Antigenic Relationship between Mugwort and Ragweed Pollens by Crossed Immunelectrophoresis

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Mugwort and ragweed pollens have been considered as important respiratory allergens in Korea. These two pollens are abundant in the air of Seoul from August through October. Many ragweed-sensitive patients have shown concurrent sensitivities to mugwort pollen. However the antigenic relationship between these two pollens has not been clarified. To observe the cross-reactivity between them, we developed polyclonal anti-mugwort and anti-ragweed antibodies by immunization on New Zealand white rabbits, and performed crossed immunelectrophoresis(CIE) with two pollen extracts. Five precipitation lines were formed by mugwort and anti-mugwort antibody. One precipitation line was formed by ragweed and anti-ragweed antibody. There was no reaction from mugwort and anti-ragweed antibody, and from ragweed and anti-mugwort antibody. These results indicate that there is no cross-antigenicity between mugwort and ragweed pollens.

Key Words : Ragweed, Mugwort, Pollen, Antigen.

INTRODUCTION

Mugwort(*Artemisia vulgaris* L.) pollen is one of the common causes of allergic reaction during late summer in northern Europe(Charpin et al., 1974 : Holopainen et al., 1979). Ragweeds(*Ambrosia* sp.) are the most significant allergenic source in autumn in North America. In Korea, the pollens of mugwort and ragweed are reported to be abundant in the air of Seoul from the end of August through September(Hong et al., 1986). They are known to be main sources of late summer-autumn respiratory allergens(Park et al., 1989).

The possibility of cross-reactivity between these two pollens has been suggested. Weber and Nelson(1985) reported that 65% of ragweed-

sensitive patients were allergic to mugwort : the patients who were allergic to ragweed showed a general tendency to respond to other Composite families. Song et al.(1992) reported that nine (81.8%) of 11 ragweed-sensitive asthmatic patients were sensitized to mugwort pollen and/or house dust mites. Park et al.(1989) reported 14(53.9%) of 26 mugwort-sensitive asthmatic patients were sensitized to ragweed.

The purpose of this study is to investigate the antigenic relationship between these two important allergenic pollens using crossed immunelectrophoresis(CIE).

MATERIALS AND METHOD

Preparation of whole pollen extracts

Mugwort and ragweed pollens were collected from flowering plants in season and prepared according to the previously described method(Park

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et al., 1989). The dried pollens were defatted with diethylether and extracted with 0.125 M NH_4HCO_3 (pH 8.3), 1 : 50 w/v, for 72 hours at 4°C, followed by centrifugation at 10,000 g at 4°C for 1 hour. Ammonium sulfate was added to the supernatant to 70% saturation with stirring. After stirring in a cold room for 18 hours, the precipitate was collected by centrifugation at 10,000 g, 4°C for 1 hour. The precipitate was dissolved in one tenth of the original volume of 0.125 M NH_4HCO_3 , dialyzed against distilled water, and lyophilized. The protein content was measured by the Bensadoun method (Bensadoun and Weinstein, 1976). The protein was 380 ug per mg of lyophilized extract of mugwort dissolved in 1 ml of phosphate buffered saline (PBS) and ragweed had 470 ug of protein in 1 mg of lyophilized extract. They were used as antigens.

Immunization

To obtain polyclonal rabbit antibodies against each extract, immunization procedure was performed (Harboe and Ingild, 1983). Ten rabbits (New Zealand White), weighing 2.5 kg received 1 cc of 1 mg/ml of each extract, mixed with the same volume of complete or incomplete Freund's adjuvant (Sigma Chemical Co., USA). Initially, on days 0, 7, 14, 21, 28, rabbits were given injections of each antigen with complete or incomplete Freund adjuvant. After 28 days, blood was drawn from the ear veins of each rabbit. Double-diffusion precipitation was performed to confirm the development of anti-mugwort and anti-ragweed antibodies after 28 days. Then, 1 ml of 1 mg/ml of each extract mixed with 1 cc of incomplete adjuvant was injected subcutaneously into each rabbit at monthly intervals for four months. On the seventh day after each injection, blood from ten rabbits were drawn, pooled, frozen at -20°C and used in CIE.

