

Production and characterization of monoclonal antibodies against major allergens of American cockroach

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Summary

From several fusion experiments between spleen cells obtained from BALB/c mice immunized with partially purified Cr-PI of American cockroach and NS-1 cells, growth was observed in many wells. Seven stable subclones secreting monoclonal antibodies (mAbs) against Cr-PI, as determined by enzyme-linked immunosorbent assay (ELISA) with high absorbance values and immunoblot analysis, were obtained. All seven mAbs were characterized as IgG1 subclass by immunodiffusion, and reacted strongly with 72 kilodaltons (kD) of Cr-PI which have been identified as a major allergen of American cockroach. Six mAbs were found to have similar epitope specificities against Cr-PI by ELISA. The remaining mAb was found to have different epitope specificities with others. Interestingly, all mAbs did not react with any components of crude extracts of Oriental and German cockroaches as determined by immunoblot analysis and ELISA. A mAb-based double-antibody sandwich ELISA was developed, and the ELISA was dose-dependent and capable of detecting as little as 140 ng of Cr-PI allergen.

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Introduction

High incidences of cockroach hypersensitivity in atopic and asthmatic populations have been reported [1–5]. The causative association of skin sensitivity to cockroach with allergic bronchial asthma has been demonstrated by bronchial provocation test [5–8]. Cockroach induces antigen-specific IgE-mediated bronchial asthma, and cockroach is one of the major causes of allergic asthma [5–10]. Allergens of American cockroach with higher (120–40 kD) and lower (39–12 kD) molecular weights have been reported [3,11,12].

It has been shown that crude extract of American cockroach (*Periplaneta americana*) contains at least 29 antigenic components and 18 allergens as revealed by crossed immunoelectrophoresis and crossed radio-immunoelectrophoresis, respectively [11]. In the same study, nine principal allergens with molecular weights ranging from 26 to 120 kD which are able to bind specific IgE have been demonstrated. Among these, two compo-

nents (molecular weights 78 and 72 kD) which bound 100% of the sera of atopic patients tested have been identified as major allergens. Cr-PI containing a large quantity of major allergens of American cockroach has been partially purified by gel filtration and ion exchange chromatography [11].

Allergen crude extracts used in diagnosis and immunotherapy of IgE-mediated allergic reactions are complex mixtures of components, of which only a small portion of materials are active allergens. The need for standardization of allergenic preparations has been recognized for many years, and a variety of methods have been proposed [13–17], but there is no consensus on the application of these methods. The recent development of hybridoma technology and the production of mAbs offers immunologic tools and a unified approach to standardization of crude extracts [18–20]. mAbs of antigenic components and allergens of ragweed pollen [19,21], rye grass [22,23], wheat flour [24], *Alternaria* [25], and house dust mite [26–28] have been produced by different investigators. However, to our knowledge, the production of mAbs against allergens of cockroach has not previously been reported. In this study, we have developed monoclonal

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mouse antibodies against the Cr-PI of American cockroach with preliminary characterization and classification of their specificities.

Materials and methods

Immunizing antigens and allergenic extracts

Crude American cockroach extract was prepared by extracting *P. americana* with CoCa's solution, and Cr-PI and Cr-P II were prepared from crude *P. americana* extract by Sephadex G-150 SF gel filtration as described previously [11]. German cockroach (*Blattella germanica*, Lot no. 0379), Oriental cockroach (*Blatta orientalis*, Lot no. 0377) and *P. americana* (Lot no. 0378) extracts were obtained from Hollister-Steir (Spokane, U.S.A.) in lyophilized form.

Production of polyclonal antibodies against Cr-PI

Young adult New Zealand white rabbits were immunized with multiple injections of 400 µg Cr-PI in complete Freund's adjuvant (CFA, Difco, Detroit, MI, U.S.A.) and were boosted at least four times with 200 µg Cr-PI in incomplete Freund's adjuvant (IFA, Difco) at intervals of 2 weeks. Serial bleeding of the rabbit antisera were performed at time intervals, tested, pooled and stored at -70°C.

