A Pilot Study of Intralymphatic Immunotherapy for House Dust Mite, Cat, and Dog Allergies

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INTRODUCTION

Intralymphatic immunotherapy (ILIT) was introduced recently as a new modality of allergen-specific immunotherapy (AIT); in this approach, only three intralymphatic injections induce marked clinical improvements as early as 4 months after the day of the first injection, and last for 3 years.¹⁶ Moreover, ILIT causes fewer and milder adverse reactions. However, clinical efficacy has been questioned.³ The efficacy and adverse effects of ILIT for Dermatophagoides farinae (Df), Dermatophagoides pteronyssinus (Dp), and dog allergens, which are indoor allergens common globally, should be investigated. Furthermore, use of multiple allergens in ILIT warrants further investigation.

In this study, we evaluated the clinical efficacy and adverse effects of ILIT using aqueous Df, Dp, dog and cat allergens or mixtures thereof in patients with allergic rhinitis.

MATERIALS AND METHODS

Study population

We enrolled subjects with AR, symptoms of which were provoked by Df, Dp, dog, and/or cat allergens. The subjects met the following enrollment criteria described below. 1) Sensitization proven by skin prick test (SPT) and serum level of allergen-specific IgE measured by ImmunoCAP® (ThermoFisher Scientific, Uppsala, Sweden). Subjects were regarded as being sensi-

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• Sang Pyo Lee and Seung Joon Choi contributed equally to this work.
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Intralymphatic Immunotherapy for Mite, Cat, and Dog Allergies

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tized to an allergen if, according to the SPT, the allergen/hista-
minal (A/H) ratio in the wheal was ≥ 1 and serum level of aller-
gen-specific IgE was ≥ 0.35 kU/L. 2 Complaints of AR symp-
toms during exposure to house dust, dog and/or cat in daily life.

Study design

At the first visit, patient eligibility was determined, informa-
tion about the study was provided to subjects, written consent
was obtained from subjects, and rescue medications including
oral antihistamine ( cetirizine) and nasal glucocorticosteroid
spray (ciclesonide) were prescribed (Supplementary Fig. 1).
The patients were also asked to administer oral antihistamines
with or without a nasal glucocorticosteroid spray, according to
the recommendation of the Allergic Rhinitis and its Impact on
Asthma (ARIA) guidelines.8

At the second visit, pretreatment status was evaluated using
questionnaires addressing allergic symptoms, and SPT, intra-
dermal test (IDT), and nasal allergen provocation test (NAPT)
results. Questionnaires used to assess allergic symptoms in-
cluded the Sinonasal Outcome Test-20 (SNOT-20),7 and Rhino-
conjunctivitis Quality of life Questionnaire (RQLQ).9 IDTs and
IDTs were performed using serial dilutions of extracts of the
sensitized allergen Df, Dp, dog and/or cat (HollisterStier, New
Orleans, LA, USA) according to the manufacturer’s instruc-
tions. In subjects with Df and/or Dp allergy, NAPTs with the
sensitized allergen were performed as previously described.11
In addition to AR symptoms during the NAPT, the mean vol-
ume (cm³) of the nasal cavity in the anterior nasal segment (vol-
ume 2-6 cm) was measured before the NAPT (baseline test)
and every 15 minutes during NAPT by acoustic rhinometry
(SRE 2000 Rhinometer; Rhinometrics, Lynge, Denmark) ac-
cording to the guidelines of the Standardization Committee
On Acoustic Rhinometry.12 Subjects were asked to stop oral cetiri-
zine (half-life: 8 h) and nasal ciclesonide spray (half-life: 3.5 h) 3
days before the second visit to ensure the validity of the SPT,
IDT, and NAPT results.

At visits 3 to 5, the study subjects received three 0.1 mL injec-
tions of their sensitized allergen extract at 4-week intervals. Us-
ing ultrasound guidance and a 25-gauge needle, aqueous aller-
gen extracts (HollisterStier, New Orleans, USA) were aseptically
injected into the superficial inguinal lymph node in the right-
side groin.1-7 Before the injections, aspiration was performed to
avoid inadvertent intravascular administration. After each in-
jection, subjects were closely monitored for 1 h with checking
of vital signs at 5-min intervals, and adverse events, if any, were
recorded. Adverse events due to previous injections were also
checked before the next injection at visits 4 and 5. Large local
reactions (swelling > 10 cm in diameter that persisted for > 24 h)
were identified, and systemic hypersensitivity reactions were
graded using the Mueller classification.9,13 At visit 3, venous pun-
ture was performed to measure the serum levels of total
IgE, allergen-specific IgE, and allergen-specific immunoglobu-
lin G4 (IgG4), and subjects were asked to rate the pain pro-
voked by intralymphatic injection and that by venous puncture
using the visual analog scale (VAS) ranging from 0 to 100 mm.1-3

