

Changes in Plasma TARC Levels during Japanese Cedar Pollen Season and Relationships with Symptom Development

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Key Words

Allergy · Japanese cedar pollinosis · Thymus and activation-regulated chemokine

Abstract

Background: Japanese cedar pollinosis (JCPsis) is an immunoglobulin E-mediated type I allergy caused by exposure to Japanese cedar pollen (JCP). Blood thymus and activation-regulated chemokine (TARC) levels are well known as an objective parameter for disease severity for several allergic disorders. The present study aims to evaluate the relationship between TARC levels and disease symptoms during the pollen season. **Methods:** Analysis was performed of results of symptom scores and blood parameters obtained from 42 JCPsis patients who participated in a probiotic (*Bifidobacterium longum* BB536) intake trial in the JCP season of 2005 (January to April), using a randomized, double-blind, placebo-controlled design. **Results:** Significant increases in plasma TARC levels were observed in subjects receiving placebo ($p < 0.05$ in February and $p < 0.01$ in March), but not in subjects receiving BB536. Increased plasma TARC levels were markedly greater in subjects who experienced severe symptoms and were thus excluded early from the intervention (placebo group: $n = 8$; BB536 group: $n = 2$). Significant differences were found in changes from baseline TARC levels in

February and March between the subjects where treatment was terminated early and the remaining ones. Among the remaining subjects, significant positive correlations were found as regards changed values of TARC compared to baseline in March and April with symptom scores recorded in the pollen season. **Conclusion:** Changed values of blood TARC in the pollen season may offer promising parameters for assessing disease severity and monitoring treatment.

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Introduction

Japanese cedar pollinosis (JCPsis) is an immunoglobulin E (IgE)-mediated type I allergy caused by exposure to Japanese cedar (*Cryptomeria japonica*) pollen (JCP). JCPsis is one of the most common allergic diseases in Japan, representing a public health issue affecting >16% of the Japanese population, and prevalence has been increasing over recent decades [1].

Although the detailed reasons for increases in allergic diseases require further evaluation, marked skewing of immune responses to the Th2 lineage is generally considered to be associated with the development of allergic disease [2]. Upon exposure to allergens, antigens are likely to be accosted by dendritic cells or macrophages, phagocy-

tosed, fragmented and then presented on the surface to allergen-specific T cells, predominantly Th2 cells. Interleukin-4 (IL-4), and probably other Th2 cytokines such as IL-5 and IL-13 secreted by Th2 cells, helps to recruit B cells, mast cells and eosinophils, all of which can produce IgE antibodies, which in turn play a central role in the induction of allergic symptomatology [2]. Accumulation of JCP-specific IgE caused by exposure to JCP is known as a cue for the development of symptoms. However, JCP-specific IgE levels do not seem to be the only key factor for onset and severity of pollinosis symptoms, since a certain population possessing JCP-IgE does not exhibit symptoms. Limited data has been available on blood markers for disease severity and treatment monitoring of JCPsis.

We previously reported that the intake of *Bifidobacterium longum* BB536 was potentially effective in relieving JCPsis symptoms and modulating the Th1/Th2 balance [3, 4]. In 2004, we performed a human trial to investigate the effects of yogurt supplemented with BB536 in the treatment of JCPsis during the pollen season. We found that some of the subjective symptoms were alleviated following BB536 intake, while decreased interferon- γ levels were particularly suppressed at the early stage of the pollen season [3]. The effect of BB536 in the treatment of JCPsis was confirmed during the 2005 pollen season by using lyophilized powders of BB536. We found significant changes in several parameters during the pollen season. These included marked increases in plasma levels of thymus and activation-regulated chemokine (TARC), a member of the CC chemokine family that is known to function by recruiting T cells, during pollen dispersion in individuals who received placebo, but not in the BB536 group [4]. Significant differences were found in changes from baseline TARC levels in February ($p = 0.038$) and March ($p = 0.031$) in the treatment group compared with the placebo group [4]. TARC levels are well known as an objective parameter for disease severity in atopic dermatitis, bronchial asthma, allergic rhinitis and other allergic disorders [5–14]. Herein we report the analysis of results supporting TARC as possibly associated with disease development and as a promising tool for monitoring JCPsis.

Methods

In the present study analyses of data were done for symptom scores and blood parameters obtained during the course of a clinical study reported by Xiao et al. [3] on the effect of BB536 on clinical symptoms of JCPsis and blood parameters related to pollen allergy.

Participants and Clinical Trial

These details have been published elsewhere [3]. Briefly, a total of 44 JCPsis patients with a clinical history of JCPsis were randomized to ingest either BB536 powder (BB536 group; 13 men, 9 women; mean age 36.0 ± 7.3 years) or placebo powder (placebo group; 13 men, 9 women; mean age 36.5 ± 8.1 years), in a randomized, double-blind design from January 17 for 13 weeks during the pollen season of 2005.

