Allergology International. 2008;57:397-403
DOI: 10.2332/allergolint.O-08-541

ORIGINAL ARTICLE

Oral Administration of Heat-Killed *Lactobacillus gasseri* OLL2809 Reduces Cedar Pollen Antigen-Induced Peritoneal Eosinophilia in Mice

Toshihiro Sashihara[1], Shuji Ikegami[1], Natsuko Sueki[1], Taketo Yamaji[1], Kohsuke Kino[1], Naoki Taketomo[1], Minoru Gotoh[2] and Kimihiro Okubo[3]

ABSTRACT

Background: *Lactobacillus gasseri* OLL2809 strongly stimulates the production of interleukin (IL)-12 (p70) by innate immune cells. Thus, it is expected to ameliorate allergic diseases. We investigated whether the oral administration of heat-killed *L. gasseri* OLL2809 suppressed eosinophilia in cedar pollen antigen-challenged mice.

Methods: BALB/c mice sensitized with Japanese cedar pollen extract were intraperitoneally challenged with the same extract. The mice were orally given heat-killed *L. gasseri* OLL2809 at doses of 0.5, 1, or 2 mg/day throughout the experimental period (21 d). After 24 hours of the challenge, the eosinophil number and cytokine levels in the peritoneal lavage fluid and the serum antigen-specific IgG levels were determined.

Results: On administering varying amounts of heat-killed *L. gasseri* OLL2809, the number of eosinophils among the total number of cells was significantly reduced in all groups. In addition, the eosinophil number significantly decreased, and the eosinophil-suppression rate significantly increased by 44% in the 2-mg group. Although the serum immunoglobulin (Ig) G2a and IgG1 levels were not affected, the IgG2a/IgG1 ratio increased significantly in the 2-mg group compared with that of the control group. Furthermore, the administration of heat-killed *L. gasseri* OLL2809 resulted in the induction of IL-2 and reduction in granulocyte-macrophage colony-stimulating factor levels in peritoneal lavage fluid.

Conclusions: We demonstrated that the oral administration of heat-killed *L. gasseri* OLL2809 suppresses eosinophilia via the modulation of Th1/Th2 balance. These observations suggested that heat-killed *L. gasseri* OLL2809 might potentially ameliorate the increased number of eosinophils in patients with Japanese cedar pollinosis.

KEY WORDS

eosinophil, *Lactobacillus gasseri*, probiotics, Th1/Th2 balance

INTRODUCTION

In the past decade, the number of patients suffering from allergic diseases such as atopic dermatitis, allergic asthma, and allergic rhinitis has increased in many countries. In Japan, in particular, approximately 15% of the population is reported to suffer from cedar pollinosis.[1] Cedar pollinosis significantly reduces the quality of life of patients and is therefore becoming a matter of serious concern in Japan.[2] While immediate hypersensitivity, including pollinosis, is generally characterized by an elevation in the immunoglobulin

[1]Food Science Institute, Division of Research and Development, Meiji Dairies Corporation, Kanagawa, [2]Department of Otolaryngology, Nippon Medical School, Chiba Hokusoh Hospital, Chiba and [3]Department of Otorhinolaryngology, Nippon Medical School, Tokyo, Japan.
Correspondence: Toshihiro Sashihara, Ph. D., Food Science Institute, Division of Research and Development, Meiji Dairies Corporation, 540 Naruda, Odawara, Kanagawa 250-0862, Japan.
Email: toshihiro_sashihara@meiji-milk.com
Received 7 February 2008. Accepted for publication 4 June 2008.

(Ig) E levels,[3] local or systemic increase in the number of eosinophils rather than increased IgE levels is related to the severity of the symptoms.[4-6] Eosinophils act as the effector cells, not only by functioning as antigen-presenting cells[7] but also by producing various cytokines/chemokines[8,9]; they penetrate and injure the local mucosa and produce chemical mediators such as major basic proteins, eosinophilic cationic proteins, and leukotrienes.[10,11]

Local and systemic increase in the eosinophil number has been attributed to a shift in the balance between type I helper T (Th1) cells and type II helper T (Th2) cells.[12-14] Th1 cells produce cytokines such as interferon-gamma (IFN-γ) to promote cellular immunity, while Th2 cells produce cytokines such as interleukin (IL)-4 and IL-5 to promote humoral immunity through IgE production.[15] IL-5, which is produced by Th2 cells as well as by eosinophils in an autocrine fashion,[16] contributes to cell differentiation, proliferation, and maturation of eosinophils, thereby stimulating eosinophilic inflammation.[17] It could therefore be possible to suppress not only IgE production but also eosinophilic inflammation by shifting the Th1/Th2 balance from Th2-dominant immunity toward Th1-dominant immunity.

Probiotics are functional foods containing potentially beneficial microorganisms.[18,19] Because they exert various health-promoting effects, including the amelioration of irritable bowel disease, constipation, and diarrhea, animal and clinical studies on probiotics have been conducted extensively.[20] For example, some strains of lactic acid bacteria, often utilized as probiotics, exhibit immunostimulatory effects in the host even when administered as heat-killed organisms.[21,22] *Lactobacillus gasseri* OLL2809 is one of the probiotic lactobacilli that have been selected from 273 strains isolated from humans on the basis of their immunostimulatory activity. We previously reported that the oral administration of heat-killed *L. gasseri* OLL2809 effectively reduces serum antigen-specific IgE levels in mice via the stimulation of IL-12 (p70) production in splenocytes.[23,24] Moreover, it shifts the Th1/Th2 balance from Th2-dominant immunity toward Th1-dominant immunity. These effects are more prominent in the case of *L. gasseri* OLL2809 even when compared with other lactobacilli, including their type strains.[23] Therefore, it is expected that heat-killed *L. gasseri* OLL2809 suppressed the increase in the accumulation of eosinophils stimulated by antigen challenge.

In this study, we investigated whether the oral administration of heat-killed *L. gasseri* OLL2809 suppressed the accumulation of eosinophils stimulated by Japanese cedar pollen extract.

METHODS

MICROORGANISM

In this study, we used *L. gasseri* OLL2809, which was isolated from the feces of Japanese subjects and previously selected on the basis of its immunostimulatory activity.[23] The organism was cultured in Lactobacilli MRS broth (Becton Dickinson, Sparks, MD) at 37℃ for 18 hours. After fermentation, the cells were harvested in a refrigerated centrifuge (10,000 × *g*, 15 minutes) and washed twice with saline solution followed by 1 wash with water. The cells were resuspended in distilled water, heat-killed at 75℃ for 60 minutes, and lyophilized.

MICE

Five-week-old specific-pathogen-free female BALB/c mice (9 or 10 per group) were purchased from Japan SLC (Shizuoka, Japan) and maintained on a standard diet (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan). The experimental protocols were approved by the Animal Care Committee of the Division of Research and Development, Meiji Dairies Corporation.

EXPERIMENTAL SCHEDULE

The animal model was prepared according to the procedure of Kaneko *et al.*,[25] with minor modifications. In brief, BALB/c mice were sensitized with an extract of Japanese cedar pollen which was diluted 50-fold with saline (pH 7.2). We subcutaneously injected 0.2 ml of the diluted extract, which contained a final concentration of 0.2 μg/ml Cry j 1, one of the major antigens in Japanese cedar pollen, into the dorsal portion of the mice on days 0, 1, 4, 6, 8, and 14 (Fig. 1). On day 20, the mice were challenged with an intraperitoneal injection of 0.2 ml of the same diluted extract. Normal (non-sensitized) mice were prepared with subcutaneous injection of saline instead of the cedar pollen extract and were challenged in the same manner as described above. Throughout the experimental period (21 days), the mice were orally given heat-killed *L. gasseri* OLL2809 by gastric gavage at doses of 0.5, 1, or 2 mg/body/day (5 × 10^8 cells/mg) in 0.5 ml distilled water. Mice in both the normal and control (sensitized and challenged with an extract of Japanese cedar pollen) groups were given only distilled water.

The mice were anaesthetized with diethyl ether 24 hours after the challenge; sera were collected from the tail veins to determine antigen-specific IgG levels. The mice were then sacrificed by cervical dislocation, and peritoneal cells were harvested to count the accumulated eosinophils.

EOSINOPHIL COUNT

Peritoneal cells were harvested from the peritoneal lavage fluid after injecting 5 ml of phosphate-buffered saline (PBS; pH 7.2) containing 1.0% fetal calf serum (Intergen, Purchase, NY). On a microscope slide, 50 μl peritoneal cell suspension that was adjusted to a cell concentration of approximately 2 × 10^5 cells/ml was smeared using Cytospin 2 apparatus (Shandon,

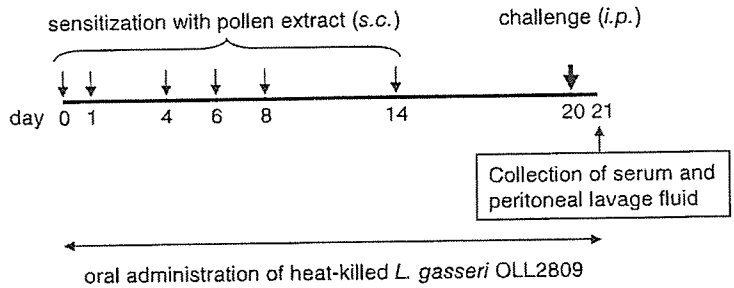

Fig. 1 Experimental schedule for the preparation of Japanese cedar pollen antigen-induced peritoneal eosinophilia in BALB/c mice. Sensitization was performed by injecting an extract of Japanese cedar pollen in the dorsal portion of mice. Normal (non-sensitized) mice were injected with saline instead of the cedar pollen extract.

Table 1 Effect of oral administration of heat-killed *Lactobacillus gasseri* OLL2809 on peritoneal eosinophilia

Groups	*n*	Total cells ($\times 10^5$ cells/ml)	Eosinophils ($\times 10^5$ cells/ml)	Relative proportion of Eosinophils (%)	Eosinophil-suppression rate (%)
Normal	10	8.5 ± 1.2	0.18 ± 0.05	1.9 ± 0.3	100 ± 1
Control	9	16.7 ± 2.1**	4.30 ± 0.70**	25.5 ± 1.6**	0 ± 17**
0.5 mg	10	18.7 ± 2.1	3.29 ± 0.54	17.2 ± 2.0##	25 ± 13
1 mg	10	19.4 ± 1.6	3.35 ± 0.35	17.6 ± 1.9#	23 ± 9
2 mg	10	15.8 ± 1.7	2.48 ± 0.35#	16.2 ± 1.6##	44 ± 9#

Data are expressed as mean ± SEM.

Cell counts indicate cell numbers in the collected peritoneal lavage fluid.

Relative proportion of eosinophils represents the number of eosinophils among the total number of cells.

**: $p < 0.01$, as compared with the normal group by using Student's *t* test.

#, ##: $p < 0.05$, 0.01, respectively, as compared with the control group by using Dunnet's multiple comparison tests.

The same experiment was performed twice, producing similar results, and 1 representative result is shown.

Pittsburgh, PA) at 700 rpm for 1 minute. After fixation and staining with Microscopy Hemacolor (Merck kGaA, Darmstadt, Germany), eosinophils were counted under a microscope. The eosinophil-suppression rate was calculated as follows: eosinophil suppression rate (%) = {1 – (number of eosinophils in individual mice in each group – mean eosinophil number in the normal group)/(mean eosinophil number in the control group – mean eosinophil number in the normal group) } × 100.

