Effect of Milk Fermented with Lactobacillus acidophilus Strain L-92 on Symptoms of Japanese Cedar Pollen Allergy: A Randomized Placebo-Controlled Trial

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Received December 7, 2004; Accepted May 1, 2005

A placebo-controlled, single-blind study was conducted to evaluate the effects of Lactobacillus acidophilus strain L-92 (L-92) on the symptoms of Japanese cedar-pollen allergy. This study was carried out during the 2002 and 2003 seasons of Japanese cedar pollination. Twenty-three in-house volunteers were asked to drink 100 ml of heat-treated milk fermented with L-92 containing $5 \times 10^{10}$ of the bacteria, twice a day, for 6 consecutive weeks. A similar study was carried out during the 2003 season for 10 weeks, but the daily dose of bacteria was $2 \times 10^{10}$. A significant improvement of the ocular symptom-medication score (SMS) was observed in 2002 and of the score of distress of life in 2003. These data show that a daily oral intake of not less than $2 \times 10^{10}$ heat-treated L-92 cells improved the symptoms of Japanese cedar pollinosis, thereby contributing to reduce the dose of concomitant medications. However, no blood parameter was significantly affected in these trials.

Key words: Lactobacillus acidophilus; Japanese cedar pollinosis; allergic disorder; placebo-controlled trial

Allergy, in the form of atopic diseases such as pollinosis, atopic eczema, allergic rhinitis and asthma, is a chronic disorder of increasing significance in developed countries.1) Since this epidemiological trend has emerged during an era of unparalleled hygiene and successful control of infection, researchers believe that the waxing of atopic diseases is etiologically linked to waning exposure to microbial agents2) in addition to the increase in level of allergen exposure.3,4) In fact, over the last 20 years, Japan has witnessed a disturbing rise in the prevalence of Japanese cedar pollinosis.

Pollen of Cryptomeria japonica (Japanese cedar), which accounts for a large part of the Taxodiaceae family, is scattered from February to March in Japan. Japanese cedar pollinosis is common in the spring and is becoming one of the most serious health issues. An important way to improve for quality of life and well-being of the population in Japan as well as in other highly industrialized countries would be to suppress the progressive increase in the number of patients with pollen allergy. It has been demonstrated that infection could modulate the incidence of allergic disorders, possibly through the elucidation of Th1-skewed phenomena that upset the Th2-dominant character of atopic diseases. The increased credibility of probiotics has been fueled in part by the hygiene hypothesis. Interest is therefore focused on their use as a source of completely safe substitutive infection. From the viewpoint of quality of life, probiotics are considered more important than occasional infections in atopic disease prevention.

Intestinal microflora, both pathogenic and commensal, show a similar ability to modulate the local immunological environment, and this local modulation can influence systemic immunological exploits. The mechanism(s) by which this immune conditioning occurs has not yet been elucidated, although the clinical implications seem to be accepted. The prophylactic effects of microbial food ingredient, including probiotics, are now being explored with growing interest as a genuine medicinal option in the management—and even primary prevention—of type-I allergic diseases. With respect to control of atopic diseases, interest has been focused on the management of environmental allergen-specific serum IgE, because serum IgE is influenced by a Th1/Th2 balance and plays a significant role in many allergic diseases. Researchers have recently reported several strains of Lactobacillus, including Lactobacillus casei strain Shirota5) and Lactobacillus plantarum strain L-137,6) as probiotics that work as modifiers of antigen-specific serum IgE in animal
models. These phenomena suggest the possible usage of these lactic acid bacteria in preventing atopic diseases, even when administered orally. At the same time, however, there have been few screening studies on serum IgE-modulating strains of lactic acid bacteria. Therefore, we do not know exactly the difference regarding their ability to suppress serum IgE among strains, species, and genera of lactic acid bacteria. Moreover, little is known about the exact mechanism(s) for the suppressive effect of these lactic acid bacteria on serum IgE. In this regard, we conducted a screening test using an animal model and found that the serum IgE modulation ability was a species-specific characteristic. The results of an experiment enabled us to select L-92 as a candidate strain with excellent IgE-lowering ability.7)

