

Serum Transforming Growth Factor- β Levels Depend on Allergen Exposure in Allergic Rhinitis

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Key Words

Transforming growth factor- β · Allergic rhinitis · Pollen season · Sublingual immunotherapy

Abstract

Background: Allergic rhinitis (AR) is characterized by inflammation sustained by dysregulated immune response. T-regulatory cells are involved in AR pathogenesis, mainly producing IL-10 and transforming growth factor (TGF)- β . Indeed, there is a functional and allergen-specific defect of T-regulatory cells in AR. However, there are no data about the influence of allergen exposure on TGF- β serum levels. Therefore, the aim of this preliminary study was to evaluate TGF- β serum levels in patients with seasonal AR. Patients were evaluated either outside the pollen season and after 1 preseasonal sublingual immunotherapy (SLIT) course (38 subjects) or during the pollen season (57 subjects). **Methods:** All patients were allergic to *Parietaria* and/or grasses alone. TGF- β was measured by a commercially available kit. Symptoms, drug use and eosinophils were evaluated. Serum allergen-specific IgG and IgA levels were also measured by the ELISA method. **Results:** TGF- β serum levels were significantly lower in patients evaluated outside the pollen season in comparison with the other 2 situations. SLIT induced the significantly highest TGF- β serum levels. There was a significant negative relationship between TGF- β and eosinophils

in patients after SLIT. IgG and IgA levels were higher in SLIT-treated patients. **Conclusion:** This preliminary study provides evidence that TGF- β serum levels are significantly dependent on allergen exposure.

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Introduction

Allergic rhinitis (AR) is characterized by an inflammatory reaction, sustained by dysregulated immune response, classically defined as Th2 polarization [1]. Indeed, peripheral blood mononuclear cells of AR patients display a predominant IL-4 production by Th2 cells as compared with interferon- γ expression by Th1 cells. It confirms the functional dichotomy between Th1 and Th2 cells [2]. This bivalent concept has been recently modified by the discovery of 2 T-lymphocyte subsets, such as T-regulatory (T_{reg}) and Th17 cells [for a review, see ref. 3–6].

T_{reg} cells release IL-10 and transforming growth factor (TGF)- β , fundamental cytokines for switching immunoglobulin production towards G and A classes, respectively. Allergic patients show an allergen-specific functional defect of T_{reg} cells that promotes Th2 polarization and IgE synthesis [7].

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1018–2438/10/1521–0066\$26.00/0

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The inhibitory cytokine TGF- β is the main cytokine responsible for inducing the switching of B lymphocytes in favor of IgA synthesis and represents a key factor in tolerogenic regulatory responses [8]. The induction of TGF- β expression has been observed in peripheral mononuclear cells following allergen-specific subcutaneous immunotherapy (SCIT) [7] and is at least partly related to the development of T_{reg} cells. Very recently, it has been reported that SCIT for pollen allergy induces an IgA response correlating with increased nasal TGF- β expression [9]. Moreover, TGF- β has multiple regulatory effects also on T-cell proliferation, antigen presentation and expression of costimulatory molecules [10]. All of them may be implicated in the response to successful immunotherapy.

However, no study investigated TGF- β serum levels in patients with seasonal AR (SAR) considering the effect of pollen exposure, such as evaluating patients during and outside the pollen season. The relevance of evaluating serum levels may be based on the concept that assessment of a cytokine at the serum level would certainly simplify clinical evaluation. Therefore, the present study aimed at investigating serum TGF- β levels in SAR patients during or outside pollen exposure and after 1 sublingual immunotherapy (SLIT) course.

Methods

Patients

The study included 2 groups of SAR patients: (1) 38 patients (21 males, 17 females, mean age 37.6 years) evaluated outside the pollen season and at the end of 1 preseasonal SLIT course; (2) 57 patients (21 males and 36 females, mean age 35.4 years) evaluated during the pollen season.

AR was diagnosed according to validated criteria proposed by the ARIA guidelines [11].

All subjects were healthy, except for AR, without any active disorder, including rhinosinusitis; asthma was excluded in all patients. All of them were allergic only to *Parietaria judaica* and/or grasses as confirmed by allergen-specific conjunctival challenge. Patients with other sensitizations and a positive history of other allergies were excluded. Allergic patients were treated only with antihistamines on demand during the pollen season.

