Effects of glucan treatment on the Th1/Th2 balance in patients with allergic rhinitis: 過敏性鼻炎 a double-blind placebo-controlled study

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ABSTRACT. ***Background.*** **Allergic rhinitis (AR) is a disease characterized by IgE-mediated, allergic inflammation of the nasal mucosa. T helper (Th) 2 cells play an important role in the development of IgE-mediated diseases such as AR, with local overproduction of Th2 cytokines (IL-4, IL-5 and IL-13) at the site of allergic inflammation. Th1 cytokines (IL-12 and IFN-γ) are known to suppress this Th2 immune response, aiding the treatment of these diseases. β-1,3-1,6-glucan (Glucan) is an immunomodulator stimulating particularly the antitumor response. An efficient antitumor stimulation can be achieved through a Th1-mediated immune response.** ***Objective.*** **The aim of this study was to investigate the effects of Glucan on the immunopathogenic processes in the microenvironment to determine if it reverses the Th2-mediated immune response in AR to Th1-mediated response.** ***Methods.*** **24** ***Olea europea*** **mono-sensitized patients with AR were randomized into Glucan and placebo groups. The Glucan group consisted of 12 patients who received Glucan treatment for 12 weeks, while the placebo group of 12 patients received placebo during the same period. A nasal provocation test (NPT) with** ***Olea europea*** **was performed at the beginning and end of treatment, and nasal lavage followed the positive NPT. IL-4, IL-5, IFN-γ and IL-12 levels and the eosinophil count (%) were measured in nasal lavage fluid (NLF) samples. Simultaneously, peripheral blood eosinophil % values were measured.** ***Results.*** **After treatment, IL-4 and IL-5 levels in NLF from the Glucan group were found to have decreased significantly ($p = 0.027$, $p = 0.04$; respectively), while IL-12 levels were found to have significantly increased ($p = 0.008$). However, IFN-γ levels had not changed. On the other hand, none of the cytokine levels had changed significantly in the placebo group following treatment. Moreover, the percentage of eosinophils in the NLF was found to have decreased significantly after treatment in the Glucan group ($p = 0.01$), while that of the placebo group did not change. Peripheral blood percentage eosinophil levels had not changed significantly in any group.** ***Conclusion.*** **Th2-originated IL-4 and IL-5 levels responsible for the allergic inflammatory response in the microenvironment of patients with AR, are decreased with Glucan while levels of Th1-originated IL-12 are increased. Moreover, eosinophils, which are important effector cells of the inflammatory response, are decreased in the microenvironment. As a result, Glucan may have a role as an adjunct to standard treatment in patients with AR.**

Keywords: allergic rhinitis, cytokine, eosinophil, glucan, nasal lavage

Allergic rhinitis (AR) is clinically defined as a symptomatic disease of the nasal mucosa caused by an IgE-mediated allergic inflammation [1]. Recent insights into the development of allergic diseases such as AR, asthma and atopic eczema are based on the functional diversity of T helper (Th) 1 and Th2 lymphocytes [2]. Th2 cells (secreting IL-4, IL-5, IL-9 and IL-13) are now considered to be responsible for the induction, as well as for many of the manifestations of atopic diseases [2, 3]. In these diseases, IL-4 plays a key role in driving the differentiation of $CD4^+$ Th precursor into Th2, and B cells into IgE-producing cells [4]. IL-5 stimulates eosinophil generation and chemotaxis; it activates mature eosinophils while prolonging their survival [5]. T cell-mediated overproduction of Th2 cytokines (IL-4, IL-5 and IL-13) locally at the site of allergic inflammation in atopic individuals, has been reported by several authors [6, 7]. Additionally, Th1 and Th2 cells can inhibit each other's function when they are activated, through several cytokines such as IL-10, IL-4 for Th2 and IFN-α IL-12 for Th1 cells [4, 8].

