Effect of Rush Immunotherapy in House-Dust-Mite (HDM)-Sensitive Adult Bronchial Asthma: Changes in In Vivo and In Vitro Responses to HDM

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An open study was conducted to evaluate the changes in in vivo and in vitro responses to house-dust-mite (HDM) after rush immunotherapy (RI). A 7-day RI protocol using an extract containing HDM allergen was administered to 12 subjects with HDM-sensitive asthma, and the effects on bronchial responsiveness and serum antibody levels were evaluated up to 16 or 20 weeks after RI. The levels of HDM-specific IgG, IgGl and IgG4 antibodies were significantly elevated from 4 or 8 weeks after RI. Provocative doses causing a 20% fall in FEV₁ (PD20) by allergen inhalation were elevated in all subjects at 16 to 20 weeks after RI. There was a high correlation between the increase in log-PD20 and the increase in the ratio of HDM-specific IgG4 to IgGl (r=0.68, p<0.05). The results suggest that RI elicits the improvement of allergen-specific bronchial responsiveness and the increase in serum antibody levels within a relatively short period.

Key words: bronchial responsiveness, IgGl antibody, IgG4 antibody

Introduction

House-dust-mite (HDM) is a major allergen for allergic bronchial asthma in many countries including Japan (1). More than 70% of Japanese asthmatics show a positive skin test to HDM and/or HDM-specific serum IgE antibody (2). Seasonal exacerbation of asthmatic symptoms usually occurs in September and October (3), and is consistent with the increase of numbers of HDM in the living environments (4).

Immunotherapy (IT) has been performed for the treatment of HDM-sensitive patients with asthma, and its efficacy has been verified (5). In most studies Dermatophagoides pteronyssinus (Dp) or D. farinae (Df) extract was used, whereas a certain house-dust (HD) extract has been exclusively and widely used for the treatment of HDM allergy in Japan.

Although IT has been generally performed according to the conventional schedule, rush immunotherapy (RI) is an alternative method of IT originally described by Freeman (6) in 1930, reaching a maintenance dose within several days. RI has been confirmed to be effective for the treatment of hypersensitivity to HDM (7), grass pollens (8), hymenoptera (9) or Alternaria (10) using relevant antigen extracts. We have previously reported that rush immunotherapy of a 7-day protocol using the HD extract can be carried out safely and securely for HDM-sensitive adult asthmatics (11). Since the maintenance dose can be reached within several days by RI, it is reasonable to expect that the effects of IT on cellular and/or humoral responses against HDM appear much earlier than conventional IT in which it takes a few months, at the earliest, to reach the maintenance dose. Bousquet and associates (7) have reported that RI using Dp extract elicits the clinical efficacy within a few weeks. They also noted the increase in Dp-specific IgG and IgG4 antibodies in sera, the decrease in basophil histamine release and the increase in the threshold dose causing an immediate asthmatic response during an antigen provocation test. However, they were unable to find any significant correlation between the clinical improvement and the changes in in vitro parameters.

In the present study the changes in serum antibody levels and bronchial responsiveness after RI were evaluated to confirm whether or not RI, using the HD extract, elicits in vivo and in vitro responses against HDM in a relatively short period. The

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correlations between the changes in airway responsiveness and immunological parameters were also evaluated.

Materials and Methods

Subjects

Twelve subjects with perennial asthma ranging in age from 18 to 59 years were enrolled in the study. Each subject fulfilled the following criteria: 1) A definite diagnosis of bronchial asthma according to the criteria of the American Thoracic Society (12), 2) no dependency on systemic corticosteroids, 3) a high titer of serum IgE antibody specific to *Dermatophagoides pteronyssinus* (Dp) and no significant level of serum IgE antibody to other common allergens, 4) a positive bronchial provocation test (BPT) by inhaling HD extract, 5) no prior immunotherapy, and 6) absence of pregnancy or chronic obstructive pulmonary disease such as chronic bronchitis or pulmonary emphysema. All eligible subjects gave informed consent before enrollment in the study. Ten HDM-sensitive asthmatics with equivalent backgrounds were examined as the controls. There was no statistically significant difference in age, sex, severity and duration of asthma, serum levels of total IgE and Dp-specific IgE, or bronchial responsiveness to HD and methacholine between RI subjects (RI group) and the controls (control group).

