The Cross-allergenecity of Pollens from *Compositae* Family :

Dendranthema grandiflorum, Artemisia vulgaris and *Taraxacum officinale*

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and *Taraxacum officinale*

Directed by Professor Jung-Won Park

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This certifies that the Master's Thesis
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ABSTRACT

The Cross-allergenecity of Pollens from *Compositae* Family : *Dendranthema grandiflorum Artemisia vulgaris* and *Taraxacum officinale*

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Background: Chrysanthemum, dandelion and mugwort belong to *Compositae (Asteraceae)* family on systemic botany. But their cross-allergenecity was not completely evaluated yet. So we investigated the clinical aspects and the cross-allergenecity of the pollens from these 3 plants.

Materials & Methods: We reviewed the medical records of 6,497 patients who have ever had skin prick test (SPT) at Severance Hospital Allergy-Asthma Clinic during last 10 years. On the basis of these clinical data, the sensitization rates of the pollens were estimated. The binding patterns of specific IgE (sIgE) were analyzed by immunoblotting. Cross-allergenecity between these pollens was evaluated by inhibition ELISA.

Results: Among 6,497 allergic patients, 17% had positive responses to one of

chrysanthemum, dandelion or mugwort. And 5.2% of them demonstrated positive reactions to all three pollen allergens. Some patients responded exclusively to one allergen (1.5% to chrysanthemum, 1.4% to dandelion and 4.5% to mugwort). Patterns of sIgE bindings to each *Compositae* familly pollen were different in immunoblotting. By mugwort pollen extracts, sIgE to chrysanthemum, dandelion and mugwort were inhibited upto 95%, 86% and 96% in inhibition ELISA with the pooled sera of atopic patients sensitized to all 3 allergens (n=6). 50% inhibitory allergen concentrations (IC50) for chrysanthemum-, dandelion- and mugwort-sIgE were not different between solid phase antigens and mugwort. However, mugwort-sIgE level was only suppressed up to 74% and 27% by chrysanthemum, dandelion, respectively. IC50 of chrysanthemum and dandelion for mugwort-sIgE were 0.3 and 57.0 mcg/mL each, while that of mugwort was 0.05 mcg/mL. Mugwort also inhibited dandelion-sIgE of the serum of atopic subject who was exclusively atopic to dandelion.

Conclusion: Chrysanthemum and dandelion pollens had extensive cross-allergenecity with mugwort. Skin test results for atopic subjects who had sensitized exclusively to chrysanthemum or dandelion were not in concordance with the CAP test or inhibition ELISA.

Key Words : *Compositae* family, mugwort, chrysanthemum, dandelion, pollen allergen, cross-allergenecity

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I. Introduction

Weed pollens, the major cause of the pollinosis in autumn, are found in the atmosphere of Seoul from the beginning of August and increase upto maximum concentration until September or October[1-4]. Among the weed pollen, mugwort was the most common sensitizer. *Humulus japonicus* and ragweed rank next to mugwort in turn[1,2,5]. Mugwort pollen, the most important weed pollen in Korea, belong to *Compositae (Asteraceae)* family[1,6]. *Asteraceae* or *Compositae* (*Aster* family) consists of 1,535 Genus, 23,000 species[6,7]. In Korea, the wild plants of *Compositae* family are about 77 Genus, 390 species including denizens[8,9].

Chrysanthemum, dandelion and mugwort belong to *Compositae (Asteraceae)* family of plant taxonomic system (Fig. 1)[6,7]. In contrast to wind-borne plant like mugwort, insect-borne plant such as chrysanthemum or dandelion is at a disadvantageous position for being an inhalant allergen[1,2,10-13]. However, there were many reports that chrysanthemum pollens induced contact dermatitis[14-18]. In Europe, the prevalence of *Compositae* contact allergy was estimated as 0.7 ~ 1.4% in general population and 4.5 ~ 14% among occupationally exposed person[18]. Chrysanthemum is considered to be a major sensitizer among cultivated *Compositae* plant in Europe (60%)[18]. Several studies referred occupational asthma or rhinitis among florists handling this[19-22], and there were a small number of experimental studies supporting chrysanthemum sensitizarion[23]. Nevertheless, clinicians have been used not only mugwort but also chrysanthemum or dandelion for skin prick test and allergen specific immunotherapy for respiratory allergy. Interestingly, a report mentioned about a close relationship of allergens of chrysanthemum and mugwort in 8 pollinosis cases of chrysanthemum grower[19].

Unlike mugwort, we didn't know the exact sensitization rate of chrysanthemum or dandelion in Korea. Although there had been some efforts to identify the constitution of pollen allergens from chrysanthemum[24-27], the analysis of allergen sequences and structures in chrysanthemum or dandelion pollens still remained to be completed. So there are little evidences for using chrysanthemum or dandelion pollens in clinical test or immunotherapy. For their cross-allergenecity was not completely evaluated yet, clinicians have had difficulties in the selection of proper pollen allergens for specific immunotherapy. So we investigated the clinical aspects and the cross-allergenecity of the pollens from these 3 plants.