The trend in immunological modulatory treatment with biological agents has recently been a topic of interest, however, it was initiated by research performed 30 years ago [9-11]. Pillemer and Ecker [12] applied the name zymosan to a yeast cell wall fraction of *Saccharomyces cerevisiae,* which produced prominent hyperplasia and hyperfunction of the reticuloendothelial system when administered to experimental animals. Glucan, a β-1,3 linked

glucopyronase polysaccharide of approximately 6.5 kD, has been isolated from zymosan and shown to be a potent activator of the macrophage/monocyte cell series, and also, responsible for most of the stimulatory effects on the reticuloendothelial system [13-15]. Results of many studies following this up have revealed antitumor properties of glucans [16-19]. The Th1 type of immune response causes an activation of macrophage functions and growth, an increase in accessory cell functions and antigen presentation, as well as strong chemotaxis in lymphocytes and leucocytes. Additionally, it leads to a strong cellular immune response against tumor cells and viruses by producing cytokines (IFN-α, IL-12) [8]. It is known that an efficient antitumor response requires a Th1-mediated immune reaction. Glucan may be causing a skewing of the immune system towards a Th1 response. Nevertheless, lentinan, which is an important source of Glucan, has been reported to be a possible immunomodulator therapy in patients with cancer of the digestive tract by decreasing the Th2-mediated immune response and converting it to a Th1-mediated response [19].

In the light of all this knowledge, we designed this study to investigate the effects of Glucan on immunopathogenic processes in the microenvironment and its ability to restore a balance between Th2- and Th1-mediated immune response in AR.

MATERIALS AND METHODS

Subjects and study design

The study was carried out according to a randomized, doubled-blind, parallel group, placebo-controlled design. A total of 26 patients with seasonal AR participated in this study. All of the patients selected for this study were sensitive only to olive pollen. The study was performed in autumn and winter, out of the pollen season, to avoid natural allergen provocation. Before the study, informed consent for the described procedures was obtained from all patients. Approval for the study was given by the local ethics committee of our hospital. Diagnosis of seasonal AR was based on history, physical examination and laboratory findings. Allergic sensitization was demonstrated by the skin prick test. Skin prick tests were performed according to the EAACI guidelines [20] for the most common inhalant allergens in Turkey, including house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), fungi (*Aspergillus fumigatus, Alternaria alternata, Cladosporium herbarum, Penicillium notatum*), grasses (*Lolium perenne, Fectuca pratensis, Phleum pratense, Poa pratensis, Dactylis glomerata*), weeds (*Plantago lanceolata, Artemisia vulgaris, Rumex acetosa, Taraxacum vulgare, Parietaria officinalis*) and trees (*Sambucus nigra, Populus alba, Ulmus scabra, Salix caprea, Fagus silvatica, Carpinus betulus, Quercus robur, Fraxinus excelsior, Olea europea*) with histamine and diluent control (Allergopharma Ltd, Reinbek, Germany). Two patients were excluded because the nasal provocation test (NPT) with *Olea europea* (ALK-Abello, Madrid, Spain) could not be performed due to the total obstruction of at least one nostril, as shown by peak nasal inspiratory flow (PNIF) measurements. The NPT with *Olea europea* was performed on 24 patients who were included in the study after having stopped their medications (oral anti-histamines and nasal topical corticosteroids for 1 week and nasal topical anti-histamines for at least 48h before NPT). After 30 min of NPT, nasal lavage was performed on all subjects for measurement of IL-4, IL-5, IFN-α, IL-12 and eosinophil count (%). Blood samples were obtained to measure blood eosinophils (%). Subjects were then randomized into two matched groups: Glucan group (n = 12) to be treated with β-1,3-1,6-glucan (Imuneks® capsule, Mustafa Nevzat Ilac San., Istanbul, Turkey), 10 mg, twice a day for 12 weeks, and the placebo group (n = 12) with the same posology and duration. Following both treatments, all subjects underwent a second NPT with *Olea europea*, together with the nasal lavage, and blood sampling.