Study Design

The clinical part of the study was conducted in Genoa. The *Parietaria* pollen season generally starts in March and lasts until October, the grass season is from April to September.

A skin prick test and blood sampling for assessment of peripheral eosinophils were performed in all patients. The skin prick test was performed according to the guidelines of the European Academy of Allergy and Clinical Immunology [12]. The allergen panel consisted of the following: house dust mites (*Dermatophagoides farinae* and *D. pteronyssinus*), cats, dogs, grasses mix, Compositae mix, *P. judaica*, birch, hazel trees, olive trees, *Alternaria tenuis*,

Cladosporium, and *Aspergillus* mix. The concentration of allergen extracts was 100 immune reactivity/ml (Stallergenes, Milan, Italy). A histamine solution in distilled water (10 mg/ml) was used as positive control, and the glycerol-buffer diluent of the allergen preparations was used as negative control. Each patient was skin tested on the volar surface of the forearm using 1-mm prick lancets (Stallergenes). The skin reaction was recorded after 15 min by evaluating the skin response rate to the inoculation of each allergen extract in comparison with the wheal given by the positive and the negative control. A wheal diameter of 3 mm was considered a positive reaction.

Patients undergoing SLIT received the pollen allergen extract (Anallergo, Florence, Italy) of *P. judaica* (the accumulate dose was 12 μ g) for at least 3 months before the pollen season, starting in early December (the so-called 'preseasonal immunotherapy'). All of them responded positively to SLIT.

The study was approved by the local ethics committee and performed with written informed consent of all participants.

Allergen-specific conjunctival challenge was performed, according to validated criteria, uniquely to confirm the diagnosis [13]; briefly, a drop containing the allergen extract of *Parietaria* or grasses (Stallergenes) was instilled in the conjunctival fornix. Redness of the conjunctiva was the primary parameter to consider the test positive [13].

Clinical Assessment

Clinical severity was also evaluated, considering nasal symptom perception and drug consumption at the end of the pollen season as previously validated [14]. The patient questionnaire included a validated visual analogue scale for quantifying the perception of nasal symptom intensity [15]. Patients had to have a visual analogue scale score for nasal symptoms ≥ 5 .

During the pollen season, use of levocetirizine drops (10 or 20, corresponding to a 2.5- or 5-mg concentration) on demand as rescue medication was permitted. However, medication was withdrawn at least 1 week before drawing blood samples. The medication scoring was as follows: not at all (0), occasionally/seldom (1), occasionally/often (2), almost daily (3), continuously with the lowest dose (4), continuously with the maximal dose (5).

Specific IgG Antibodies

Determination of allergen-specific total IgG antibodies was performed on all sera against the causal allergens: grasses, and *Parietaria judaica* in a single laboratory session (Phadia AB, Uppsala, Sweden) using an IgG ImmunoCAP FEIA assay described previously [16]. Briefly, the method consists of the following steps: the antigen of interest, covalently coupled to ImmunoCAP, reacted with the specific IgG antibodies in the diluted patient serum specimen. After washing away nonspecific IgG, enzyme-labeled antibodies against IgG were added to form a complex. After incubation, unbound enzyme-anti-IgG was washed away and the bound complex was then incubated with a developing agent. After stopping the reaction, the fluorescence of the eluate was measured. The higher the response value, the more specific IgG antibodies were present in the specimen. To evaluate the test results, the response for the patient samples was transformed to concentrations with the use of a calibration curve (specific IgG 1:100 diluted sample 2–200 mg_A/l, where A represents antigen-specific antibodies).

Before assaying, all sera had to be diluted 1:100 with specific IgG sample diluent. The dilution step can be performed manually or automatically using ImmunoCAP 100.

ImmunoCAP specific IgG calibrator strip was used for determination of total IgG, and values were expressed as mg/l. The detection limit for IgG was <0.02 mg_A/l.

