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## Characteristics of Five Monoclonal Antibodies to Major Allergens of the Short Ragweed Pollen

Horng-Der Shen, Lu-Yin Chang, Song-Nan Su, Shou-Hwa Han

Department of Medical Research, Veterans General Hospital, Taipei, Taiwan, Republic of China

**Abstract.** Monoclonal antibodies against major allergens of the short ragweed pollen were produced by fusion of NS-1 cells with splenic cells from BaLB/C mice that had been immunized separately with the major allergens, AgE-B+E-C and AgK, of the short ragweed pollen. These monoclonal antibodies were detected by enzyme-linked immunosorbent assay and further characterized by immunoblot analysis using the crude extract and highly purified allergens of the ragweed pollen. Three monoclonal antibodies obtained by immunization with AgE-B+E-C, designated as 36-6, 27-2 and 48-5, reacted mainly with the beta (36-6) and alpha (27-2) subunit of AgE and both AgE and AgK (48-5), respectively. Two monoclonal antibodies obtained by immunization with AgK, 4-7 and 8-5, had a similar reactivity with AgE-B+E-C, AgK, AgK-A and AgK-B. In addition, however, antibody 4-7 also reacted with AgE-B<sub>2</sub> as well as the 36- and the 24- to 22-kilodalton antigens of the crude extract. All 5 monoclonal antibodies were characterized as IgG1 subclass. Besides, monoclonal antibody 48-5 also showed cross-reactivity to components of sage pollen. Beyond academic interests, the monoclonal antibodies described here may also be useful in clinical allergy.

### Introduction

IgE-mediated allergic disease constitutes a common clinical problem [1]. The allergenic extracts used in clinical diagnosis, therapy and research of associated diseases are complex mixtures containing many components only some of which are true allergens [2]. Most approaches to the standardization of allergenic extracts do not quantitate individual allergenic components [3]. Furthermore, clinical experience has shown that patients who are allergic to some allergens are, as a rule, also allergic to some other sources of allergens [4, 5]. Partial immunochemical identity between molecules in the same or different allergenic extracts has also been demonstrated in different reports [2, 6-9]. Cross-reactivity among different crude allergen preparations therefore constitutes another problem in the diagnosis and treatment of allergic diseases. Application of monoclonal antibody technology in this field may therefore provide a suitable primary standard for use in the standardization and quality control of allergenic crude extracts [10, 11]. Suitable monoclonal antibodies against al-

lergens may also be useful in the study of the specificity of allergenic molecules in crude extracts and cross-reactivity between molecules in different allergen sources [12-14].

In this study, we generated 5 monoclonal antibodies against the major allergens, AgE and AgK, of the short ragweed (*Ambrosia elatior*) pollen and characterized the antigen(s) recognized by these monoclonal antibodies by immunoblot technique. Cross-reactivity among different pollen antigens using these monoclonal antibodies was also analyzed.

### Materials and Methods

#### Allergenic Extracts

Defatted pollens of short ragweed (*A. elatior*), mixed sage (*Artemisia* sp.), *Plantago major*, *Xanthium strumarium*, *Amaranthus spinosus*, *Chenopodium ambrosioides*, *Rumex crispus japonicus*, *Acacia confusa*, *Eucalyptus robusta*, *Zea mays* and *Miscanthus floridulus* were obtained from International Biologicals (Piedmont, Okla., USA). Allergenic extracts were prepared by extracting each pollen with phosphate-buffered saline (PBS), pH 8.0, for 72 h at 4°C with constant shaking. After filtration through a Whatman

No. 1 filter and centrifugation at 800 g for 30 min at 4°C, the supernatant was dialyzed against distilled water. After sterile filtration (0.2 µm; Sartorius, Hayward, Calif., USA), 1-ml lots of antigen were stored at 4°C.

Highly purified ragweed allergens, antigen E-B and E-C (AgE-B+E-C), antigen E-B<sub>2</sub> (AgE-B<sub>2</sub>), antigen K (AgK), antigen K-A (AgK-A), antigen K-B (AgK-B), antigen Ra3 (AgRa3) and antigen Ra5 (AgRa5) were obtained from the National Institutes of Health (NIH; Bethesda, Md., USA).