Nasal allergen provocation test

Subjects were allowed to rest for 15 min to ensure that equilibration of the nasal mucosa with the environmental conditions of the laboratory was achieved. Symptom scores and PNIF values measured with a PNIF meter device (Clement Clarke Int Ltd, Essex, UK), which were reported to reveal quite specific and sensitive results in the NPT, were recorded initially as a baseline [21, 22]. Specific allergen extract was insufflated into each nostril using spray bottles containing incremental concentrations of allergen (2, 4, and 8 BU/mL); these insufflations delivered 0.16, 0.32, and 0.64 μg of allergen, respectively. At each insufflation, the nasal reaction obtained was recorded according to a symptom score technique. Patients were challenged with incremental doses of the allergen (0.016, 0.032, and 0.064 μg in each nostril) until a composite symptom score of 6 or more accompanied by a 20% or more decrease in PNIF was obtained. The clinical criteria for a positive NPT result included symptom assessment through the use of a semiquantitative method similar to that used by others [23, 24]. Briefly, 3 major nasal symptoms (sneezing, rhinorrea, and nasal obstruction) were recorded 10 minutes after nasal challenge, according to a scale ranging from 0 (no symptoms) to 3 (severe). A total nasal symptom score was calculated by adding these scores; the maximum possible score was 9 for each subject. A composite symptom score of 6 or more was the clinical criterion for a positive response to the NPT. In addition to this, a 20% or more decrease in PNIF was the laboratory criterion for a positive response to NPT. Cumulative doses of allergen required to induce a nasal response varied from 0.016 to 0.112 μg for each nostril.

Nasal lavage

Nasal lavages were performed 30 min after NPT. The subject, in the sitting position, was instructed to extend the neck approximately 30 degrees from the horizontal and to refrain from breathing or swallowing. The nasal cavity was washed with 10 mL of physiological saline, warmed to 37 °C, by reciprocating the piston of the syringe 10 times on each side while the subject did not breathe or swallow. The subject flexed the neck and expelled the nasal lavage fluid (NLF) into a collection pot 10 seconds after each lavage. All NLF was filtered through a 52 μm nylon filter

to remove mucin, and the filtrate was centrifuged at 4 °C for 10 min at 1000*g*. The supernatant was stored at -80 °C until assayed for cytokine levels. The sediment was subjected to cell count measurement.

Measurements of cytokines and eosinophil count

Levels of cytokines in NLF supernatant were measured using commercially available ELISA kits (IL-4, Catalog # KHC0041; IL-5, Catalog # KHC0051; IFN-α Catalog # KHC4021; IL-12, Catalog # KAC1561. BioSource International, Inc., Camarillo, CA, USA) following the manufacturer's instructions. Levels of these mediators below the sensitivity of the assay were 2 pg/mL for IL-4, 4 pg/mL for IL-5, 4 pg/mL for IFN-α, 1.5 pg/mL for IL-12.

The sediment from the NLF was resuspended in 1 mL RPMI 1640 (Gibco BRL, Life Technologies Ltd, Paisley, UK) containing 10% FCS. An equal volume of 16% N-acetylcysteine in calcium-free, Hank's balanced salt solution (Gibco BRL, Life Technologies Ltd, Paisley, UK) was added to each sample and samples were then incubated for 45 minutes at 37 °C to disaggregate remnant mucus and clumps. Differential counts were performed on cytospin slides stained with DiffQuick (DADE S.p.A. Rome, Italy) and examined by means of light microscopy. Pre-treatment and post-treatment percentage eosinophil values were used for statistical evaluation. Additionally, the peripheral blood eosinophil count (%) of each subject was recorded by a hemocounter pre- and post-treatment.

Statistical assessment

The significance of differences between within-group and inter-group comparisons were determined using the Wilcoxon signed rank's test and the Mann-Whitney *U* test, respectively. Correlations were evaluated by the Pearson's correlation test. Differences were considered significant when the p-value was less than 0.05.

RESULTS

The study was completed with 24 patients. The demographical, disease and SPT characteristics of our patients are summarized in *table 1*. There were no differences in these characteristics between the groups.

Mean allergen dosage ± SEM required for a positive nasal response in the Glucan group increased minimally from 0.04 ± 0.008 μg at the pre-treatment period to 0.056 ± 0.011 μg at the post-treatment period. However, the difference between the two values was not statistically significant (p = 0.21) *(figure 1)*. Similarly, the mean allergen dosage required for a positive nasal response in the placebo group did not display a significant change at the pre-treatment and post-treatment periods (0.04 ± 0.011 μg and 0.037 ± 0.005 μg; respectively) (p = 0.78) *(figure 1)*.

