Effect of Local Nasal Immunotherapy on Nasal Blockage in Pollen-Induced Allergic Rhinitis of Guinea Pigs

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ABSTRACT
Background: As a non-injection route for immunotherapy, local nasal immunotherapy has been examined in allergic rhinitis patients. However, it is unclear how the immunotherapy affects sneezing, biphasic nasal blockage and nasal hyperresponsiveness. Thus, we evaluated the therapeutic effects of nasal immunotherapy on the symptoms of guinea pig allergic rhinitis. Additionally, we also evaluated whether the immunotherapy relieved pollen-induced allergic conjunctivitis.

Methods: Sensitized animals were repeatedly challenged by pollen inhalation once every week. After the 7th challenge, the pollen extract was intranasally administered 6 times a week until the 30th challenge. Sneezing frequency was counted after each of the challenges. As an indicator of nasal blockage, changes in specific airway resistance were measured. Nasal hyperresponsiveness was assessed by measuring leukotriene D4-induced nasal blockage. Additionally, during the immunotherapy, we applied pollen onto the ocular surface to induce the allergic conjunctivitis symptoms.

Results: At the 11th–30th challenges, the nasal immunotherapy showed inhibition or a tendency to inhibit the biphasic nasal blockage although the inhibitions were variable at respective challenges. The development of nasal hyperresponsiveness was markedly suppressed by the immunotherapy. Nevertheless, neither sneezing nor antigen-specific IgE antibody production was substantially influenced by the immunotherapy. On the other hand, the nasal immunotherapy did not affect the induction of allergic conjunctivitis symptoms.

Conclusions: Local nasal immunotherapy may be clinically useful for allergic nasal blockage associated with nasal hyperresponsiveness. The mechanisms responsible for this effectiveness might not be related to IgE production. Additionally, the effectiveness for nasal tissue was dissociated from that seen for the ocular tissue.

KEY WORDS
allergic rhinitis, animal model, local nasal immunotherapy, nasal blockage, pollenosis

INTRODUCTION
In order to relieve the symptoms of pollenosis, specific immunotherapies have been developed that use subcutaneous administration of specific pollen antigens.1 While subcutaneous immunotherapy has become an established therapeutic modality, it is also well known that subcutaneous injections of antigen can potentially result in the occurrence of life-threatening anaphylactic reactions.2 Thus, to avoid such occurrences but still be able to provide specific immunotherapy, oral immunotherapy has been investigated and clinically tested in patients with allergic rhinitis.3 However, generally higher doses of the allergen are needed when administered orally, which in some cases can induce a cytotoxic effect on the digestive tract.3

Another alternative non-injection route for immunotherapy is the use of local nasal immunotherapy. This administration method has been examined in patients with allergic rhinitis.4,6 Local immunotherapy has an advantage in that only a small amount of the allergen is required as compared to that needed for the oral immunotherapeutic methods.3 In most of the
Fig. 1 Schedule for sensitization and challenge with Japanese cedar pollen, and intranasal instillations of the pollen extract for the intranasal immunotherapy in guinea pigs. S: Sensitization with the pollen extract + Al(OH)$_3$ twice a day.

clinical examinations that have investigated local nasal immunotherapy, the therapy has been shown to be effective in the induction of allergic nasal signs.$^4$-$^8$ However, due to the limitations of these previous clinical studies, it is not clear as to how the immunotherapy is able to affect the various nasal allergic symptoms, such as sneezing, biphasic nasal blockage and nasal hyperresponsiveness to non-specific stimuli. Thus, there is a need to examine the effects on local nasal mucosa using an experimental animal model of allergic rhinitis that has symptoms similar to those seen in clinical settings, and generate results that will help to elucidate the mechanisms underlying the therapeutic effect.

We have established a Japanese cedar pollen-induced allergic rhinitis guinea pig model.$^9$-$^{12}$ In this model, after a pollen inhalation challenge, there is immediate sneezing and biphasically induced nasal blockage.$^{10}$ One of the characteristic features of experimental allergic rhinitis is that there is a markedly increased nasal responsiveness to intranasally instilled histamine or leukotriene (LT) D$_4$.$^{11}$-$^{12}$ Therefore, in the present study we tried to evaluate the effectiveness of local nasal immunotherapy in our experimental model. We also assessed whether nasal immunotherapy affected allergic conjunctivitis induced by dropping a pollen suspension into the eyes of test subjects.

METHODS

ANIMALS

Male, 3-week-old Hartley guinea pigs were purchased from Japan SLC (Hamamatsu, Japan). The animals were housed in an air-conditioned room at a temperature of 23 ± 1°C and 60 ± 10% humidity with the lights on from 8:00 a.m. to 8:00 p.m. Animals were fed a standard laboratory diet and given water ad libitum. The first sensitization was started 2 weeks after the arrival of the guinea pigs.