Study design

All subjects were allowed to continue to take daily anti-asthma medications which were the combination of sustained-release theophylline, oral β-agonist and/or β-agonist inhaler, disodium cromoglycate, ketotifen, or beclomethasone dipropionate inhaler. These medications had been used without any change in the dose for more than six months prior to the study. Bronchial responsiveness to both the HD extract and methacholine were examined twice: Prior to enrollment in both groups, and 16 to 20 weeks after RI in the RI group or enrollment in the control group. Peripheral blood was collected before, one day, 4, 8, 12 and 16 weeks after RI in the RI group, and before and 16 to 20 weeks after RI in the RI group or methacholine were examined twice: Prior to enrollment in both study. Bronchial provocation test (BPT) by inhaling HD extract, 5) no prior immunotherapy, and 6) absence of pregnancy or chronic obstructive pulmonary disease such as chronic bronchitis or pulmonary emphysema. All eligible subjects gave informed consent before enrollment in the study. Ten HDM-sensitive asthmatics with equivalent backgrounds were examined as the controls. There was no statistically significant difference in age, sex, severity and duration of asthma, serum levels of total IgE and Dp-specific IgE, or bronchial responsiveness to HD and methacholine between RI subjects (RI group) and the controls (control group).

**HD extract for RI**

Vials of HD extract were purchased from Torii Pharmaceutical Co. (Tokyo, Japan). HD extract was dissolved with physiological saline containing 0.5% phenol at the concentrations of 1/10, 1/100 and 1/1,000 (W/V). Weight per volume (W/V) indicates the weight (g) of delipidized HD per the volume (ml) of extracting buffer. Further diluted HD solution was prepared with use of the same solution.

**Schedule of RI**

Each subject of the RI group was hospitalized in order to receive RI. RI was performed according to the method previously reported (Table 1) (11). In brief, the initial concentration of injection was a solution diluted 1/10 of the threshold concentration determined by skin test titration. Each subject received three to six subcutaneous shots daily, at one to two hour intervals. In order to prevent the appearance of systemic or local reaction, the following medications were administered during RI in addition to anti-asthma drugs; three mg oforal mequitazine, twice or three times daily, and inhalation of both 20 mg of disodium cromoglycate and 2.5 mg of salbutamol hemisulfate, four times daily. The final dose was set at 0.5 ml of 1/10 solution unless systemic reaction was provoked. When a systemic reaction such as an asthmatic attack or generalized urticaria occurred, patients were carefully examined and promptly received an appropriate therapy, thereafter, the dose of the next injection was reduced to half of the previous injection. After confirming the safety of that dose, it was determined to be the maintenance dose. After discharge, injection of the maintenance dose was repeated every two weeks in the outpatient clinic.

**Bronchial provocation test (BPT)**

BPT using HD extract was performed employing the standardized method of the Japanese Society of Allergology. In brief, HD extract was diluted from 1/10 (W/V) to 1/10,000 serially by physiological saline. Aerosol was delivered by a nebulizer (Devilbiss Model 646, Devilbiss, Somerset, PA, U.S.A.) at five L/min with compressed air, and inhaled by tidal breathing while a nose clip was set. Spirometry was performed using an autoSpirometer (PK-Morgan, Kent, England). After the baseline FEV1 was measured, physiological saline was inhaled to check the absence of non-specific bronchoconstriction. Thereafter, 1/10,000 HD solution was inhaled for two minutes, and FEV1 was measured ten minutes later. Unless the fall in FEV1 was more than 20% of the baseline value, the ten-fold concentrated solution was inhaled. This procedure was repeated until

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**Table 1. The Schedule of Rush Immunotherapy with HD Extract**

<table>
<thead>
<tr>
<th>Day</th>
<th>Time</th>
<th>W/V*</th>
<th>ml</th>
<th>Day</th>
<th>Time</th>
<th>W/V</th>
<th>ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9 am</td>
<td>10 -6</td>
<td>0.05</td>
<td>4</td>
<td>9 am</td>
<td>10 -3</td>
<td>0.25</td>
</tr>
<tr>
<td>10 am</td>
<td>0.10</td>
<td>10 am</td>
<td>0.35</td>
<td>11 am</td>
<td>0.20</td>
<td>11 am</td>
<td>0.50</td>
</tr>
<tr>
<td>1 pm</td>
<td>0.35</td>
<td>1 pm</td>
<td>10 -3</td>
<td>0.05</td>
<td>3 pm</td>
<td>10 -4</td>
<td>0.05</td>
</tr>
<tr>
<td>5 pm</td>
<td>10 -2</td>
<td>5 pm</td>
<td>0.10</td>
<td>10 -1</td>
<td>1 pm</td>
<td>0.50</td>
<td>1 pm</td>
</tr>
<tr>
<td>3 pm</td>
<td>0.15</td>
<td>3 pm</td>
<td>0.10</td>
<td>10 am</td>
<td>0.35</td>
<td>7 am</td>
<td>0.30</td>
</tr>
<tr>
<td>10 am</td>
<td>0.35</td>
<td>10 am</td>
<td>0.40</td>
<td>11 am</td>
<td>0.50</td>
<td>10 am</td>
<td>0.15</td>
</tr>
<tr>
<td>5 pm</td>
<td>10 -3</td>
<td>5 pm</td>
<td>0.05</td>
<td>11 am</td>
<td>0.50</td>
<td>11 am</td>
<td>0.50</td>
</tr>
</tbody>
</table>