It is now considered that a prophylactic treatment with probiotics is useful to promote oral allergen tolerance with promising clinical results from small children with food allergy8) and to improve the symptoms of atopic diseases. In humans, the perinatal administration of Lactobacillus rhamnosus strain GG has suppressed the incidence of atopic eczema in children at risk.9) This fact shows that probiotics of the genus Lactobacillus might have a clinical impact on atopic diseases. However, one study has shown no beneficial effects of probiotics on pollinosis.10) The present study was conducted to evaluate the effect of L-92 on the prevalence of Japanese cedar polinosis, level of the serum antigen-specific IgE antibody and allergic symptoms.

Materials and Methods

Subjects. During the 2002 season, 23 in-house volunteers (research staff at the R&D Center and staff members of the head office of Calpis Co., Ltd.) allergic to Japanese cedar pollen, 26–48 years old, participated in this study. All of them had the typical symptoms of Japanese cedar polinosis and were positive for IgE against Japanese cedar pollen. They were randomized into the intervention and placebo groups. No significant difference between the placebo and L-92 intervention groups (daily dose of 1 × 10^11 count) in terms of age, mean duration of polinosis, severity of symptoms and serum anti-Japanese cedar pollen IgE level (Tables 1 and 2) was detected. The volunteers in both groups lived in Kanagawa Prefecture and the Tokyo metropolitan area, less than 40 km from the pollen-counting site (Sagamihara National Hospital, Sagamihara-shi, Kanagawa, Japan). Thus, exposure to pollen was similar for the two groups of volunteers.

In 2003, 20 in-house volunteers participated in a second study, and we evaluated the effectiveness of a reduced daily amount (2 × 10^10 count) of orally-administered L-92 on the severity of symptoms of cedar pollinosis. There was no significant difference between the two groups of participants in terms of age, mean duration of polinosis, severity of symptoms and serum anti-cedar pollen IgE level (Tables 1 and 2).

Preparation of the active and placebo samples for clinical test. In the 2002 clinical test, L-92 fermented milk and placebo acidified milk were prepared. To 15% (w/v) of reconstituted skim milk was added 0.1% (w/v) yeast extract, and the mixture pasteurized at 95°C for 5 min. Then, 0.01% (w/v) of a lyophilized L-92 starter was inoculated into the pre-fermentation mixture, and the mixture cultured overnight at 37°C. The resulting L-92-fermented milk was mixed with sweetener and flavour, and the final acidity (lactic acid equivalent) of the resulting mixture was adjusted to 1.1% (w/v) with lactic acid. Placebo acidified milk was prepared by simply adding lactic acid to the pre-fermentation mixture to a final acidity of 1.1% (w/v). The final non-fat solid (SNF) in both samples was 8.1% (w/v). Thereafter, both samples were dispensed into 100-ml bottles and heat-treated at 75°C for 10 min.

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Table 1. Clinical Characteristics of the L-92 and Placebo Groups of Patients

<table>
<thead>
<tr>
<th></th>
<th>Study in 2002</th>
<th>Study in 2003</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>L-92</td>
<td>placebo</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Sex (m:f)</td>
<td>10:2</td>
<td>7:4</td>
</tr>
<tr>
<td>Mean age (years ± S.D.)</td>
<td>30.6 ± 5.3</td>
<td>34.1 ± 6.2</td>
</tr>
<tr>
<td>Mean duration of Japanese cedar pollinosis (years ± S.D.)</td>
<td>12.9 ± 5.3</td>
<td>15.0 ± 8.8</td>
</tr>
<tr>
<td>Severity of ocular symptoms at baseline (mean score ± S.D.)</td>
<td>2.3 ± 1.2</td>
<td>2.6 ± 1.2</td>
</tr>
<tr>
<td>Severity of nasal symptoms at baseline (mean score ± S.D.)</td>
<td>2.7 ± 1.2</td>
<td>2.7 ± 1.4</td>
</tr>
</tbody>
</table>

Table 2. Serum Total Immunoglobulin E (IgE) Level, IgE Specific For Japanese Cedar Pollen and Th1/Th2 Balance in Peripheral Blood of Volunteers in the L-92 Group and Placebo Group Prior to the Study (mean ± S.E.)