Immunization and production of mAbs to Cr-PI

BALB/c mice were immunized intraperitoneally with 30 µg of Cr-PI in CFA and were boosted three times with 20 µg Cr-PI in IFA at intervals of 10 days. Four days before fusion, the mice were injected intravenously with 20 µg of Cr-PI. Cell fusion was performed essentially as described by Galfre and Milstein [29]. Spleen cells were washed once with RPMI 1640 medium, pH 7.5 containing 25 mM HEPES, 2 mM glutamine, 10 U/ml penicillin and 100 ng/ml streptomycin (RPMI-GPS, Gibco, Grand Island, NY, U.S.A.) After spleen cells were treated with Gey's haemolytic medium, cells were mixed at a 7:1 ratio with murine plasmacytoma NS-1 cells in 35% polyethylene glycol (PEG, molecular weight 3000-4000, Gibco). Following fusion, the cells were centrifuged at 200 × g for 5 min and washed once with RPMI-GPS. Cells were resuspended in RPMI-GPS containing 10% foetal bovine serum (Gibco), 0.1 mM hypoxanthine and 0.016 mM thymidine (HT, Gibco). Cells were then plated into 24-well tissue culture plates (Falcon, Lincoln Park, U.S.A.) at about 5 × 10⁵ cells/well and fed 24 hr later with HT medium containing 0.43 µM aminopterin (HAT, Gibco). Cultures were then fed again with HAT medium at 2-day intervals for 6 days. Hybrid cells were screened for antibody production by ELISA.

Screening and determination of antigen specificity of the mAbs by ELISA

An ELISA was used to screen culture supernatants and determine antigen specificity of the mAbs. Microtitre plates (Immunolon II, Daynatech, Alexandria, VA, U.S.A.) were either coated with 100 µl of *P. americana* (20 µg/ml), *B. orientalis* (20 µg/ml), *B. germanica* (20 µg/ml), Cr-PI (2.0 µg/ml), or Cr-P II (4.0 µg/ml) in 0.1 M sodium carbonate buffer, pH 9.6 for 2 hr at 37°C. After incubation, coated wells were washed with phosphate-buffered saline containing 0.05% Tween 20, pH 7.4 (PBS/Tween). The wells were filled with 2% bovine serum albumin (BSA) and then incubated overnight at 4°C. After washing with PBS/Tween, wells were incubated with 50 µl of culture supernatants at room temperature for 2 hr, and wells were washed as before. Peroxidase-conjugated anti-mouse immunoglobulin (100 µl of 1:2000, Zymed laboratories, San Francisco, CA, U.S.A.) was reacted with the wells for 2 hr at room temperature. Wells were washed with PBS/Tween, and then the reaction was developed by using 100 µl of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS, 55 mg/ml in 0.1 M sodium citrate buffer, pH 4.2 containing 0.03% of H₂O₂). After 30 min incubation, the reaction was stopped with 50 µl of 5% sodium dodecyl sulphate (SDS), and the optical density was determined at 415 nm on an EAR 400 AT Easy Reader (SLT-Labinstruments, Salzburg, Austria).

Purification of mAbs

Hybridomas were cloned in HT medium twice by limiting dilution and then expanded further gradually. The isotype of each mAb was determined by immunodiffusion with rabbit anti-mouse class/subclass-specific antisera (The Binding Site Ltd, Birmingham, U.K.). mAb was purified by Protein A-agarose affinity chromatography using Affi-Gel Protein A MAPS II kit (BioRad, Richmond, CA, U.S.A.) according to the method described by the manufacturer. Briefly, 5 ml of Affi-gel Protein A was packed into a 1 × 10 cm column, equilibrated with 30 ml of binding buffer (pH 9.0). Hybridoma culture supernatant was applied to the column which was then washed with binding buffer until the absorbance of the effluent at 280 nm was zero. Immunoglobulin was eluted with 30 ml of elution buffer (pH 3.0), and each fraction was monitored for absorbance at 280 nm. The eluted IgG fractions were collected in 1.0 M Tris-HCl, pH 9.0, dialysed against 0.05 M PBS, pH 7.4, overnight at 4°C and finally lyophilized. Concentration of immunoglobulin was determined by immunodiffusion with anti-mouse IgG antisera (The Binding Site Ltd).

