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Original article

Immunological parameters in prophylactic sublingual immunotherapy in asymptomatic subjects sensitized to Japanese cedar pollen

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ABSTRACT

Background: This study aims to examine the immunological parameters, focusing IL-10 productivity, in prophylactic sublingual immunotherapy (SLIT) in asymptomatic subjects sensitized to Japanese cedar pollen (JCP).

Methods: This study was conducted as part of a randomized, double-blind, placebo-controlled, multiple center trial, and was performed for two consecutive pollen seasons in 2012 and 2013. The present results were based only on our institution. We recruited 29 participants with specific IgE against JCP of at class 2 and higher levels without history of the pollinosis symptoms at the time of JCP scattering. The SLIT group received standardized JCP extract for five months over the pollen season. We observed and judged development of the symptoms in the pollen season. The percentage of IL-10 producing $CD4^+$ T (Trl) cells, B cells and monocytes were analyzed by flow cytometry. JCP specific IgE and total IgE were also measured.

Results: The ratio of development of cedar pollinosis was significantly lower in the SLIT group compared to the placebo group in 2013. In 2012, the percentage of circulating Tr1 cells and IL-10 producing monocytes significantly increased in the SLIT group. In 2013, the percentage of circulating Tr1 cells and IL-10 producing B cells increased significantly in the SLIT group. The percentage of circulating IL-10 producing monocytes significantly decreased in the placebo group.

Conclusions: Prophylactic SLIT is effective for prevention of the development of pollinosis. Induction of IL-10 producing T cells, B cells and monocytes is an important mechanism of SLIT for prevention of pollinosis in asymptomatic but sensitized subjects.

Introduction

Japanese cedar pollinosis is an allergic disease specific to Japan with a high prevalence estimated to be 26.5%, which has increased by 10% over the past ten years.[1] Seasonal allergic rhinitis induced by cedar pollen takes a chronic course in the majority of middle-aged patients.[2] Remission rarely occurs, especially in the younger generation.

Sublingual immunotherapy (SLIT) is safer than conventional percutaneous antigen-specific immunotherapy, and is the only treatment which can completely cure the disease. It has been shown that SLIT is effective and safe in the treatment of cedar pollinosis by a randomized, placebo-controlled, double-blind study.[3]

About 20% of asymptomatic subjects sensitized to this pollen develop symptoms in the pollen scattering season.[4] Thus, it is important to prevent the development of pollinosis in these asymptomatic, sensitized subjects. To determine whether SLIT can prevent the development of pollinosis in sensitized subjects who have no history of pollinosis, a randomized, placebo-controlled, double blind trail was carried out over two pollinosis seasons in 2012 and 2013 in multiple facilities in Japan.

The mechanism of action of SLIT is not completely understood. However, IL-10 is critical for the induction of specific T cell tolerance and the increase in IL-10 production by monocytes and T cells during inflammatory responses or after SLIT may influence effector

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cells involved in allergic responses. Increased IL-10 production following specific immunotherapy causes anergy in peripheral T cells, and regulates specific IgE and IgG4 production towards normal IgG4-related immunity.[5] Low IL-10 productivity by monocytes and T cells is closely related to sensitivity to multiple allergens and resistance to allergic diseases.[6,7] Augmentation of constitutive IL-10 production from the immune system is a potential therapeutic approach for allergic disorders. Thus we hypothesized that IL-10 may play an important role in prophylactic SLIT for asymptomatic sensitized subjects.

We also examined the ratio of specific IgE against total IgE (sIgE/tIgE), because the evidence concerning the relationship between this ratio and the efficacy of SLIT is conflicting. Di Lorenzo et al. reported that a high sIgE/tIgE ratio was associated with an effective response in sublingual and subcutaneous immunotherapy in monosensitized patients for the following allergens: grass, *Parietaria judaica*, *Olea europea* and house dust mite.[8] On the other hand, Fujimura et al. reported that the sIgE/tIgE ratio before treatment correlated with the symptom-medication score in the SLIT group and that patients with low sIgE/tIgE ratios were more responsive to SLIT in treatment for Japanese pollinosis.[9]

We examined the immunological parameters, including IL-10 productivity, in prophylactic SLIT in asymptomatic subjects sensitized to Japanese cedar pollen.