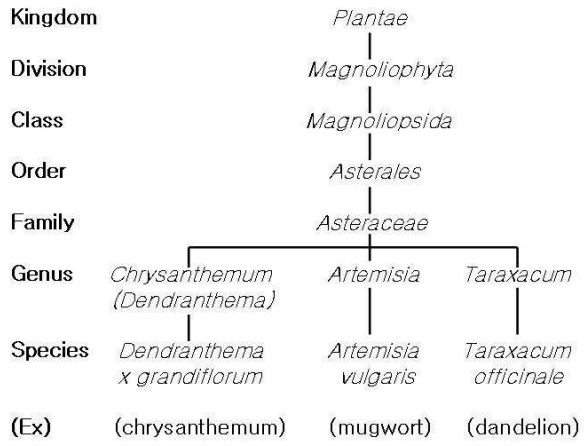

Figure 1. The Plant Taxonomic Classification of *Compositae* Family: chrysanthemum (*Dendranthema x grandiflorum*, PUMA), *Taraxacum officinale* and *Artemisia vulgaris*

II. Materials and Methods

1. Materials

A. Patients group for estimating the sensitization rate

We evaluated 6,497 patients who have ever had skin prick test (SPT) at Severance Hospital Allergy-Asthma Clinic during last 10 years (1995~2005). On the basis of this analysis, the sensitization rates of 3 *Compositae* family pollens were estimated.

B. Pollens of three *Compositae* family plants: chrysanthemum (*Dendranthema x grandiflorum*), *Taraxacum officinale* and *Artemisia vulgaris*

Chrysanthemum pollens were obtained from 20 bunches of white or yellow colored chrysanthemum (*Dendranthema x grandiflorum* : spray chrysanthemum , PUMA variety). Those flowers were picked up from March to May and full-bloomed at delivery. On 15 ~ 20^{th} day after delivery, we shook the chrysanthemum pollens out on the Petri dishes. Then we made 10 times diluted (volume) suspension with phosphate buffered saline (PBS, pH 7.4). This suspension was incubated and kept being shaken in cold room during three days, and dialyzed with Spectra / Por® membrane (3,500: MWCO) for 3 days. After that, we quantified protein contents of chrysanthemum, dandelion and mugwort pollen extracts by Bradford method. The extracts of dandelion (*Taraxacum officinale, Lot 3000-6849#143)*) and mugwort *(Artemisia vulgaris, Lot. 30009982#106)* were kindly provided by Allergopharma (Reinbek, Germany). Remnant chrysanthemum pollens were lyophilized for further studies.

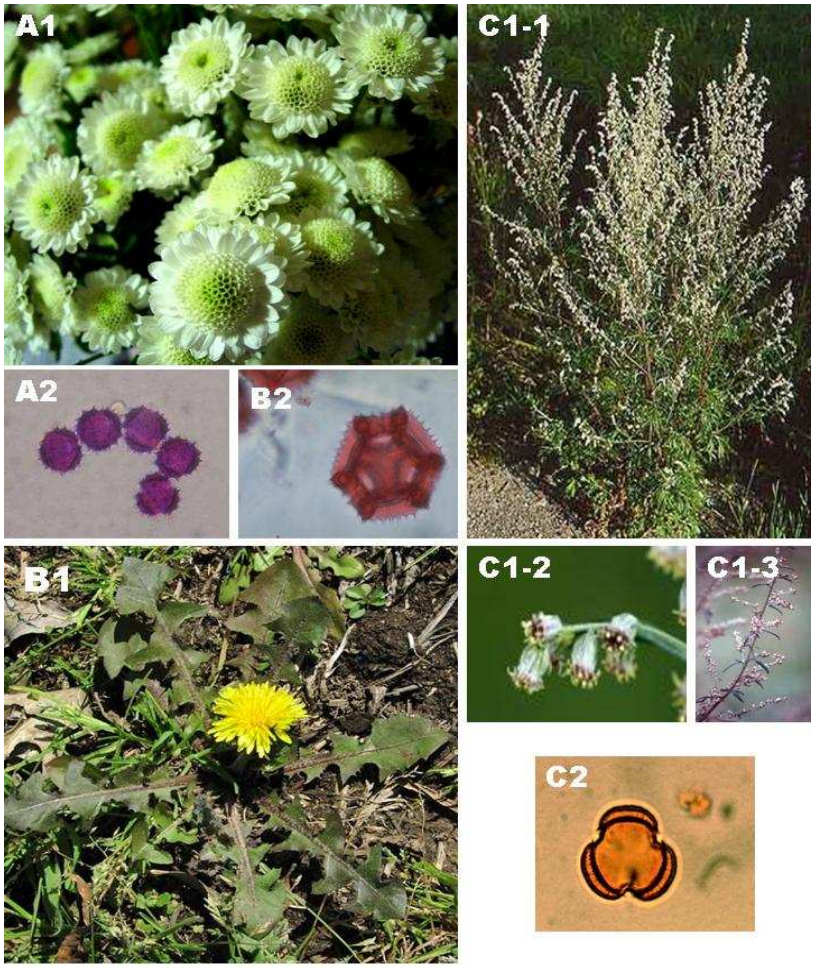

Figure 2. The flowers of *Dendranthema x grandiflorum* (A-1), *Taraxacum officinale* (B-1) and *Artemisia vulgaris* (C1-1,2,3) and their corresponding pollens (A2, B2, C2)

C. Patients

Among the 6,497 patients, we selected 8 patients who were sensitized to mugwort, dandelion and chrysanthemum pollens. Their sera were used for analysis of cross-allergenicity of *Compositae* family pollens. Demographic features, skin prick test (SPT) and specific IgE (sIgE)

results of atopic patient whose sera were used for analysis of cross-allergenecity were shown in Table 1. Other 6, 11, and 9 atopic patients who were exclusively sensitized to mugwort or chrysanthemum or dandelion were also recruited for analysis of cross-allergenecity. Wheal size that is 3 mm larger than negative control was considered as positive response to skin prick test (Allergopharma, Reinbek, Germany). Specific IgE for 3 allergens were measured by UniCAP® 100 (Pharmacia Diagnostics AB, Uppsala, Sweden).