<table>
<thead>
<tr>
<th></th>
<th>Study in 2002</th>
<th>Study in 2003</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L-92</td>
<td>placebo</td>
</tr>
<tr>
<td>Serum IgE level (IU/ml)</td>
<td>322.3 ± 161.4</td>
<td>186.9 ± 95.0</td>
</tr>
<tr>
<td>IgE specific for Japanese cedar pollen (UA/ml)</td>
<td>14.5 ± 5.6</td>
<td>14.1 ± 5.6</td>
</tr>
<tr>
<td>Th1/Th2 balance</td>
<td>12.9 ± 2.2</td>
<td>15.0 ± 5.8</td>
</tr>
</tbody>
</table>
In the 2003 clinical trial, similar active and placebo samples were prepared to a final acidity of 0.55% and SNF of 3.4%.

Study design. In both 2002 and 2003, the study was single-blind and placebo-controlled. The 2002 study was carried out from February 8 to April 7. Volunteers were randomized after a familiarization period (from February 8 to February 19) into the intervention and placebo groups. Those in the intervention group (2 women and 10 men) were told to drink two bottles a day of 100 ml of sterilized milk fermented with L-92 \((5 \times 10^{10} \text{ count/100 ml per bottle})\), and those in the placebo group (4 women and 7 men) had to take the same amount of a sterilized pre-fermented mixture of the same acidity adjusted with lactic acid for 6 weeks (between February 20 and April 2). The subjects were instructed to keep symptom and medication diary throughout the experimental period.

The 2003 study was carried out between January 7 and April 15. Subjects in the intervention group (10 men) were asked to drink every day 100 ml of sterilized milk fermented by L-92 that contained \(2 \times 10^{10} \text{ count}\), and those in the placebo group (4 women and 6 men) were asked to drink the same amount of a sterilized pre-fermented mixture prepared with the same acidity for 10 weeks (from January 21 to March 31). During the study period, a physician examined their nasal cavity 6 times, and blood samples were obtained 6 times. The subjects were instructed to keep a symptom and medication diary during the study period. These studies were carried out in accordance with instructions of the Declaration of Helsinki.

Count of pollen. Data on pollen floating in the air around Sagamihara City area were kindly provided by Dr. H. Yasueda of Sagamihara National Hospital (Sagamihara-shi, Kanagawa, Japan). These data were obtained by using a Durham pollen sampler placed on the roof of Sagamihara National Hospital and are expressed as grains per cm².

Evaluation of subjective symptoms. Nasal and ocular symptoms described by the patients were classified as follows: sneezing, runny nose, stuffy nose, itchy eyes, and watery eyes, each being scored from 0 to 4 (Table 3). The highest score for sneezing, runny nose and stuffy nose was used as the nasal symptom score, and the highest score of itchy eyes and watery eyes was used as the ocular symptom score. Although no medication to prevent the allergy itself was administered before the pollen season, the volunteers in both studies were concomitantly treated with commercial and/or prescribed medicine(s) to relieve their symptoms, and use of drugs was scored from 0 to 3 (Table 4). The sum of the symptom and medication scores was used as the symptom-medication score (SMS). This score, defined by the Japanese Society for Allergology, was used to assess the severity of the symptoms of Japanese cedar pollinosis.

Medical examination. A physician (HK) examined each subject to determine the severity of symptoms of Japanese cedar pollinosis. The swelling and color of the nasal mucosa, and amount and aspect of snivel were also scored from 0 to 3 (Table 5).

### Table 3. Scoring of Symptoms

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Severity of symptom by its score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Sneezing(^a)</td>
<td>0</td>
</tr>
<tr>
<td>Runny nose(^b)</td>
<td>0</td>
</tr>
<tr>
<td>Stuffy nose</td>
<td>None</td>
</tr>
<tr>
<td>Watery eyes</td>
<td>None</td>
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</tbody>
</table>

\(^a\)Mean number of sneezing attacks in a day.  
\(^b\)Mean number of nose blows in a day.