Specific IgA Antibodies

Specific IgA ImmunoCAP (Phadia) was experimentally used for research purpose only (measured for those pollens, using the fluor enzyme immunoassay FEIA). The calibrators are traceable to the International Reference Preparation (IRP) 67/86 for Human Serum Immunoglobulins A, G and M from the World Health Organization (WHO). The measuring range for a diluted sample is 1.0–100 mg_A/l. The detection limit is <0.01 mg_A/l.

TGF-β Measurement

This part of the study was conducted in Pavia. The human TGF-β₁ immunoassay (Quantikine TGF-β Immunoassay, R&D Systems Inc., Milan, Italy) employs the quantitative sandwich enzyme immunoassay technique and was performed according to the manufacturer's instructions (more details are reported on http://www.rndsystems.com/molecule_detail.aspx?m=2151) and expressed as ng/ml.

Statistical Analysis

Descriptive statistics were first performed and quantitative parameters were reported as the mean, median (MD) and standard deviation (SD), as well as the 25th and 75th percentile. The nonparametric Wilcoxon test was used to compare samples. Pearson's test was used for evaluating the relationships. All tests were two sided and a p value <0.05 was considered statistically significant. The package S-Plus (MathSoft Corp.) was used for all analyses.

Results

The 2 groups were well matched concerning age, gender, clinical severity and sensitization.

The first group consisted of 28 patients sensitized to both *Parietaria* and grasses, 5 to *Parietaria* and 5 to grasses. The second group consisted of 42 patients sensitized to both allergens, 8 to *Parietaria* and 7 to grasses.

The patients evaluated outside the pollen season had a mean TGF-β value of 19.24 ng/ml (MD 4.6, SD 29.16; 25th percentile 3.19, 75th percentile 20.66). The patients evaluated at the end of 1 preseasonal SLIT course had a mean TGF-β value of 46.66 ng/ml (MD 52.48, SD 35.54; 25th 5.34, 75th 70.7).

The patients evaluated during the pollen season had a mean TGF-β value of 24.2 ng/ml (MD 22.9, SD 11.83; 25th 17.35, 75th 27.92). Intergroup analysis shows that there was a significant difference between the outside and the inside group (p = 0.0001), between outside and SLIT-

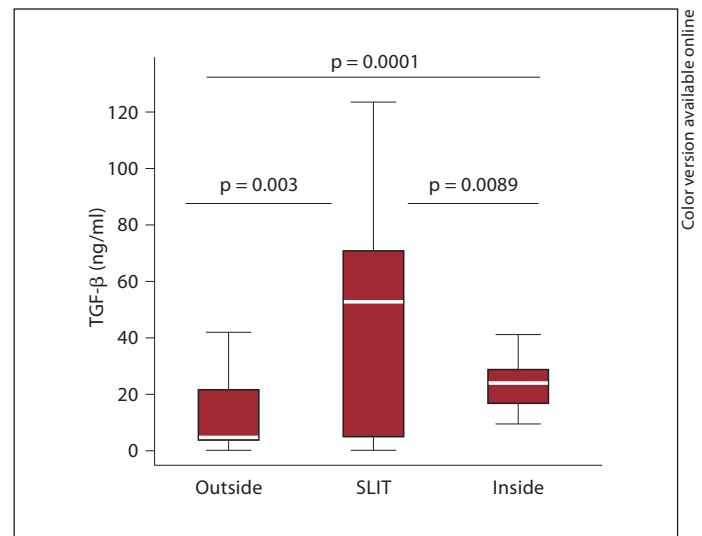


Fig. 1. TGF-β distribution in patients evaluated outside the pollen season, during the pollen season and at the end of a preseasonal SLIT course. Values are represented as the median (horizontal line) and the 25th and 75th percentile (box); whiskers extend from the highest to the lowest values and p values between the groups.

treated patients (p = 0.0004) and between the inside and the SLIT group (p = 0.0089), as reported in figure 1.

There were no relationships among TGF-β, symptoms and drug use (data not shown). However, there was a significant negative relationship between TGF-β and eosinophils in patients treated with SLIT, as reported in figure 2.