Table 1. The demographics of patients sensitized to all three *Compositae* family pollen allergens

Patient	Sex	Age	Diagnosis	Skin Prick Test (mean wheal size: mm)			sIgE (KIU/L)		
				Sagebrush (mugwort)	Chrysan-themum	Dandelion	Mugwort	Chrysan-themum	Dandelion
A1	M	31	ARC, BA	15	21	5	93.4	85.4	42.3
A2	M	42	ARC	17	15	10	14.6	9.4	5.2
A3	F	32	AR	6	14	13	9.6	5.9	5.2
A4	M	19	AR, AD	15	7	11	26.8	14.0	17.5
A5	F	21	AR, AD	11	14	14	2.3	1.1	1.5
A6	F	66	AR	12	16	10	5.8	4.1	2.8
A7	M	21	AR	13	6	12	3.9	2.7	2.1
A8	F	59	AR	5	4	5	1.4	0.9	0.9

ARC: allergic rhinoconjunctivitis, BA: bronchial asthma, AR: allergic rhinitis, AD: atopic dermatitis

Table 2. The demographics of patients exclusively atopic to mugwort in SPT

Patient	Sex	Age	Diagnosis	Skin Prick Test (mean wheal size: mm)			sIgE (KIU/L)		
				Sagebrush (mugwort)	Chrysan-themum	Dandelion	Mugwort	Chrysan-themum	Dandelion
M1	F	41	AR	8	0	0	3.93	2.47	0.79
M2	F	34	BA	9	0	0	2.87	2.47	1.23
M3	M	42	BA	8	0	0	3.12	3.24	1.50
M4	M	22	AR	10	0	0	0.65	0.58	0.39
M5	M	41	AR	6	0	0	<0.35	<0.35	<0.35
M6	F	28	BA	12	0	0	0.70	<0.35	<0.35

Table 3. The demographics of patients exclusively atopic to chrysanthemum in SPT

Patient	Sex	Age	Diagnosis	Skin Prick Test (mean wheal size: mm)			sIgE (KIU/L)		
				Sagebrush (mugwort)	Chrysan-themum	Dandelion	Mugwort	Chrysan-themum	Dandelion
C1	F	42	AR	0	11	0	<0.35	0.52	0.38
C2	M	21	BA	0	5	0	<0.35	<0.35	<0.35
C3	M	63	BA	0	4	0	1.40	1.40	1.60
C4	M	34	BA	0	4	0	<0.35	<0.35	<0.35-
C5	M	42	AR, Urticaria	0	4	0	<0.35	<0.35	<0.35-
C6	M	30	EB	0	4	0	0.50	0.47	0.35-
C7	M	45	EB, Chr S	0	4	0	<0.35	<0.35	<0.35-
C8	F	23	BA	0	4	0	<0.35	<0.35	<0.35-
C9	F	24	BA	0	4	0	<0.35	<0.35	<0.35-
C10	M	34	BA	0	4	0	0.69	0.65	0.57-
C11	M	30	AR	0	4	0	0.37	<0.35	0.41

EB: eosinophilic bronchitis, ChrS: chronic sinusitis

Table 4. The demographics of patients exclusively atopic to dandelion in SPT

Patient	Sex	Age	Diagnosis	Skin Prick Test (mean wheal size: mm)			sIgE (KIU/L)		
				Sagebrush (mugwort)	Chrysan-themum	Dandelion	Mugwort	Chrysan-themum	Dandelion
D1	M	36	BA	0	0	6	<0.35	<0.35	<0.35
D2	F	47	AR	0	0	4	<0.35	<0.35	<0.35
D3	M	30	AR, Urticaria	0	0	5	<0.35	<0.35	<0.35
D4	F	28	AR, Urticaria, Dermographism	0	0	4	0.63	0.68	<0.35
D5	M	41	AR	0	0	4	0.37	0.41	0.41
D6	F	35	AR	0	0	4	<0.35	<0.35	1.78
D7	F	49	BA	0	0	4	0.46	0.42	<0.35
D8	M	39	BA	0	0	4	<0.35	<0.35	<0.35
D9	F	55	AR, Urticaria	0	0	4	<0.35	<0.35	<0.35

2. Methods

A. SDS-PAGE of chrysanthemum, dandelion and mugwort pollen allergens

We performed SDS-PAGE (Sodium dodecyl sulfate polyacryl amide gel electrophoresis) according to Laemmli ‘s protocol (1970). Molecular weight marker (BenchMark™ Prestined Protein Ladder; Invitrogen Corporation, Carlsbad, CA, USA) and each pollen allergen mixed with SDS sample buffer was boiled in 100°C water for 5 minutes. We made SDS-polyacryl amide gel (13.5%) and purified this gel by electrophoresis (50V, 10 min). After that, 15 μL of each pollen allergen extracts were injected in turn. Electrophoresis was done with this (50 V - 30 min for stacking gel (5% acryl amide gel), 180 V - 2 hrs for separation gel (13.5%)). After electrophoresis, the gel was stained with Coomassie brilliant blue (0.1% Coomassie brilliant blue R 250, 10% glacial acetic acid, 45% methanol) and destained it with 10% glacial acetic acid and 45% methanol.