#### Development of Monoclonal Antibodies

BaLB/C mice were immunized and boosted intraperitoneally with 80 µg of AgE-B+E-C or AgK in complete and incomplete Freund's adjuvant, respectively, at a 1-month interval. Two weeks after the booster injection, 80 µg of the same antigen(s) in saline were injected intravenously. Spleen cells were removed 3 days after the last intravenous injection and fused with murine plasmacytoma NS-1 cells in the presence of polyethylene glycol (molecular weight 1,500 daltons; Merck). Antibody-producing hybrid cells were screened by enzyme-linked immunosorbent assay (ELISA). Cells from positive wells were subcloned at least twice by limiting dilution and then further expanded. The isotype of the monoclonal antibodies was determined by ELISA with class-specific antisera (Zymed Laboratories, San Francisco, Calif., USA).

#### ELISA

Wells of flexible polyvinyl microtiter plates (Costar, Cambridge, Mass., USA) were coated with AgE-B+E-C or AgK by the addition of 50 µl of a 1-µg/ml solution in carbonate buffer, pH 9.6. The crude extract of short ragweed pollen was coated in the same condition with a 200-µg/ml solution. After incubation at 37°C for 2 h and three washes with 10 mM PBS, pH 7.4, containing 0.05% (v/v) Tween 20 (PBST), 250 µl of 3% bovine serum albumin (Calbiochem, San Diego, Calif., USA) in PBS was added to each well and left overnight at 4°C. Plates were rewashed thrice and 50 µl of culture supernatant was added to each well and incubated for 1 h at 37°C. After washing with PBST, 50 µl of alkaline phosphatase-conjugated rabbit anti-mouse IgG (whole molecule; Sigma, St. Louis, Mo., USA) was added to each well and incubated for 1 h at 37°C. Wells were washed as above and 50 µl of substrate solution (1 mg/ml *p*-nitrophenyl phosphate in 10% diethanolamine buffer, pH 9.8) was added to each well for 30 min at 37°C. Color change was detected by measurement of the absorbance at 405 nm on a Titertek Multiskan (Flow Laboratories, McLean, Va., USA). Culture supernatant of NS-1 cells was used as negative control.

#### Immunoblot Analysis

Proteins from the crude extracts of various pollens and the highly purified allergens of the short ragweed pollen mentioned above were prepared for regular and/or sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli [15] and separated on 10% acrylamide gels. The molecular weight standards used in this study were obtained from Pharmacia (Uppsala, Sweden). The standard preparation contained phosphorylase-b, 94 kilodaltons (kD); bovine serum albumin, 67 kD; ovalbumin, 43 kD; carbonic anhydrase, 30 kD; soybean trypsin inhibitor, 20.1 kD, and alpha-lactalbumin, 14.4 kD. When electrophoresis was completed, the gels were removed and the separated proteins were either stained with Coomassie blue R-250 or electrophoretically transferred to nitrocellulose paper (NCP, 0.45 µm; BA 85;

**Table I.** Reactivity of monoclonal antibodies against AgE-B + E-C, AgK and the crude extract of the short ragweed pollen

| Monoclonal antibodies | AgE-B + E-C | AgK | Crude extract |
|-----------------------|-------------|-----|---------------|
| 36-6                  | ++          | -   | +             |
| 27-2                  | +++         | +   | ++            |
| 48-5                  | +++         | +++ | +++           |
| 4-7                   | ++          | +++ | ++            |
| 8-5                   | +           | +++ | ++            |

All assays were carried out using undiluted tissue culture supernatants by enzyme-linked immunosorbent method. +, ++, +++ = different intensities of the absorbance of the supernatants at 405 nm if they were greater than the absorbance of the culture supernatant of NS-1 cells and were less than 0.5, between 0.5 and 1.5, and greater than 1.5, respectively. - = negative color reaction and indicates that the absorbance of the supernatant at 405 nm was less than or equal to that of NS-1 cells.