MEASUREMENT OF CYTOKINES AND Cry j 1-SPECIFIC IgG1, IgG2a, AND IgE LEVELS

The concentrations of 8 cytokines in the peritoneal lavage fluid were simultaneously determined using Mouse Cytokine Th1/Th2 Panel (Bio-Rad, Hercules, CA) and Bio-Plex 200 system (Bio-Rad). Transforming growth factor (TGF)-β1 levels were measured using a commercially available enzyme-linked immunosorbent assay kit (Promega, Mannheim, Germany). The detection limit of TGF-β1 was 15.6 pg/ml. We strictly followed the procedures recommended by the manufacturer.

Serum Cry j 1-specific IgG1, IgG2a, and IgE levels were measured, as described by Kozutsumi *et al.*[26]

STATISTICAL ANALYSIS

Data were expressed as mean ± standard error of the mean (SEM). Statistical differences between the normal and control groups were analyzed with Student's *t* test or Mann-Whitney's U test. Differences between the control group and the groups given heat-killed *L. gasseri* OLL2809 were analyzed with Dunnet's multiple comparison tests. The differences were considered significant when the *p* value was less than 0.05.

RESULTS

EFFECT OF *L. gasseri* OLL2809 ON PERITONEAL ACCUMULATION OF EOSINOPHILS

We first examined the effect of sensitization with Japanese cedar pollen extract on the accumulation of cells in the peritoneal cavity. As summarized in Table 1, the total number of cells and eosinophils in the normal group was 8.5 ± 1.2 × 10^5 and 0.18 ± 0.05 × 10^5 cells/ml respectively, while that in the control group was 16.7 ± 2.1 × 10^5 and 4.30 ± 0.70 × 10^5 cells/ml re-

Table 2 Effect of oral administration of heat-killed *Lactobacillus gasseri* OLL2809 on serum anti-Cry j 1-specific IgG1, serum anti-Cry j 1-specific IgG2a (pg/ml) and their ratio

			Lactobacillus gasseri OLL2809		
Groups	Normal	Control	0.5 mg	1 mg	2 mg
n	10	9	10	10	10
IgG2a	221 ± 29	168 ± 28	197 ± 2	203 ± 3	226 ± 61
IgG1	138 ± 21	219 ± 33	140 ± 2	144 ± 2	153 ± 8
IgG2a/IgG1	1.39 ± 0.01	0.75 ± 0.02*	1.40 ± 0.01	1.42 ± 0.03	1.81 ± 0.46#

Data are expressed as mean ± SEM.

*: $p < 0.05$, as compared with the normal group by using Student's *t* test.

#: $p < 0.05$, as compared with the control group by using Dunnet's multiple comparison tests.

The same experiment was performed twice, producing similar results, and 1 representative result is shown.

spectively. Thus, the total number of cells, the number of eosinophils, and the relative proportion of eosinophils in the control group increased significantly ($p < 0.01$) as compared with the findings in the normal group. The relative proportion of eosinophils increased from 1.9 ± 0.3% in the normal group to 25.5 ± 1.6% in the control group. It could therefore be confirmed that the eosinophil-accumulation model was successfully prepared.

When varying amounts of heat-killed *L. gasseri* OLL2809 were given to the eosinophilia- induced, the relative proportion of eosinophils reduced significantly ($p < 0.05$ or 0.01) in all groups compared with that of the control group. While there was no difference in the total number of cells in the groups subjected to allergen sensitization and challenge, the eosinophil number decreased significantly in the 2-mg group compared with that of the control group, and the eosinophil-suppression rate was significantly increased by 44%.

These results indicated that the oral administration of heat-killed *L. gasseri* OLL2809 to the cedar pollen-challenged mice reduced eosinophil accumulation in the peritoneal cavity.

EFFECT OF *L. gasseri* OLL2809 ON SERUM ANTI-Cry j 1-SPECIFIC IgG2a/IgG1 RATIO

To further investigate the effects administering of heat-killed *L. gasseri* OLL2809, the levels of serum anti-Cry j 1-specific IgG1 and anti-Cry j 1-specific IgG2a were measured. No distinct differences could be observed in these levels even between the normal and control groups (Table 2). However, the ratio between the IgG2a and IgG1 levels was significantly ($p < 0.05$) lower in the control group than in the normal group. On the other hand, this ratio was significantly higher ($p < 0.05$) in the 2-mg group. These data clearly demonstrated that in the eosinophilia-induced mice the administration of heat-killed *L. gasseri* OLL2809 promoted a shift in the systemic Th1/Th2 balance from Th2-dominant immunity toward Th1-dominant immunity. The serum anti-Cry j 1-specific IgE levels were under the detection limit in all groups.

EFFECT OF *L. gasseri* OLL2809 ON CYTOKINE LEVELS IN PERITONEAL LAVAGE FLUID

We determined the serum levels of cytokines related to Th1/Th2 balance, such as those of IL-2, IL-4, IL-5, IL-10, IL-12 (p70), IFN-γ, TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), and TGF-β1. Although IL-5 and GM-CSF levels significantly ($p < 0.05$) increased in the control group compared with those in the normal group, no significant changes were found in the groups given heat-killed *L. gasseri* OLL2809 when compared with the control group (data not shown).

We have therefore analyzed cytokine levels in peritoneal lavage fluid. In contrast to serum cytokine levels, while most cytokine levels in the peritoneal lavage fluid in the normal group were under the detection limit, they were significantly increased in the control group except for TGF-β1 (Table 3). This observation suggested that not only eosinophils but also other mononuclear cells, including both Th1 and Th2 cells, migrated to local inflammatory sites and were activated by the intraperitoneally injected antigens.

Oral administration of heat-killed *L. gasseri* OLL2809 resulted in the induction of a Th1-derived cytokine, specifically, IL-2 which was observed in the 0.5- and 1-mg groups. On the other hand, the levels of GM-CSF, which enhances the proliferation of eosinophils,[27] were lower in the 1- and 2-mg groups than in the control group. In particular, the GM-CSF level showed a positive correlation to the number of eosinophils in the peritoneal lavage fluid; and the correlation was statistically significant (Fig. 2A, $p < 0.01$). Although the change in IL-5 levels was not statistically significant, it was comparable to that of the GM-CSF levels since the IL-5 levels decreased as the amount of the administered *L. gasseri* OLL2809 increased. Moreover, these levels were positively correlated with the eosinophil number as well as the GM-CSF level (Fig. 2B, $p < 0.01$). TGF-β1 was not de-

Table 3 Effect of oral administration of heat-killed *Lactobacillus gasseri* OLL2809 on the production of cytokines in the peritoneal lavage fluid

Groups	Normal	Control	*Lactobacillus gasseri* OLL2809		
			0.5 mg	1 mg	2 mg
n	10	9	10	10	10
IL-2	2.2 ± 0.7	8.0 ± 0.8**	12.7 ± 0.6#	11.3 ± 0.8#	9.7 ± 1.0
IL-4	n.d.	48.4 ± 15.3 **	40.9 ± 14.8	21.7 ± 3.5	29.3 ± 13.3
IL-5	n.d.	173 ± 42 **	184 ± 57	114 ± 33	76 ± 8
IL-10	n.d.	12.2 ± 1.5 **	14.3 ± 3.1	13.6 ± 1.3	10.2 ± 2.5
IL-12 (p70)	2.5 ± 0.9	17.5 ± 2.7 **	24.1 ± 5.5	36.1 ± 14.5	20.3 ± 3.0
GM-CSF	n.d.	40.6 ± 7.0 **	29.1 ± 8.5	15.7 ± 6.4#	10.5 ± 3.4#
IFN-γ	n.d.	5.04 ± 2.34 **	1.34 ± 1.17	8.65 ± 8.65	n.d.
TNF-α	4 ± 2	75 ± 12 **	100 ± 14	104 ± 27	54 ± 6
TGF-β1	n.d.	n.d.	n.d.	n.d.	n.d.

Data are expressed as mean ± SEM (pg/ml).

n.d.: not detected.

**: $p < 0.01$, as compared with the normal group by using Mann-Whitney's U test.

#: $p < 0.05$, as compared with the control group by using Dunnet's multiple comparison tests.

The same experiment was performed twice, producing similar results, and 1 representative result is shown.

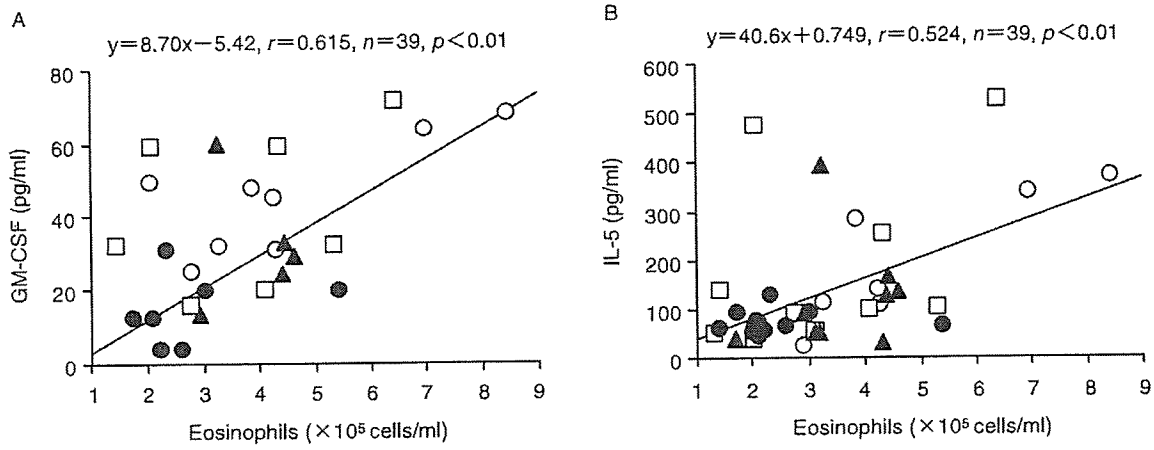

Fig. 2 Correlation between the number of eosinophils and GM-CSF (**A**) or IL-5 (**B**) levels in peritoneal lavage fluid. Symbols: ○, Control; □, 0.5 mg; ▲, 1 mg; and ●, 2 mg groups.

tected in any of the mice examined in this study.

In general, these cytokine-expression profiles strongly suggested that the suppression of eosinophilia in peritoneal lavage fluid was associated with the local Th1/Th2 balance.

DISCUSSION

We previously reported that the oral administration of heat-killed *L. gasseri* OLL2809 to ovalbumin-sensitized mice reduced the serum ovalbumin-specific IgE levels, which was associated with the enhancement of IL-12 (p70) production in splenocytes and the reduction of IL-4 production in both splenocytes and mesenteric lymph node cells.[23] In this study, we demonstrated that heat-killed *L. gasseri* OLL2809 suppressed not only antigen-specific IgE levels, as reported previously,[23] but also the local accumulation of eosinophils in mice sensitized with Japanese cedar pollen extract. In addition, this effect was found to be associated with a shift in the local and systemic Th1/Th2 balance, as demonstrated by cytokine levels in the peritoneal lavage fluid and the serum-Cry j 1-specific IgG2a/IgG1 ratio, respectively.