*Weight per volume.*
FEV\textsubscript{1} decreased more than 20% of the baseline FEV\textsubscript{1}. At the end of the test, 20 mg or 30 mg of oral prednisolone was administered in order to prevent a possible late asthmatic response. Every BPT was started between 9 and 11 am.

The threshold value of BPT was expressed as a logarithm of the provocative dose causing a 20% fall in FEV\textsubscript{1} (PD\textsubscript{20}). When the 1/10,000 solution was inhaled for one minute, one point was given as PD\textsubscript{20}. If the fall of FEV\textsubscript{1} did not reach 20% after inhalation of the 1/10 solution, 2,222 points were given.

**Methacholine challenge test**

Non-specific bronchial responsiveness to methacholine was measured by the oscillation method using the Astograph (TCK-6100, Chest Co., Tokyo, Japan), according to the method described by Takishima and associates (13). The minimum cumulative dose of methacholine (D\textsubscript{min}) required to induce a two-fold increase of respiratory resistance was calculated as a parameter expressing non-specific bronchial responsiveness. Every test was started between 2 and 3 pm.

Both BPT and the methacholine challenge test were performed only when a subject did not show any asthmatic symptom. Prior to each test, bronchodilators were withdrawn for at least 12 hours, beclomethasone dipropionate, disodium cromoglycate, and ketotifen for at least two weeks, respectively.

**Measurement of antibodies and IgE-binding factor in sera**

Serum IgE level and D\textsubscript{p}-specific IgE antibody were measured by radioimmunoassay (RIA) kits (Pharmacia, Uppsala, Sweden). D\textsubscript{p}-specific IgG antibody was measured by enzyme-linked immunosorbent assay (ELISA) using a D\textsubscript{p}-specific IgG test kit (Shionogi Pharmaceutical Co., Osaka, Japan). D\textsubscript{p}-specific IgG subclasses were assayed by the conventional solid-phase ELISA method with use of lyophilized *Dermatophagoides* extract (Torii Pharmaceutical Co.), peroxidase-conjugated anti-human IgG1 and IgG4 monoclonal antibody (Shionogi Pharmaceutical Co.), and reference sera for D\textsubscript{p}-specific IgG1 and IgG4 antibody (Shionogi Pharmaceutical Co.).

The amount of IgE-binding factor (IgE BF) was measured by a solid-phase sandwich radioimmunoassay using two monoclonal antibodies specific for FceRII on RPMI 8866 cells as previously reported (14).

**Statistical analysis**

All data were expressed as mean±SD. Statistical analysis was performed using Student’s paired t-test for changes in clinical scores and immunological parameters, and Wilcoxon’s signed-rank test for changes in PD\textsubscript{20} and D\textsubscript{min}. A difference was considered to be significant when the p-value was less than 0.05.

**Results**

**Treatment courses**

All subjects were able to receive 1/10 solution of HD extract as a maintenance dose within seven to ten days (Table 2).