Crossed immunoelectrophoresis (CIE)

CIE was performed according to the previously described method (Lowenstein 1978). Twelve ml of agarose (agarose A (pharmacia 17-0422-01) with tris-barbiturate buffer (pH 8.6)) was poured over the whole surface of the 84X94mm glass plate (LKB) and was allowed to cool and solidified. A gel plate was then laid in the bottom of the plate holder. The punch with outer diameters of 4.0 mm was inserted through the hole in the plate and pushed gently down into the gel to make a cylindrical cut. Ten ul

of each allergen extract (10 mg/ml) was applied into each well. First dimension electrophoresis was performed with Multiphor II unit (LKB) for 1.5 hr at 10 V/cm field strength. After the electrophoresis, the gel was cut out along the middle of gel and transferred on to a new plate. Eight ml of agarose gel was mixed at 54°C with 0.9 ml of rabbit antibodies to mugwort or ragweed. Washing the gel with saline, second electrophoresis was then run for 18 hours at 2 V/cm field strength. During electrophoresis, the plate was placed on a flat surface, wet with distilled water, and covered with one wet and five dry filter papers. A 1 kg weight was laid on the top of the plate. After three minutes, the upper five filter papers were replaced and this process was repeated twice. The gel was washed and pressed twice more and dried to a hard and thin film. The gel was placed into the staining solution (1 g Coomassie blue R 250 (BRL 5528 UA) in of destaining solutions for five minutes, then rinsed with water and destained about 10 to 20 minutes in destaining solution (ethanol (95%), 40 mol/l glacial acid and distilled water).

RESULTS

Crossed immunoelectrophoresis (CIE) finding

Five precipitation lines were formed between

Fig. 1. Crossed immunoelectrophoresis finding between mugwort (MG) and rabbit anti-mugwort (AM) antibody.

mugwort and anti-mugwort antibody(Fig. 1). CIE with ragweed and anti-ragweed antibody revealed one precipitation line as shown in Fig. 2. CIE of mugwort and anti-ragweed antibody revealed no precipitation lines as shown in Fig. 3. CIE with ragweed and anti-mugwort antibody revealed no precipitation lines as shown in Fig. 4.

Fig. 2. Crossed immunoelectrophoresis finding between ragweed(RG)and rabbit anti-ragweed(AR) antibody.

Fig. 3. Crossed immunoelectrophoresis finding between mugwort(MG)and rabbit anti-ragweed(AR) antibody.

Fig. 4. Crossed immunoelectrophoresis finding between ragweed(RG)and rabbit anti-mugwort(AM) antibody.

DISCUSSION

Mugwort and ragweed have been considered as important respiratory allergens in Korea. The results of skin prick tests with Korean pollen extracts, commonly found in the air of Seoul, revealed that the incidence of sensitization to *Artemisia* (Art.) species- (sagebrush) pollen was 14.2%, and sensitization to ragweed was 10.4% (Kim et al., 1987). Park et al. (1989) reported 26 cases of atopic bronchial asthma induced by Korean *Artemisia* spp. pollen extracts. Song et al. (1992) reported 11 cases of ragweed-sensitive asthma, confirmed by bronchoprovocation tests.

A few studies have been performed to characterize mugwort pollen extracts with regard to IgE-binding components and their chemical or biochemical compositions. Recently, Ipsen et al. (1985) have identified 10 allergens from *Art. vulgaris* pollen and Paulsen et al. (1985) have observed seven allergens among 18 antigens by crossed radioimmunoelectrophoresis (CRIE). Nilsen and Paulsen (1986) has partially purified and defined Ag 7 as the major allergen from *Art. vulgaris* extracts. Jaggi and Gangal (1987) have purified one important allergen with a molecular weight of 14,300 daltons from *Art. scoparia* pollens. Park et al. (1989) reported two bands (37,000, 23,000 bands) which showed the highest IgE-binding property, and 12 bands bound to IgE antibody from 50% of the sera

tested in the molecular range of 10,000 and 85,000 daltons by 10% sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), western blotting and autoradiography. However, allergens from ragweed pollens have already been characterized and purified by many investigators (King TP and Norman PS, 1962; King TP et al., 1964; King TP et al., 1976; Underdown, 1969; Klapper et al., 1980; Vidusek et al., 1985; Roebber et al., 1983).