B. Immunoblotting for chrysanthemum, dandelion and mugwort pollen allergen-sIgE

We performed SDS-PAGE for each of three pollen allergens according to above method and obtained gels. Then the gels were transferred to nitrocellulose membrane (Hybond™ -C Extra, 8 x 8 cm; Amersham Biosciences, Buckinghamshir, UK) with transfer buffer (distilled water 2 L + Tris 5.8 g + Glycine 29 g + MetOH 200 mL) at room temperature and 200 V for 2 hours. After this process, we confirmed the transferred protein bands on nitrocellulose membranes by PonceauS staining. Then the membrane was cut into several strips. Blocking with 2 mL/lane of 5% skimmed milk (diluted by PBS-T solution) was done for 1 hour. And the strips were incubated overnight

with 6-fold diluted patient sera. Ten-minute washing with 2 mL/lane of PBST solution (Phosphate buffered saline and 0.1% Tween20) was done by three times. Then sIgEs were detected by alkaline phosphatase conjugated goat anti-human IgE antibodies (SIGMA, St. Louis, MO, USA; #A3525) diluted to 1:1,000. After then, 2 times PBS-T rinsing was done for 10 minutes each. Then 1.5 mL/lane of TBS-AP buffer (1 M Tris + 1 M NaCl + 1 M MgCl + distilled water) was applied to each lane for 5 minutes and discarded. Finally, color reactions were developed with NBT/BCIP (Promega, Madison, WI, USA; S380C 14968004 and S381C 17814602) in TBS-AP buffer and incubated with agitation at room temperature. Distilled water was used for stopping color reaction.

C. Inhibition ELISA test for evaluation of cross-allergenecity among pollens from three *Compositae* family plants

Inhibition ELISA was done for investigating the cross-allergenecity of pollens from three *Compositae* family plants (chrysanthemum, dandelion and mugwort) by following procedures. At first, three allergens were diluted with 0.1 M carbonate buffer to 10 mcg/mL concentration. These diluted allergens were distributed into 96 well-plate (Costar, Cambridge, MA, USA) by 50 μL/well respectively and incubated for 18 hrs at 4°C.

On the other hand, the pooled sera of patients sensitized to all three *Compositae* family pollen allergens (n=6) or exclusively atopic to chrysanthemum or dandelion (n=9 each) were made. And we made these pooled sera 1:4 diluted with 1% BSA (bovine serum albumin; AMRESCO, Solon, OH, USA)-PBST. Then each of three *Compositae* family pollen allergens extracts (20 mcg/mL concentration) was diluted 4 fold serially, so the inhibitory concentrations of these extracts ranged from 20 mcg/mL to 0.02 mcg/mL. These serially diluted allergen

extracts were mixed with the pooled sera and incubated for 12 hrs at 4°C. After these steps, we added 200 μL/well of 1% BSA-PBST(1% bovine serum albumin, 137 mM NaCl, 1.8 mM KH_2PO_4, 10 mM Na_2HPO_4, 27 mM KCl, 0.1% Tween 20, pH 7.4) into each well of allergen-coated plates for blocking of non-specific protein bindings and incubated it for 1 hour.

Then we distributed the serially inhibited serum-allergen mixtures, previously described, into the wells of allergen-coated plate by 50 μL/well respectively and incubated for 1 hour at room temperature. After incubation, we washed these plates with PBS-T solution 3 times by 150 μL/well for each time and incubated these with 1:1,000 (v/v) diluted biotinylated goat anti-human IgE (Vector, CA, USA) for 30 minutes. Following the washing of these with PBS-T solution (3 times by 150 μL/well for each time), streptavidin-peroxidase (Sigma, St. Louis, MO, USA) 1:1,000 (v/v) diluents were reacted with these for 30 minutes. After this reaction, 5 times PBS-T washing were done in the same manner. Finally, the color reaction was developed with 3,3,5,5-tetramethyl-benzidine(TMB)-peroxidase (KPL, Gaithersburg, MD, USA) mixed with H_2O_2 was added by 100 μL/well for 5 minutes at room temperature, and the reaction was then stopped with 1% H_2O_4. Optical densities (OD) were measured by microplate reader 600 (Dynatec lab, Alexandria, Virginia, USA) at 450 nm.

III. Results

1. The sensitization rates to three *Compositae* family allergens in skin prick test

Among 6,497 allergic patients who had SPT, 17.0% showed positive responses in at least one of chrysanthemum, dandelion or mugwort. 5.2% demonstrated positive reactions to all three pollen allergens. Some patients responded exclusively to 1 allergen (1.5% to chrysanthemum, 1.4% to dandelion and 4.5% to mugwort)(Fig. 3).