Eosinophils from patients with allergies, such as asthma, exhibit increased migratory responses, adhesiveness, and degranulation compared with those from normal subjects.[27] The priming of the activation of eosinophils is caused by IL-5 and GM-CSF.[27-29] On the other hand, IL-2, which has been widely known to aid T cell proliferation and clonal expansion,[30] has an

inhibitory effect on the migration of eosinophils toward chemotactic factors.[31,32] Therefore, the cytokine-expression profiles observed in the peritoneal lavage fluid appeared to correspond to the serum-Cry j 1-specific IgG2a/IgG1 ratio and the eosinophil number. Heat-killed *L. gasseri* OLL2809 strongly stimulate the production of IL-12 (p70) by murine macrophages and dendritic cells[23,24] and was therefore expected to induce the proliferation of Th1 cells from naïve T cells, consequently affecting the Th1/Th2 balance. However, both IL-2 and GM-CSF are secreted by various cells other than Th1 and Th2 cells, including eosinophils, in an autocrine fashion[9]; therefore we could not determine whether the suppression of eosinophilia was caused by the modification of these cytokine levels or as a consequence of a reduction in eosinophil number due to other factors.

Recently, numerous studies have indicated that regulatory T cells are strongly associated with allergic disorders.[33] Regulatory T cells are subsets of T cells and are involved in the maintenance of peripheral self-tolerance by actively suppressing the activation and proliferation of autoreactive T cells.[33] Several types of regulatory T cells have been characterized; Th3 and Tr1 cells have been implicated in regulatory T cell function through the production of immunosuppressive cytokines such as TGF-β and IL-10.[33] As shown in this study, the levels of nearly all cytokines in the peritoneal lavage fluid were significantly higher in the control group than in the normal group. This clearly indicates that not only Th2 cells but also Th1 cells were attracted to the inflammatory site and were activated by sensitization with the Japanese cedar pollen antigens. However, since TGF-β1 levels in the peritoneal lavage fluid were under the detection limit, and no obvious influence could be seen even in the normal and control groups, and in addition, IL-10 levels were not affected by the administration of heat-killed *L. gasseri* OLL2809, it can therefore be stated that the results of this experiment do not implicate regulatory T cells in the reduction of eosinophilia.

The effective component in *L. gasseri* OLL2809 has not been isolated and therefore much remains controversial. Nevertheless, peptidoglycan is likely to be one of the most effective components, as reported previously.[23,24] The effectiveness of microbial peptidoglycan has been reported even in epidemiological research since it has been found to be inversely associated with the prevalence of allergic disorders.[34] Recently, it is becoming clear that commensal microflora are recognized by toll-like receptors (TLRs) expressed on the intestinal epithelial cells, including microfold (M) cells in Peyer's patches.[35,36] Signaling cascades are initiated via TLRs to activate natural immune responses and induce the production of cytokines such as TNF-α and IL-12 (p70).[37,38] It can therefore be speculated that the administered heat-killed *L. gasseri* OLL2809 was incorporated into these immune cells at specific sites, inducing immunomodulatory activity, as shown in this study. Further studies are required to investigate the effective component of *L. gasseri* OLL2809 and to discuss this issue in detail.

In conclusion, we have shown that the oral administration of heat-killed *L. gasseri* OLL2809 suppresses the peritoneal accumulation of eosinophils in mice sensitized with Japanese cedar pollen extract. This effect was the result of a shift in the balance between Th1 and Th2 cells. These observations suggest that heat-killed *L. gasseri* OLL2809 can potentially ameliorate the clinical symptoms of Japanese cedar pollinosis. However, in the allergic animal model used in this study, sensitization and antigen challenge were not performed in the nasal mucosa, where both the inductive and effecter phases of immune reaction occur in pollinosis; which was conducted to clearly observe the effect of *L. gasseri* OLL2809. In addition, the above mentioned methods did not induce allergic symptoms such as scratching and sneezing. In our next study, we hope to determine whether heat-killed *L. gasseri* OLL2809 ameliorates allergic symptoms in allergic rhinitis animal models and investigate in further detail the mechanism by which it affects Th1/Th2 balance.

ACKNOWLEDGEMENTS

We thank Drs. Masako Tsunematsu and Daisuke Kozutsumi for providing valuable suggestions and technical assistance for measuring the peritoneal eosinophils and serum anti-Cry j 1-specific Igs.

REFERENCES

1. Okuda M. Epidemiology of Japanese cedar pollinosis throughout Japan. *Ann Allergy Asthma Immunol* 2003;**91**: 288-96.
2. Okubo K, Gotoh M, Shimada K, Ritsu M, Okuda M, Crawford B. Fexofenadine improves the quality of life and work productivity in Japanese patients with seasonal allergic rhinitis during the peak cedar pollinosis season. *Int Arch Allergy Immunol* 2005;**136**:148-54.
3. Dreborg S. The implications of nomenclature. *Ann Allergy Asthma Immunol* 2002;**89**:83-5.
4. Adachi T, Motojima S, Hirata A, Fukuda T, Makino S. Eosinophil viability-enhancing activity in sputum from patients with bronchial asthma, contributions of interleukin-5 and granulocyte/macrophage colony-stimulating factor. *Am J Respir Crit Care Med* 1995;**151**:618-23.
5. Prehn A, Seger RA, Faber J *et al.* The relationship of serum-eosinophil cationic protein and eosinophil count to disease activity in children with bronchial asthma. *Pediatr Allergy Immunol* 1998;9:197-203.
6. Ciprandi G, Vizzaccaro A, Cirillo I, Tosca M, Massolo A, Passalacqua G. Nasal eosinophils display the best correlation with symptoms, pulmonary function and inflammation in allergic rhinitis. *Int Arch Allergy Immunol* 2005; **136**:266-72.
7. Weller PF, Rand TH, Barrett T, Elovic A, Wong DT, Finberg RW. Accessory cell function of human eosinophils. HLA-dependent, MHC-restricted antigen-presentation and IL-1 alpha expression. *J Immunol* 1993;**150**:2554-62.

8. Ying S, Meng Q, Taborda-Barata L *et al.* Human eosinophils express messenger RNA encoding RANTES and strore and release biologically active RANTES protein. *Eur J Immunol* 1996;**26**:70-6.
9. Woerly G, Roger N, Loiseau S, Dombrowicz D, Capron A, Capron M. Expression of CD28 and CD86 by human eosinophils and role in the secretion of type 1 cytokines (interleukin 2 and interferon gamma): inhibition by immunoglobulin a complexes. *J Exp Med* 1999;**190**:487-95.
10. Gleich GJ. Mechanisms of eosinophil-associated inflammation. *J Allergy Clin Immunol* 2000;**105**:651-63.
11. Piliponsky AM, Gleich GJ, Bar I, Levi-Schaffer F. Effects of eosinophils on mast cells: a new pathway for the perpetuation of allergic inflammation. *Mol Immunol* 2002;**38**: 1369-72.
12. Shirakawa T, Enomoto T, Shimizu S, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. *Science* 1997;**275**:77-9.
13. Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG. Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999;**353**: 196-200.
14. Hopkin JM. The rise of atopy and links to infection. *Allergy* 2002;**57**:5-9.
15. Kidd P. Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. *Altern Med Rev* 2003;**8**:223-46.
16. Huang CD, Wang CH, Liu CY *et al.* Eosinophils from asthmatics release IL-5 in an autocrine fashion to prevent apoptosis through upregulation of Bcl-2 expression. *J Asthma* 2005;**42**:395-403.
17. Greenfeder S, Umland SP, Cuss FM, Chapman RW, Egan RW. Th2 cytokines and asthma. The role of interleukin-5 in allergic eosinophilic disease. *Respir Res* 2001;**2**:71-9.
18. Fuller R. Probiotics in man and animals. *J Appl Bacteriol* 1989;**66**:365-78.
19. Salminen S, Bouley C, Boutron-Ruault MC *et al.* Functional food science and gastrointestinal physiology and function. *Br J Nutr* 1998;**80**:S147-71.
20. Rastall RA, Gibson GR, Gill HS *et al.* Modulation of microbial ecology of the human colon by probiotics, prebiotics and synbiotics to enhance human health: an overview of enabling science and potential applications. *FEMS Microbiol Ecol* 2005;**52**:145-52.
21. Cross ML, Gill HS. Can immunoregulatory lactic acid bacteria be used as dietary supplements to limit allergies? *Int Arch Allergy Immunol* 2001;**125**:112-9.
22. Cross ML, Stevenson LM, Gill HS. Anti-allergy properties of fermented foods: an important immunoregulatory mechanism of lactic acid bacteria? *Int Immunopharmacol* 2001;**1**:891-901.
23. Sashihara T, Sueki N, Ikegami S. An analysis of heat-killed lactic acid bacteria in alleviating allergic diseases. *J Dairy Sci* 2006;**89**:2846-55.
24. Sashihara T, Sueki N, Furuichi K, Ikegami S. Effect of growth conditions of *Lactobacillus gasseri* OLL2809 on the immunostimulatory activity for production of interleukin-12 (p70) by murine splenocytes. *Int J Food Microbiol* 2007;**120**:274-81.
25. Kaneko M, Hitoshi Y, Takatsu K, Matumoto S. Role of interleukin-5 in local accumulation of eosinophils in mouse allergic peritonitis. *Int Arch Allergy Appl Immunol* 1991;**96**:41-5.
26. Kozutsumi D, Tsunematsu M, Yamaji T, Murakami R, Yokoyama M, Kino K. PS80 interferes with the antiallergic effect of Cry-consensus peptide, a novel recombinant peptide for immunotherapy of Japanese cedar pollinosis, at very low concentration through modulation of Th1/Th2 balance. *Immunollogy* 2006;**118**:392-401.
27. Lampinen M, Carlson M, Håkansson LD, Venge P. Cytokine-regulated accumulation of eosinophils in inflammatory disease. *Allergy* 2004;**59**:793-805.
28. Bruijnzeel PL, Rihs S, Virchow JC Jr, Warringa RA, Moser R, Walker C. Early activation or "priming" of eosinophils in asthma. *Schweiz Med Wochenschr* 1992;**122**:298-301.
29. Stout BA, Bates ME, Liu LY, Farrington NN, Bertics PJ. IL-5 and granulocyte-macrophage colony-stimulating factor activate STAT3 and STAT5 and promote Pim-1 and Cyclin D3 protein expression in human eosinophils. *J Immunol* 2004;**173**:6409-17.
30. Linsley PS, Brady W, Grosmaire L, Aruffo A, Damle NK, Ledbetter JA. Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 mRNA accumulation. *J Exp Med* 1991;**173**:721-30.
31. Levi-Schaffer F, Barkans J, Newman TM *et al.* Identification of interleukin-2 in human peripheral blood eosinophils. *Immunology* 1996;**87**:155-61.
32. Lampinen M, Håkansson L, Venge P. Interleukin-2 inhibits eosinophil migration but is counteracted by IL-5 priming. *Clin Exp Allergy* 2001;**31**:249-58.
33. Chatila TA. Role of regulatory T cells in human diseases. *J Allergy Clin Immunol* 2005;**116**:949-59.
34. van Strien RT, Engel R, Holst O *et al.* Microbial exposure of rural school children, as assessed by levels of *N*-acetylmuramic acid in mattress dust, and its association with respiratory health. *J Allergy Clin Immunol* 2004;**113**:860-7.
35. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. *Cell* 2004;**118**:229-41.
36. Tyrer P, Foxwell AR, Cripps AW, Apicella MA, Kyd JM. Microbial pattern recognition receptors mediate M-cell uptake of a gram-negative bacterium. *Infect Immun* 2006; **74**:625-31.
37. Miettinen M, Lehtonen A, Julkunen I, Matikainen S. Lactobacilli and Streptococci activate NF-κB and STAT signaling pathways in human macrophages. *J Immunol* 2000; **164**:3733-40.
38. Michelsen KS, Aicher A, Mohaupt M *et al.* The role of toll-like receptors (TLRs) in bacteria-induced maturation of murine dendritic cells (DCs). *J Biol Chem* 2001;**276**: 25680-6.