Cross-reactivity between mugwort pollen and other inhalant allergens has been reported. Park et al. (1990) suggested some cross-reactivities between ricebran extracts and mugwort and ragweed pollens by ricebran radioimmunoassay (RIA) inhibition test. Moreover, some cross-reactivity between mugwort and food allergens has been suggested by several investigators (Wuthrich and Hofer, 1984; Dietschi et al., 1987). They reported significantly higher positive rates on skin test to food allergens such as celery, carrot and spices in mugwort-sensitive allergy patients. Park and Kim (1992) reported the possibility of cross-allergenicity between sunflower seed extracts and mugwort pollens. Subiza (1989) suggested the cross-reactivity between chamomille, mugwort, and ragweed pollens.

In Korea, ragweed and mugwort pollens are abundant in the air of Seoul in the same season, and many ragweed-sensitive patients have shown concurrent positive responses on skin prick tests to mugwort pollen. Lowenstein (1980) has demonstrated that short ragweed and mugwort have a partial antigenic identity of 33% by RAST inhibition test. Leiferman et al. (1976) has demonstrated by RAST inhibition that mugwort does not inhibit the ragweed-RAST; moreover, the ragweed AgE (Amb) was not present in mugwort extract (Krilis et al., 1981). However RAST inhibition test is known to be inadequate in identifying cross-antigenicity between two antigens. In this study, CIE between mugwort and anti-ragweed antibody, and between ragweed and anti-mugwort antibody showed no precipitation line.

In this study, mugwort and anti-mugwort antibody showed only five precipitation lines, which was fewer than those of other investigations (Ipsen et al., 1985). But this was more than in Brandys' study, which revealed four antigens. The reason for this is not clear. Comparative study among six mugwort pollen extracts including our mugwort extracts revealed very similar SDS-PAGE patterns or IgE binding patterns on autoradiography (Brandys et al., 1993). Therefore,

we cannot speculate any differences in antigens. To determine the possible differences in polyclonal antibodies, further investigations perhaps with modification of the immunization schedule are needed.

These results suggest that there is no cross-reactivity between mugwort and ragweed pollens.

REFERENCES

- Bensadoun A, Weinstein D: *Assay of proteins in the presence of interfering materials. Annal Biochem* 70: 241-50, 1976.
- Brandys J, Grimsoen A, Nilsen BM, Paulsen BS, Park HS, Hong CS: *Cross-reactivity between pollen extracts from six Artemisia species. 1993 (in publish).*
- Charpin J, Surinych R, Frankland AW: *Atlas of European allergenic pollens. Sandoz, 1974.*
- Dietsch R, Wuthrich B, Johansson SGO: *Zum sogenannten "Sellerie-Karotten-Beifuß-Gewürzsyndrom" RAST-Resultate mit neuen Gewürzdiscs. Z Hautkr* 62: 524-31, 1987.
- Harboe NM, Ingild A: *Immunization, isolation of immunoglobulins and antibody titer determination. Scand J Immunol* 17: 345-51, 1983.
- Hong CS, Hwang Y, Oh SH, Kim HJ, Huh KB, Lee SY: *Survey of the airborne pollens in Seoul, Korea. Yonsei Med J* 27: 114-20, 1986.
- Holopainen E, Salo OP, Tarkkainen E: *The most important allergens in allergic rhinitis. Acta Otolaryngol* 360: 16-8, 1979.
- Ipsen N, Formgren N, Lowenstein H, Ingemann L: *Immunochemical and biological characterization of a Mugwort (Artemisia vulgaris) pollen extract. Allergy* 40: 289-94, 1985.
- Jaggi KS, Gangal S: *Isolation and identification of pollen allergens of Artemisia scoparia. J Allergy Clin Immunol* 80: 562-72, 1987.
- Kim YL, Lee KS, Oh SH, Moon BS, Park HS, Hong CS: *A study of allergic skin tests with Korean pollen extract. Yonsei Med J* 28: 112-8, 1987.
- King TP: *Chemical and biological properties of some atopic allergens. Adv Immunol* 23: 77-105, 1976.
- King TP, Norman PS: *Isolation studies of allergens from ragweed pollen. Biochemistry* 1: 709-20, 1962.
- King TP, Norman PS, Connel JJ: *Isolation and characterization of allergens from ragweed pollen. Biochemistry* 3: 458-68, 1964.
- Klapper DG, Goodfriend L, Capra JD: *The amino acid sequence of ragweed allergen Ra 3. Biochemistry* 19: 5729-34, 1980.
- Krilis S, Baldo BA, Raison RL, Callard RE, Basten A: *Standardization of antigen E in ragweed pollen extracts using a monoclonal antibody-based enzyme immunoassay. J Allergy Clin Immunol* 71: 261-5, 1983.
- Leiferman KM, Gleich GJ, Jones RT: *The cross-reactivity*