During the study, participants were instructed to record subjective symptoms, self-care measures, and medication in accordance with the guidelines of the Nasal Allergy Clinic 2002, Japan. They were allowed to use self-care measures such as wearing a mask, eye drops or local nasal sprays when they felt the need. The use of a prescribed pollinosis medication, which was set as a drop-out criterion, was permitted when the subject felt the need, but further symptom evaluation and blood sampling were then stopped and these participants were regarded as 'early-terminated'.

Physical examination and blood sampling were performed before and during intervention. Two participants in the placebo group did not attend blood sampling for personal reasons. Blood samples were drawn from an antecubital vein after overnight fasting. Plasma TARC levels were analyzed using a human ELISA kit (R&D Systems, Minn., USA).

Details of study outcomes have been published elsewhere [3]. In summary, we observed a significant difference in the number of subjects who were early-terminated due to severe symptoms and pollinosis medication between groups (placebo group: 9 of 22 subjects; BB536 group: 2 of 22 subjects; $p = 0.0057$). Subjective symptom scores for rhinorrhea and nasal blockage were significantly reduced in the BB536 group compared with the placebo group. As a total of nasal, ocular and throat symptom scores, composite scores were significantly lower in the BB536 group compared with the placebo group. We observed significant increases in JCP-specific IgE levels in April and significant decreases of interferon- γ levels along with pollen dispersion in placebo and BB536 groups. Significant increases from baseline TARC levels were found in the placebo group in February ($p < 0.05$) and March ($p < 0.01$), but not in the BB536 group. Significant differences were found in changes from baseline TARC levels in February ($p = 0.038$) and March ($p = 0.031$) in the BB536 group compared with the placebo group. Furthermore, significant increases from baseline were noted in eosinophil rates with pollen dispersion among JCPsis subjects. These results indicated that a Th2-skewed immune response occurred along with pollen dispersion in JCPsis subjects, and BB536 intake modulated the immune response.

Statistical Analysis

All statistical analyses were performed using SPSS version 14 software. Symptom scores were averaged within each week for each subject and changes in score were calculated compared to week 1. Weekly total scores for sneezing, rhinorrhea, nasal blockage, nasal itching, eye symptoms and throat symptoms were totaled as composite scores (scale 0–168) [3]. Areas under the curve (AUCs) were then calculated using changes in score for weeks 5–13 (pollen season).

The Mann-Whitney U test and Wilcoxon test were used to analyze between-group and within-group differences, respectively. Spearman's correlation tests were applied for correlation anal-

Table 1. Changes in serum TARC levels among remaining and early-terminated JCPsis subjects during the pollen season

Group	Serum TARC levels, pg/ml			
	ratios to baselines			
	baseline (January)	February	March	April
JCPsis subjects in placebo group				
Remaining	59.3 (35.0–83.5) (n = 12)	1.07 (0.76–1.39) (n = 12)	1.21 (0.71–1.71) (n = 12)	1.13 (0.68–1.59) (n = 12)
Early-terminated	64.0 (30.8–97.3) (n = 8)	1.84 (1.18–2.50) (n = 8) ^{+, *}	2.23 (1.72–2.75) (n = 6) ^{+, *}	n.d. (n = 0)
JCPsis subjects in both placebo and BB536 group				
Remaining	74.3 (52.1–98.6) (n = 32)	0.92 (0.76–1.08) (n = 32)	1.07 (0.88–1.27) (n = 32)	0.94 (0.72–1.15) (n = 32)
Early-terminated	64.6 (39.3–89.4) (n = 10)	1.73 (1.19–2.28) (n = 10) ^{++, **}	2.20 (1.83–2.56) (n = 8) ^{+, ***}	n.d. (n = 0)

Data are presented as geomeans (95% CIs). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. remaining (Mann-Whitney U test after logarithmic transformation); ⁺ $p < 0.05$; ⁺⁺ $p < 0.01$ vs. baseline (Wilcoxon test). n.d. = Not determined.

Table 2. Correlation of blood TARC levels with subjective symptom scores among the individuals with JCPsis during the pollen season

	All (n = 32)	ρ value	Placebo group (n = 12)	ρ value	BB536 group (n = 20)	ρ value
AUCs of composite symptom scores	229.9 (154.9–304.8)		301.7 (158.0–445.4)		195.3 (107.5–283.0)	
Serum TARC, pg/ml						
January Measured values	74.7 (53.2–96.2)	0.232	61.1 (38.9–83.2)	0.210	84.2 (52.5–115.9)	0.165
February Measured values	68.2 (49.3–87.2)	0.025	63.4 (50.5–76.3)	0.230	71.3 (41.6–101.0)	0.034
Ratio to baseline (Jan.)	0.9 (0.8–1.1)	0.202	1.0 (0.7–1.3)	0.307	0.8 (0.7–1.0)	0.027
March Measured values	80.6 (62.9–98.4)	0.065	74.9 (51.0–98.8)	0.349	84.3 (58.6–109.9)	0.009
Ratio to baseline (Jan.)	1.1 (0.9–1.3)	0.458**	1.2 (0.8–1.7)	0.636*	1.0 (0.8–1.2)	0.466*
April Measured values	70.4 (57.5–83.3)	0.053	68.9 (49.2–88.6)	0.462	71.3 (53.2–89.5)	0.103
Ratio to baseline (Jan.)	0.9*	0.410*	1.1 (0.7–1.5)	0.650*	0.8 (0.6–1.1)	0.259

Data are presented as geomeans (95% CIs). * $p < 0.05$; ** $p < 0.01$ (significant correlation with AUCs of composite symptom scores, Spearman's correlation test). Jan. = January.

yses. Blood data after termination of early-terminated subjects were treated as lost data. As distributions of some parameters were skewed, comparisons were made after logarithmic transformation. Limit values were used in analyses of blood parameters for data under detection limits. Values of $p < 0.05$ were considered statistically significant.