Assaying for epitope specificity of the mAbs by ELISA

Epitope specificity of the mAbs to Cr-PI was analysed by an ELISA [30] based on the consideration that an antigen bound with a monoclonal antibody (mAb₂) was unable to recognize a surface-attached first monoclonal antibody (mAb₁), when the mAb₁ has the same epitope specificity as the mAb₂. Microtitre plates were coated with 50 µl of first purified mAb (2.5 µg/ml), diluted in sodium carbonate buffer, per well for 2 hr at 37°C and washed after each step of incubation with PBS/Tween. Fifty microlitres of a constant concentration of Cr-PI (10 µg/ml) were pre-incubated with 50 µl of serial diluted second purified mAb (10 µg/ml) for 1 hr at 25°C, and 50 µl of the pre-incubated mixture were then subsequently added to the wells coated with mAb₁. After 1-hr incubation at 25°C, Cr-PI bound to the first mAb was determined by rabbit anti-Cr-PI antibodies (100 µl of 1:8000) followed by goat anti-rabbit IgG-peroxidase (100 µl of 1:1000, Sigma, St Louis, MO, U.S.A.). The reaction was developed, stopped and detected as described.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting

Proteins were separated by SDS-PAGE under denatured conditions with a discontinuous buffer according to the method of Laemmli [31] as previously described [11]. After SDS-PAGE, protein bands were transferred onto 0.45 µm nitrocellulose (NC) membrane by the method of Towbin *et al.* [32] as described by Ford *et al.* [33]. NC membranes were washed in 10% methanol in Tris-buffered saline (TBS, 20 mM Tris, 0.5 M NaCl, pH 7.5) for 1 hr with rocking, and then incubated for 1 hr at 25°C with 3% gelatin in TBS. After three washes with TBS containing 0.05% Tween 20, the blots were incubated overnight at 4°C either with NS-1 or hybridoma culture supernatants (1:20), purified mAb (15 µg/ml) or rabbit anti-Cr-PI (15 µg/ml) diluted in TBS containing 0.05% Tween and 0.5% gelatin, washed, and then incubated with peroxidase-labelled rabbit anti-mouse IgG (1:2000) or goat anti-rabbit IgG (1:8000, Bio-Rad) for 1 hr at 25°C. After an additional three washes the strips were immersed in a substrate solution of 0.05% 4-chloro-1-naphthol (Bio-Rad) and 0.03% H₂O₂ in TBS. After 30 min at 25°C, the blots were rinsed in distilled water and air dried.

Detection of Cr-PI allergens in cockroach extracts with enzyme-labelled mAb

Alkaline phosphatase (Sigma) was conjugated to the affinity-purified IgG fraction of mAb E-4 by the method of Avrameas [34]. A double-antibody sandwich ELISA was performed by using affinity-purified mAb. Briefly,

Table 1. Optical densities of culture supernatants of NS-1 and hybridomas against *B. orientalis*, *B. germanica*, *P. americana*, CR-PI and Cr-P11

Mono-clonal antibodies	<i>B. orientalis</i>	<i>B. germanica</i>	<i>P. americana</i>	Cr-PI	Cr-P11
NS-1	0.012	0.011	0.011	0.010	0.011
A-2	0.013	0.011	1.208	1.109	0.013
D-3	0.012	0.010	1.075	1.026	0.012
E-4	0.012	0.011	1.050	1.034	0.013
F-4	0.012	0.010	1.048	1.008	0.012
F-6	0.012	0.011	1.043	1.025	0.011
G-1	0.012	0.012	1.103	1.083	0.010
H-1	0.011	0.012	1.068	1.031	0.012