Eosinophil count

There were no differences in the pre-treatment values for the mean eosinophil ± SEM count in NLF between the Glucan and placebo groups (15.33 ± 0.90%, 13.50 ± 1.18%; respectively) (p = 0.23) *(figure 2)*. The eosinophil count in the NLF did not differ between the two groups post-treatment either (11.83 ± 1.06% for the Glucan group *versus* 14.17 ± 0.87% for the placebo group) (p = 0.12) *(figure 2)*. However, eosinophil count in NLF from the Glucan group decreased significantly after treatment (p = 0.01) *(figure 2)*. The percentage of eosinophils in the NLF did not differ between the pre-treatment and post-treatment period in the placebo group (p = 0.53) *(figure 2)*. Decreases in IL-5 values were significantly correlated with the decrease in the eosinophil count in the NLF following treatment in the glucan group ($r = 0.676$; p = 0.016), while there was no correlation with the changes in other cytokines.

The peripheral blood eosinophil count was very similar in the two groups before treatment. (5.75 ± 0.63% for Glucan group *versus* 5.58 ± 0.5% for the placebo group) (p = 0.86). Similarly, the peripheral eosinophil count was very similar for the groups after treatment, (5.83 ± 0.56% for the Glucan group *versus* 5.33 ± 0.33% for the placebo group) (p = 0.46). Comparison of peripheral eosinophil counts within the groups did not demonstrate any significant difference for either group (p = 0.8, p = 0.56; respectively).

Results for the cytokines

Results for all cytokines are summarized in *table 2* as mean±SEM. All cytokine levels in NLF before treatment and in the Glucan group did not differ from the values found in the placebo group. However, the IL-12 level in the Glucan group was significantly higher than that in the placebo group (p = 0.014) *(figure 3d)*.

Comparison of IL-4 and IL-5 levels within the Glucan group before and after treatment revealed a significant decrease after treatment (p = 0.027, p = 0.04; respectively) *(figure 3a,b)*. We observed that the IL-12 level increased significantly in the Glucan group after treatment (p = 0.008). However, we found that IFN- α levels in the Glucan group before and after treatment did not change

Table 1
Demographical, disease and SPT characteristics of our patients. NS, p-value not significant

	Glucan group	**Placebo group**	**p-value**
	(n = 12)	**(n = 12)**	
Age (year) (mean ± SEM)	33.5 ± 3.34	32.25 ± 3.75	NS
Gender (F/M)	5/7	6/6	NS
Disease type	Seasonal AR	Seasonal AR	
Disease duration (year) (mean ± SEM)	8.08 ± 0.72	7.42 ± 0.79	NS
SPT result *Olea europea/Others*	12/0	12/0	

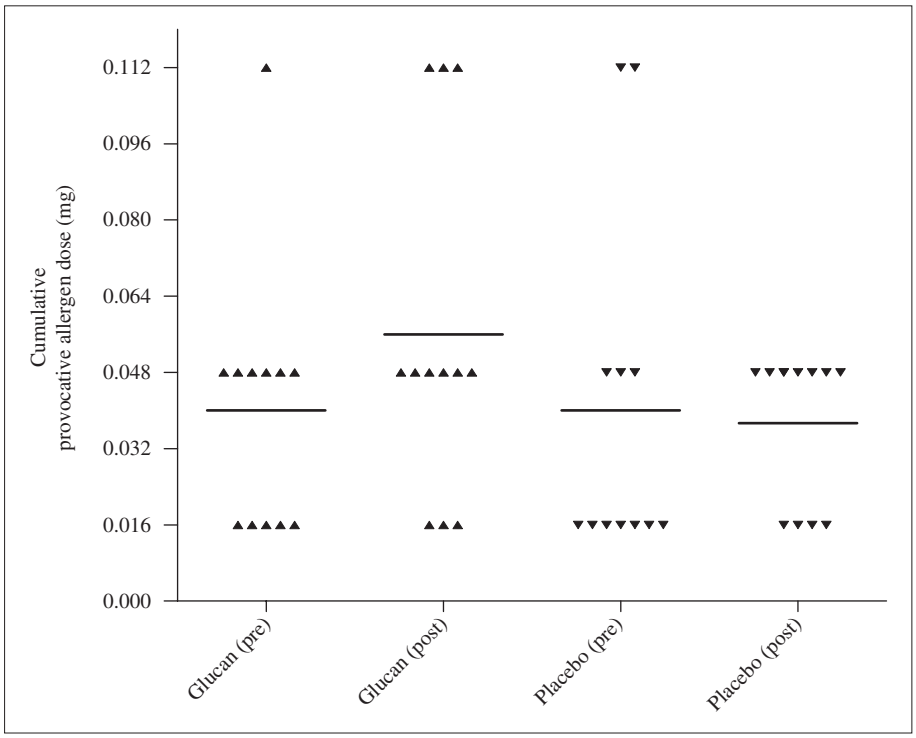

Figure 1
Cumulative allergen dosage for each nostril required for a positive nasal response in the Glucan and placebo group during the pre-treatment and post-treatment periods.
In the Glucan group, this dosage increased minimally from the pre-treatment period to the post-treatment period. In the placebo group, this dosage did not display any change at the pre-treatment or post-treatment periods. See text for p-values. Pre = Pre-treatment; Post = post-treatment.