- of IgE antibodies with pollen allergens. *J Allergy Clin Immunol* 58 : 140-8, 1976.
- Lowenstein H : Qualitative immunoelectrophoretic methods as a tool for the analysis and isolation of allergens. *Prog Allergy* 25 : 1-62, 1978.
- Lowenstein H : Cross reactions among pollen antigens. *Allergy* 35 : 198-200, 1980.
- Nilsen BM, Paulsen S : Allergens in pollen from Mugwort (*Artemisia vulgaris*). *Int Arch Allergy Appl Immunol* 80 : 113-21, 1986.
- Park HS, Kim YJ : Sunflower seed allergy : Cross-reactivity with mugwort pollen. *J Kor Soc Allergy* 13 : 75, 1993.
- Park HS, Hong CS, Choi HJ, Hahm KS : Identification and partial purification of pollen allergen from *Artemisia princeps*. *Yonsei Med J* 30 : 348-54, 1989.
- Park HS, Hong CS, Kim JW : Rice bran allergens : Particular relationships with pollens. *J Kor Soc Allergy* 10 : 104-10, 1990.
- Paulsen BS, Nesje LFG, Wold JK : Allergens in pollen from Mugwort (*Artemisia vulgaris* L.). *Int Arch Allergy Appl Immunol* 78 : 206-12, 1985.
- Roebber M, Hussain R, Klapper DG, Marsh DG : Isolation and properties of a new short ragweed pollen allergen. *Ra* 6. *J Immunol* 131 : 706-11, 1983.
- Song KS, Lee NH, Lee SK, Kim JM, Hong CS : Bronchial provocation test with ragweed pollen extracts. *J Kor Soc Inter Med* : 131, 1992(abstract).
- Subiza J, Subiza JL, Hinojosa M, Garse R, Jerez M, Valdivieso R, Subiza E : Anaphylactic reaction after the ingestion of chamomille tea : A study of cross-reactivity with other composite pollens. *J Allergy Clin Immunol* 84 : 353-58, 1989.
- Underdown BJ, Goodfriend L : Isolation and characterization of an allergen from short ragweed pollen. *Biochemistry* 8 : 980-9, 1969.
- Vidusek DA, Roeberts MF, Goodfriend LL : 500-MHz HNMR studies of ragweed allergen *Ra* 5. *Biochemistry* 24 : 2747-53, 1985.
- Weber RW, Nelson HS : Pollen allergens and their interrelationships. *Clin Rev Allergy* 3 : 291-318, 1985.
- Wuthrich B, Hofer T : Nahrungs mittelallergie das sellerie-Beifu B-Gewurz syndrom. *Dtsch Med Wschr* 109:981-6, 1984.