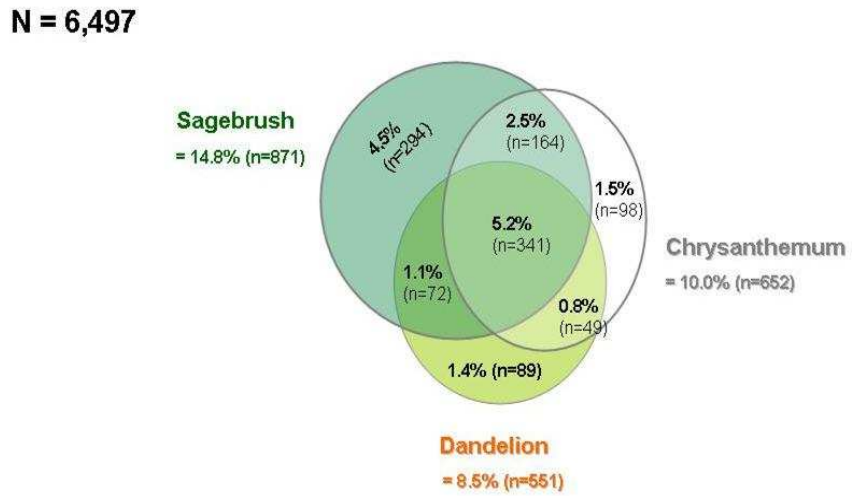

Figure 3. The proportion of positive responders to mugwort, chrysanthemum and dandelion in 6,497 allergic patients who underwent skin prick test

2. SDS-PAGE and sIgE Immunoblotting for chrysanthemum, dandelion and mugwort pollen allergens

Proteins of chrysanthemum, mugwort and dandelion pollens were separated by SDS-PAGE, and specific IgE immunoblottings were followed using the patients' sera atopic to all or only one of three pollens. The individual patterns of specific IgE bindings to each *Compositae* familly pollens were different (Fig. 4S).

Among the six sera atopic to all three *Compositae* pollens, three (patient A1,2,4 of Table 1) showed relatively strong specific IgE bindings to each pollens (Fig. 4A1,2,4). For mugwort, chrysanthemum and dandelion, sIgE bindings were strong at 20 ~ 25 kD area (Fig. 4). In one serum, mugwort-sIgE bindings were also prominent at 36~62 kD, 70 ~ 173 kD area (Fig. 4A1). Dandelion sIgE bound to 13 kD area in one case (Fig. 4D6). The sera of the six allergic patients exclusively atopic to mugwort on skin test showed very poor concordance with CAP test (Table 2). Among them, only one case demonstrated enhanced sIgE binding to mugwort and chrysanthemum at 22 ~ 23 kD area in immunoblotting (Fig. 4M2). The serum of an allergic patient exclusively atopic to chrysanthemum failed to show any sIgE binding (Fig. 4C1). But the serum atopic to dandelion only demonstrated prominent sIgE bindings at 13 kD and 110 kD area (Fig. 4D6).

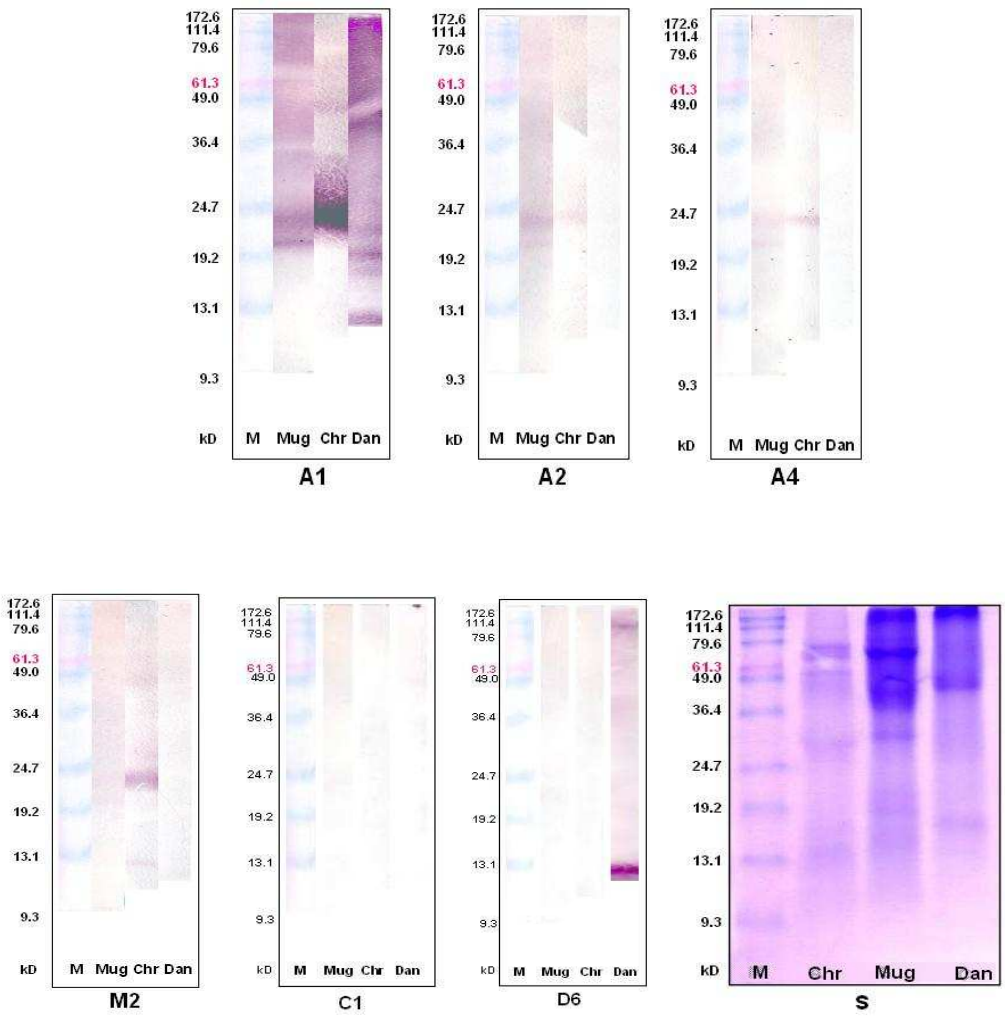

Figure 4. The binding patterns of sIgE to pollen allergens from three *Compositae* family by immunoblotting with the atopic sera sensitized to all three pollens (A1, A2, A4) or with the serum exclusively sensitized to mugwort (M2) or chrysanthemum (C1) or dandelion (D6). SDS-PAGE features of 3 pollens were also shown (S). M: marker, Mug: mugwort, Chr: chrysanthemum, Dan: dandelion.