The initial dose of allergen was a 1,000-fold dilution of the max-
imal concentration of allergen extract for subcutaneous immu-
notherapy (SCIT) (initial concentration: 30 AU/mL for Df or
Dp; 10 AU/mL for cat hair; and 1:1/10 weight/volume (w/v) for
dog hair/dander; HollisterStier) in a volume of 0.1 mL. After the
first injection, the allergen concentration was escalated 3-fold
on the day of the second injection, and 10-fold on the day of the
third injection, if there was no or mild local or systemic hyper-
sensitivity reaction. The allergen concentration did not change
on the day of second or third injection if there was a moderate
local or systemic reaction. The allergen concentration was de-
creased by 3- to 1,000-fold from the previous concentration if
there was a severe local or systemic reaction. When 2 or more
allergens were injected into the inguinal lymph node, the aller-
gen mixture was produced in a volume of 0.1 mL to preserve
the target concentration of each allergen.

At visits 6 and 7, posttreatment status was evaluated in a man-
ner similar to that at the first visit and blood sampling was per-
formed, respectively. Adverse events after the third injections
were also checked at visit 6.

The study was approved by our Institutional Review Board
and monitored by our Human Research Protection Committee.
This study was registered in an open-access trials registry (Clini-
calTrials.gov identifier: NCT02301884).

Statistical analysis

Statistical analysis was performed using PASW 20.0 (SPSS Inc.
Chicago, IL, USA). Continuous variables were analyzed by
paired Man-Whitney U test, whereas categorical variables were
analyzed by Fisher’s exact test. A P value < 0.05 was deemed to
indicate statistical significance.

RESULTS

Informed consent was obtained from a total of 24 subjects;
however, 4 did not attend a further visit (Supplementary Fig. 2).
Therefore, pre-ILIT evaluation of 20 subjects was carried out,
but 5 dropped out due to lack of time to participate in this
study, and 2 due to occurrence of anaphylaxis during SPT and
IDT with Df and Dp allergens, And 2 due to lack of time after
the first injection of ILIT. The post-ILIT evaluation was thus
performed in 11 subjects. The demographic characteristics of
the subjects are shown in Table 1.

Local and systemic adverse effects during ILIT

The pain of intralymphatic injection was comparable to that
of venous puncture (Supplementary Fig. 3). Seven subjects
complained of mild local or systemic reaction (grade 0-1 in the
Mueller classification); however, four experienced large local re-


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actions or moderate-to-severe systemic reactions of grade 2-3 in the Mueller classification (Table 2). Despite those severe reactions, they strongly desired to undergo further ILIT as scheduled, so that additional injections were performed using 3-, 100-, or 1,000-fold dilutions of the concentration previously applied.

AR symptoms, quality of life, and prescription of rescue medication

The SNOT-20 and RQLQ scores were significantly decreased 4 months (P=0.012 and P=0.007, respectively) and 1 year (P=0.047 and P=0.009, respectively) after ILIT compared with the baseline levels (Figure).

In general, rescue medications, with the exception of antihistamine eye drops, were prescribed less frequently after ILIT. The frequency of nasal corticosteroid spray prescription was significantly reduced 4 months after the first injection of ILIT (P=0.04; data not shown).

Nasal reactivity to house dust mites in NAPTs

Nasal symptoms during nasal challenge with house dust mite allergens in NAPTs were significantly reduced 1 year after ILIT (P<0.05; Supplementary Fig. 4). The decrease in nasal cavity volume during NAPTs was also alleviated 1 year after ILIT; however, the magnitude of the decrease was not significant.

Skin reactivity to allergens in the SPT and IDT

Skin reactivity to allergens in the SPT and IDT was generally increased after ILIT, albeit without statistical significance. Skin reactivity to Dp in the SPT was significantly increased 1 year after ILIT (P<0.05; data not shown).

Serum total IgE and serum allergen-specific IgE and IgG4

Serum levels of allergen-specific IgE to Df and Dp were significantly increased 4 months after ILIT (P<0.05), but they de-
creased 1 year after ILIT ($P<0.05$; Supplementary Fig. 5). The serum level of allergen-specific IgG4 to Df showed a similar trend; however, it was not significantly different 4 months and 1 year after ILIT compared with baseline. The serum level of al-