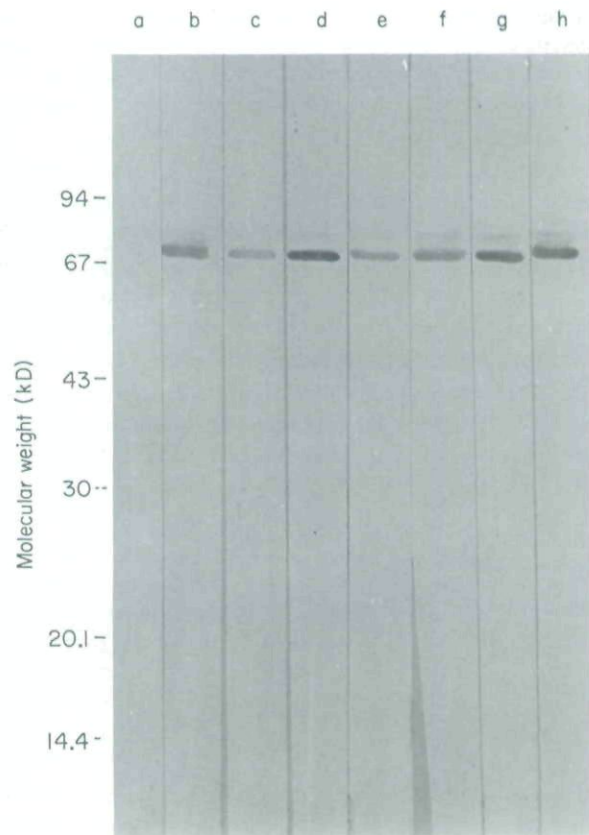


Fig. 1. Immunoblot analysis of monoclonal antibodies to Cr-PI. Cr-PI (10 µg) separated by SDS-PAGE and electrophoretically transblotted onto NC membrane. After incubation with NS-1 (strip a) or hybridoma culture supernatants (strips b, A-2; c, D-3; d, E-4; e, F-4; f, F-6; g, G-1; and h, H-1), the blots were incubated with anti-mouse IgG peroxidase conjugate and detected by adding 4-chloro-1-naphthol and H₂O₂.

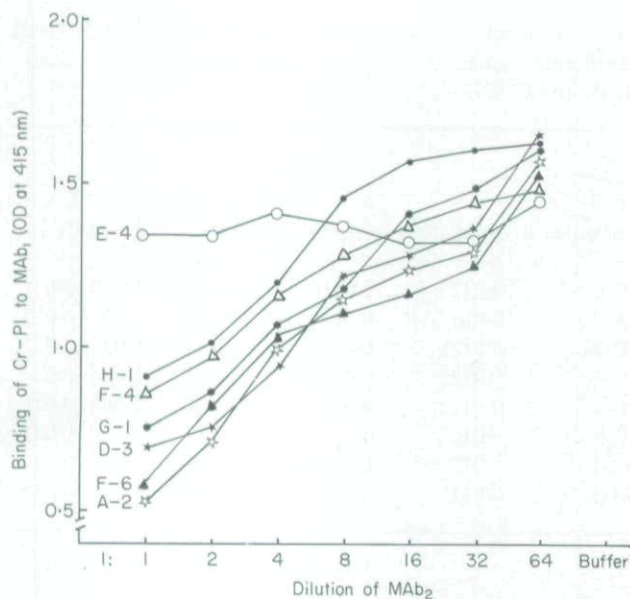


Fig. 2. Determination of the epitope specificities of monoclonal antibodies by ELISA. Microtitre plates were coated with purified mAb A-2. Cr-PI was pre-incubated with serial dilutions of purified mAbs. Pre-incubated mixtures were added and Cr-PI bound to microtitre plates was detected by ELISA as described.

microtitre plates were coated with purified IgG fraction of mAb A-2 (750 ng/well) and blocked with 2% BSA overnight at 4°C. After washing, 100 µl of test allergens were incubated for 2 hr at room temperature, and 100 µl of E-4 mAb-AP conjugate were added. After 2 hr incubation at room temperature, 100 µl of *p*-nitrophenyl phosphate solution (100 mg in 100 ml of diethanolamine buffer, pH 9.8, Sigma) were added and incubated for 30 min at room temperature. Absorbances were read at 405 nm on an EAR 400 AT Easy Reader.

Protein determination

Protein concentration was by Coomassie brilliant blue G-250 protein-dye binding method of Bradford [35] using BSA as a standard.

Results

Cell fusion between spleen cells of BALB/c mice immunized with Cr-PI and NS-1 cells resulted in a series of hybridomas specific for Cr-PI. These cell cultures were subcloned twice, and seven stable cell lines secreting mAbs against Cr-PI, as determined by ELISA with high values of absorbance (Table 1), were obtained and expanded *in vitro*. These mAbs designated as A-2, D-3, E-4, F-4, F-6, G-1 and H-8 were chosen for further studies. All seven mAbs were characterized as IgG1 sub-