3. Inhibition ELISA test for evaluation of cross-allergenecity among pollens from three *Compositae* family plants

By mugwort, sIgE to chrysanthemum, dandelion and mugwort were inhibited up-to 95%, 86% and 96% in inhibition ELISA with the pooled atopic serum (n=6) sensitized to all 3 allergens (Fig. 5A,B,C).

50% inhibitory allergen concentrations (IC50) for chrysanthemum-, dandelion- and mugwort-sIgE were not different between solid phase antigen and mugwort. However, mugwort-sIgE level was only suppressed upto 74% and 27% by chrysanthemum, and dandelion respectively. IC50 of chrysanthemum and dandelion for inhibition of mugwort-sIgE were 0.3 and 57.0 mcg/mL each, while that of mugwort was 0.05 mcg/mL.

Mugwort also inhibited dandelion-sIgE of one serum (patient D6 in Table 4) exclusively atopic to dandelion (Fig. 5D).

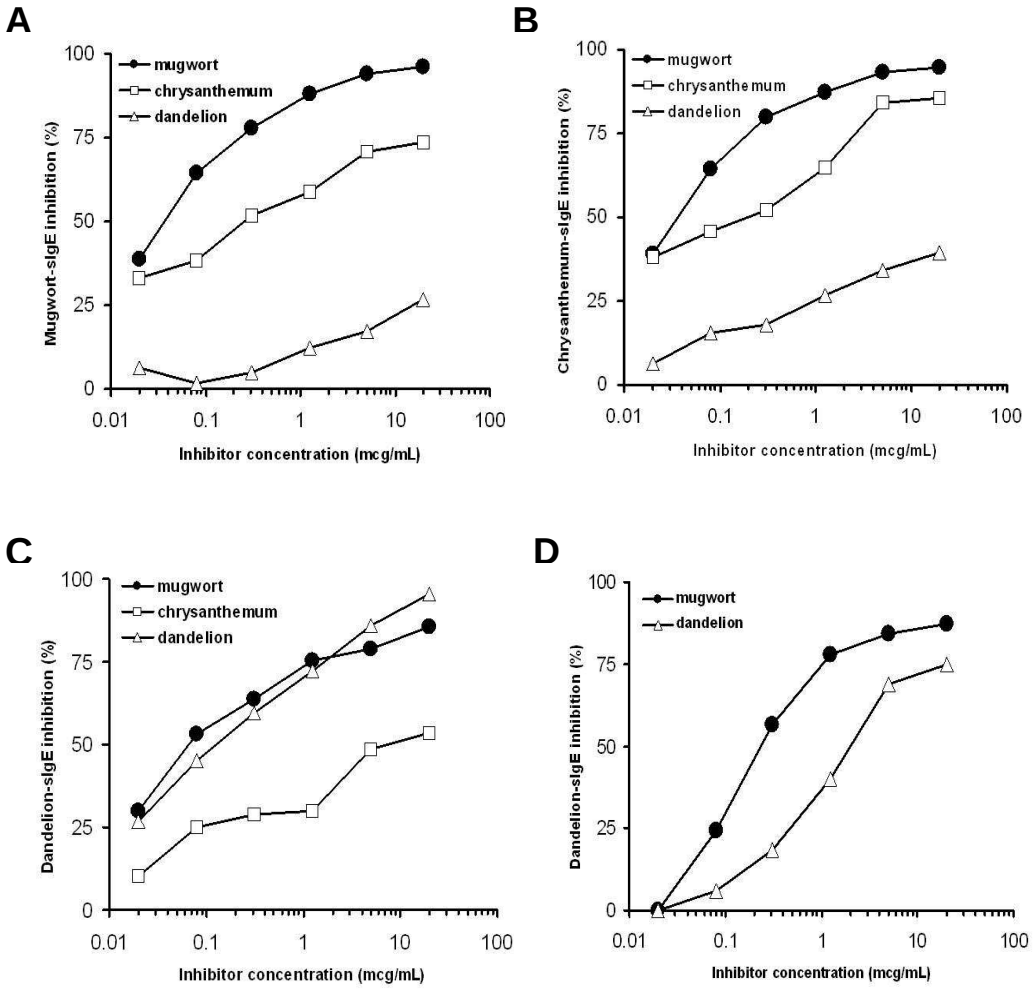

Figure 5. ELISA inhibition of three pollens (chrysanthemum, dandelion and mugwort) using the pooled atopic sera sensitized to all three pollens (A, B, C) or the serum exclusively sensitized to dandelion (D). Solid phase allergens were murgwort (A), chrysanthemum (B) and dandelion (C, D).