### Table 4. Scoring of Medicines

<table>
<thead>
<tr>
<th>Medicine</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral-antihistamines</td>
<td>1</td>
</tr>
<tr>
<td>Oral histamine release inhibitors</td>
<td>1</td>
</tr>
<tr>
<td>Nose drops (excluding focal steroids)</td>
<td>1</td>
</tr>
<tr>
<td>Eye drops (excluding focal steroids)</td>
<td>1</td>
</tr>
<tr>
<td>Focal administration of steroids</td>
<td>2</td>
</tr>
<tr>
<td>Oral-antihistamines and focal steroids</td>
<td>3</td>
</tr>
</tbody>
</table>

Prescribed medications and commercial drugs recorded on daily cards were scored according to the description in this Table.

### Table 5. Scoring of Symptoms of Nasal Cavity Findings

<table>
<thead>
<tr>
<th>Finding</th>
<th>Severity of finding by score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Nasal mucosa</td>
<td>swelling</td>
</tr>
<tr>
<td>color</td>
<td>normal</td>
</tr>
<tr>
<td>Snivel in nasal cavity</td>
<td>amount</td>
</tr>
<tr>
<td>aspect</td>
<td>none</td>
</tr>
</tbody>
</table>
**Blood examination.** Blood samples were examined to determine total-IgE, anti-Japanese cedar pollen IgE, anti-house dust IgE, anti-mite IgE, anti-cypress IgE, Th1 percentage, Th2 percentage and Th1/Th2 ratio. All blood tests were performed by SRL, Inc., Tokyo, Japan.

**Statistical analysis.** The symptom score and symptom-medication score are each expressed as daily averages. An analysis of variance for split-plot design was used to evaluate the statistical significance of the data expressed as a weekly average of the daily mean score for subjective symptoms, these being considered as continuous variables, Th1 and Th2 percentages, Th1/Th2 ratio, and concentration of serum antibodies. Scheffé’s test was then applied as a multiple-comparison procedure.

Friedman’s test was applied to analyze the physician’s evaluation of nasal cavity findings. When statistical significance was observed, the Steel-Dwass test was used to determine the level of significance of the difference between each time point.

**Results**

**Study done during the 2002 season**

**Count of pollen**

Figure 1A shows the daily averages of the Japanese cedar pollen count during the 2002 season. The total Japanese cedar pollen dispersed during the 2002 season was 8952.8 grains per cm²; during this study period, it was 8871.6 grains per cm².

**Subjective symptom**

The effect of orally administered L-92 on the symptoms was examined by comparing the time-course changes in the mean SMS value for the L-92 group and that for the placebo group during the experimental period. Mean ocular SMS for the L-92 group became significantly lower than that for the placebo group as time progressed (Fig. 1B), although no significant difference was observed in the scores for sneezing, runny nose, stuffy nose, itchy eyes, watery eyes and nasal SMS (data not shown). However, the symptom scores for itchy eyes did approach significance, being lower in the L-92 group (P < 0.1).

**Blood examination**

The percentage of Th2 cells increased as the amount of Japanese cedar pollen increased. On the other hand, the percentage of Th1 cells remained constant throughout the experimental period; therefore, the Th1/Th2 ratio decreased with time. However, there was no significant difference between the two groups in these measurements (data not shown).

The total serum IgE and anti-Japanese cedar pollen-IgE levels also rose with time. No significant differences were observed between the two groups in these measurements. The time-dependent changes in anti-Japanese cedar pollen-IgE levels are shown in Fig. 1C. No significant difference was observed between the two groups.

**Study done during the 2003 season**

**Count of pollen**

Figure 2A shows the daily averages of the Japanese cedar pollen count during the 2003 season. The total Japanese cedar pollen dispersed during the 2003 season was 8205.6 grains per cm², while it was 8133.6 grains per cm² during the study period.

**Subjective symptom**

Distress in the life of the subjects during the period of ingesting L-92 fermented milk was significantly less than that of the placebo group (Fig. 2b). Other measurements of subjective symptoms did not show any significant differences throughout the experimental period (data not shown).