Schleicher & Schuell, Dassel, FRG) essentially as described by Towbin et al. [16]. The NCP and gel were electrophoresed in transfer buffer [25 mM Tris, 192 mM glycine and 20% (v/v) methanol, pH 8.3] under constant voltage at 90 V and 4°C for 1.5 h using TE 50 Transphor (Hoefer, San Francisco, Calif., USA). After electrophoresis, the NCP was stained either immunochemically or with Coomassie blue R-250. Immunochemical staining was performed by first blocking the NCP overnight with 3% (w/v) gelatin in Tris-buffered saline (TBS; 20 mM Tris, 500 mM NaCl, pH 7.5) at 4°C. All subsequent manipulations of the NCP were done at room temperature. After three washes with TTBS (0.05% Tween 20 in TBS), the blots were incubated with hybridoma supernatants diluted 1:10 in TTBS for 1 h. For control experiments, culture supernatants of NS-1 cells were used. The blots were washed three times with TTBS for 10 min each and then incubated in a 1:3,000 dilution in TTBS of affinity-purified, horseradish peroxidase-labeled goat anti-mouse IgG(H+L) (Bio-Rad, Richmond, Calif., USA) for 1 h. The blots were washed as described above and developed in a substrate solution containing 0.05% (w/v) 4-chloro-1-naphthol (Bio-Rad) and 0.015% (v/v) hydrogen peroxide. After 30 min, the blots were rinsed in distilled water and air-dried. Antigen-antibody binding was identified by deposition of purple stain.

## Results

### Monoclonal Antibodies Specific for Major Allergens of the Short Ragweed Pollen

Cell fusion between spleen cells obtained from BaLB/C mice immunized with purified AgE-B+E-C or AgK and NS-1 cells resulted in a series of hybridomas secreting monoclonal antibodies specific for major allergens of the short ragweed pollen. Five monoclonal antibodies designated as 36-6, 27-2, 48-5, 4-7 and 8-5 were chosen for further studies. The mono-

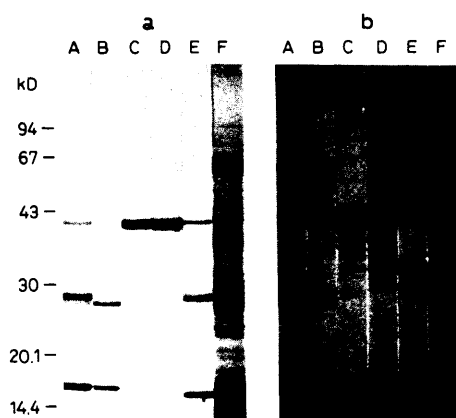


**Fig. 1.** Protein profiles of purified allergens and the crude extract of the short ragweed pollen separated by 10% SDS-PAGE. **a** Gel stained with Coomassie blue R-250. **b** Transfers of SDS-PAGE-separated components to nitrocellulose filters and stained with Coomassie blue R-250. Lanes A-F: AgE-B+E-C (A), AgE-B<sub>2</sub> (B), AgK (C), AgK-A (D), AgK-B (E) and the crude extract of the short ragweed pollen (F).

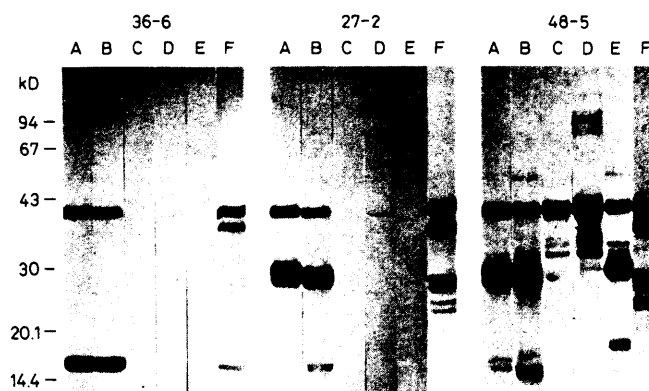


**Fig. 2.** Immunoblot profiles of monoclonal antibodies (36-6, 27-2 and 48-5) to purified allergens and the crude extract of the short ragweed pollen separated by SDS-PAGE. Lanes A-F: AgE-B+E-C (A), AgE-B<sub>2</sub> (B), AgK (C), AgK-A (D), AgK-B (E) and the crude extract of the short ragweed pollen (F).

clonal antibodies 36-6, 27-2 and 48-5 were derived from mice immunized with AgE-B+E-C. Besides reactivities with AgE-B+E-C, monoclonal antibody 27-2 showed very weak and 48-5 showed strong binding activity to purified AgK which had been coated directly onto a polyvinyl microtiter plate (table I). Another two monoclonal antibodies, 4-7 and 8-5, derived from mice immunized with AgK, reacted with both AgE-B+E-C and AgK (table I). These two antibodies showed lower binding activity to AgE-B+E-C than to AgK. All 5 monoclonal antibodies were characterized as IgG1 subclass and contained a kappa light chain.