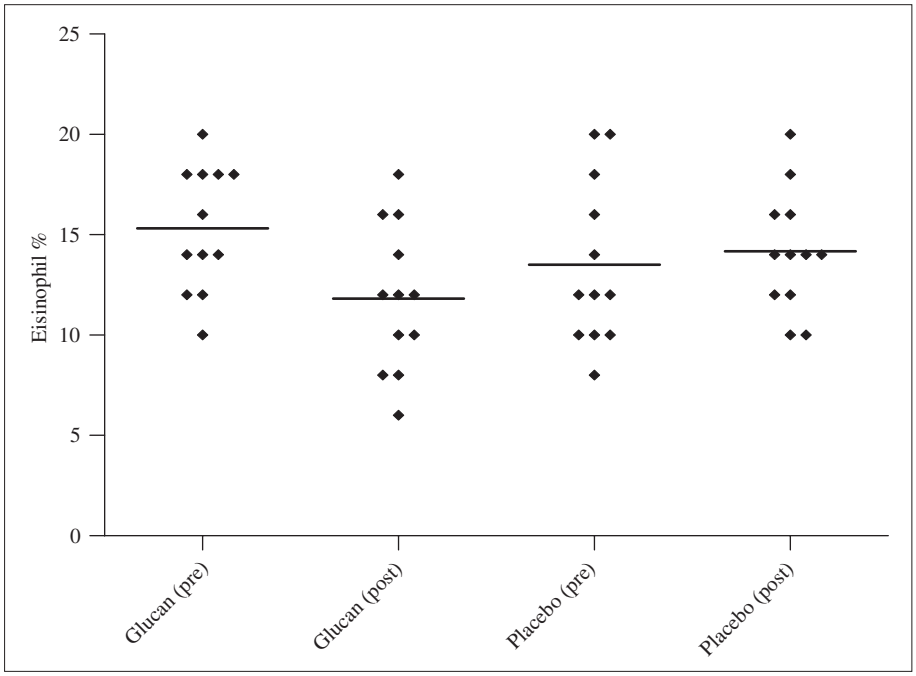

Figure 2
Eosinophil (%) values in the nasal lavage fluid of the Glucan and placebo groups before and after treatment.
See text for p-values. Pre = pre-treatment; Post = post-treatment.

significantly ($p = 0.1$) *(figure 3c)*. Additionally, cytokine levels obtained before treatment were seen not to change significantly after treatment in the placebo group.
Changes in any cytokine level with treatment in the Glucan group were found not to be correlated with changes in any other cytokine.

DISCUSSION

Allergic rhinitis is a disease characterized by an IgE-mediated inflammation, which presents with a Th2-mediated immune response [1]. Eosinophils especially, play an important role in the allergic inflammatory pro-

Table 2
Mean±SEM cytokine levels in nasal lavage fluid of the Glucan and placebo groups before and after treatment

					p-value			
	Pre-treatment		**Post-treatment**		**Within group**		**Intergroup**	
	Glucan	Placebo	Glucan	Placebo	Glucan	Placebo	Pre-treatment	Post-treatment
IL-4 (pg/mL)	5.48 ± 0.92	4.63 ± 0.69	3.66 ± 0.64	4.45 ± 0.85	0.027	0.622	0.624	0.272
IL-5 (pg/mL)	8.58 ± 1.58	6.78 ± 0.69	5.81 ± 0.83	6.57 ± 0.68	0.04	0.753	0.707	0.214
IFN-α (pg/mL)	6.19 ± 1.18	6.13 ± 0.58	7.83 ± 1.22	6.85 ± 0.80	0.1	0.384	0.386	0.84
IL-12 (pg/mL)	11.08 ± 2.43	10.48 ± 1.51	17.31 ± 2.75	9.97 ± 1.54	0.008	0.8	0.773	0.014