**Table 2. Treatment schedule and local/systemic reactions**

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Injection No.</th>
<th>ILIT dose* (AU/mL or w/v)</th>
<th>Local reaction</th>
<th>Systemic reaction</th>
<th>Mueller classification†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Dog 1:1/10</td>
<td>None</td>
<td>None</td>
<td>Grade 0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Dog 1:3/10</td>
<td>Heating sensation, edema, itching</td>
<td>None</td>
<td>Grade 0</td>
</tr>
<tr>
<td>3</td>
<td>Dog 1:1</td>
<td>Erythema, itching</td>
<td>None</td>
<td>None</td>
<td>Grade 0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Df 30, Dp 30 Dog 1:1/10, Cat 10</td>
<td>None</td>
<td>Generalized itching</td>
<td>Grade 1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Df 100, Dp 100 Dog 1:3/10, Cat 30</td>
<td>Pain, wheal, erythema</td>
<td>None</td>
<td>Grade 0</td>
</tr>
<tr>
<td>3</td>
<td>Df 300, Dp 300 Dog 1:1, Cat 100</td>
<td>Pain, wheal, flare, edema</td>
<td>None</td>
<td>None</td>
<td>Grade 0</td>
</tr>
<tr>
<td>3</td>
<td>Df 30, Dp 30 Dog 1:1/10, Cat 10</td>
<td>Edema, itching</td>
<td>Urticaria, generalized itching</td>
<td>Grade 1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Df 30, Dp 30 Dog 1:1/10, Cat 10</td>
<td>Pain, wheal, erythema</td>
<td>Mild headache</td>
<td>Grade 0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Df 100, Dp 100 Dog 1:3/10, Cat 30</td>
<td>Heating sensation, wheal, erythema, edema, itching</td>
<td>None</td>
<td>Grade 0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>Df 30</td>
<td>Pain</td>
<td>None</td>
<td>Grade 0</td>
</tr>
<tr>
<td>2</td>
<td>Df 100</td>
<td>Heating sensation</td>
<td>None</td>
<td>Grade 0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Df 300</td>
<td>Pain</td>
<td>None</td>
<td>Grade 0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>Df 30, Dp 30</td>
<td>None</td>
<td>Anaphylaxis</td>
<td>Grade 3</td>
</tr>
<tr>
<td>2</td>
<td>Df 0.03, Dp 0.03</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Grade 0</td>
</tr>
<tr>
<td>3</td>
<td>Df 0.1, Dp 0.1</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Grade 0</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>Df 30, Dp 30</td>
<td>Heating sensation, edema, itching</td>
<td>Delayed-typed dyspnea &amp; wheezing</td>
<td>Grade 0</td>
</tr>
<tr>
<td>2</td>
<td>Df 100, Dp 100</td>
<td>None</td>
<td>Anaphylaxis</td>
<td>Grade 3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Df 1, Dp 1</td>
<td>None</td>
<td>None</td>
<td>Grade 0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>Df 30, Dp 30 Cat 10</td>
<td>Pain</td>
<td>RLQ pain</td>
<td>Grade 0</td>
</tr>
<tr>
<td>2</td>
<td>Df 100, Dp 100 Cat 30</td>
<td>Pain</td>
<td>RLQ pain</td>
<td>Grade 0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Df 300, Dp 300 Cat 100</td>
<td>Pain</td>
<td>RLQ pain</td>
<td>Grade 0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>Dp 30</td>
<td>None</td>
<td>Diarrhea</td>
<td>Grade 0</td>
</tr>
<tr>
<td>2</td>
<td>Dp 100</td>
<td>None</td>
<td>Fever, chilling, headache</td>
<td>Grade 0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Dp 100</td>
<td>Erythema, itching</td>
<td>None</td>
<td>Grade 0</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>Df 30, Dp 30</td>
<td>Myalgia</td>
<td>None</td>
<td>Grade 0</td>
</tr>
<tr>
<td>2</td>
<td>Df 100, Dp 100</td>
<td>Pain</td>
<td>None</td>
<td>Grade 0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Df 300, Dp 300</td>
<td>Pain</td>
<td>None</td>
<td>Grade 0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>Df 30, Dp 30</td>
<td>None</td>
<td>None</td>
<td>Grade 0</td>
</tr>
<tr>
<td>2</td>
<td>Df 100, Dp 100</td>
<td>Severe edema and erythema with itching and heating sensation</td>
<td>None</td>
<td>Grade 0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Df 30, Dp 30</td>
<td>Severe edema and erythema with itching and heating sensation</td>
<td>None</td>
<td>Grade 0</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>Df 30, Dp 30 Dog 1:1/10</td>
<td>None</td>
<td>None</td>
<td>Grade 0</td>
</tr>
<tr>
<td>2</td>
<td>Df 100, Dp 100 Dog 1:3/10</td>
<td>None</td>
<td>None</td>
<td>Grade 0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Df 300, Dp 300 Dog 1:1</td>
<td>None</td>
<td>Urticaria, whole body itching, febrile sensation, tightness</td>
<td>Grade 2</td>
<td></td>
</tr>
</tbody>
</table>