Original Paper

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2009;149:239–245
DOI: 10.1159/000199719

Received: June 9, 2008
Accepted after revision: October 21, 2008
Published online: February 12, 2009

Re-Treatment with Omalizumab at One Year Interval for Japanese Cedar Pollen-Induced Seasonal Allergic Rhinitis Is Effective and Well Tolerated

Satoshi Ogino[a] Toshikazu Nagakura[b] Kimihiro Okubo[c] Norio Sato[d]
Masao Takahashi[d] Takeru Ishikawa[e]

[a]Graduate School of Allied Health Science, Faculty of Medicine, Osaka University, Osaka,
[b]Allergy and Internal Medicine, Yoga Allergy Clinic, [c]Department of Otorhinolaryngology, Nippon Medical School,
and [d]Clinical Development, Novartis Pharma KK, Tokyo, and [e]Kumamoto University School of Medicine,
Kumamoto, Japan

Key Words
Omalizumab · Seasonal allergic rhinitis · Anti-IgE antibody

Abstract
Background: Seasonal allergic rhinitis (SAR) induced by Japanese cedar pollens is a serious problem in Japan. Omalizumab, a humanized monoclonal anti-IgE antibody, improves symptoms associated with SAR, but a study comprehensively investigating the clinical efficacy, safety and pharmacological effects of omalizumab re-treatment has not yet been conducted. ***Methods:*** The open-label, 12-week study was carried out in 34 patients who had been treated with omalizumab in the core study conducted in the previous Japanese cedar pollen season. The study plan including study period, efficacy and safety endpoints, as well as dose regimen, was designed to be the same as in the core study. Omalizumab was administered subcutaneously every 2 or 4 weeks based on the serum IgE level and body weight of each patient. ***Results:*** Time course changes in daily nasal symptom medication scores as well as in daily ocular symptom medication scores throughout the pollen period were comparable with those in the omalizumab group in the core study. Serum free IgE levels decreased to below the target level in all patients and were equal to those in the omalizumab group in the core study. The adverse reaction profiles were similar to those in the core study. In addition, the overall incidence of drug-related adverse events and injection site reactions in the re-treatment study did not increase compared with those in the omalizumab group in the core study. There were no serious adverse events, and no anti-omalizumab antibodies were detected. ***Conclusion:*** Omalizumab was effective and safe when consecutively readministered in the second Japanese cedar pollen season.

Introduction

Seasonal allergic rhinitis (SAR), especially Japanese cedar pollen-induced SAR, is a considerable medical problem in Japan, afflicting approximately 20% of the Japanese people [1]. Symptoms can range from mild to seriously debilitating and can influence the quality of life by causing fatigue, headache, cognitive impairment and other systemic symptoms. Such symptoms can result in the loss of work productivity and in missed school days [2].

Omalizumab is a recombinant humanized monoclonal anti-IgE antibody currently approved in the US and Europe for treating inadequately controlled allergic asthma which selectively binds to the Cε3 domain of IgE that interacts with IgE receptors on effector cells [3]. This an-

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1018–2438/09/1493–0239$26.00/0

Accessible online at:
www.karger.com/iaa

Correspondence to: Prof. Dr. Satoshi Ogino
Graduate School of Allied Health Science
Faculty of Medicine, Osaka University
1-7, Yamadaoka, Suita-shi, Osaka 565-0871 (Japan)
Tel./Fax +81 6 6879 2543, E-Mail ogino@sahs.med.osaka-u.ac.jp

Table 1. Dosing tables for omalizumab administered every 4 (A) or every 2 weeks (B)

	Baseline IgE IU/ml	Body weight, kg				
		≥30–60	>60–70	>70–80	>80–90	>90–150
A	≥30–100	150	150	150	150	300
	>100–200	300	300	300	300	
	>200–300	300				
B	≥30–100					
	>100–200					225
	>200–300		225	225	225	300
	>300–400	225	225	300	300	
	>400–500	300	300	375	375	
	>500–600	300	375			
	>600–700	375				

Omalizumab doses are given in mg/dose.

tibody blocks the binding of IgE to high-affinity receptors, thereby preventing the IgE-mediated cellular responses [4].

We have previously demonstrated the noticeable clinical effects of this novel antibody on Japanese cedar pollen-induced moderate to severe SAR against placebo [5] and suplatast tosilate [6]. Because Japanese cedar pollen-induced SAR develops between February and April every year, we further tried to investigate the clinical efficacy, safety and pharmacological effects of re-treatment with omalizumab.

This open-label re-treatment study was an extension of the placebo-controlled core study which had been conducted in the previous year [5] and was undertaken during the following Japanese cedar pollen season in patients who had received omalizumab in the placebo-controlled core study. This is the first study to comprehensively investigate the clinical efficacy, safety and pharmacological effects of omalizumab re-treatment, and we have compared the results obtained from this re-treatment study with those from the core study.

Materials and Methods

Participants

Patients who met the following criteria were considered eligible for enrollment: (1) having received omalizumab in the core study conducted in the previous Japanese cedar pollen season; (2) serum total IgE levels of 30–700 IU/ml and body weights of 30–150 kg at baseline.

Patients who had a history or clinical status of the following were excluded from the study: (1) specific immunotherapy to Japanese cedar pollens in the previous 2 years; (2) severe anaphylactoid or anaphylactic reactions; (3) active or recent development (within 3 months prior to the study onset) of any other type(s) of rhinitis; (4) positive reaction to omalizumab in the skin test at screening; (5) pregnant/nursing women, and (6) serious medical conditions (e.g., cancer, hepatic failure and renal failure).

The present study was conducted in compliance with the current good clinical practice, and the protocol was approved by each institutional ethical committee. Prior to the onset of the study, written informed consent was obtained from all the patients who were enrolled.

Study Design

This open-label study was conducted in 2 regions of Japan (Tokyo and Osaka) between October 2002 and April 2003 and consisted of a 4-week screening period, a 12-week treatment period and a 12-week follow-up period after final dosing. The study design including study period, efficacy and safety endpoints, as well as dose regimen, was the same as in the core study.

Daily pollen counts were measured in Tokyo and Osaka. The first day of the pollen period was defined as the first of 2 consecutive days when ≥1 grain/cm^2 was counted; the last day of the pollen period was the first of 3 consecutive days when no grain was counted.

Doses and Administration

Omalizumab (150 or 300 mg every 4 weeks, or 225, 300 or 375 mg every 2 weeks) was administered to patients subcutaneously based on their serum total IgE level and body weight at baseline (table 1), which ensured a minimum dose of 0.016 mg/kg/IgE (IU/ml) every 4 weeks [7]. The initial dose was administered at least 1 month prior to the expected starting date of the pollen period.

The following drugs were permitted as rescue medications: for nasal use, clemastine fumarate (tablet), sodium cromoglycate (nose drop), naphazoline nitrate (nose drop), and for ocular use, sodium cromoglycate (eye drop). Agents, which were prohibited for use as concomitant drugs, were leukotriene antagonists, corticosteroids, antihistamines (except for rescue medications), anticholinergic agents, nasal vasoconstrictors (except for rescue medications), tricyclic antidepressants, and monoamine oxidase inhibitors. Specific immunotherapy was prohibited.

Evaluation of Efficacy

Patients enrolled were requested to fill in the patient diary in order to describe their 7 rhinoconjunctival symptoms (sneezing, itchy nose, runny nose, stuffy nose, itchy eyes, watery eyes, and red eyes) according to the 4-point scale (0 = none; 1 = mild; 2 = moderate; 3 = severe) and to document their rescue medication use, if any. Regarding each rescue medication, its usage was scored as 1 point regardless of dose and frequency.

The daily nasal symptom medication score (0–15 points) consisted of the sum of the daily nasal symptom severity score (0–12 points) and the daily nasal rescue medication score (0–3 points). The daily ocular symptom medication score (0–10 points) also consisted of the sum of the daily ocular symptom severity score (0–9 points) and the daily ocular rescue medication score (0–1 point).

Serum Total and Free IgE Levels

Blood samples were collected for the measurement of total and free IgE at baseline and at 2, 4, 8 and 12 weeks of the treatment period. Total and free IgE were measured by ELISA, as previously described [8].

Evaluation of Safety

Adverse events were examined throughout the treatment period. Clinical laboratory tests were conducted at baseline and at 2, 4, 8 and 12 weeks of the treatment period. Anti-omalizumab antibodies (IgG subtype) were measured by using solid-phase ELISA at baseline, as well as 12 weeks after the final dosing [9].

Statistical Analysis

In efficacy and serum free IgE analyses, patients who completed both the re-treatment and core studies without protocol violations were included. In safety and serum total IgE analyses, data from the patients in the core study who also enrolled in the re-treatment study were used to compare the re-treatment study data. Comparisons of serum total IgE were made using Wilcoxon's signed rank sum test. A 2-tailed p value <0.05 was considered statistically significant.

The safety and tolerability of the study drugs were summarized by appropriate descriptive methods.

Table 2. Patient characteristics in the omalizumab group (n = 34)

Gender	
Male/female	20/14
Age, years	
Mean ± SD	32.4 ± 11.9
Range	21 – 64
History of SAR induced by Japanese cedar pollens, years	
Mean ± SD	12.5 ± 6.2
Range	5 – 36
Specific IgE levels against Japanese cedar pollens (CAP-RAST)[1]	
Class 2 (0.70–3.49 UA/ml)	7
Class 3 (3.50–17.49 UA/ml)	18
Class 4 (17.50–49.99 UA/ml)	9
Serum total IgE levels at baseline, IU/ml	
Mean ± SD	127.8 ± 110.9
Range	29.4 – 447.8

[1] Specific IgE levels against Japanese cedar pollens at baseline were categorized into 7 groups (classes 0–6), and a class ≥2 group was assessed to be positive against the allergen.

Results

Of the 48 patients treated with omalizumab in the core study, 34 patients entered this study. None of the patients showed a positive reaction to omalizumab in the skin test at screening. Only 1 patient discontinued the study due to adverse events, but none of the patients discontinued the study because of unsatisfactory therapeutic effect. Patient characteristics are shown in table 2. Thirty-one patients completed both the re-treatment and core study without protocol violations.