This animal study was approved by the Experimental Animal Research Committee at the Kyoto Pharmaceutical University.

ANTIGEN AND ADJUVANT

Japanese cedar (Cryptomeria japonica) pollen was harvested in Gifu and Shiga prefectures (Japan) in 1998. Al (OH)$_3$ gels were prepared with 0.25 N NaOH and 0.25 N Al$_2$(SO$_4$)$_3$, as has been previously described.$^{13}$

The cedar pollen extract used for the sensitization was prepared as follows. The pollen was suspended in PBS at 100 mg/ml followed by vigorous stirring at 4°C for 48 hours. The suspension was then centrifuged and the supernatant collected. Protein concentration in the supernatant was measured using the method of Bensadoun and Weinstein.$^{14}$ The extract was combined with an Al(OH)$_3$ suspension to achieve a concentration of 100 μg protein/100 mg Al (OH)$_3$/ml.

The pollen extract used for the local nasal immunotherapy was prepared by centrifuging (4°C, 31,000 x g, 30 minutes) the pollen suspension (100 mg/ml) after stirring for 48 hours. The supernatant collected was then used for intranasal administration for the immunotherapy.

SENSITIZATION AND CHALLENGE (Fig. 1)

Using a previously described method,$^{10}$ guinea pigs were sensitized by bilateral intranasal instillation of the cedar pollen extract that was adsorbed onto the Al(OH)$_3$ gel at a concentration of 0.3 μg protein/0.3 mg Al(OH)$_3$/$3$ μl each nostril twice a day for 7 days. To prevent rapid elimination of the antigen by ciliary movement, the upper airway mucosal surface was anesthetized through the use of a 2-min inhalation of a mist of 4% lidocaine hydrochloride solution (MP Biomedicals, Solon, OH, USA) that was generated by an ultrasonic nebulizer (NE-U12, Omron, Osaka, Japan) prior to each sensitization session. The sensitized animals were then intranasally challenged once
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EFFECT ON THE BIPHASIC NASAL BLOCKAGE

The 7th pollen inhalation challenge induced biphasic nasal blockage (Fig. 2A), as has been previously reported. Starting on the 1st day after the 7th challenge, the pollen extract was intranasally administered 6 times a week. Figure 2B–E show the effect of the local intranasal immunotherapy on the biphasic nasal blockage for the 9th–30th inhalation challenges. Early (EPR) and late phase responses (LPR) are expressed as the area under the response curve (AUC) for the changes in sRaw at 0–2 hours and 3–6 hours after the challenge, respectively. The nasal immunotherapy significantly inhibited both the EPR and LPR at the 11th challenge (Fig. 2C). The tendency to inhibit the nasal blockage was observed at the 16th and 30th challenges, whereas the inhibitions were variable, and not statistically significant (Fig. 2D, E).

EFFECTS ON SNEEZING

As previously reported, induction of sneezing occurred within 1 hour after the respective pollen inhalation challenges, with sneezing frequencies of 10–20 times/hour. In contrast to the effects seen for the nasal blockage, the nasal immunotherapy did not substantially affect the frequency of sneezing that was induced at the 9th–30th inhalation challenges (Fig. 3A–D).

EFFECT ON THE DEVELOPMENT OF NASAL HYPERRESPONSIVENESS TO LTD₄

Consistent with our previous findings, nasal responsiveness to LTD₄ in the control group was markedly
Local Immunotherapy for Pollenosis

Fig. 3 Effect of the nasal immunotherapy on the occurrence of sneezing induced by the 9th (A), 11th (B), 16th (C) and 30th (D) inhalation challenges with Japanese cedar pollen in sensitized guinea pigs. As described in Fig. 1, extract derived from 0.003 or 0.3 mg pollen was intranasally administered. During the first hour after each challenge, sneezing frequency was counted. Each column represents the mean ± S.E. of 7 or 8 animals.

higher as compared with the sensitized non-challenged guinea pig 2 days after the 16th and 30th challenges (Fig. 4A, B). The development of nasal hyperresponsiveness after the challenges was almost completely suppressed by the local nasal immunotherapy (Fig. 4A, B).

LACK OF INDUCTION OF NASAL SYMPTOMS AFTER INTRANASAL INSTILLATION OF THE EXTRACT DURING IMMUNOTHERAPY
The effect of instillation of the extract during immunotherapy on induction of sneezing and nasal blockage was assessed on days 15 and 86 after the start of the immunotherapy. As shown in Table 1, 2, neither nasal blockage at 1 and 4 hours after the instillation nor sneezing within 1 hour was induced in 0.003 and 0.3 mg pollen-derived extract-treated groups.