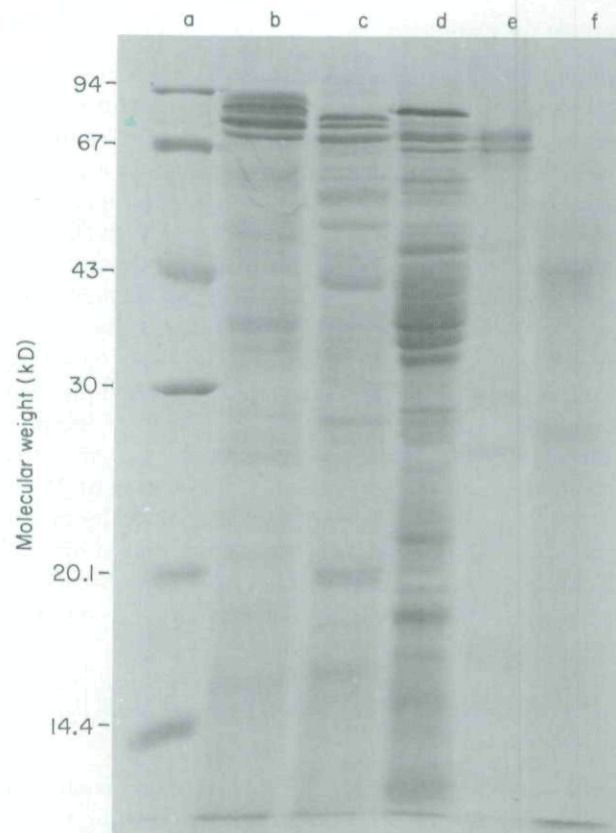


Fig. 3. SDS-PAGE analysis of crude *B. orientalis*, *B. germanica*, *P. americana* extracts, Cr-PI and Cr-P11. MWs were determined from a standard mixture of proteins of known MW; a, low markers: phosphorylase-b (94 kD), BSA (67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), soybean trypsin inhibitor (20.1 kD) and α -lactalbumin (14.4 kD); b, crude *B. orientalis* (40 µg); c, crude *B. germanica* (40 µg); d, crude *P. americana* (40 µg); e, Cr-PI (10 µg); and f, Cr-P11 (20 µg).

class by immunodiffusion. Immunoblot analyses were performed with Cr-PI to determine the specificity of these mAbs. Culture supernatant of NS-1 was included as negative control and showed no reactivity with any components of Cr-PI (strip a, Fig. 1). The immunoblotting results showed that all seven mAbs bound weakly to 78 kD and strongly to 72 kD proteins (Fig. 1) which have been identified as major allergens of American cockroach by immunoblotting with human IgE antibodies [11].

Epitope specificity of the mAbs to Cr-PI was analysed by an ELISA as described above. The results are presented in Fig. 2. The results in Fig. 2 show that A-2, D-3, F-4, F-6, G-1, and H-1 inhibited the binding of A-2, indicating that these antibodies recognize the same binding site. However, mAb E-4 did not inhibit Cr-PI binding and therefore indicates that A-2 and E-4 recognize the different epitope.

Fig. 4. Immunoblot analysis of crude *B. orientalis*, *B. germanica*, *P. americana* extracts, Cr-PI and Cr-P II. SDS-PAGE separated components as described in Fig. 3 were electrotransferred onto NC membranes and followed by immunodetection with rabbit anti-Cr-PI (strips a-e), purified mAb A-2 (strips f-j) and mAb E-4 (strips k-o). a, f and k, *B. orientalis*; b, g and l, *B. germanica*; c, h and m, *P. americana*; d, i and n, Cr-PI; e, j and o, Cr-P II.