**Blood examination**

The changes in the data from the blood examination was similar to that observed during the 2002 season. The percentage of Th1 cells in the L-92 group was also higher, and thus the Th1/Th2 ratio was higher (Fig. 2C). However, no significant difference was apparent between the two groups in either parameter.

**Medical examination of the nasal cavity**

The scores for swelling and color of the mucosa in the inferior nasal concha approached significance with reduced values (P < 0.1) in the L-92 group. However, no significant differences in the scores of the other parameters were apparent between the two groups (data not shown). The amount and nature of pituita did not differ either between the two groups (data not shown).

**Discussion**

The prevalence of Japanese cedar pollinosis has been estimated to be 5 to 20% in various target populations in Japan. This is one example of the progressive increase in frequency of atopic disease in developed countries. There are many hypotheses that could explain this increase. Among them, it has been suggested that alterations in the intestinal microflora resulting from changes in diet and hygiene could be the cause, because the microflora is considered to affect the host’s Th1/Th2 balance. In a recent case-control study, Matricardi et al. have indicated that inappropriate stimulation by commensal intestinal bacteria or pathogens affecting gut-associated lymphoid tissue (GALT) enhanced the risk of atopy. The induction of a Th1-polarized immune status and subsequent inhibition of IgE production is associated with a reduction in the risk of developing atopic diseases.

Probiotics are thought to be one of the tools for establishing a Th1 predominance through changes in the composition of the host’s intestinal microflora by their affects or by their direct action towards GALT. Recent studies have also documented a relationship between the establishment of intestinal microorganisms or its process and resistance to the development of allergy. An analysis of the intestinal microflora in fecal samples from 2-year-old children in Sweden and Estonia in-
**Fig. 1.** Clinical Trial during the 2002 Season.

A. Japanese cedar pollen dispersed around the Sagamihara City area. Data were kindly provided by Dr. Yasueda of Sagamihara National Hospital. B. Time-dependent changes in daily average ocular SMS during the study. C. Time-dependent changes in the daily average of serum anti-Japanese cedar pollen IgE levels during the study.
Fig. 2. Clinical Trial during the 2003 Season.
A. Japanese cedar pollen dispersed around the Sagamihara City area. Data were kindly provided by Dr. Yasueda of the Sagamihara National Hospital. B. Time-dependent changes in the daily average scores of distress of life during the study. C. Time-dependent changes in the Th1 and Th2 populations in peripheral blood. Each point and bar represent the mean value ± standard error of the mean.
dicated a lower rate of colonization by lactobacilli in allergic than in non-allergic children, although the proportion of aerobic bacteria to the total bacterial count, especially coliforms and Staphylococcus aureus, was elevated in the intestinal microflora of the allergic children. Furthermore, lower titers of microbial short-chain fatty acids, with the exception of Clostridium difficile—associated F-caproic acid, have been measured in the feces of allergic infants. Based on these findings, Kalliomäki et al. have analyzed the composition of intestinal microflora in infants at a high risk of developing atopic diseases. There is room for discussion about the analytical methodology used, but they have claimed that a reduced ratio of bifidobacteria and clostridia was characteristic of those subjects in whom atopy was developing. The trial in particular presents plausible evidence for the critical neonatal role of indigenous intestinal microflora in explaining an atopy-restrictive or -permissive immunological situation. These experimental and epidemiological findings manifest the attracting possibility that manipulation of host intestinal microflora by any means represents a viable therapeutic alternative for managing atopic diseases. Further examination will prove whether this possibility is valid. However, this possible manipulation has so far been limited to the use of probiotics and prebiotics. In addition, it may be considered that orally administered bacterial cells, which are of exogenous origin, even if dead, are simply taken up by GALT and stimulate the host systemic immune system without inducing any significant alteration in the composition of the intestinal microflora.

In accordance with these hypotheses, we screened lactic acid bacteria for their capacity to alter the composition of human intestinal microflora and reduce the serum antigen-specific IgE level in a mouse model. L-92 was found to have a significant impact on the intestinal microflora; in particular, we observed a reduction in the incidence of Clostridium perfringens and an increment of incidence and number of Lactobacillus in feces. In addition, an outstanding serum antigen-specific IgE-lowering effect of orally administered L-92 has been observed in animal studies.