<table>
<thead>
<tr>
<th>Case</th>
<th>Maintenance dose</th>
<th>Days*</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10^7×0.2 ml</td>
<td>9</td>
<td>Local skin reaction***, urticaria</td>
</tr>
<tr>
<td>2</td>
<td>10^7×0.5 ml</td>
<td>7</td>
<td>Local skin reaction, asthma (mild attack)</td>
</tr>
<tr>
<td>3</td>
<td>10^7×0.2 ml</td>
<td>7</td>
<td>Local skin reaction, asthma (mild attack)</td>
</tr>
<tr>
<td>4</td>
<td>10^7×0.1 ml</td>
<td>7</td>
<td>Local skin reaction, asthma (moderate attack)</td>
</tr>
<tr>
<td>5</td>
<td>10^7×0.1 ml</td>
<td>9</td>
<td>Local skin reaction, asthma (severe attack)</td>
</tr>
<tr>
<td>6</td>
<td>10^7×0.5 ml</td>
<td>6</td>
<td>Local skin reaction</td>
</tr>
<tr>
<td>7</td>
<td>10^7×0.1 ml</td>
<td>7</td>
<td>Local skin reaction, asthma (mild attack)</td>
</tr>
<tr>
<td>8</td>
<td>10^7×0.5 ml</td>
<td>7</td>
<td>Local skin reaction</td>
</tr>
<tr>
<td>9</td>
<td>10^7×0.1 ml</td>
<td>8</td>
<td>Local skin reaction</td>
</tr>
<tr>
<td>10</td>
<td>10^7×0.3 ml</td>
<td>8</td>
<td>Local skin reaction</td>
</tr>
<tr>
<td>11</td>
<td>10^7×0.5 ml</td>
<td>8</td>
<td>Local skin reaction</td>
</tr>
<tr>
<td>12</td>
<td>10^7×0.5 ml</td>
<td>10</td>
<td>Local skin reaction, asthma (moderate attack)</td>
</tr>
</tbody>
</table>

*Days required for reaching the maintenance dose. **Local skin reaction included swelling, redness and itching.

Generalized urticaria appeared in one subject, then oral prednisolone and chlorpheniramine maleate were given resulting in a complete remission. Asthmatic attacks occurred in five subjects: Two subjects with a mild attack were treated with salbutamol nebulization. Two with a moderate attack and one with a severe attack were treated with salbutamol nebulization, and intravenous administration of aminophylline and hydrocortisone. All asthmatic attacks occurred within 30 minutes after the injection of 1/10 solution, and improved within one hour after the stated treatment. No systemic reaction occurred in the maintenance immunotherapy at the outpatient clinic.

**BPT by HD extract**

In the RI group, values of log-PD\textsubscript{20} at 16 to 20 weeks after RI were elevated in all subjects as compared with those before RI (Fig. 1). The mean values before and after RI were 1.59±0.72 (mean±SD) and 2.52±0.62, respectively, and a statistically significant difference occurred (p<0.001). However, there was no statistically significant change in the control group (2.27±0.40 vs 2.61±0.47).

**Methacholine challenge test**

In the RI group, the mean values of D\textsubscript{min} before and after RI were 0.91±0.81 and 2.91±4.64, respectively (Fig. 1). In the control group 0.94±0.69 and 1.89±2.39, respectively. No statistically significant difference occurred in the RI group nor in the control group.

**Serum IgE level**

In the RI group, the mean levels of serum IgE before and after RI were 16 weeks after RI were 585±466 and 950±833 IU/ml, respectively, and a statistically significant difference occurred (p<0.05) (Fig. 2). There was no significant change in serum IgE level in the control group (1,028±1,544 vs 970±1,477 IU/ml).
Rush Immunotherapy in Atopic Asthma

Fig. 1. Changes in the values of log-PD_{20} in BPT using HD extract (upper) and the minimum cumulative dose of methacholine (Dmin) (lower). BPT: bronchial provocation test, HD: house dust, PD_{20}: provocative dose causing a 20% fall in forced expiratory volume in one second, RI: rush immunotherapy.

IgE BF
The pre-treatment levels of serum IgE BF were 500 to 675 (576±57) pg/ml in the RI group and 395 to 765 (600±134) pg/ml in the control group (Fig. 2). Those levels at 16 weeks in the RI group and the control group were 420 to 760 (550±127) pg/ml and 275 to 845 (631±219).pg/ml, respectively. The levels decreased in seven of 12 subjects (58%) in the RI group and two of eight (25%) in the control group. However, no statistically significant difference occurred in either group.

HDM-specific IgE antibody
In the RI group, the mean levels of Dp-specific IgE antibody before, and at 0, 4, 8, 12 and 16 weeks after RI were 80.1±74.0, 77.2±70.2, 188.6±187.1, 170.0±172.5, 167.5±136.3 and 179.3±166.7 PRU/ml, respectively (Fig. 3). Statistically significant elevations, compared with before RI, occurred at 12 and 16 weeks after RI (p<0.05 and p<0.02, respectively). In the RI group, a subject showed an obvious reduction of Dp-specific IgE antibody, and no obvious change was observed in the other three. There was no significant change in the control group (172.4±214.4 vs 172.1±214.4 PRU/ml).