#### *Protein Profiles of the Short Ragweed Pollen Antigens*

For further analysis of the specificity of these monoclonal antibodies, immunoblot analyses were performed with purified AgE-B+E-C, AgE-B<sub>2</sub>, AgK, AgK-A, AgK-B and the crude extract of the short ragweed pollen. Figure 1a depicts the SDS-PAGE pattern and figure 1b shows the electrophoretically transferred nitrocellulose membrane pattern of purified allergens and the crude pollen extract. Using a multi-step chromatographic purification procedure, ragweed AgE and AgK were isolated in multiple isoelectric forms, designated AgE-A, AgE-B, AgE-C and AgE-D, in order of increasing acidity [17, 18]. AgE-A exists as a single-chain polypeptide of about 38 kD in ragweed pollen. The AgE-B and AgE-C forms are

suggested to be derived from AgE-A by naturally-occurring limited proteolysis during isolation procedures [19]. In our experiment, AgE-B+E-C obtained from the Research Resources Branch of the NIH showed two chains of about 26 (alpha subunit) and 16 kD (beta subunit) and the native 39-kD form in SDS-PAGE (fig. 1a; lane A). AgE-B<sub>2</sub> (fig. 1a; lane B) is suggested to be a product of limited trypsin digestion of AgE-A, and is chemically indistinguishable from the natural AgE-B form. Purified AgK-A (fig. 1a; lane D) and AgK-B (fig. 1a; lane E) were prepared under the same conditions as AgE-A and AgE-B, respectively [19]. The crude extract of short ragweed pollen showed at least 25 bands in the SDS-PAGE gel system (fig. 1a; lane F). Components with molecular weights of 64–62, 39, 36, 28–26, 24, 16 and 13 kD were predominant.

#### *Antigen Specificity of the Monoclonal Antibodies*

The culture supernatant of each hybridoma was incubated with nitrocellulose membrane on which purified allergens and the crude ragweed pollen extract had been electrophoretically transferred. The results are summarized in table II. Monoclonal antibody 36-6 reacted with the natural, single polypeptide form of AgE and the  $\beta$  subunit of this purified allergen (fig. 2; 36-6, lanes A, B). In addition to these two components, antibody 36-6 also recognized an additional antigen with a molecular weight of 37 kD in the crude pollen preparation (fig. 2; 36-6, lane F). Like antibody

**Table II.** Characterization of monoclonal antibodies (MAb) to major allergens of the short ragweed pollen by immunoblot analysis

| Antigens                                         | MAb derived from BalB/C mice immunized with |                |                |                |     |
|--------------------------------------------------|---------------------------------------------|----------------|----------------|----------------|-----|
|                                                  | AgE-B + E-C                                 |                |                | AgK            |     |
|                                                  | 36-6                                        | 27-2           | 48-5           | 4-7            | 8-5 |
| <b>AgE-B + E-C</b>                               |                                             |                |                |                |     |
| 39 kD (native form)                              | +                                           | +              | +              | +              | +   |
| 26 kD ( $\alpha$ subunit)                        | -                                           | +              | +              | +              | +   |
| 16 kD ( $\beta$ subunit)                         | +                                           | + <sup>w</sup> | + <sup>w</sup> | -              | -   |
| <b>AgE-B<sub>2</sub></b>                         |                                             |                |                |                |     |
| 39 kD (native form)                              | +                                           | +              | +              | +              | -   |
| 26 kD ( $\alpha$ subunit)                        | -                                           | +              | +              | +              | -   |
| 16 kD ( $\beta$ subunit)                         | +                                           | + <sup>w</sup> | + <sup>w</sup> | -              | -   |
| <b>AgK</b>                                       |                                             |                |                |                |     |
| 39 kD (native form)                              | -                                           | -              | +              | +              | +   |
| <b>AgK-A</b>                                     |                                             |                |                |                |     |
| 39 kD (native form)                              | -                                           | + <sup>w</sup> | +              | +              | +   |
| <b>AgK-B</b>                                     |                                             |                |                |                |     |
| 39 kD (native form)                              | -                                           | -              | +              | +              | +   |
| 26 kD ( $\alpha$ subunit)                        | -                                           | -              | +              | +              | +   |
| 16 kD ( $\beta$ subunit)                         | -                                           | -              | + <sup>w</sup> | -              | -   |
| <b>Crude extract of the short ragweed pollen</b> |                                             |                |                |                |     |
| 39 kD                                            | +                                           | +              | +              | +              | +   |
| 37 kD                                            | +                                           | -              | -              | -              | -   |
| 36 kD                                            | -                                           | +              | +              | +              | -   |
| 28-26 kD                                         | -                                           | +              | +              | +              | +   |
| 24-22 kD                                         | -                                           | +              | +              | + <sup>w</sup> | -   |
| 16 kD                                            | +                                           | -              | -              | -              | -   |