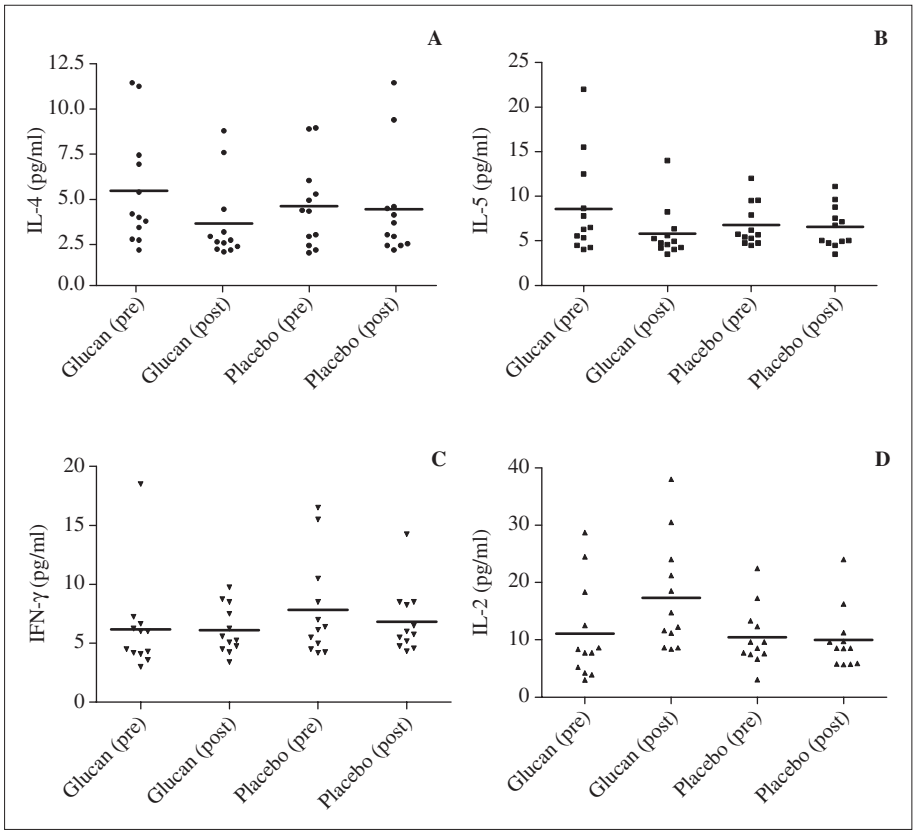

Figure 3
Cytokine levels in nasal lavage fluid of Glucan and placebo groups before and after treatment.
See text for p-values. Pre = pre-treatment; Post = post-treatment.

cess. Specific IgE synthesis is known to be mediated by IL-4 and IL-13 in patients with AR [1]. Previous studies have found that Th2-originated cytokines predominated in the evaluation of the NLF and in the activated peripheral blood mononuclear cell supernatant of patients with AR when compared to the non-atopic subjects [25-29]. Evaluation of treatment of AR and other diseases with a similar allergic nature requires the determination of the reversal of this cytokine pattern to one which originates from Th1 [30]. We also detected a decrease in the levels of Th2-originated IL-4 and IL-5 in NLF with Glucan treatment in this study. These results suggest that the Th2-mediated immune response is inhibited, especially in the microenvironment. During their extensive investigations of the Th1 and Th2 phenomena, Murata's group administered lentinan to mice [31]. Lentinan is a β-1,3-D-glucan from the medicinal mushroom *Lentinus edodes*. This molecule closely resembles other glucans typically liberated from fungal cell walls during digestion or in the course of an immune response. When they gave lentinan intraperitoneally to mice, the macrophage glutathione status as well as their capacity to produce IL-12 improved, thus orientating them toward Th1 immunity. This effect could be turned off by depleting glutathione, and then restoring it using alpha-lipoic acid, an antioxidant nutrient that replenishes glutathione [31]. Murata and colleagues also collected Th cell populations from the mice following lentinan exposure, assessed their cytokine production capacity, and found that they had shifted toward Th1 production instead of Th2 [31]. Similarly, the decrease in eosinophils that play an essential role in the immunopathogenesis of AR, in NLF following treatment, may be due to the inhibitory role of Glucan in allergic immunopathogenesis. Correlation of the decrease in IL-5 levels and eosinophil counts in NLF indicates that the changes in immunological parameters that we detected coexist with the changes in inflammatory parameters. It was not surprising to find that, in the NLF, the treatment-mediated decrease in IL-5, which is a stimu-

lator of eosinophil generation and chemotaxis and which prolongs the survival of eosinophils, parallels the decrease in the level of eosinophils after treatment [5]. Absence of a change in the eosinophil count in peripheral blood indicates that the immunological change mediated by treatment may not be systemic.