HDM-specific IgG antibody
In the RI group, mean levels of Dp-specific IgG antibody before, and at 0, 4, 8, 12 and 16 weeks after RI were 263.3±127.1, 364.2±261.9, 674.6±236.0, 614.9±206.1, 632.8±308.6 and 635.3±336.4 U/ml, respectively (Fig. 4). Statistically significant elevations, compared with before RI, occurred from 4 weeks after RI, (p<0.001 at 4 and 8 weeks, p<0.002 at 12 weeks, and p<0.01 at 16 weeks). There was no significant change in the control group (308.1±256.4 vs 313.5±199.3 U/ml).

HDM-specific IgG1 and IgG4 antibodies
In the RI group, mean levels of Dp-specific IgG1 antibody before, and at 0, 4, 8, 12 and 16 weeks after RI were 172.4±176.3, 174.3±142.6, 860.9±1314.8, 534.6±495.5, 463.9±346.8 and 396.2±291.8 U/ml, respectively (Fig. 5). Statistically significant elevations, compared with before RI, occurred from 8 weeks after RI (p<0.02 at 12 weeks, and p<0.05 at 8 and 16 weeks). However, in four subjects Dp-specific IgG1 decreased at 16 weeks after RI. Mean levels of Dp-specific IgG4 before,
and at 0, 4, 8, 12 and 16 weeks after RI were 89.8±59.9, 100.3±29.3, 311.0±174.2, 321.5±193.2, 300.5±161.7 and 314.2±175.7 U/ml, respectively (Fig. 6). Statistically significant elevations, compared with before RI, occurred from 4 weeks after RI (p<0.01 at 4 and 8 weeks, and p<0.001 at 12 and 16 weeks). There was no significant correlation between the cumulative antigen dose and the level of Dp-specific IgG1 or IgG4 (data not shown). No significant change in either IgG subclass occurred in the control group (IgG1; 135.5±145.6 vs 154.6±169.8 U/ml, IgG4; 47.4±62.4 vs 36.4±35.1 U/ml).

Correlation between BPT and each antibody level

Correlations between the increase in log-PD_{20} and the increase in each antibody level were examined in the RI group. Among arithmetic or logarithmic variables including each isotype and subclass, alone or in combination such as IgG1/IgE, IgG4/IgE and so on, a significant correlation was found only in the logarithmic ratio of Dp-specific IgG4/IgG1 after RI to that before RI (r=0.68, p<0.05) (Fig. 7). In the control group, no significant correlation was found in any antibody level (data not shown).
Discussion

The results of the present study demonstrated that RI using the HD extract can elicit the improvement of bronchial responsiveness as well as the increase in specific antibody levels against HDM during a relatively short period. Among antibody isotypes, IgG, IgG1 and IgG4 antibody levels distinctly increased within one or two months after RI. The apparent elevation of threshold values in BPT was observed within four or five months in all subjects in the RI group while non-specific bronchial responsiveness against methacholine did not change. Improvement of threshold in immediate asthmatic response (IAR) of BPT after RI has previously been noted by Goldstein and Chai (15) and by Bousquet and associates (7). IAR is
considered to be primarily triggered by bronchospastic mediators released from mast cells in bronchial mucosa through the cross-linking of IgE by antigen. Therefore, the attenuation of antigen-specific bronchial responsiveness after RI would be due to the decrease in the releasability of mast cells. Regarding IgE, the levels of Dp-specific IgE antibody increased approximately two-fold after RI compared to baseline. If the increase in the antibody level were limited to Dp-specific IgE, it would have enhanced IAR. However, the increase in Dp-specific IgE was accompanied by the increase in total IgE level and the ratio of Dp-specific IgE to total IgE did not change significantly (data not shown). This may imply that the amount of mast cell-bound Dp-specific IgE would not increase due to interference by non-specific IgE.