The patients evaluated outside the pollen season had a mean IgG value of 4.86 mg/l (MD 4.75, SD 3.22; 25th 3.1, 75th 5.68) and a mean IgA value of 1.24 mg/l (MD 0.95, SD 0.5; 25th 0.9, 75th 1.52). The patients evaluated at the end of 1 preseasonal SLIT course had a mean IgG value of 11.27 mg/l (MD 7.98, SD 8.84; 25th 4.76, 75th 17.4) and a mean IgA value 3.67 mg/l (MD 2.57, SD 7.71; 25th 1.66, 75th 3.54).

The patients evaluated during the pollen season had a mean IgG value of 6.06 mg/l (MD 5.49, SD 3.60; 25th 4.04, 75th 7.92) and a mean IgA value of 2.03 mg/l (MD 1.61, SD 1.19; 25th 1.12, 75th 2.74). The intergroup analysis shows that there was a significant difference in IgG levels between the outside and SLIT group (p = 0.0001) and between the inside and SLIT group (p = 0.0078), as reported in figure 3a. Moreover, there was a significant difference in IgA levels between the outside and the inside group (p = 0.0003), between outside and SLIT-treated

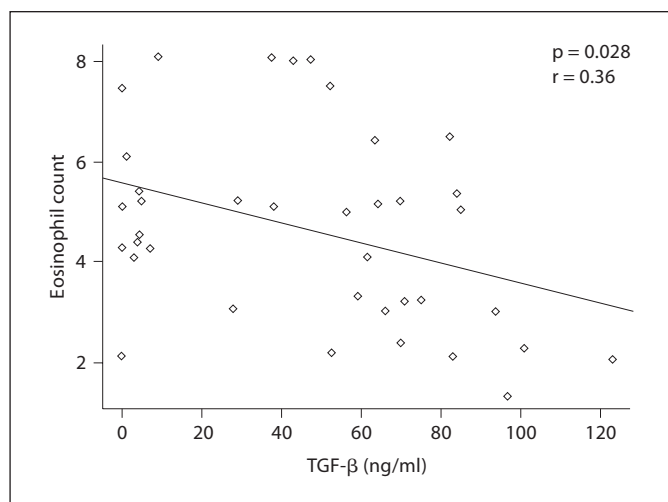


Fig. 2. Correlation, expressed with Pearson's correlation coefficient (r) and p value, between TGF- β and eosinophil counts in SLIT-treated patients.

patients ($p < 0.0001$), and between the inside and the SLIT group ($p = 0.0093$), as reported in figure 3b.

There was a significant relationship between TGF- β and IgG ($p = 0.06$, $r = 0.248$) in patients evaluated during the pollen season. Moreover, there was a significant relationship between TGF- β and IgA in patients evaluated outside the pollen season ($p = 0.0118$, $r = 0.414$) and in patients evaluated during the pollen season ($p = 0.049$, $r = 0.268$; data not shown).

Discussion

AR is characterized by an inflammatory response consequent to Th2 polarization. Th2-dependent cytokines promote, maintain and amplify allergic inflammation. Recruited inflammatory cells include mast cells, eosinophils and neutrophils. More recently, new data support a role for T_{reg} cells in the mechanism of successful allergen immunotherapy. Indeed, allergic patients have an allergen-specific functional defect of T_{reg} cells [17]. Therefore, production of T_{reg} -dependent cytokines such as TGF- β and IL-10 is thought to be relevant for the function of immune tolerance. SLIT has been associated with an upregulation of IL-10 [17, 18]. IL-10 has a number of downregulatory effects on key cells involved in allergic inflammation and induces IgG4 production. TGF- β exerts multiple regulatory effects on T-cell proliferation, antigen presentation and expression of costimulatory molecules. Furthermore,