IV. Discussion

Mugwort (*Artemisia vulgaris*), a wind-borne weed, represents an allergenic pollen species frequently encountered especially in autumn[1,2,10,11]. So many studies have been done for identification of mugwort pollen allergens[28 - 42].

Although coincidental positive skin responses to three *Compositae* Family plants (mugwort, chrysanthemum and dandelion) had been relatively common in clinical situation[19,20,43], investigations for their cross-allergenecities were rarely done.

Chrysanthemum and dandelion are common flowers commercially available or wildly grown, so there had been some reports about chrysanthemum or dandelion pollen allergy mainly related with florist occupation[14-23,43,44]. Though most of the reports were regarding to contact allergy or dermatitis[14-18,43,44], de Jong et al described 14 consecutive patients with various complaints due to the handling of flowers[20]. And extensive cross-sensitization was seen to pollens of several members of the *Compositae* family (e.g., Matricaria, chrysanthemum, solidago) and to pollens of the *Amaryllidaceae* family (Alstroemeria and Narcissus) [20]. Homemade flower extracts could be used to confirm IgE-mediated flower allergy and they suggested that mugwort could be used as a screening test for possible flower allergy[20]. Groenewoud et al reported 20.2% sensitization to one or more of 7 different members of the *Chrysanthemum* family pollen extracts among 104 greenhouse workers[21]. Interestingly, main symptoms of greenhouse workers were rhinitis or conjunctivitis and more than 85% of them were atopic[21]. In addition, frequent cross-sensitizations between chrysanthemum and mugwort were also observed in several studies[19, 21,43].

So we postulated that the frequent concordances of mugwort, chrysanthemum and dandelion on skin prick test in general population nothing to do with horticultural professions might be associated with the cross-allergene-

cities between them. According to our statistic analysis (n=6,497) for last 10 years, the concordant sensitization rate of them was 5.2% (n=341). Among them, at least one pollen allergen was sensitized in 1,107 patients (17.0%). Mugwort showed 14.8% sensitization rate. Chrysanthemum (10.0%) and dandelion (8.5%) ranked next. There were also some groups atopic to only one pollen species (4.5% for mugwort, 1.5% for chrysanthemum and 1.4% for dandelion) (Fig. 3). We could confirm the relatively frequent co-sensitization of three *Compositae* pollens and high sensitization rate to mugwort. And these statistical results gave us a clue for using mugwort as a screening test for *Compositae* pollens allergies.

Thus far, earlier *in vitro* studies demonstrated the IgE-binding capacity of a mugwort-allergen of 27-29 kD termed Art v 1 by means of immunoblotting[28]. At present, Art v 1 was identified as a major mugwort allergen and a modular glycoprotein with a defensin-like and a hydroxyproline-rich domain[35,37,38, 40-42]. Moreover, additional allergens, such as Art v 2 (35 kD), Art v 3 (12 kD; lipid transfer protein), Art v 4 (14 kD; profilin), Art v 5 (10 kD; polcalcin) and Art v 6 (44 kD; pectate lyase, Amb a1 homologue), were identified[29-32, 36, 42, 45]. In Korea, Park et al reported sixteen pollen allergen fractions (36 ~ 39kD, 22 ~ 23 kD (85%), 69 ~ 71 kD (80%), 56 ~ 58 kD (75%)) of *Artemisia lavandulae-folia*[1]. In our SDS-PAGE result (Fig. 4S), we could observe very strong protein bands at various molecular weight similar to the above-mentioned major allerens of mugwort.

However, the identifications of chrysanthemum and dandelion pollen allergens were unaccomplished yet, so we could not gather enough information for analyzing our data completely. Several protein fractions had analogous molecular weight to Art v 1 (27 ~ 29 kD), Art v 4 (14 kD), 60 ~ 85 kD mugwort allergens in molecular weight. And we could also observe some enhanced protein bands of dandelion pollen extracts with similar molecular weights to Art v 1, Art v 4 or Art v 6.

In the pooled atopic serum sensitized to all 3 allergens, specific IgE (sIgE)

to chrysanthemum, dandelion and mugwort were markedly inhibited by mugwort. However, mugwort-sIgE was partially suppressed by chrysanthemum, dandelion. Mugwort also inhibited dandelion-sIgE of one serum exclusively atopic to dandelion significantly. These results confirmed that chrysanthemum and dandelion pollens have extensive cross-allergenecity with mugwort. And we could certify that atopic sensitizations exclusive to chrysanthemum or dandelion in skin prick test rarely showed concordance with the findings of CAP test or inhibition ELISA.

There were some previous reports that mugwort showed cross-allergenecities with other species because of common lipid transfer protein allergens like Art v 3 (12 kD) [36, 44, 46-49]. And restricted cross-reactions could be attributed to the defensin-like Art v 1 family from mugwort[42]. Extensive cross-reactivity within weeds and between other allergenic plants is likely caused by three families of widely distributed pan-allergens: the profilins, the polcalcins, and the lipid transfer proteins (nsLTPs)[42]. For example, ragweed and mugwort pollen contain the pan-allergen profilin and calcium-binding proteins, which are responsible for extensive cross-reactivity among pollen-sensitized patients[42]. Some studies related to *Compositae* dermatitis suggested that sesquiterpene lactones were the main sensitizers of the *Compositae* family, and either thiophenes or benzofuran derivates also possessed not only phototoxic activity but also some sensitizing properties[18,44]. Such previous reports may provide us with the clue for finding the causative allergens of cross-reactivity between *Compositae* family pollens.