The pollen period started at the beginning of February and continued to the end of April, which was the same as in the previous year (fig. 1). The total amount of Japanese cedar pollens during the season in Tokyo and Osaka was as follows: re-treatment study, 3,542 grains/cm^2 for Tokyo and 1,855 grains/cm^2 for Osaka; core study, 5,648 grains/cm^2 for Tokyo and 913 grains/cm^2 for Osaka.

All patients received the first administration at least 3 weeks prior to the starting date of the pollen period.

Daily Nasal and Ocular Symptom Medication Score

Time course changes in daily nasal and ocular symptom medication scores are shown in figure 1a, b. Daily nasal symptom medication scores as well as daily ocular symptom medication scores throughout the pollen period were comparable with those in the omalizumab group in the core study and were markedly lower than those in the placebo group in the core study. Similar results were obtained with respect to mean daily nasal symptom medication scores as well as to mean daily ocular symptom medication scores during the pollen period (table 3). The use of the rescue medications was infrequent and was comparable with that in the omalizumab group in the core study (data not shown).

Baseline Serum Total IgE Levels

Baseline serum total IgE levels were comparable with those in the omalizumab group in the core study (median 88.6 IU/ml, range 29.4–447.8, for the re-treatment study, and median 82.5 IU/ml, range 36.2–441.0, for the core study; p = 0.09; fig. 2).

Serum Free IgE Levels

Serum free IgE levels decreased markedly, compared with the baseline levels, to below the target (50 ng/ml) at 2, 4, 8 and 12 weeks of the treatment period in all patients (range 4.2–36.7 ng/ml; fig. 3). Serum free IgE levels at 4 and 12 weeks were equal to those in the omalizumab group in the core study.

Safety

Re-treatment with omalizumab was generally well tolerated. The overall incidence of drug-related adverse events in the re-treatment study was lower than that in both the omalizumab group (table 4) and the placebo

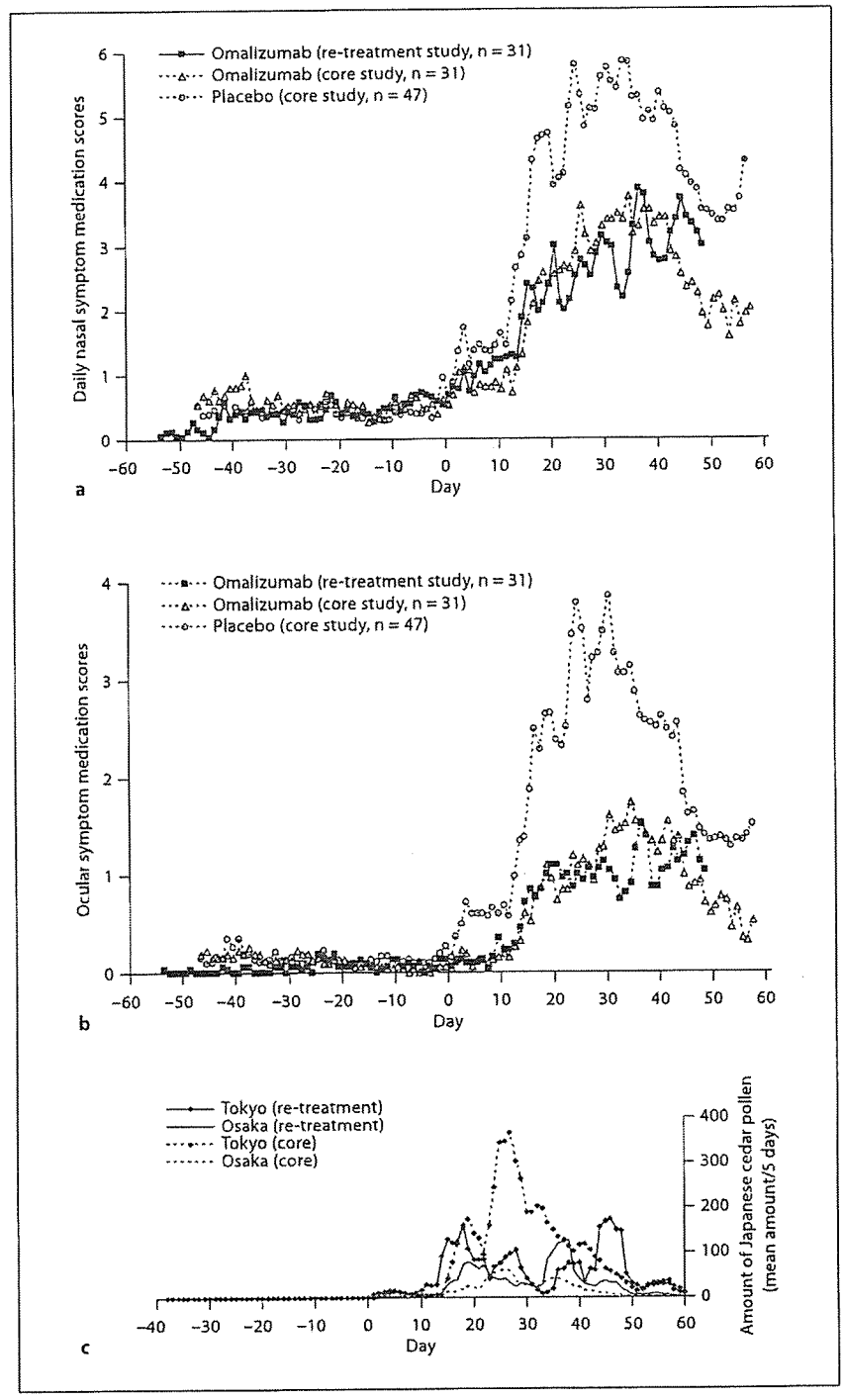

Fig. 1. Time course changes in daily nasal symptom medication score (**a**), daily ocular symptom medication score (**b**), and amount of Japanese cedar pollen (**c**). Day 0 represents the start day of the pollen period in Tokyo and Osaka.

Table 3. Mean daily nasal and ocular symptom medication scores (mean ± SE)

	Re-treatment study	Core study	
	omalizumab (n = 31)	omalizumab (n = 31)	placebo (n = 47)
Mean daily nasal symptom medication scores	2.345 ± 0.2736	2.206 ± 0.2723	3.753 ± 0.2484
Mean daily ocular symptom medication scores	0.820 ± 0.1459	0.795 ± 0.1562	1.904 ± 0.1642

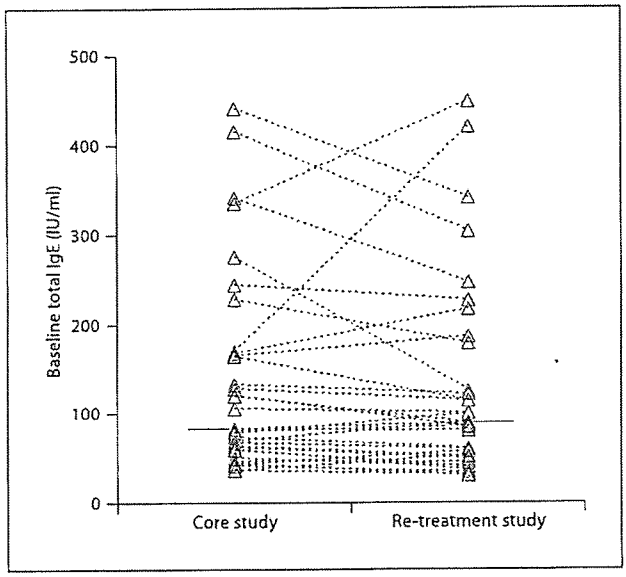

Fig. 2. Comparison of serum total IgE level of each patient (n = 34) at baseline between the re-treatment study and the core study (omalizumab group). Each symbol shows an individual value; horizontal bars are median values.

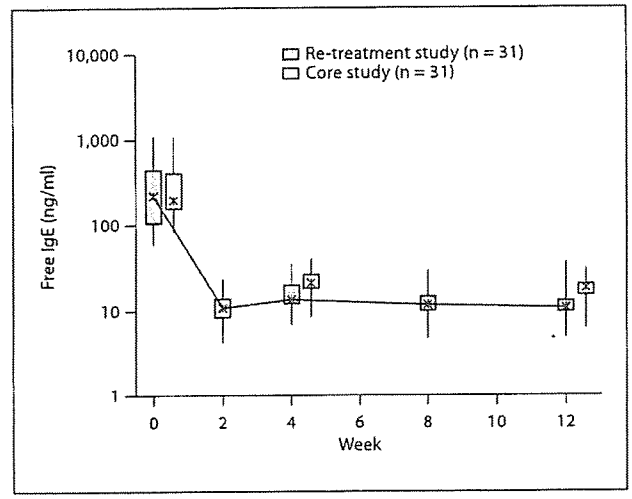

Fig. 3. Time course changes in serum free IgE levels (n = 31) in the re-treatment study (grey boxes) and in the core study (omalizumab group, white boxes). The boxes include the interval between the 25th and 75th percentiles. The extremes represent the minimum and maximum values. The median is shown as an asterisk. Day 0 represents the first day of therapy with omalizumab.

group (20.0%, 10/50) in the core study. The most frequently reported drug-related adverse events in both studies were injection site reactions, but the incidence rate in the re-treatment study was lower than that in the omalizumab group and higher than that in the placebo group (8.0%, 4/50) in the core study.

One patient discontinued treatment because of severe adverse events (injection site pain and injection site discomfort) which developed 23 days after the first dose of omalizumab and disappeared without additional treatment. The investigator considered their relations with the drug not denied because the causes were unclear. All adverse events except those two were of mild degree in the re-treatment study.

There were no anaphylactic reactions, and neither evidence of immune complex disease nor clinically important abnormalities in vital signs and laboratory tests were found. No anti-omalizumab antibodies were detected.

Discussion

This is the first study comprehensively investigating the clinical efficacy, safety and pharmacological effects of omalizumab re-treatment. Because Japanese cedar pollen-induced SAR develops between February and April every year, the results in this study seem valuable for the clinical use of omalizumab.

Daily nasal as well as ocular symptom medication scores throughout the pollen period were comparable with those in the omalizumab group and were markedly lower than those in the placebo group in the core study. As the amount of Japanese cedar pollen scattering varies with place and year, those in the re-treatment and core studies were within the usual range in each area. Though there are limitations in the direct comparison between the efficacy results of the 2 studies, the results that daily nasal as well as ocular symptom medication scores in the re-treatment study were comparable with those in the omalizumab group in the core study suggest that there is no evidence of a loss of efficacy with re-treatment in the clinical use of omalizumab.

Re-treatment with omalizumab was generally well tolerated. The overall incidence of drug-related adverse events in the re-treatment study did not increase compared with the core study. In addition, the incidence rate of injection site reactions in the re-treatment study, which were most frequently reported adverse events, did not increase either compared with that in the omalizumab group in the core study. Only 1 patient discontinued treatment because of severe adverse events (injection site pain and injection site discomfort) whose causalities with the drug were not denied, but they disappeared without additional treatment. All adverse events except those two were of mild degree. No anti-omalizumab antibodies and no significant immune complex-mediated disorders were detected. Furthermore, no cases of anaphylaxis were reported. Therefore, the safety profile of omalizumab in the re-treatment of SAR seems favorable and does not represent an increased risk of adverse events, which corresponds to the preliminary data reported by Nayak et al. [10].