To verify the possibility of primary or secondary prevention of atopic sensitization by orally administered L-92, we evaluated oral bacteriotherapy with L-92 in volunteers sensitive to Japanese cedar pollen. Heat-treated L-92 was employed to subtract the effect of L-92 by the activation of Th2 cells; what is called Th2-polarized immune impairment. In fact, we observed a significant increase in the number of Th2 cells in peripheral blood associated with exposure to Japanese cedar pollen prior to the rise in the level of serum anti-Japanese cedar pollen IgE. The Th1 and Th2 populations, Th1/Th2 ratio, and anti-Japanese cedar pollen IgE did not seem to be significantly affected by an oral

Data concerning the prophylactic effects of probiotics consumption on allergy remain controversial. However, Majamaa and Isolauri have shown a significant amelioration of clinical symptoms and immunological parameters in a randomized, placebo-controlled trial of probiotic therapy in infants with atopic eczema and cow’s milk allergy. Indeed, the addition of live L. rhamnosus GG to the hydrolyzed whey formula fed to patients on a strict bovine milk protein-elimination diet accelerated the resolution of eczema and drastically reduced the signal of intestinal inflammation. A subsequent study has demonstrated a correlation between these improvements in clinical score with changes related specifically to allergic inflammation. The most clear evidence for the potential of probiotics in a clinical context is a double-blind, randomized, placebo-controlled trial in which lactobacilli given prenatally to pregnant women with a high risk of atopic eczema, allergic rhinitis, or asthma resulted in the symptom level of the probiotic group being one-half that of the placebo group. This suggests that the ingestion of a probiotic or a particular set of commensal organisms may have a profound systemic effect on several of the manifestations of allergy and asthma.

Regardless of inconclusive mechanism details, probiotics represent a therapeutic paradigm that, unlike conventional modalities, attends to the epidemiology, and possibly the etiology, of atopy. For this reason, probiotic intervention during infantile microbial colonization of the gut, and the articulation of an intrinsic immunologic “disposition” antithetical to Th2 polarization, may be a realistic consideration for the primary prevention of allergy and asthma. Probiotics, which are established from commensal microflora or of other origin to specifically induce Th1 cells, promote potentially anti-allergenic processes by balancing the Th1/Th2 ratio. L-92 is a probiotic strain which effectively suppresses the allergen-specific serum IgE level in mice. L-92 might behave as an effective counter-regulator of Th2-skewed immunity in patients with pollinosis when orally administered.
intake of L-92. However, these data do not necessarily indicate that L-92 did not influence the Th1/Th2 ratio and IgE production, because the number of volunteers might have been insufficient. Another possible explanation for the anti-allergic effect of L-92 is related to the functional status of regulatory T cells. The use of probiotics in allergy prevention is further supported by the results of studies showing that the oral administration of lactobacilli to atopic children enhanced the production of transforming growth factor β (TGF-β) and interleukin 10 (IL-10) in vivo.24,29 Findings from clinical and experimental studies indicate that these anti-inflammatory cytokines play a significant role, possibly more essential than that of Th1 inducers, in the prevention of atopic diseases.30,31 In present study, little difference in Th1/Th2 ratio and the anti-Japanese cedar pollen IgE level was observed between the control and L-92 groups. This fact suggests that regulatory T cells or the Th3 sub-population might play a critical role in the course of expression of the anti-allergic effect of L-92. Further investigation is needed to elucidate the mechanism(s) for the anti-allergic effect of orally administered L-92.

It could be argued that the milk fermented by L-92 that was used in this study was heat-treated. It is considered that heat-killed L-92 cells cannot affect the host’s intestinal microflora. Therefore, the underlying mechanism(s) for the anti-allergic effect of orally administered L-92 may be explained by direct action on the host systemic immune system.

The effect of L-92 on other kinds of type-I allergy such as chronic allergic rhinitis and the mechanism(s) for the anti-allergic action of L-92 should be investigated in future studies.

Acknowledgment

We thank Dr. H. Yasueda of Sagamihara National Hospital, for providing the data on pollen dispersion in the air around the Sagamihara area, and for helpful suggestions.

References