*The initial dose of allergen was a 1,000-fold dilution of the maximal concentration of allergen extract for subcutaneous immunotherapy (initial concentration: 30 AU/mL for Df or Dp; 10 AU/mL for cat hair; and 1:1/10 weight/volume (w/v) for dog hair/dander, HollisterStier, New Orleans, USA) in a volume of 0.1 mL. After the first injection, the allergen concentration was escalated 3-fold on the day of the second injection, and 10-fold on the day of the third injection, if there was no or mild local or systemic hypersensitivity reaction. The allergen concentration did not change on the day of second or third injection if there was a moderate local or systemic reaction. The allergen concentration was decreased by 3- to 1,000-fold from the previous concentration if there was a severe local or systemic reaction. *Systemic hypersensitivity reactions were graded using the Mueller classification. ILIT, intralymphatic immunotherapy; AU/mL, allergy units/mL (for Df, Dp, and cat allergens); w/v, weight/volume (for dog allergen); Df, Dermatophagoides farinae; Dp, Dermatophagoides pteronyssinus; RLQ, right lower quadrant of the abdomen.
Allergen specific IgG4 to Dp was significantly increased 1 year after ILIT compared with baseline ($P<0.05$). Neither the serum level of allergen-specific IgE and IgG4 to dog and cat nor the serum total IgE level changed significantly after ILIT (data not shown).

**DISCUSSION**

All previous studies of ILIT have reported that ILIT causes only mild adverse effects.\(^1\,^3\) However, we observed that ILIT could cause severe adverse reactions even at very low concentrations that were not expected to cause serious reactions in SCIT. We therefore propose that AIT of the lymph nodes is not entirely safe, as they are connected to the systemic circulation through the thoracic duct, and ILIT can cause severe adverse reactions even when very low doses of allergens are used.

In subjects who showed moderate-to-severe systemic reactions (subjects 5, 6, and 11), ILIT using Df and Dp at concentrations that, according to SPTs, led to A/H ratios in wheals of more than 1 caused systemic reactions (Supplementary Table 1). We thus suggest that the allergen concentration be reduced in hypersensitized patients, as recommended by the manufacturer of SCIT. In detail, we propose that SPTs be performed with serial dilutions of allergens, that the initial dose of allergen in ILIT not exceed the maximal concentration leading to an A/H ratio in wheals of less than 1, and that we carefully monitor patients undergoing ILIT with allergens at doses exceeding this concentration.

No severe reaction occurred in 2 patients (subject numbers 1 and 7 in Supplementary Table 1), although the allergen dose used in ILIT exceeded the above-mentioned concentration. Regarding severe local and systemic reactions to ILIT, we must also consider other factors such as the type and preparation of allergen and patient clinical characteristics other than hypersensitization.

Like most previous studies of ILIT, the symptoms of AR and quality of life in this study were improved as early as 4 months after the first injection of ILIT, and lasted for 1 year.\(^1\,^6\) In NAPTs, nasal reactivity to HDM allergens was decreased after ILIT as previously described.\(^1\,^3\,^5\) Furthermore, the serum levels of allergen-specific IgE and IgG1 to Df and Dp were increased 4 months after ILIT, being consistent with the results of previous studies.\(^3\,^1\,^1\,^1\) However, these levels were again decreased 1 year after ILIT. Previous studies have reported decreased allergen-specific IgE levels 3 years after ILIT\(^2\) and decreased allergen-specific IgG1 levels 1 year after ILIT.\(^1\) Regarding dog and cat allergens, we failed to observe any significant change in the level of allergen-specific IgE or IgG4 due to an inadequate number of subjects. Unlike previous reports,\(^1\,^3\,^6\) skin reactivity to allergens in SPT and IDT generally increased after ILIT in this study.

This study has several limitations. First, it is not placebo-controlled. Therefore, the effects of other factors—including phar- macotherapy, allergen avoidance or other lifestyle modifications, natural course, and subject expectations—should be considered. Additionally, application of ILIT using multiple allergens might have hampered interpretation of the results of this study.

Despite these limitations, our study provides useful information on ILIT. First, the findings suggest that ILIT can provoke serious local or systemic reactions and that a reduced allergen dose, especially of aqueous allergen extracts, should be applied in hypersensitized patients. Secondly, this study is the first of ILIT to evaluate Df, Dp, and dog allergens, which are prevalent globally. Thirdly, this is to our knowledge the first study to use multiple allergens in ILIT.

In conclusion, ILIT can rapidly improve AR symptoms and reduce the frequency of prescription of rescue medication, and this effect lasts for 1 year. However, in hypersensitized patient, ILIT can also cause severe systemic and/or local hypersensitivity reactions when performed using aqueous allergen extract.

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