In terms of acquired resistance to molecular targeting drugs, development of antibodies to the drug would be one of the possible mechanisms [11], but other mechanisms such as overexpression of the target molecule [12], decreased sensitivity of the drug induced by mutations in the target molecule [13, 14] and increased metabolism/excretion of the drug [15] could also cause resistance to the drug. Since omalizumab acts as a neutralizing antibody by binding to IgE at the same site as high-affinity receptor, to compare serum free IgE levels in the re-treatment study with those in the core study was thought to be important. Consistent with the efficacy results, serum free IgE levels in the re-treatment study decreased markedly to <50 ng/ml at 2, 4, 8 and 12 weeks of the treatment period in all patients and were equal to those in the omalizumab group in the core study.

Table 4. Drug-related adverse events

	Omalizumab (n = 34)	
	re-treatment study	previous study
Total number of patients with drug-related adverse event	5 (14.7)	15 (44.1)
General disorders and administration site conditions	5 (14.7)	11 (32.4)
Injection site		
Reactions	5 (14.7)	10 (29.4)
Erythema	3 (8.8)	5 (14.7)
Hemorrhage	1 (2.9)	0
Induration	0	1 (2.9)
Edema	0	2 (5.9)
Pain	2 (5.9)	2 (5.9)
Pruritus	0	2 (5.9)
Swelling	2 (5.9)	6 (17.6)
Discomfort	1 (2.9)	0
Feeling hot	0	1 (2.9)
Investigations	0	4 (11.8)
Blood bilirubin increased	0	1 (2.9)
Neutrophil count decreased	0	1 (2.9)
White blood cell count		
Decreased	0	1 (2.9)
Increased	0	2 (5.9)
Musculoskeletal and connective tissue disorders	1 (2.9)	0
Muscle stiffness	1 (2.9)	0

Figures in parentheses are percentages.

Though there are a lot of reports showing that treatment with omalizumab reduces serum free IgE levels [16] and downregulates the expression of high-affinity receptor for IgE on basophils [17] and mast cells [18], its effect on IgE synthesis is unclear. It was demonstrated that treatment with omalizumab in asthma patients induced significant inhibition of stimulated IgE release from peripheral blood mononuclear cells and reduction in the number of circulating B cells [19]. In contrast, Corren et al. [20] reported that omalizumab treatment for 6 months in rhinitis patients did not have an effect on IgE-secreting B cells and ε mRNA in nasal lavage fluid. The results of this study show that serum total IgE levels at baseline in the re-treatment study were comparable with those in the core study imply that a 12-week treatment with omalizumab does not modulate IgE synthesis.

As already mentioned, Nayak et al. [10] have previously reported the tolerability and pharmacological effects (but not the clinical efficacy) of re-treatment with oma-

lizumab, but there are some differences in the study design. According to the report of Nayak et al. [10], the dose regimen in the re-treatment study (300 mg every 3 or 4 weeks) was different from that in the core study (50, 150 or 300 mg every 3 or 4 weeks), and both dose regimens could not ensure a minimum effective dose of 0.016 mg/kg/IgE (IU/ml) every 4 weeks. In this study, the study plan was designed to be the same as in the core study, including study period, efficacy and safety endpoints, as well as dose regimen, which was approved for allergic asthma in the US and ensures the minimum effective dose [7]. From the aspect of safety evaluation, the amount of exposure to omalizumab per patient in the study of Nayak et al. [10] was thought to be relatively low compared with this study due to the difference in dose regimen. Though the number of patients in this study was smaller than that in the study of Nayak et al. [10], we investigated the total effects of re-treatment with omalizumab more precisely, under a condition closer to clinical practice.

In conclusion, re-treatment with omalizumab was effective and well tolerated in patients with moderate to severe Japanese cedar pollen-induced SAR. Therefore, omalizumab represents a new promising treatment for patients with SAR induced by Japanese cedar pollens.

Acknowledgment

This study was supported by Novartis Pharma KK, Tokyo, Japan.

References

1 Okuda M: Epidemiology of Japanese cedar pollinosis throughout Japan. Ann Allergy Asthma Immunol 2003;91:288–296.
2 Okubo K, Gotoh M, Shimada K, Ritsu M, Okuda M, Crawford B: Fexofenadine improves the quality of life and work productivity in Japanese patients with seasonal allergic rhinitis during the peak cedar pollinosis season. Int Arch Allergy Immunol 2005;136:148–154.
3 Presta LG, Lahr SJ, Shields RL, Porter JP, Gorman CM, Fendly BM, Jardieu PM: Humanization of an antibody directed against IgE. J Immunol 1993;151:2623–2632.
4 Shields RL, Whether WR, Zioncheck K, O'Connell L, Fendly B, Presta LG, Thomas D, Saban R, Jardieu P: Inhibition of allergic reactions with antibody to IgE. Int Arch Allergy Immunol 1995;107:308–312.
5 Okubo K, Ogino S, Nagakura T, Ishikawa T: Omalizumab is effective and safe in the treatment of Japanese cedar pollen-induced seasonal allergic rhinitis. Allergol Int 2006; 55:379–386.
6 Nagakura T, Ogino S, Okubo K, Sato N, Takahashi M, Ishikawa T: Omalizumab is more effective than suplatast tosilate in the treatment of Japanese cedar pollen-induced seasonal allergic rhinitis. Clin Exp Allergy 2008;38:329–337.
7 Strunk RC, Bloomberg GR: Omalizumab for asthma. N Engl J Med 2006;354:2689–2695.
8 Ädelroth E, Rak S, Haahtela T, Aasand G, Rosenhall L, Zetterstrom O, Byrne A, Champain K, Thirlwell J, Cioppa GD, Sandström T: Recombinant humanized mAb-E25, an anti-IgE mAb, in birch pollen-induced seasonal allergic rhinitis. J Allergy Clin Immunol 2000;106:253–259.
9 Casale TB, Bernstein IL, Busse WW, LaForce CF, Tinkelman DG, Stoltz RR, Dockhorn RJ, Reimann J, Su JQ, Fick RB Jr, Adelman DC: Use of an anti-IgE humanized monoclonal antibody in ragweed-induced allergic rhinitis. J Allergy Clin Immunol 1997;100:110–121.
10 Nayak A, Casale T, Miller SD, Condemi J, McAlary M, Fowler-Taylor A, Della Cioppa G, Gupta N: Tolerability of retreatment with omalizumab, a recombinant humanized monoclonal anti-IgE antibody, during a second ragweed pollen season in patients with seasonal allergic rhinitis. Allergy Asthma Proc 2003;24:323–329.
11 Haraoui B, Cameron L, Ouellet M, White B: Anti-infliximab antibodies in patients with rheumatoid arthritis who require higher doses of infliximab to achieve or maintain a clinical response. J Rheumatol 2006;33:31–36.
12 le Coutre P, Tassi E, Varella-Garcia M, Barni R, Mologni L, Cabrita G, Marchesi E, Supino R, Gambacorti-Passerini C: Induction of resistance to the Abelson inhibitor STI571 in human leukemic cells through gene amplification. Blood 2000;95:1758–1766.
13 Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, Sawyers CL: Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 2001;293:876–880.
14 Pedersen MW, Meltorn M, Damstrup L, Poulsen HS: The type III epidermal growth factor receptor mutation. Ann Oncol 2001; 12:745–760.
15 Mahon FX, Belloc F, Lagarde V, Chollet C, Moreau-Gaudry F, Reiffers J, Goldman JM, Melo JV: MDR1 gene overexpression confers resistance to imatinib mesylate in leukemia cell line models. Blood 2003;101:2368–2373.
16 Milgrom H, Fick RB Jr, Su JQ, Reimann JD, Bush RK, Watrous ML, Metzger WJ: Treatment of allergic asthma with monoclonal anti-IgE antibody. rhuMAb-E25 Study Group. N Engl J Med 1999;341:1966–1973.
17 MacGlashan DW Jr, Bochner BS, Adelman DC, Jardieu PM, Togias A, McKenzie-White J, Sterbinsky SA, Hamilton RG, Lichtenstein LM: Down-regulation of FcεRI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. J Immunol 1997;158:1438–1445.
18 Beck LA, Marcotte GV, MacGlashan DW, Togias A, Saini S: Omalizumab-induced reductions in mast cell FcεRI expression and function. J Allergy Clin Immunol 2004;114: 527–530.
19 Hanf G, Brachmann I, Kleine-Tebbe J, Seybold J, Kunkel G, Suttorp N, Noga O: Omalizumab decreased IgE-release and induced changes in cellular immunity in patients with allergic asthma. Allergy 2006;61:1141–1144.
20 Corren J, Díaz-Sanchez D, Saxon A, Deniz Y, Reimann J, Sinclair D, Davancaze T, Adelman D: Effects of omalizumab, a humanized monoclonal anti-IgE antibody, on nasal reactivity to allergen and local IgE synthesis. Ann Allergy Asthma Immunol 2004;93: 243–248.

Allergology International. 2008;57:205-209
DOI: 10.2332/allergolint.R-08-164

REVIEW ARTICLE

Anti-IgE Antibody Therapy for Japanese Cedar Pollinosis: Omalizumab Update

Kimihiro Okubo[1] and Toshikazu Nagakura[2]

ABSTRACT

Seasonal allergic rhinitis (SAR) induced by Japanese cedar pollens is a substantial problem in Japan. Omalizumab, a novel humanized monoclonal anti-immunoglobulin E (IgE) antibody, has already been proven to reduce symptoms associated with SAR. To investigate the safety and efficacy of omalizumab in the treatment of patients with Japanese cedar pollen-induced SAR compared to placebo or anti-allergic drug, two randomized, double-blind studies were conducted in Japan. Omalizumab (150, 225, 300, or 375 mg) or placebo was administered subcutaneously every 2 or 4 weeks based on serum total IgE and body weight at baseline. IPD was administered 300 mg per day through the season. Primary and all secondary efficacy variable scores were significantly lower in the omalizumab group than in the placebo group ($P < .01$) and IPD, Th2 cytokine inhibitor group ($P < .01$). Omalizumab was effective and safe in the treatment of SAR induced by Japanese cedar pollens. And the methods of increasing effects by combining omalizumab with antibody-specific immunotherapy are being considered. These strategy is more effective than immune-therapy alone.

KEY WORDS

anti-IgE therapy, asthma, immunotherapy, omalizumab, seasonal allergic rhinitis

INTRODUCTION

Allergic rhinitis including hay fever is a disorder in which sensitization occurs through the inhalation of antigens (induction phase), and a local immune reaction then takes place between the antigen-specific IgE thereby produced and the antigens which have invaded the nasal mucosa (effecter phase). Treatment for allergic rhinitis depends on suppressing the flow of this allergic reaction at some point. Antigen-specific immunotherapy has its point of effect earlier than midway between the induction phase and effecter phase of allergic reaction, unlike general allergy medications (antihistamines, chemical mediator release inhibitors, leukotriene receptor antagonists, etc.). The anti-IgE antibody omalizumab is also such a drug whose point of action differs from previous allergy medications.