The signs + and - indicate the presence or absence of the deposition of purple stain, respectively; +<sup>w</sup> indicates a weaker color reaction.

36-6, monoclonal antibody 27-2 also reacted with the natural form of AgE, but showed very strong reactivity to the  $\alpha$  subunit and weak reactivity to the  $\beta$  subunit of AgE (fig. 2; 27-2, lanes A, B). With very weak binding activity to AgK as detected in the ELISA, monoclonal antibody 27-2 also showed a very weak band with a molecular weight of 39 kD in the AgK-A preparation (fig. 2; 27-2, lane D). This may be due to the minor contamination of AgE in the AgK preparations. Monoclonal antibody 48-5 showed the same immunoblot pattern as antibody 27-2 to AgE-B+E-C and AgE-B<sub>2</sub> (fig. 2; 48-5, lanes A, B). Besides, 48-5

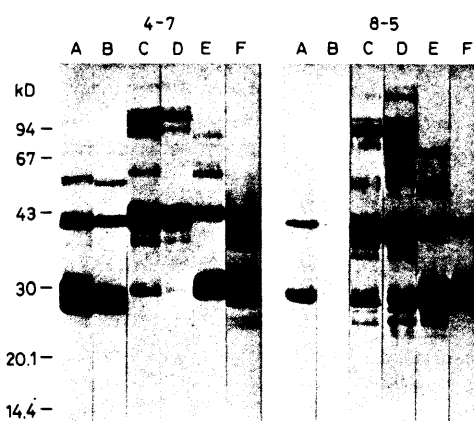


**Fig. 3.** Immunoblot profiles of monoclonal antibodies (4-7 and 8-5) to purified allergens and the crude extract of the short ragweed pollen separated by SDS-PAGE. Lanes A-F: AgE-B+E-C (A), AgE-B<sub>2</sub> (B), AgK (C), AgK-A (D), AgK-B (E) and the crude extract of the short ragweed pollen (F).

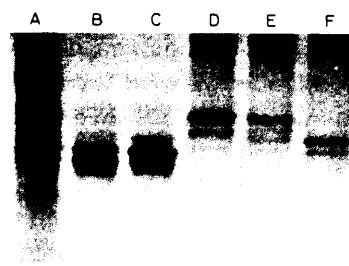


**Fig. 4.** Coomassie blue R-250-stained protein profiles of purified allergens and the crude extract of the short ragweed pollen separated by 10% regular PAGE. Lanes A-F: AgE-B+E-C (A), AgE-B<sub>2</sub> (B), AgK (C), AgK-A (D), AgK-B (E) and the crude extract of the short ragweed pollen (F).

also showed strong binding activity to AgK, AgK-A and AgK-B (fig. 2; 48-5, lanes C-E). These two monoclonal antibodies showed at least 6 bands in the immunoelectropherogram of the crude ragweed pollen extract (fig. 2; 27-2, lane F and 48-5, lane F). Components with molecular weights of 39 and 28-26 kD seemed to correspond to the native and the  $\alpha$  subunit of major allergens AgE and AgK. Components with molecular weights of 36 and 24-22 kD may represent other components in the crude extract which shared an antigenic determinant with both major allergens of short ragweed pollen.

