FACS-isolated populations of human regulatory CD4+CD25+ T cells. Clin Immunol. 2005; 115: 10-18. CrossRef PubMed

[5] Suri-Payer E, Amar AZ, Thornton AM, Shevach EM. CD4+CD25+ T cells inhibit both the inductive and effector function of autoreactive T cells and represent a unique lineage of immunoregulatory cells. J Immunol. 1998; 160: 1212-1218. PubMed

[6] Lewkowich IP, Herman NS, Schleifer KW, Dance MP, Chen BL, Diengner KM, Squires AA, Shah JS, Köhl J, Belkaid Y, Wills-Karp M. CD4+CD25+ T cells protect against experimentally induced asthma and alter pulmonary dendritic cell phenotype and function. J Exp Med. 2005; 202: 1549-1561. CrossRef PubMed

[7] Kearley J, Barker JE, Robinson DS, Lloyd CM. Resolution of airway inflammation and hyperreactivity after in-vivo transfer of CD4+CD25+ regulatory T cells is interleukin 10 dependent. J Exp Med. 2005; 202: 1539-1547. CrossRef PubMed

[8] Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Crameri R, Thunberg EM, AK, Valenta R, Fiebig H, Kegel C, Dirsch R, Schmidt-Weber CB, Blaser K, Akdis CA. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergenspecific T regulatory 1 and T helper 2 cells. J Exp Med. 2004; 199: 1567-1575. CrossRef PubMed

[9] Ling EM, Smith T, Nguyen XD, Pridgeon C, Dallman M, Arbery J, Carr VA, Robinson DS. Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. Lancet. 2004; 363: 608-615. CrossRef PubMed

[10] Karagiannidis C, Akdis M, Holopainen P, Woolley NJ, Hense G, Rückert B, Mantel PY, Menz G, Akdis CA, Blaser K, Schmidt-Weber CB. Glucocorticoids upregulate FOXP3 expression and regulatory T cells in asthma. J Allergy Clin Immunol. 2004; 114: 1425-1433. CrossRef PubMed

[11] Shi HZ, Li S, Xie ZF, Qin XJ, Qin X, Zhong XN. Regulatory CD4+CD25+ T lymphocytes in peripheral blood from patients with atopic asthma. Clin Immunol. 2004; 113: 172-178. CrossRef PubMed

[12] Bellinghausen I, Klostermann B, Knap J, Saloga J. Human CD4+CD25+ T cells derived from the majority of atopic donors are able to suppress TH1 and TH2 cytokine production. J Allergy Clin Immunol. 2003; 111: 862-868. CrossRef PubMed

[13] Grindebach H, Wing K, Andersson AC, Suri-Payer E, Rak S, Rudin A. Defective suppression of Th2 cytokines by CD4CD25 regulatory T cells in birch allergies during birch pollen season. Clin Exp Allergy. 2004; 34: 1364-1372. CrossRef PubMed

[14] BiasinfilI, Villena E, Rogakon a, Pellegrini S, Bacic M, Compolati E, Braido F, Le Grazie C, Canonica GW, Passalacqua G. Effects of mometasone furoate on the quality of life: a randomized placebo-controlled trial in persistent allergic rhinitis and intermittent asthma using the Rhinasthma questionnaire. Clin Exp Allergy. 2011; 41: 417-423. CrossRef PubMed

[15] Kay AB. Allergy and allergic diseases. First of two parts. N Engl J Med. 2001; 344: 30-37. CrossRef PubMed

[16] El Biaze M, Boniface S, Koscher V, Manessier E, Daguy P, Milhe F, Ramadour M, Versefoel D, Maguen A. T cell activation, from atopy to asthma: more a paradox than a paradigm. Allergy. 2003; 58: 844-853. CrossRef PubMed

[17] Upham JW, Holt BJ, Baron-Hay MJ, Yabuhara A, Hales BJ, Thomas WR, Loh RK, O’Keefe PT, Palmer L, Le Souef PN, et al. Inhalant allergen-specific T-cell reactivity is detectable in close to 100% of atopic and normal individuals: covert responses are unmasked by serum-free medium. Clin Exp Allergy. 1995; 25: 634-642. CrossRef PubMed