Although the diminished releasability of mast cells in IAR could be explained by several mechanisms, one possible explanation would be the synthesis of antigen-specific blocking antibodies. It has been hypothesized that specific IgG4 antibody acts as the blocking antibody, although its exact roles in allergic diseases still remain controversial (16, 17). If the IgG4 antibody acts as the blocking antibody, the increase in the IgG4 antibody level ought to be reflected in the magnitude of IAR. From this point of view, we found a high correlation between the improvement of threshold values in BPT and the increase in the ratio of Dp-specific IgG4 to IgG1. Similar findings have recently been demonstrated by Einarsson and associates (18). They demonstrated that the ratio of Df-specific IgG4 to IgG1 increased linearly up to a year after initiation IT and the mean increase in G4/G1 was 3.4-fold compared to baseline. In addition, they observed that such an increase was associated with the increase in the ratio of the threshold dose in the conjunctival provocation test after IT to that before IT. In contrast, poor correlation between the ratio of antigen-specific IgG4 to IgG1 and the clinical improvement has also been reported: One with use of grass pollen (19) and another with Cladosporium (20). The reason for such a discrepancy remains unclear, but might be partly due to the difference in antibody-isotype induction between HDM and grass pollen or mold. Nevertheless, the present results may support the protective role of IgG4 antibody in immediate allergic reaction, however, it should be confirmed by increasing the number of subjects.

Other suppressive mechanisms could also be induced by IT. One we found was that levels of IgE BF in sera tended to decrease after RI. The functional roles of IgE BF have been reported in recent years: IgE BF enhances IgE production by interleukin-4 (IL-4) (21). By contrast, IgE BF inhibits the synthesis of chemical mediators by CD23-positive cells via blocking the binding of IgE to the receptor (22). The net effect of IgE BF on the overall allergic reaction is still unknown, however, provided that the levels of IgE BF in serum reflects the amount of cell-associated CD23 molecules, the reduction of IgE BF in sera might implicate the reduction of the number of CD23 on the cell surface or the number of CD23 positive cells, leading to the attenuation of FceRII-mediated allergic reaction. However, it is unable to attribute the decrease of IgE BF to the clinical effectiveness of RI at the present time as it was not a consistent finding at 16 to 20 weeks after RI. The significance of the alteration of serum IgE BF level would need to be evaluated after a relatively long period, and that study is currently underway.

Suppression of antigen-specific T cell response is another putative mechanism of IT. IT induced the suppression of IL-2 production by relevant antigen (23). Recently eosinophils have been regarded as pivotal cells in the pathophysiology of bronchial asthma (24). Human T cells with a profile analogous to murine Th2 secrete IL-3, IL-5 and GM-CSF. These cytokines are known to be responsible for eosinophil differentiation, proliferation, chemotaxis and activation (25). Therefore it is conceivable that IT suppresses the production of these eosinophilopoietic cytokines. Indeed, IT decreased the eosinophil influx into the airway by exposure to antigens (26).

In concert with this, we observed that IT reduced the production of eosinophil chemotactic activity from peripheral blood mononuclear cells (unpublished data).

The hospitalization is mandatory in our RI protocol as the injection is repeated several times per day for a week, and it is necessary to observe patients carefully after each injection and treat immediately once systemic reactions occur. In addition, it is expected that asthmatic conditions become more stable in the hospital than at home by the removal of house-dust exposure, which would reduce the risk of the occurrence of systemic reactions.

The incidence of systemic reactions such as asthma or generalized urticaria was about 42% during the increasing of the dose. Meanwhile, no systemic reaction occurred during the maintenance immunotherapy in the outpatient clinic. Hejaoui and associates reported that systemic reactions in their 3-day
protocol was seen in 36% of subjects, followed by a much lower incidence using concomitant preventive therapy or a 14 day-step protocol (27, 28). The difference in the incidence of systemic reactions between their study and the present study could be caused by the difference in the maintenance dose. They set the maintenance dose in every subject at 3,000 biological units (BU) where injection of 4,000 BU caused systemic reaction in more than half of the subjects (7). We aimed at setting the maintenance dose individually by increasing the dose up to 0.5 ml of 1/10 solution maximally unless a systemic reaction appeared. Nevertheless, the optimal fixed-dose with a lower incidence of undesirable systemic reaction has to be determined and employed in future studies.

We used an HD extract instead of HDM extracts. The HD extract (Torii HD extract\(^\text{a}\)) has been exclusively and widely used in Japan for the treatment of HDM allergy. It contains a certain amount of major mite antigens, and lot-to-lot variability has been minimized by titrating the intradermal responses in HDM-sensitive subjects. Thus, such qualities of the extract would afford the rationale for the use in IT until standardized HDM extract is approved for therapeutic use in Japan in the future.

In conclusion, the present study suggests that RI elicits the improvement of allergen-specific bronchial responsiveness and the increase in specific antibody levels within a relatively short period. The attenuated bronchial responsiveness to HDM was correlated with the increase in the ratio of HDM-specific IgG4 to IgG1.

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