The increase in IL-12, which is secreted from Th1, in the NLF of patients receiving Glucan treatment, indicates that the systemic use of Glucan in patients with AR may convert the immune response towards Th1. There are earlier, cytokine-based studies involving allergen-specific immunotherapy, which demonstrated that a Th1-mediated immune response predominated with this treatment [30]. Therefore, the increase in IL-12 levels that we have detected may indicate the predominance of a Th1 immunological response. Saito *et al.* have reported that Glucan increases IL-12 levels *in vitro* and decreases Ig-E synthesis *in vivo*, inhibiting the Th2-mediated immune response, similar to our results [32]. Authors in this previous study have concluded that glucans can be used in the prevention and treatment of allergic diseases. However, the absence of a change in the levels of IFN-α, which is also secreted from Th1, may be the result of the lack of an adequate time period during which the pattern of this cytokine may alter. Nevertheless, previous research on AR patients receiving allergen-specific immunotherapy revealed that changes in levels of IFN-α manifested only during the second year of treatment [33].

Although insignificant, increases in the provocative allergen dose required to induce a positive nasal response in the group receiving Glucan treatment suggest that Glucan may suppress the allergen-mediated response, along with changes in microenvironment.

In conclusion, Glucan treatment may be used as an adjunct to treatment in patients with AR because of the immunological modulation it causes.