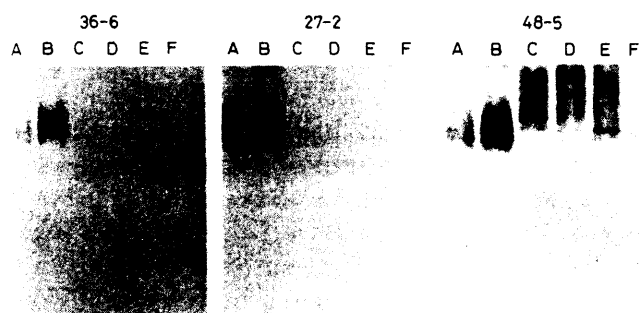


Fig. 5. Immunoblot profiles of monoclonal antibodies (36-6, 27-2 and 48-5) to purified allergens and the crude extract of the short ragweed pollen separated by regular PAGE. Lanes A-F: AgE-B+E-C (A), AgE-B<sub>2</sub> (B), AgK (C), AgK-A (D), AgK-B (E) and the crude extract of the short ragweed pollen (F).

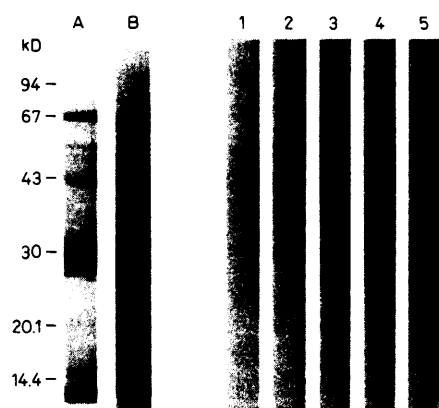


Fig. 6. Immunoblot profiles of monoclonal antibodies to the crude extract of the mixed sage pollens separated by 10% SDS-PAGE. Lane A: gel stained with Coomassie blue R-250; lane B: transfer of SDS-PAGE-separated sage pollen components to nitrocellulose filter and stained with Coomassie blue R-250; lanes 1-5 are monoclonal antibodies 36-6, 27-2, 48-5, 4-7 and 8-5, respectively.

Figure 3 depicts immunoblot results showing the specificity of monoclonal antibodies 4-7 and 8-5 which were obtained from mice immunized with AgK. These two antibodies reacted with AgE-B+E-C and AgK as shown in the ELISA tests (table I). Antibody 4-7 showed the same immunoblot pattern as antibody 48-5, except that it did not show the weak band at the position of the  $\beta$  subunit of AgE as antibody 48-5. Another interesting finding is that, though antibody 8-5 had an immunoblot pattern similar to antibody 4-7, it did not react with either AgE-B<sub>2</sub> or antigens with molecular weights of 36 and 24-22 kD in the crude extract of short ragweed pollen. Since AgE-B<sub>2</sub> is produced by limited trypsin digestion of AgE-A and is chemically indistinguishable from the natural AgE-B form, results shown here indicate that some

changes in antigenicity occurred during the digestion procedure, and such changes can only be detected by using antibodies with a high specificity such as monoclonal antibody 8-5 shown here.

We also used regular PAGE to check if the antigenicity of the antigens studied had been changed by protein denaturation in the SDS-PAGE system. Figure 4 shows a regular PAGE gel pattern of the purified allergens and the crude extract of the ragweed pollen. It is evident in figure 5 that monoclonal antibodies 36-6, 27-2 and 48-5 reacted with AgE-B+E-C and AgE-B<sub>2</sub>. In addition, antibody 48-5 also reacted with AgK, AgK-A and AgK-B. These three antibodies showed very weak reactions with the crude pollen extract. Monoclonal antibodies 4-7 and 8-5 also showed a similar reaction pattern in regular and in the SDS-PAGE systems (data not shown), and antibody 8-5 did not react with AgE-B<sub>2</sub> in both systems.

None of the 5 monoclonal antibodies described in this study reacted with two other allergens, AgRa3 and AgRa5, of the short ragweed pollen as determined by immunoblot analysis using both regular and SDS-PAGE systems (data not shown).

#### Cross-Reactivity among Different Pollen Antigens Determined by Monoclonal Antibodies

Since there is cross-reactivity among different crude allergen preparations, we examined the antigenic relationship between short ragweed and other pollens using the monoclonal antibodies described. Two tree pollens, two grass pollens and 6 weed pollens were included in this study. Our results show that only antigens in the crude extract of mixed sage pollens were reactive with monoclonal antibody 48-5 (fig. 6). On analysis of cross-reactivity using *Artemisia* pollens from Allergon AB (Sweden), antibody 48-5 was found to react with crude extracts of *A. tridentata*, *A. frigida* and *A. vulgaris*. The same immunoblot pattern as shown in figure 6 was observed (data not shown).