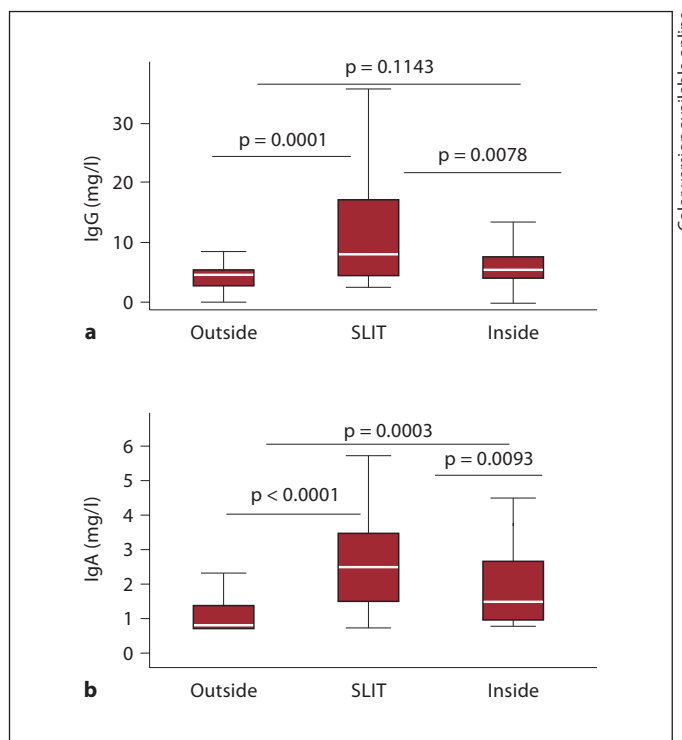


Fig. 3. IgG (a) and IgA (b) distribution in patients evaluated outside the pollen season, at the end of a preseasonal SLIT course and during the pollen season. Values are represented as the median (horizontal line) and the 25th and 75th percentile (box); whiskers extend from the highest to the lowest values and p values between the groups.

TGF- β drives the production of IgA. Increased production of TGF- β has been demonstrated following SCIT [7, 9]; however, there are no data concerning SLIT effects on this cytokine. Very recently, it has been reported that there is a significant positive relationship between allergen-specific serum IgA and TGF- β serum levels in allergic patients [19]. However, there are no data concerning the possible effect of allergen exposure on TGF- β serum levels. Therefore, the present study aimed at investigating whether TGF- β could depend on the level of allergen exposure.

The findings present evidence that serum TGF- β levels may be dependent on the allergen exposure quantity. Indeed, SAR patients evaluated outside the pollen season showed the lowest values. SAR patients evaluated during the pollen season, such as with symptoms and inflammation, showed significantly higher levels. In addition, SAR patients after SLIT treatment had the highest levels. In fact, SLIT is based on the assumption of administering very elevated doses of the causal allergen extract. Indeed,

the SLIT doses are a lot higher than the natural allergen exposure. In this regard, this study demonstrates that serum TGF- β levels well depend on the quantity of allergen exposure, which is maximal during SLIT.

On the other hand, this study has to be considered preliminary as there are some limitations. Firstly, the patient groups differ; further studies should evaluate the same patients both outside and during the pollen season. Secondly, the number of patients should be more conspicuous and the study should be conducted in several allergy centers. Thirdly, a placebo-controlled trial should be performed to evaluate the SLIT effect on TGF- β .

However, this study provides an interesting outcome: TGF- β is negatively related with eosinophils after SLIT. This phenomenon adds new information about the mechanism of action of SLIT, which might explain the anti-inflammatory activity exerted by SLIT, so TGF- β might be considered a 'protective' factor. In addition, this point confirms a very recent study demonstrating that TGF- β is positively related with serum IgA which in turn is positively related with low symptom severity [19]. In this regard, our study shows that there is a different pattern of both allergen-specific IgG and IgA in the patient groups.

In particular, patients after SLIT had the highest levels of both immunoglobulin classes, whereas patients before SLIT had lower levels than patients evaluated during the pollen season. These findings confirm the role of allergen exposure also on immunoglobulin production. This outcome is reinforced by the direct relationship between TGF- β and immunoglobulin.

However, further studies should investigate a possible relationship between the increase in TGF- β and the improvement in clinical parameters after SLIT. In this way, the clinical relevance of these preliminary findings could be confirmed.

In conclusion, our study provides the first evidence that serum TGF- β levels are significantly dependent on allergen exposure and confirms previous studies conducted in patients treated with SCIT.