INFLUENCE ON Cry j 1-AND Cry j 2-SPECIFIC IgE PRODUCTION
Large amounts of both Cry j 1- and Cry j 2-specific IgEs were detected in the sera at the 15th and 29th inhalation challenges. Local nasal immunotherapy had no effect on the production of IgE (Fig. 5A, B).

EFFECT ON POLLEN-INDUCED ALLERGIC CONJUNCTIVITIS
We also assessed whether local nasal immunotherapy suppressed pollen-induced allergic conjunctivitis. There was an apparent increase in the CIS even at the 1st ophthalmic challenge with the pollen, with the increase peaking at 0.5 hours. The scratching frequency was also increased within 1 hour after the 1st challenge. Both responses tended to be intensified at the subsequent 2nd and 3rd challenges. However, the nasal immunotherapy did not affect the induction of the allergic conjunctivitis symptoms (Fig. 6A–F). The amount of albumin in the ophthalmic lavage fluid, which is an indicator of plasma extravasation, was increased after the 3rd pollen challenge in the eye. This increase in the albumin amount was not affected by the nasal immunotherapy (Fig. 6G).

DISCUSSION
In the present study, therapeutic effects of nasal immunotherapy on the induction of allergic rhinitis symptoms were evaluated in guinea pigs. Results indicated that biphasic nasal blockage, which consists of both EPR and LPR, was suppressed by nasal immunotherapy at the 11th–30th challenges, although the influences were variable at respective challenges and not apparent. Nasal responsiveness to intranasal application of LTD₄ was markedly increased in the control animals at the 16th and 30th challenges. Interestingly, the development of nasal hyperresponsiveness was completely inhibited by the immunotherapy. Nevertheless, neither sneezing nor Cry j 1- and Cry j 2-specific IgE production was influenced by the therapy. On the other hand, after an intranasal administration of 0.003 and 0.3 mg pollen-derived extract, there were no nasal symptoms in the sensitized guinea pigs, as shown by the sRAW and sneezing fre-
Fig. 4 Effect of the nasal immunotherapy on the increase of nasal responsiveness to leukotriene (LT) D4 after the 16th (A) and 30th (B) inhalation challenges with Japanese cedar pollen in sensitized guinea pigs. Extract derived from 0.003 or 0.3 mg pollen was intranasally administered as described in Fig. 1. The nasal responsiveness to intranasal instillation of 0.01 and 1 μM LTD4 was assessed 2 days after the challenges. Each point represents the mean ± S.E. of 7 or 8 animals. *p < 0.05, **p < 0.01 and ***p < 0.001 vs. control, †p < 0.05, ††p < 0.01 and †††p < 0.001 vs. sensitized-non-challenged group.

Table 1 Lack of induction of nasal blockage after intranasal instillation of the pollen extract for the immunotherapy

<table>
<thead>
<tr>
<th>Day</th>
<th>Changes in sRaw at 1 hour [cmH2O x ml/(ml/sec)]</th>
<th></th>
<th>Changes in sRaw at 4 hours [cmH2O x ml/(ml/sec)]</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>0.003 mg</td>
<td>0.3 mg</td>
<td>Control</td>
</tr>
<tr>
<td>15</td>
<td>0.19 ± 0.12</td>
<td>0.30 ± 0.14</td>
<td>0.24 ± 0.10</td>
<td>0.23 ± 0.15</td>
</tr>
<tr>
<td>86</td>
<td>0.05 ± 0.09</td>
<td>0.06 ± 0.14</td>
<td>−0.04 ± 0.15</td>
<td>0.03 ± 0.12</td>
</tr>
</tbody>
</table>

Extract derived from 0.003 or 0.3 mg pollen was intranasally administered as described in Fig. 1. Specific airway resistance (sRaw) was measured before, and 1 and 4 hours after the intranasal instillation of the extract on days 15 and 86 after the start of the immunotherapy. Each value represents the mean ± S.E. of 8 animals.

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The present results are essentially similar to our previous study that examined the therapeutic effect of oral immunotherapy in this experimental allergic rhinitis model.17 In our oral immunotherapy study, 1 mg pollen-derived extract was orally administered until the 30th challenge and was found to be effective in biphasic nasal blockage and in the development of nasal hyperresponsiveness, although no effects were noted on IgE production or on the occurrence of sneezing.17 The magnitudes of the inhibition in the development of the nasal hyperresponsiveness by the nasal immunotherapy when using 0.003 and 0.3 mg pollen-induced extract in the present study were almost equipotent to that seen in our previous oral immunotherapy study that used a 1 mg pollen-derived extract.17 Although we have not examined the dose-dependent effect of the oral immunotherapy, local nasal immunotherapy is effective at relatively low doses of antigen, similar to that which has been reported in clinical trials.3 In most local nasal immunotherapy clinical trials for allergic rhinitis, nasal immunotherapy effectively relieved the symptoms of allergic rhinitis, whereas allergen-specific IgE production was not inhibited.4-8 Our present results are consistent with these clinical

quency measurements. Thus, the effects of the nasal immunotherapy may not be due to a decreased anaphylactic reaction that had been influenced by repeated antigen-antibody reactions at the nasal mucosa.