Results

Analysis was performed to compare TARC levels between the early-terminated (n = 10; placebo group: n = 8; BB536 group: n = 2) and the remaining subjects (n = 32, placebo group: n = 12; BB536 group: n = 20). No differences were found in baseline levels, but marked increases were observed in February and March for early-terminated subjects (table 1). Significant intergroup differences

in changed TARC levels were seen between the remaining and early-terminated subjects in February and March.

Among the remaining subjects, no significant correlations were found for baseline (January) levels and levels measured in February, March and April between TARC levels and AUCs of composite scores (table 2). A significant correlation was noted between changes from baseline for serum TARC and AUCs of composite scores for all subjects (n = 32; $\rho = 0.489$, $p = 0.005$ in March; $\rho = 0.428$, $p = 0.016$ in April), the placebo group (n = 12; $\rho = 0.673$, $p = 0.023$ in March; $\rho = 0.6366$, $p = 0.035$ in April) and the BB536 group (n = 20; $\rho = 0.46$, $p = 0.038$ in March).

Discussion

We showed herein that elevated plasma TARC levels during the pollen season are possibly associated with disease severity of JCPsis. We found that (1) plasma TARC levels in JCPsis subjects increased significantly during the JCP season in the placebo group, but not in the group administered a probiotic strain, BB536; (2) there were significant differences in changed values from baseline at weeks 4 and 8 between the placebo group and BB536 group; (3) plasma TARC level increased markedly more in subjects who experienced more severe symptoms during the pollen season, and (4) there were significant positive correlations between changed levels of plasma TARC and symptom scores during the pollen season.

TARC is a member of the CC chemokine family and is expressed transiently in phytohemagglutinin-stimulated peripheral blood mononuclear cells and constitutively and selectively in the thymus [15]. The CCR4 TARC receptor is selectively expressed on Th2 cells and TARC is related to the induction of Th2-type responses [16, 17]. In vitro studies have shown that TARC induces selective migration of lymphocytes with the Th2 phenotype [16–19]. The TARC level is well known as an objective parameter for disease severity in atopic dermatitis and represents a promising tool for treatment monitoring [5–7]. Elevation of blood TARC levels has been observed in individuals with cigarette smoke-induced pulmonary inflammation, nonepisodic angioedema associated with eosinophilia, and exercise-induced bronchoconstriction in asthma [8–10]. Plasma TARC levels might also offer a useful diagnostic marker for bronchial asthma and allergic rhinitis [11, 12]. Terada et al. [13] showed that nasal epithelial cells derived from allergic rhinitis subjects release higher concentrations of TARC than those of subjects without allergy. The authors also demonstrated that TARC production could be induced by Th2-type cytokines, especially in the combination with TNF- α , in human nasal epithelial cells, and the induction was at a significantly higher

level in allergic rhinitis subjects compared with nonallergic subjects. Further, Nomura et al. [14] reported that IL-13 induced TARC mRNA expression and protein production in human peripheral blood mononuclear cells; the level of TARC from allergic rhinitis patients was significantly higher than that from healthy volunteers.

However, to the best of our knowledge, few studies have reported on the importance of blood TARC levels in seasonal allergic diseases. Our results showed that elevated plasma levels, but not baseline or measured TARC levels during the pollen season are possibly associated with disease severity. However, a correlation between the measured levels of plasma TARC and symptom scores during the pollen season was not found. The discrepancy between our results and other studies that have investigated patients with perennial allergic diseases may be explained as follows. First, baseline levels of plasma TARC measured before the pollen season indicate prior priming and are not associated with symptoms. Second, elevated levels of plasma TARC upon exposure represent the degree of sensitization to pollen exposure and are important for showing disease. Further, variations might exist in baseline levels due to the possible inclusion in the present study of some patients with perennial allergic disorders, since patients with seasonal allergic disorders commonly experience perennial allergic diseases. In supporting our results, Ogawa et al. [20] observed a tendency for increased blood TARC levels in a limited number of JCPsis subjects during the pollen season from March 8 to April 5, 2005.

In conclusion, our results support the possible association of TARC with JCPsis and suggest that elevated plasma TARC levels in the JCP pollen season may offer a promising parameter for determining disease severity and monitoring treatment. However, future scale-up studies are needed to evaluate the role of TARC in allergic rhinitis. Screening of patients to exclude those with perennial symptoms should give more insight into the role of TARC in JCPsis.

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