THE KNOWLEDGE OF ANTI-IgE ANTIBODY THERAPY

The anti-IgE antibody (omalizumab) produces its effect by binding to IgE which is not bound to mast cells, inhibiting it from binding to mast cells. Since omalizumab does not affect T cells, unlike conventional immunotherapy it is not a curative therapy, but it is a groundbreaking drug in its application of an immunological concept.[1]

This drug is under application in Japan as indicated for severe asthma. There is certainly much evidence for asthma and this is good news for inadequately controlled asthma. In the field of asthma the concept of response to omalizumab has already been published.[2] Currently research is focused on cost-effectiveness. Medical expenses for asthma in America in 2002 (direct and indirect expenses) totaled 14 billion dollar (direct medical expenses: 3.1 billion dollar for hospitalization, 4.6 billion dollar for drugs). The cost of omalizumab to improve QOL is 821,000 dollars, and at present cost-effectiveness is not good. If it could be reduced to 200 dollars or less, it is calculated that cost-effectiveness would increase.[3] However, in correspondence, D. Revicki argues that the purely medical cost-effectiveness which is not based

[1]Department of Otorhinolaryngology, Nippon Medical School and [2]Yoga Allergy Clinic, Tokyo, Japan.
Correspondence: Kimihiro Okubo, MD, PhD, Department of Otorhinolaryngology, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8603, Japan.
Email: ent-kimi@nms.ac.jp
Received 22 April 2008.

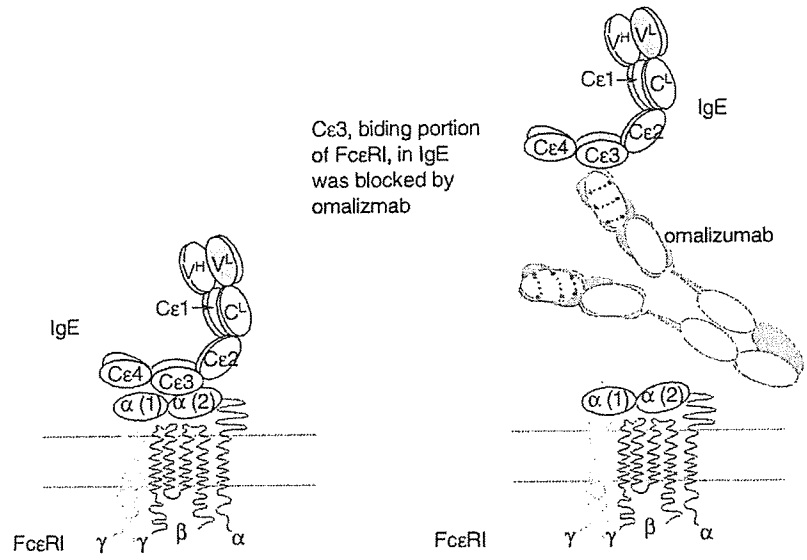

Fig. 1 The inhibition mechanism of allergic disease by omalizumab. Omalizumab was humanized monoclonal antibody against C epsilon 3 portion of Fc epsilon receptor I. Omalizumab blocks the binding between IgE and Fc epsilon receptor I, so allergic symptom must be reduced by this mechanism.

on the Asthma Policy Model is better.[4] The cost-effectiveness will be a large issue in using omalizumab for all allergic disorders in the future.

ANTI-IgE ANTIBODY

In 1991, the American company Genentech produced an antibody specific to Cε3 that binds to Fcε receptors, in the constant region of human IgE. Omalizumab is a humanized monoclonal antibody using mouse monoclonal antibody as a base, retaining the antigen-specific region and replacing the other fragments with human IgGlκ.

When this antibody binds to free IgE in blood by an antigen-antibody reaction between the Fcε receptor and the Cε3 binding site, an IgE–anti-IgE complex is formed, and as a result, free IgE is decreased (Fig. 1). Therefore, IgE that binds to mast cells is decreased, so that even if antigens invade, binding to mast cells to form cross-linking is inhibited and allergic reaction is controlled. Another effect is to inhibit the differentiation of B cells into IgE-producing cells. This is thought to be because it reacts with membrane-binding IgE on B cells, inhibiting mRNA expression of the ε chain. In actual animal experiments, IgE-producing B cells are virtually eliminated.[5]

EFFECTS OF ANTI-IgE ANTIBODY THERAPY IN THE US AND EUROPE

In the West, clinical trials of anti-IgE antibody therapy using omalizumab have been conducted for several years by subcutaneous injection. The target disorders are allergic rhinitis and atopic asthma, and clinical trials are being conducted in Japan for the same targets. In the West, the trials for hay fever differ from asthma, being conducted at single doses of 150 mg or 300 mg. Casale *et al.* have reported on a double-blind comparative trial of those dose levels plus a placebo and 50 mg for a total of 4 groups, using American patients with ragweed pollinosis.[6] The condition of 300 mg group was better than the placebo group throughout the pollen dispersal season and at the peak of pollen dispersal. Lower dose levels also showed effects, and dose relationship was observed. Omalizumab also showed significant improvement by the RQLQ (Juniper's QOL questionnaire). Similar results have been obtained for birch pollinosis in the West, and reduction in drugs for emergency use is being evaluated (Fig. 2).[7]

EFFECTS ON JAPANESE CEDAR POLLINOSIS IN JAPAN

Clinical trials were conducted on Japanese cedar pollinosis in Japan in 2002 and 2003. The trials of 2002 and 2003 were placebo-controlled comparative study and comparative study with an anti-allergy drug, respectively.

The placebo-controlled study used a dose concept of considering the level of omalizumab which can eliminate IgE systemically, as with asthma in Japan, in contrast to the overseas studies which have a set dosage of 300 mg. The amount of omalizumab was set from body weight and IgE level immediately before administration in December as 0.0016 mg/kg/

IgE (IU/mL) and revised every 4 weeks. Therefore, IgE was uniformly reduced to the detection limit (50 ng/mL) in the administration group. Results of the study showed omalizumab significantly reduced the nasal symptom medication score by about 40% for e, and significantly reduced ocular symptoms by 50% (Fig. 3). Individual symptoms of Japanese cedar pollinosis (itchy nose, sneezing, runny nose, stuffy nose, itchy eyes, watery eyes, red eyes) were all significantly alleviated (Fig. 4). Both nasal and ocular symptoms were decreased significantly more than in the placebo group. The major adverse event was pain at the injection site. And one case of ulcerative colitis was reported, but the colitis manifestation is not thought to be responsible omalizumab application.[8]

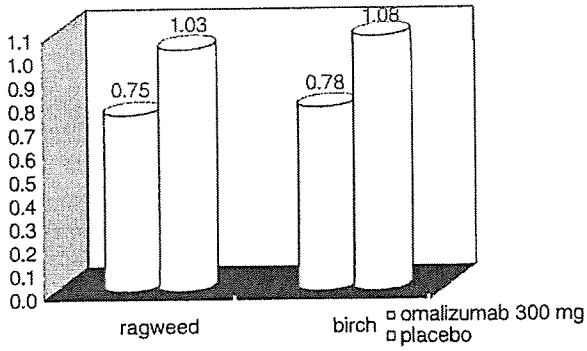

Fig. 2 Overseas study of omalizumab for ragweed or birch pollinosis. Daily nasal severity score in the placebo controlled study of the patients with ragweed pollinosis (reference 6), and birch pollinosis (reference 7). Significant reduction of daily nasal severity score in omalizumab group (yellow bar) compared to that in placebo (purple bar) at both studies.

In 2003, an active control study was conducted with IPD, Th2 cytokine inhibitor. Because the results of the placebo-controlled study were satisfactory, the first active control study, double dummy comparative controlled trial, of omalizumab in the worldwide was conducted. Dose levels were set to compare omalizumab and IPD using the dose concept. 300 mg per day of IPD was administered initial treatment from beginning of February, and through the season. The nasal symptom medication score during the pollen dispersal season was 30% lower than IPD (Fig. 5). In individual symptoms, omalizumab was more effective than IPD for sneezing, runny nose and stuffy nose, although there were no significant differences in itchy nose or ocular symptoms. Omalizumab was more effective to the same degree through the pollinating season including the high pollen dispersal season, and there were no adverse reactions.[9]

Omalizumab is currently under application at the Ministry of Health, Labour and Welfare for severe asthma, but application for hay fever has not been made. This may be because it is not a life-threatening illness, and since there are many patients, recogni-

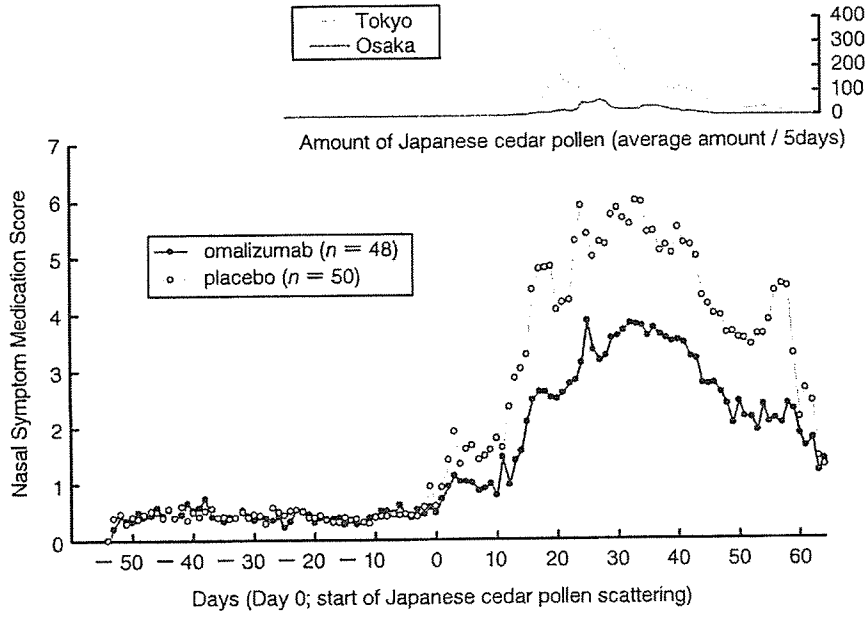

Fig. 3 The change in daily nasal symptom medication score in patients with Japanese cedar pollinosis in 2002. The nasal symptom medication score was significantly reduced in omalizumab group (filled square) in Japanese cedar pollen dispersing season in Tokyo and Osaka compared to the score in placebo (open square).

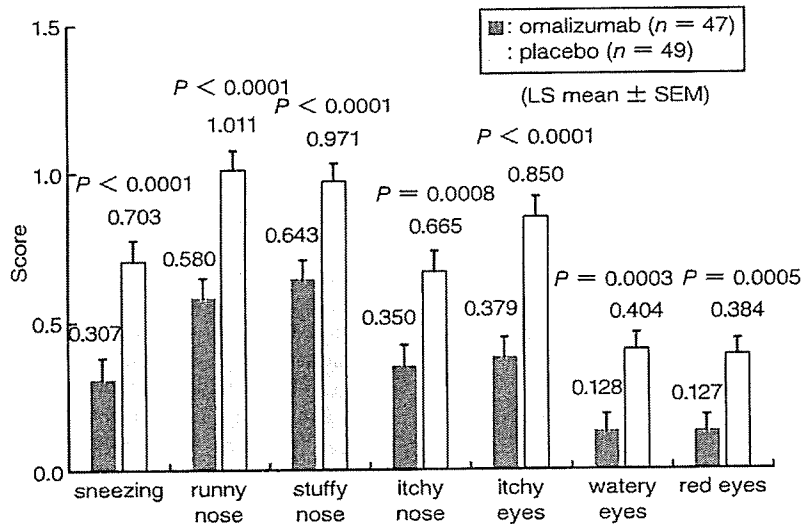

Fig. 4 The change in daily nasal and ocular symptom score in patients with Japanese cedar pollinosis in 2002. All symptoms score, sneezing, runny nose, stuffy nose, itchy nose, itchy eyes, watery eyes, and red eyes, were significant reduced in omalizumab group (purple bar) compared with the score in placebo (yellow bar), especially in eye symptoms.