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findings. We have also previously reported that the sneezing which was induced within 1 hour after challenge was almost completely suppressed by treatment with anti-histaminics. This indicates that the histamine that is released from the mast cells immediately after the pollen inhalation plays a major role in the occurrence of sneezing. Nevertheless, local nasal immunotherapy inhibited neither the induction of sneezing nor the increase of Cry j 1- and Cry j 2-specific IgE levels. These findings are similar to the results from our previous experiment that assessed the effects of oral immunotherapy, and indicate that nasal immunotherapy has no inhibitory effect on the IgE-mediated mast cell activation at the nasal mucosa.

Table 2  Lack of induction of sneezing after intranasal instillation of the pollen extract for the immunotherapy

<table>
<thead>
<tr>
<th>Day</th>
<th>Sneezing frequency (times/hour)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>15</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>86</td>
<td>0.8 ± 0.4</td>
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</table>

Extract derived from 0.003 or 0.3 mg pollen was intranasally administered as described in Fig. 1. Sneezing frequency was counted 0–1 hour after the intranasal instillation of the extract on days 15 and 86 after the start of the immunotherapy. Each value represents the mean ± S.E. of 8 animals.

However, presently there are no clear mechanisms that can explain the effectiveness of the local nasal immunotherapy on the development of nasal hyperresponsiveness other than by inhibition of IgE production and mast cell activation. However, the effectiveness of the nasal immunotherapy was similar to the results we obtained for the oral immunotherapy in our previous study. Thus, it can be speculated that even though the treatments for the intranasally and orally applied allergens were different in the nasal- and gut-associated lymphoid tissues, respectively, hyporesponsiveness to the allergen may be required for both of these immunotherapy cases. Because recognition of allergen by a specific IgE may not be affected by the immunotherapy described above, it can be speculated that antigen recognition by antigen-presenting cells may be decreased, thus leading to suppression of the nasal hyperresponsiveness. Further detailed analyses need to be conducted in order to elucidate these mechanisms.

On the other hand, the pollen-induced allergic conjunctivitis was not affected by the nasal immunotherapy even though there was sustained effectiveness in the nasal tissue. The dissociation of the effectiveness of the nasal immunotherapy between the nasal and conjunctivitis tissues has also been reported in a previous clinical examination. It may well be that the nasal immunotherapy is effective only on the local or-
Fig. 6  Effect of the nasal immunotherapy on the Japanese pollen-induced allergic conjunctivitis in the sensitized guinea pigs. Extract derived from 0.003 or 0.3 mg pollen was intranasally administered as described in Fig. 1. During the nasal immunotherapy (1 day before the 26th, 27th and 28th challenges), animals were challenged by dropping a pollen suspension in each eye for a total of 3 times. (A, B and C) The magnitude of conjunctival edema and redness were macroscopically judged at 0 – 5 hours after the respective 1st – 3rd ophthalmic challenges, and expressed as the conjunctivitis intensity score (CIS). (D, E and F) Scratching frequencies at 0 – 0.5 hours after the respective 1st – 3rd challenges were counted. (G) Albumin amounts in the ophthalmic lavage fluid that was collected before and 0.5 hour after the 3rd challenge were measured by an enzyme-linked immunosorbent assay. Each point or column represents the mean ± S.E. of 7 or 8 animals.

gans, which are controlled by the nasal-associated lymphoid tissue. However, in one of our previous studies, oral immunotherapy, which may affect the systemic immune system, was also found to be effective for nasal allergic symptoms even though it did not have any effect on the conjunctival allergies (unpublished data). Thus, the immunological induction mechanisms underlying the nasal blockage and hyperresponsiveness might be largely different from those in allergic conjunctivitis. We have previously reported that scratching incidence, increases in CIS, and albumin leakage are all immediate allergic responses that peak at around 30 minutes after a pollen challenge. These findings suggest that these responses are part of an IgE-dependent allergy, much like the case of sneezing that is seen in the allergic
rhinitis model. These results also provide further support for the rationale that we can exclude the effects on IgE-mediated mast cell activation as being a part of the mechanism of this immunotherapy.

In conclusion, there was effective suppression of both the biphasic nasal blockage and the development of nasal hyperresponsiveness induced by the subsequent pollen inhalation challenges in sensitized guinea pigs undergoing intranasal administration of pollen extract 6 times a week, although there was no suppression of the sneezing. This inhibition was not related to serum antigen-specific IgE levels. The effectiveness of this local nasal immunotherapy might be due to a nasal hyporesponsiveness mechanism to the specific allergen.

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