Skin tests reflect the clinically relevant sensitization to a higher degree than *in vitro* findings[1]. However, we are doubtful whether positive skin responses to chrysanthemum or dandelion of mugwort-sensitized patients are due to real sensitizations or positive reactions caused by cross-allergenecity, for the majority of sera sensitized to chrysanthemum or dandelion in skin prick test failed to show positive CAP test results. Of course, we should consider the superior sensitivity of skin prick test, but there had already been many reports

on pollen cross-reactivity between mugwort and others including tree, grass and weed species[42, 50-54]. Moreover, chrysanthemum and dandelion are insect-borne plants so their larger, stickier pollens have lower chances for reaching respiratory tracts than mugwort pollen. In addition, we confirmed the extensive cross-allergenecities of above three pollen species with the respiratory allergic sera co-sensitized to all three pollen species.

From these, we infer that mugwort sensitization was very important in the allergic patients co-sensitized to the pollens from chrysanthemum and dandelion which belong to the same *Compositae* family. And we should approach allergic patients mono-sensitized to chrysanthemum or dandelion from the aspect of mugwort cross-allergenecity too. We also need to complete the analysis of some protein fractions peculiar to only one pollen species. These further investigations will provide us with the more concrete background information for choosing proper allergens in specific immunotherapy to pollinosis due to *Compositae* family.

V. Conclusion

We found that chrysanthemum and dandelion pollens have extensive cross-allergenecity with mugwort. Atopic sensitizations exclusive to chrysanthemum or dandelion were also found in skin prick test, but such sensitizations failed to show concordance with the results of CAP test & inhibition ELISA. Moreover, such observations were made from the general population beyond the confines of horticultural workers. So we suggest that mugwort pollen allergens are most important in the pollinosis patients who are co- or exclusively sensitized to these three *Compositae* family plants. These results provide us with background information for selecting proper allergens in specific immunotherapy to pollinosis from *Compositae* family.

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ABSTRACT [in Korean] 국문요약

국화과 꽃가루의 교차항원성 :

Dendranthema grandiflorum, Artemisia vulgaris

그리고 *Taraxacum officinale*

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연구배경 및 목적: 국화, 민들레 그리고 쑥은 식물 분류학상 국화과에 속한다. 하지만 이들의 교차 항원성에 대해서는 아직 완전히 밝혀져 있지 않다. 그러므로, 본 연구에서는 이 3종의 국화과 식물 꽃가루와 관련된 임상적 측면과 그 교차 항원성에 대해 연구하였다.

연구재료 및 방법: 세브란스병원 알레르기-천식 클리닉에서 10년 동안 피부 반응검사를 받은 바 있는 6,497명의 알레르기 환자를 평가하였다. 이 임상자료를 기초로, 국화과 식물 3종(국화, 쑥, 민들레)의 꽃가루 알레르겐에 대한 감작률과 그 교차반응성을 추정하였다. Immunoblotting을 시행하여 이들 3종의 꽃가루 알레르겐 특이 IgE의 결합양상을 분석하였다. 그리고, 아토피 혈청들을 이용한 ELISA 억제 검사를 시행하여 국화과 3종 꽃가루간의 교차항원성에 대해 연구하였다.

연구 결과: 피부 반응검사를 받은 바 있는 6,497명의 알레르기 환자들 중, 17%에서 국화, 민들레, 혹은 쑥 중 최소한 1가지 이상에 대해 양성 반응을 보였다. 5.2%는 3종 모두에 대해 양성 반응을 나타냈다. 일부 환자들은 오직 1종의 알레르겐에 대해서만 반응하였다(국화 1.5%, 민들레 1.4%, 그리고 쑥 4.5%).

Immunoblotting에서 각각의 국화과 식물 꽃가루 알레르겐들에 결합하는 특이 IgE의 개인적 양상이 다르게 나타났다. 특이 IgE 결합의 이러한 차이들은 꽃가루 종류와 여러 가지 감작 양상에 따라 달리 나타났다. 세가지 알레르겐 모두에 대해 감작된 아토피 혼합혈청(n=6)을 이용하여 시행한 ELISA 억제 검사 결과, 쑥 항원(알레르겐)에 의해 국화, 민들레 및 쑥 특이 IgE가 최대 95%, 86% 및 96%까지 억제 되었다. 고형상 항원(solid phase antigen)과 쑥 꽃가루 항원 사이의 국화, 민들레, 그리고 쑥 특이 IgE에 대한 50%억제 항원농도(IC50)는 차이를 보이지 않았다. 하지만, 쑥 특이 IgE 농도는 국화나 민들레 꽃가루 항원에 의해서 단지 74%와 27%씩만 억제되었고, 50% 억제 농도(IC50)는 각각 0.3 및 57.0 mcg/mL였으나 쑥은 0.05 mcg/mL였다. 쑥 꽃가루 항원은 민들레에 대해서만 감작된 혼합혈청 내의 민들레 특이 IgE도 억제하였다.

결론: 국화 그리고 민들레 꽃가루 항원은 쑥 꽃가루와 강한 교차항원성이 있었다. 피부단자 검사에서 국화 혹은 민들레 중 단 한 가지에만 감작된 경우 또한 발견되었으나 이들은 CAP 검사나 ELISA 억제시험 결과와 일치된 결과를 보여주지 못했다.

핵심 되는 말 : 국화과, 쑥, 국화, 민들레, 꽃가루 알레르겐, 교차 항원성