[18] Tili S, Dickason R, Huston D, Humbert M, Robinson D, Larché M, Durham S, Kay AB, Corrigan C. IL-5 secretion by allergen-stimulated CD4+ T cells in primary culture: relationship to expression of allergic disease. J Allergy Clin Immunol. 1997; 99: 563-569. CrossRef PubMed

[19] Shi HZ, Qin XJ. CD4CD25 regulatory T lymphocytes in allergy and asthma. Allergy. 2005; 60: 986-995. CrossRef PubMed

[20] Leavings MK, Sangregorio R, Sartirana C, Moschini AL, Battaglia M, Orban PC, Roncarolo MG. Human CD25+CD4+ T suppressor cell clones produce transforming growth factor beta, but not interleukin 10, and are distinct from type 1 T regulatory cells. J Exp Med. 2002; 196: 1335-1346. CrossRef PubMed

[21] Danke NA, Koelle DM, Yee C, Beheray S, Kwok WW. Autoreactive T cells in healthy individuals. J Immunol. 2004; 172: 5967-5972. CrossRef PubMed

[22] Itoh M, Takahashi T, Sakaguchi N, Kuniyasu Y, Shimizu J, Otsuka F, Sakaguchi S. Thymus and autoimmunity. production of CD25+CD4+ naturally anergic and suppressive T cells as a key function of the thymus in maintaining immunological self-tolerance. J Immunol. 1999; 162: 5317-5326. PubMed

[23] Kuniyasu Y, Takahashi T, Itoh M, Shimizu J, Toda G, Sakaguchi S. Naturally anergic and suppressive CD25+CD4+ T cells as a functionally and phenotypically distinct immunoregulatory T cell subpopulation. Int Immunol. 2000; 12: 1145-1155. CrossRef PubMed

[24] Sakaguchi S. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. Nat Immunol. 2005; 6: 345-352. CrossRef PubMed

[25] Xue K, Zhou Y, Xiong S, Xiong W, Tang T. Analysis of CD4+CD25+ regulatory T cells and Foxp3 mRNA in the peripheral blood of patients with asthma. J Huazhong Univ Sci Technol Med Sci. 2007; 27: 31-33. CrossRef PubMed

[26] Shi YH, Shi GC, Wan HW, Jiang LH, Ai XY, Zhu HX, Tang W, Ma JY, Jin XY, Zhang BY. Coexistence of Th1/Th2 and Th17/Treg imbalances in patients with allergic asthma. Chin Med J (Engl). 2011; 124: 1951-1956. PubMed

[27] Ling EM, Smith T, Nguyen XD, Pridgeon C, Dallman M, Arbery J, Carr VA, Robinson DS. Relation of CD4+CD25+ regulatory T-cell suppres-
ation of allergen-driven T-cell activation to atopic status and expression of allergic disease. Lancet. 2004; 363: 608-615. CrossRef PubMed

[28] Han D, Wang C, Lou W, Gu Y, Wang Y, Zhang L. Allergen-specific IL-10-secreting type I T regulatory cells, but not CD4(+)CD25(+)Foxp3(+) T cells, are decreased in peripheral blood of patients with persistent allergic rhinitis. Clin Immunol. 2010; 136: 292-301. CrossRef PubMed

[29] Hoffmann HJ, Malling TM, Topcu A, Ryder LP, Nielsen KR, Varming K, Dahl R, Omland O, Sigsgaard T. CD4dimCD25bright Treg cell frequencies above a standardized gating threshold are similar in asthmatics and controls. Cytometry A. 2007; 71: 371-378. CrossRef PubMed

[30] Cheng X, Lou W, Wang C, Zhang W, Han D, Zhang L. FOXP3-marked IL-17a-producing regulatory T cells are increased in patients with allergic rhinitis. Acta Otolaryngol. 2012; 132: 1311-1317. CrossRef PubMed

[31] Anderson AE, Mackerness KJ, Aizen M, Carr VA, Nguyen D, Du Pre F, Durham SR, Robinson DS. Seasonal changes in suppressive capacity of CD4+ CD25+ T cells from expansion of effector T cells among the CD25+ population. Clin Exp Allergy. 2009; 39: 1693-1699. CrossRef PubMed

[32] Lee JH, Yu HH, Wang LC, Yang YH, Lin YT, Chi-ang BL. The levels of CD4+CD25+ regulatory T cells in paediatric patients with allergic rinitis and bronchial asthma. Clin Exp Immunol. 2007; 148: 53-63. CrossRef PubMed