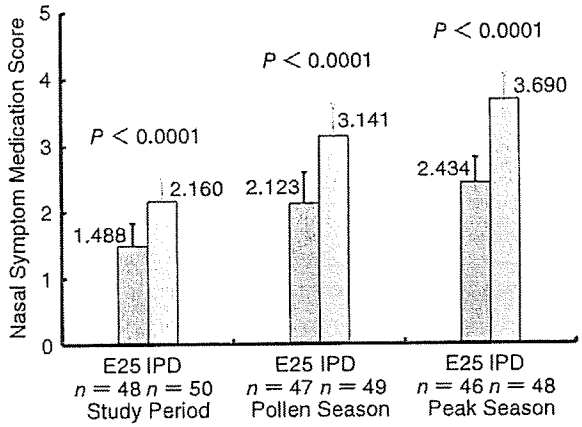

Fig. 5 The daily nasal symptom medication score in comparative study between omalizumab (E25) and IPD for patients with Japanese cedar pollinosis in 2003. Nasal symptom medication score was evaluated in three periods such as study period, pollen dispersing period (pollen season), peak pollen dispersing period (peak season). In all three period, nasal symptom medication score with omalizumab (E25) group (grey bar) was reduced compared to the score with the placebo (orange bar).

tion of the indication could lead to improper use. At any rate, application for allergic rhinitis, including Japanese cedar pollinosis has not been made in any country, but if with advances in technology lower costs for antibody manufacturing and means of reduction to safer numbers can be attained, the treatment for hay fever will become broader in Japan in the future.

OTHER USES FOR ANTI-IgE THERAPY

Overseas, methods of increasing effects by combining omalizumab with antibody-specific immunotherapy are being considered. Wahn *et al.* conducted an RCT in children with sensitization to birch and grass pollen using immunotherapy for either in combination with omalizumab, and the symptom scores for each immunotherapy were further reduced by half.[10] In a study of the same group, leukotriene release by antigen stimulation after the pollen dispersal season that was not reduced by immunotherapy alone was suppressed. However, this suppression reverts back one month after treatment.[11] Klunker *et al.* described combination with rush immunotherapy against ragweed pollinosis. In this study omalizumab administration was begun 9 weeks before the start of rush immunotherapy, with improved effects. This idea is in accordance with the idea of administering omalizumab before the advent of symptoms in the Japanese clinical study. There were no differences in the symptom scores of omalizumab alone and rush immunotherapy combined with omalizumab, but the ability of B cells to bind with allergen-IgE complexes was inhibited more than by rush immunotherapy alone. That is, omalizumab was shown to inhibit CD23 expression in B cells for 30 weeks after completion of immunotherapy at week 42.[12]

A study on nasal polyps was also conducted. Penn

et al. performed endoscopic surgery on nasal polyps complicated by allergic asthma and allergic rhinosinusitis, and observed the course of omalizumab administration thereafter. Recurrence was 25% with and without omalizumab (1/4), but the number of cases was small, and the effects have not been completely verified.[13,14]

THE FUTURE

In the field of allergies there are few illnesses in which IgE does not play a role, and the time may be coming when the use of omalizumab will be considered for all disorders whose pathology has some involvement of IgE. There are many problems that need to be overcome, including that of cost. However, as it is, reduction of IgE can improve the clinical condition in almost all allergic disorders, and we must demonstrate in Japan as well that the current application of omalizumab can be expanded to other indications.

REFERENCES

1. Presta LG, Lahr SJ, Shields RL *et al.* Humanization of an antibody directed against IgE. *J Immunol* 1993;**151**:2623-32.
2. Humbert M, Berger W, Rapatz G, Turk F. Add-on omalizmab improves day-to-day symptoms in inadequately controlled severe persistent allergic asthma. *Allergy* 2008;**63**: 592-6.
3. Wu AC, Paltiel AD, Kuntz KM, Weiss ST, Fuhlbrigge AL. Cost-effectiveness of omalizumab in adults with severe asthma: results from the Asthma Policy Model. *J Allergy Clin Immunol* 2007;**120**:1146-52.
4. Revicki D, Brown R, Dale P. Questioning the economic evaluation of omalizumab. *J Allergy Clin Immunol* 2008; **121**:1514.
5. MacGlashan DW Jr, Bochner BS, Adelman DC *et al.* Down-regulation of FcεRI expression on basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J Immunol* 1997;**158**:1438-45.
6. Casale TB, Bernstein IL, Busse WW *et al.* Use of an anti-IgE humanized monoclonal antibody in ragweed-induced allergic rhinitis. *J Allergy Clin Immunol* 1997;**100**:110-21.
7. Adelroth E, Rak S, Haahtela T *et al.* Recombinant mAb-E25, an anti-IgE mAb, in birch pollen-induced seasonal allergic rhinitis. *J Allergy Clin Immunol* 2000;**106**:253-9.
8. Okubo K, Ogino S, Nagakura T, Ishikawa T. Omalizumab is effective and safe in the treatment of Japanese cedar pollen-induced seasonal allergic rhinitis. *Allergol Int* 2006; **55**:379-86.
9. Nagakura T, Ogino S, Okubo K, Sato N, Takahashi M, Ishikawa T. Omalizumab is more effective than suplatast tosilate in the treatment of Japanese cedar pollen-induced seasonal allergic rhinitis. *Clin Exp Allergy* 2008;**38**:329-37.
10. Kuehr J, Brauburger J, Zielen S *et al.* Efficacy of combination treatment with anti-IgE plus specific immunotherapy in polysensitized children and adolescents with seasonal allergic rhinitis. *J Allergy Clin Immunol* 2002;**109**:274-80.
11. Kopp MV, Stenglein S, Kamin W *et al.* Omalizumab (Xolair) in children with seasonal allergic rhinitis: Leukotorien release as a potential in vivo parameter to monitor therapeutic effects. *Pediatr Allergy Immunol* 2007;**18**:523-7.
12. Klunker S, Saggar LR, Sayfert-Margolis V *et al.* Combination treatment with omalizumab and rash immunotherapy for ragweed-induced allergic rhinitis: Inhibition of IgE-facilitated allergen binding. *J Allergy Clin Immunol* 2007; **120**:688-95.
13. Penn R, Mikula S. The role of anti-IgE immunoglobulin therapy in nasal polyposis: A pilot study. *Am J Rhinol* 2007;**21**:428-32.
14. Grundmann SA, Hemfort PB, Lugar TA, Brehler R. Anti-IgE(omalizumab): a new therapeutic approach for chronic rhinosinusitis. *J Allergy Clin Immunol* 2008;**121**:257-8.

リアルタイムモニター飛散数の情報のあり方の研究と舌下ペプチド・アジュバント療法の臨床研究
スギ花粉症のアレルゲン免疫療法における導入療法改良の試み

研究分担者　永田　真　埼玉医科大学呼吸器内科　教授
研究協力者　山口剛史　埼玉医科大学呼吸器内科　助教
研究協力者　中込一之　埼玉医科大学呼吸器内科　講師
研究協力者　杣　知行　埼玉医科大学呼吸器内科　講師

研究要旨

スギ花粉症における舌下ペプチド・アジュバンド療法開発の初期段階として、まずアレルゲン免疫療法自体を花粉症の正規治療として確立・普及させる必要がある。アレルゲン免疫療法が普及しない理由のひとつとして、標準法では頻回の通院を余儀なくされることが問題である。そこでまず我々は、通院回数を減らすことと、そして治療の確実性向上を求めて、1 日に数回アレルゲン注射を反復して短期間で導入療法を完遂するクラスター方式免疫療法を試みた。11 例の成人スギ花粉症患者に前処置としてヒスタミンH1 受容体拮抗薬を内服させた後、標準化スギ・アレルゲンを 1 時間ごとに 1 日 3 回皮下注射を反復し、5 週で 2000　JAU/ml の 0.1ml にまで到達させることを目標として行った。11 例中 10 例で目標維持量に到達できた。スギ標準化アレルゲンを用いたクラスター方式での免疫療法の導入は、短期間で高濃度の維持量に達せられる有用な治療手段であると考える。かかるプロトコールは舌下療法やペプチド療法などの今後の修飾免疫療法への応用も可能と考える。

E. 結論

スギ標準化アレルゲンを用いたクラスター方式免疫療法は、短期間で高濃度の維持量に達せられる治療手段である。安全性の点で現在の治療用アレルゲンでは課題を残したが、今後、舌下あるいはペプチド免疫療法における治療スケジュールの検討上、ひとつのオプションとして応用可能であると考える。

A. 研究目的

本研究班全体としてのテーマではリアルタイムモニター花粉数の情報のあり方に加えて、アレルゲン免疫療法の新規のアプローチである舌下ペプチド・アジュバンド療法の臨床研究が主眼となっている。舌下ペプチド療法を開発普及させる初期段階の土台づくりとして、まずアレルゲン免疫療法自体を普及させ、これが日本の花粉症の正規治療として、欧米諸国の状況なみに一般化させることが、きわめて重要な前提条件であると考えられる。我が国においては、スギ花粉症の治療用標準アレルゲンが開発されたにも関らず、患者の大多数は薬物による対症療法のみを受けており、アレルゲン免疫療法の恩恵を享受している患者は極めて限られているのが現状である。その理由として、アレルゲン免疫療法を施行できる専門医や施設が日本国においては極めて限定的であって、著しく不足していること、またこれまでの通常の導入療法の施行法では頻回の通院を余儀なくされ、治療の完遂性の側面においても必ずしも充分ではないことが問題点であるものと認識される。そのためには現行の施行方法を改良することによって、短期間で、しかも確実に、アレルゲン投与量を十分な効果の期待できる高用量にまで到達せしめることができるようなアプローチの開発が必要とおもわれる。かかる試みのひとつとして、我々は、1 日に数回アレルゲン注射を反復して短期間で導入療法を完遂するクラスター方式免疫療法を試みた。

B. 研究方法

対象は平均年齢 40 歳の 11 例の成人スギ花粉症患者であった。通年性鼻炎あるいは気管支喘息の合併症例を原則として除外することとした。なお結果的には間欠型の軽症気管支喘息合併例が 1 例あった。この症例は臨床的には発作がなく安定した状態にあり、呼吸機能的にも閉塞性換気障害は存在しなかった。患者には、事前に説明書、同意書によるインフォームドコンセントを取得した。

クラスター方式アレルゲン免疫療法は、我々が以前にダニ・アレルゲンに感作されているアレルギー性成人気管支喘息患者を対象として、ダニを主成分とするハウスダストを用いて行った方法に準じて施行した。すなわちまず、導入