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REFERENCES

1. Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 2001; 108(Suppl 5): 147.
2. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. *Nat* 1996; 383: 787.
3. Murphy KM, Reiner SL. The lineage decisions of helper T cells. *Nat Rev Immunol* 2002; 2: 933.
4. Romagnani S. Human Th1 and Th2 subsets: regulation of differentiation and role in protection and immunopathology. *Int Arch Allergy Immunol* 1992; 98: 279.
5. Wills-Karp M, Chiaramonte M. Interleukin-13 in asthma. *Curr Opin Pulm Med* 2003; 9: 21.
6. Romagnani S, Maggi E, Parronchi P, Macchia D, Piccinni MP, Ricci M. Increased number of Th2-like CD4 T cells in target organs and in the allergen-specific repertoire of allergic patients. Possible role of IL-4 production by non-T cells. *Int Arch Allergy Appl Immunol* 1991; 94: 133.
7. Huang SK, Xiao HQ, Kleine-Tebbe J, *et al.* IL-13 expression at the sites of allergen challenge in patients with asthma. *J Immunol* 1995; 155: 2688.
8. Belardelli F. Role of interferons and other cytokines in the regulation of the immune response. *APMIS* 1995; 103: 161.
9. Ghaffar A, Cullet RT, Woodruff MFA. Further analysis of the antitumor effect *in vitro* of peritoneal exudate cells from mice treated with Corynebacterium parvum. *Br J Cancer* 1975; 31: 15.
10. Mohr SJ, Chirigos MA, Fuhrman FS, Pryor JW. Pyran copolymer as an effective adjuvant to chemotherapy against a murine leukemia and solid tumor. *Cancer Res* 1975; 35: 3750.
11. Morahan PS, Kaplan AM. Macrophage activation and anti-tumor activity of biologic and synthetic agents. *Intern J Cancer* 1976; 17: 82.
12. Pillemer L, Ecker EE. Anticomplementary factor in fresh yeast. *J Biol Chem* 1941; 137: 139.
13. Di Carlo FJ, Fiore JV. On the composition of zymosan. *Sci* 1958; 127: 756.
14. Riggi SJ, Di Luzio NR. Identification of a reticuloendothelial stimulatory agent in zymosan. *Am J Physiol* 1961; 200: 297.
15. Wooles WR, Di Luzio NR. Reticuloendothelial function and the immune response. *Science* 1963; 142: 1078.
16. Stewart CC, Valeriote FA, Perez A. Preliminary observations on the effect of Glucan in combination with radiation and chemotherapy in four murine tumors. *Cancer Treat Rep* 1978; 62: 1867.
17. Luzio NR, Williams DL, McNamee RB, Malshet VG. Comparative evaluation of the tumor inhibitory and antibacterial activity of solubilized and particulate Glucan. *Recent Results Cancer Res* 1980; 75: 165.
18. Kasai S, Fujimoto S, Nitta K, Baba H, Kunimoto T. Antitumor activity of polymorphonuclear leukocytes activated by a β-1,3-**D**-Glucan. *J Phamacobio-Dyn* 1991; 14: 519.
19. Yoshino S, Tabata T, Hazama S, *et al.* Immunoregulatory effects of the antitumor polysaccharide lentinan on Th1/Th2 balance in patients with digestive cancers. *Anticancer Res* 2000; 20: 4707.
20. EAACI-guideline, Dreborg S, Frew A. EAACI Subcommittee on Skin Tests. Position paper. Allergen standardization and skin tests. *Allergy* 1993; 48(suppl. 14): 49.
21. Hellgren J, Jarlstedt J, Dimberg L, Toren K, Karlsson G. A study of some current methods for assessment of nasal histamine reactivity. *Clin Otolaryngol* 1997; 22: 536.
22. Hagemann H, Bauer PC, Costabel U. Comparability of various measurement methods in nasal provocation with allergens. *Pneumol* 2002; 56: 363.
23. Sim TC, Grant A, Hilsmeir KA, Fukuda Y, Alam R. Proinflammatory cytokines in nasal secretions of allergic subjects after antigen challenge. *Am J Respir Crit Care Med* 1994; 149: 339.
24. Pedersen B, Dahal R, Richards DH, Jacques LA, Larsen BB, Pichler W, *et al.* Once daily fluticasone propionate aqueous nasal spray controls symptoms of most patients with seasonal allergic rhinitis. *Allergy* 1995; 50: 794.
25. Benson M, Strannegard IL, Wennergren G, Strannegard O. Low levels of interferon-gamma in nasal fluid accompany raised levels of T-helper 2 cytokines in children with ongoing allergic rhinitis. *Pediatr Allergy Immunol* 2000; 11: 20.
26. Benson M, Strannegard IL, Wennergren G, Strannegard O. Cytokines in nasal fluids from school children with seasonal allergic rhinitis. *Pediatr Allergy Immunol* 1997; 8: 143.
27. Rapp J, Kosa L, Halasz A, Kereki E, Borzsonyi L. Levels of interleukin-4, interleukin-5, tryptase and eosinophil cationic protein of nasal lavage fluid in pollen allergic rhinitis. *Orv Hetil* 2000; 141: 1919.
28. Moverare R, Elfman L, Stalenheim G, Bjornsson E. Study of the Th1/Th2 balance, including IL-10 production, in cultures of peripheral blood mononuclear cells from birch-pollen-allergic patients. *Allergy* 2000; 55: 171.

29. Moverare R, Rak S, Elfman L. Allergen-specific increase in interleukin-(IL)-4 and IL-5 secretion from peripheral blood mononuclear cells during birch-pollen immunotherapy. *Allergy* 1998; 53: 275.

30. Durham SR. New insights into the mechanisms of immunotherapy. *Eur Arch Otorhinolaryngol* 1995; 252(Suppl 1): 64.

31. Murata Y, Shimamura T, Tagami T, Takatsuki F, Hamuro J. The skewing to Th1 induced by lentinan is directed through the distinctive cytokine production by macrophages with elevated intracellular glutathione content. *Int Immunopharmacol* 2002; 2: 673.

32. Saito K, Yajima T, Nishimura H, Aiba K, Ishimitsu R, Matsuguchi T, *et al.* Soluble branched beta-(1,4)glucans from Acetobacter species show strong activities to induce interleukin-12 in vitro and inhibit T-helper 2 cellular response with immunoglobulin E production in vivo. *J Biol Chem* 2003; 278: 38571.

33. Oda N, Yamashita N, Minoguchi K, Takeno M, Kaneko S, Sakane T, *et al.* Long-term analysis of allergen-specific T cell clones from patients with asthma treated with allergen rush immunotherapy. *Cell Immunol* 1998; 190: 43.