Acknowledgments

The authors wish to thank Cristina Torre (Clinica Pediatrica, Fondazione IRCCS Policlinico S. Matteo) for her outstanding technical support and Vania Giunta (Dipartimento di Informatica e Sistemistica, Università di Pavia) for data analysis.

References

- Ciprandi G, Cirillo I, Klersy C, Vizzaccaro A, Tosca MA, Marseglia GL: The response to decongestion test is associated with sensitizations and allergic inflammation. *Ann Allergy Asthma Immunol* 2006;96:431–436.
- Chatila TA, Li N, Garcia-Lloret M, Kim HJ, Choi YS, et al: T-cell effector pathways in allergic diseases: transcriptional mechanisms and therapeutic targets. *J Allergy Clin Immunol* 2008;121:1311–1322.
- Umetsu DT, DeKruyff RH: The regulation of allergy and asthma. *Immunol Rev* 2006;212:238–255.
- Romagnani S: Regulation of the T cell response. *Clin Exp Allergy* 2006;36:1357–1366.
- Schmidt-Weber CB, Akdis M, Akdis CA: Th17 cells in the big picture of immunology. *J Allergy Clin Immunol* 2007;120:247–254.
- Wang YH, Liu YJ: The IL-17 cytokine family and their role in allergic inflammation. *Curr Opin Immunol* 2008;20:1–6.
- Jutel M, Akdis M, Budak F, et al: IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 2003;33:1205–1214.
- Schmidt-Weber CB, Blaser K: Regulation and role of TGF- β in immune tolerance induction and inflammation. *Curr Opin Immunol* 2004;16:709–716.
- Pilette C, Nouri-Aria KT, Jacobson MR, Wilcock LK, Detry B, Walker SM, et al: Grass pollen immunotherapy induces an allergen-specific IgA2 antibody response associated with mucosal TGF- β expression. *J Immunol* 2007;178:4658–4666.
- James LK, Durham SR: Update on mechanisms of allergen injection immunotherapy. *Clin Exp Allergy* 2008;38:1074–1088.
- Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al: Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA₂LEN and Allergen). *Allergy* 2008;63(suppl 86):8–160.
- Dreborg S, Backman A, Basomba A: Skin tests used in type I allergy testing. Position paper. *Allergy* 1989;44(suppl 10):1–59.
- Ciprandi G, Buscaglia S, Pesce GP, Villaggio B, Bagnasco M: Allergic subjects express intercellular adhesion molecule-1 (ICAM-1 or CD54) on epithelial cells of conjunctiva after allergen challenge. *J Allergy Clin Immunol* 1993;91:783–791.
- Bousquet PJ, Combescure C, Neukirch F, Klossek JM, Mechin H, Daures JP, Bousquet J: Visual analog scales can assess the severity of rhinitis graded according to ARIA guidelines. *Allergy* 2007;62:367–372.
- Ciprandi G, Contini P, Fenoglio D, Sormani MP, Negrini S, Puppo F, Indiveri F: Relationship between soluble HLA-G and HLA-A, -B, -C serum levels and IFN-gamma production after sublingual immunotherapy in patients with allergic rhinitis. *Hum Immunol* 2008;69:510–512.
- Meier P, Muller U: Evaluation of IgG RAST FEIA for the assay of venom-specific IgG antibodies during venom immunotherapy. *Int Arch Allergy Immunol* 1998;117:46–51.
- Ciprandi G, Fenoglio D, Cirillo I, Vizzaccaro A, Ferrero A, Tosca MA, Puppo F: Induction of interleukin 10 by sublingual immunotherapy for house dust mites: a preliminary report. *Ann Allergy Asthma Immunol* 2005;95:38–44.
- Cosmi L, Santarlasci V, Angeli R, Liotta F, Maggi E, Frosali F, et al: Sublingual immunotherapy with *Dermatophagoides* monomeric allergoid down-regulates allergen-specific immunoglobulin E and increases both interferon- γ and interleukin-10 production. *Clin Exp Allergy* 2006;36:261–272.
- Ciprandi G, De Amici M, Tosca MA, Negrini S, Puppo F, Marseglia G: Immunoglobulin production pattern is allergen-specific in polysensitized patients. *Int J Immunopathol Pharmacol*, in press.