#### Discussion

Short ragweed pollen is a major cause of late summer hay fever in North America. The major allergens of this pollen, AgE and AgK, which represent approximately 6 and 3%, respectively, of the protein content of the short ragweed pollen, have been studied with regard to a number of physicochemical char-

acteristics. Much of the work was done by King and colleagues [2, 17–20], who established the molecular weights, described the partial immunological identity of these two major allergens and found that the two-chain form of AgE which was obtained from the multi-step chromatographic purification, was antigenically and allergenically indistinguishable from the native single-chain form with the molecular weight of 38 kD.

In this study, we produced 5 monoclonal antibodies to the major allergens, AgE and AgK, of the short ragweed pollen. We found that it is the  $\alpha$  subunit which contains the common determinant group and is responsible for the partial immunological identity of AgE and AgK described by King et al. [18]. Furthermore, both  $\alpha$  and  $\beta$  subunits of AgE had specific antigenic determinants which were recognized by monoclonal antibodies 27-2 and 36-6, respectively, and were not shared by AgK.

Another interesting finding is that monoclonal antibody 8-5 did not react with AgE-B<sub>2</sub> which was produced by limited trypsin digestion of AgE-A. Besides, this antibody also showed weaker reactivity with AgE-B+E-C than the reaction of other antibodies with AgE as shown in the immunoblot analyses. It is possible that monoclonal antibody 8-5 did not react with AgE, and the weaker reactivity observed in the experiments was due to the minor contamination of AgK in the AgE-B+E-C preparation. Another possibility is that the determinant site in AgE recognized by antibody 8-5 may have been altered by trypsin digestion, such that there are at least two common determinant groups in AgE shared by both AgE and AgK. One such determinant group is trypsin-resistant and recognized by monoclonal antibodies 48-5 and 4-7, while the other is trypsin-sensitive and not recognized by antibody 8-5 after trypsin digestion. In the crude extract of the short ragweed pollen, components with molecular weights of 39 and 28–26 kD contained both common determinant groups mentioned above, while components with molecular weights of 36 and 24–22 kD contained only one common determinant site recognized by antibodies 48-5 and 4-7 but not by antibody 8-5.

It seems that the presence of a common allergenic determinant in several proteins is a common feature in allergenic extracts. King et al. [20] have hypothesized that the high allergenic activities of major allergens, AgE and AgK, are due to their possession of determinant groups common to other proteins in ragweed pollen. It is based on immunodiffusion experi-

ment results that AgE and ragweed allergen BPA-R with a molecular weight of 28 kD possess a common antigenic determinant [21]. In this study, since purified BPA-R was not available, it could not be determined if any of the components shared common antigenic determinants with AgE corresponding to this allergen. With the exception of AgE and AgK, these components may yet represent other allergens to be identified. In addition, results from our study also show that most of the major protein components in the crude pollen extract were recognized by monoclonal antibodies which reacted with both allergens AgE and AgK of the short ragweed pollen.

Since cross-reactivity is a well-recognized problem in the diagnosis and treatment of allergic diseases, we studied this property among different pollen extracts using the monoclonal antibodies. Except for mixed sage pollen, short ragweed did not show cross-reactivity with two tree, two grass and 5 other weed pollens which are widely distributed in Taiwan. This is in accordance with the previous finding that there is a low degree of identity between ragweed and sage pollens [7, 8]. Since the sage pollen induces severe allergic problems in North Europe [22, 23] it would be worthwhile to further study their allergenic properties. Of the 5 monoclonal antibodies reported in this study, only antibody 48-5 cross-reacted with the sage pollen. This suggests that the antigenic determinant sites recognized by antibodies 48-5 and 4-7 are different though they show similar immunoblot patterns to antigens of the short ragweed pollen.

Monoclonal antibodies described are being used in isolation and standardization procedures of allergens in complex mixtures. They are also being used in electroblotting analyses to detect both qualitative and quantitative differences between different ragweed pollen batches.

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Correspondence to: Horng-Der Shen  
Department of Medical Research  
Veterans General Hospital  
Shih-Pai  
Taipei, 11217, Taiwan (Republic of China)