Methods

Study population

This study was conducted as part of a randomized, double-blind, placebo-controlled, multiple center trial in asymptomatic subjects sensitized to Japanese cedar pollen (JCP), and the present results were based only on our institution. The study was performed for two pollen seasons from December 2011 to April 2013. We recruited 29 participants with IgE specific to JCP of at class 2 and higher without history of symptomatic pollinosis during JCP scattering. Japanese cedar pollen-specific IgE titers and total IgE in the serum were measured by CAP-FEIA (fluorescent enzyme immunoassay) (Phadia, Tokyo, Japan) before the study. Participants who were pregnant, breastfeeding or suffering from chronic rhinosinusitis were excluded.

Ethics statement

This study adhered to the tenets of the Declaration of Helsinki, and was approved by Mie University, Graduate School of Medicine Ethical Committee (No. 2283). A written informed consent was obtained from each subject before study.

Clinical protocols

The enrolled candidates were randomized into two groups by age and the levels of Cry j 1-specific IgE. The SLIT group received standardized JCP extract (Torii Pharmaceutical Co. Ltd., Tokyo, Japan),[10] and the placebo group received an inactive placebo. The protocol consisted of treatments with graded courses of the extract in 50% glycerol, followed by maintenance therapy.[11] Briefly, the extracts were graded in two concentrations: 200 and 2000 JAU/ml. From early December, the subjects received increasing doses beginning with 0.2 ml of the 200 JAU/ml vial and increasing by 0.2 ml every second day until reaching the maintenance dose of 1.0 ml of the 2000 JAU/ml for two weeks. From the third week, they received the maintenance dose of 1.0 ml of the 2000 JAU/ml daily until the end of April in the following year. The vaccine was taken sublingually, kept for 2 min without a retention reagent and then swallowed. The subjects in the placebo group received inactive 50% glycerol in saline.

Clinical symptoms and safety measurements

The subjects completed a pollinosis diary to record their nasal and eye symptoms and their use of symptom-reducing drugs. Development of the symptoms was determined on the basis of the pollinosis diary and a nasal provocation test performed at the end of April. The total amounts of pollen scattered from the Japanese cedar and Japanese cypress (*Chamaecyparis obtusa*) in Tsu city, Mie Prefecture, were 7031 and 16,578 grains/cm^2 during 2012 and 2013 pollen seasons, respectively.

Total and antigen-specific immunoglobulin titer

The levels of Cry j 1-specific IgE and total IgE in serum were measured by CAP-FEIA (fluorescent enzyme immunoassay) (Phadia, Tokyo, Japan).

Blood samples and PBMC culture

Peripheral blood was obtained from each subject before and after treatment (December and April) each year. Peripheral blood mononuclear cells (PBMC) were isolated from 10 ml of heparinized venous blood by density gradient centrifugation using Ficoll 1077 (Sigma, St. Louis, MO, USA). PBMC were cultured in RPMI 1640 medium (Nikken Bio Medical Laboratory, Kyoto, Japan) containing L-glutamine supplemented with 100 U/mL penicillin, 100 U/mL streptomycin (Invitrogen, Carlsbad, CA, USA) and l0% Human AB serum (Gemini Bio-Products, West Sacramento, CA, USA). Cells were plated onto 24-well tissue culture plates at a density of 2×10^6 cells/mL/well and were incubated with 10 JAU of Cry j1 (Torii, Tokyo, Japan) for 8 h at 37 °C in an atmosphere of 5% CO_2. Endotoxin level was confirmed to be less than 0.1 ng/μg (1 EU/μg) of the protein in Cry j1.

IL-10 staining in T cells, B cells and monocytes

After 8 h cultivation with antigens, PBMC were collected and incubated with PE-conjugated IL-10 secretion assay kit according to the manufacturer's instructions (Miltenyi Biotec, Auburn, CA, USA). Cells were also co-stained with anti-CD4-FITC and CD19-PECy5 antibodies, or anti-CD14-FITC antibody (eBioscience, Sam Diego, CA, USA). The percentage of IL-10 producing $CD4^+$ T cells, B cells and monocytes were determined using an Accuri C6 flow cytometer (Becton Dickinson, Mansfield, MA).

Statistical analysis

Two-group comparisons were performed using a Wilcoxon test or Mann–Whitney *U*-test to determine the significance of differences, or using an unpaired *t*-test as indicated. A p value of less than 0.05 was considered statistically significant.

Results

Clinical effects

Two subjects withdrew during the course of the study. The demographic characteristics of the 27 subjects before treatment are shown in Table 1. Clinical data from participants in 2012 and 2013 are shown in Table 2. As shown in Table 1, mean age of placebo group is higher compared to that of SLIT group; however, IL-10 production form T cells or monocytes is unchanged among