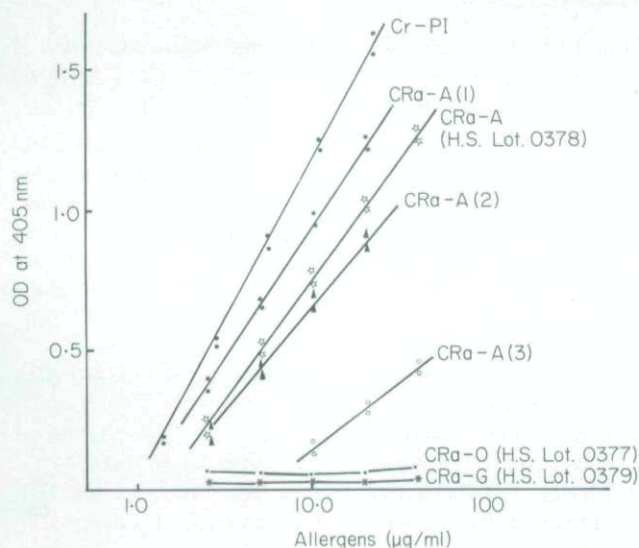
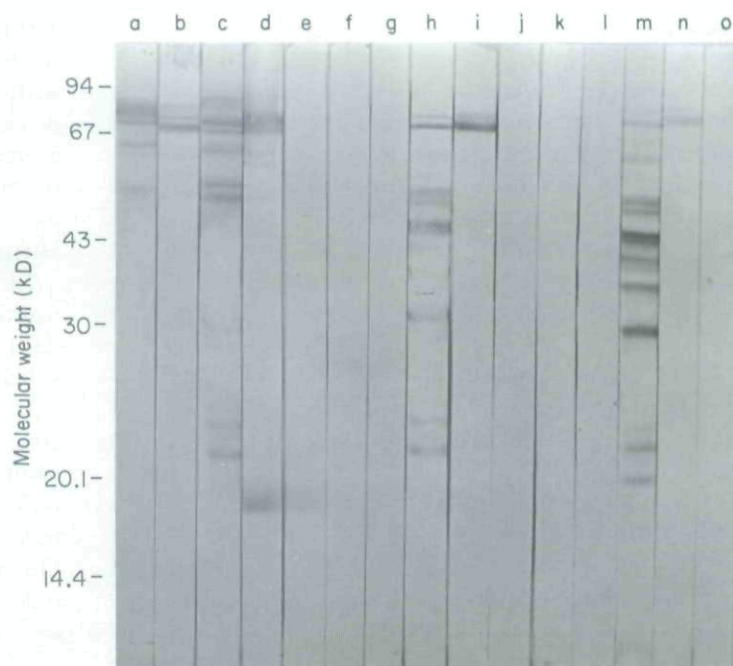


Fig. 5. Dose-response curves with different allergen preparations in mAb based Cr-PI ELISA.

The SDS-PAGE patterns of *P. americana*, *B. orientalis*, *B. germanica* extracts, Cr-PI and Cr-P II are demonstrated in Fig. 3. As A-2 and E-4 recognize the different epitope, as determined by ELISA, they were selected for the further studies. Purified mAbs (A-2 and E-4) or rabbit anti-Cr-PI were incubated with NC membrane onto which SDS-PAGE-separated crude cockroach extracts, Cr-PI and Cr-P II had been electrophoretically transferred. The results are shown in Fig. 4. Polyclonal rabbit

anti-Cr-PI bound more than six components of each of crude *B. orientalis*, *B. germanica*, *P. americana* and Cr-PI (Fig. 4, strips a-d) except Cr-P II (Fig. 4, strip e). Both mAbs, A-2 and E-4, bound only to 72 kD and 78 kD components of Cr-PI (Fig. 4, strips i and n). Both A-2 and E-4 reacted with components of 78, 72, 65, 62, 58, 52, 45, 42, 38, 35, 24 and 22 kD proteins of crude *P. americana* (Fig. 4, strips h and m). In addition, E-4 also bound 60, 49 and 26 kD of crude *P. americana* (Fig. 4, strip m). However, both mAbs, A-2 and E-4, did not react with any components of crude *B. orientalis*, *B. germanica* and Cr-P II (Fig. 4, strips f, g, j, k, l and o).

A mAb-based double-antibody sandwich ELISA was developed to detect the Cr-PI allergen in cockroach extracts. Allergen binding to mAb A-2 coupled to a microtitre plate was determined by E-4 mAb labelled with alkaline phosphatase in the ELISA system. The ELISA was dose-dependent and capable of detecting as little as 140 ng of Cr-PI allergen (Fig. 5). Crude *P. americana*, *B. orientalis*, *B. germanica* extracts from the same manufacturer and *P. americana* from different batches extracted in our laboratory were examined, and the results are shown in Fig. 5. Among the three homologous *P. americana* preparations, one reacted strongly with mAb while the remaining two reacted intermediately and weakly in the double-mAb sandwich ELISA. By using the same method, commercially prepared *P. americana* extract (H.S. Lot 0378) was found to react strongly with mAb but not with *B. orientalis* (H.S. Lot 0377) and *B. germanica* extracts (H.S. Lot 0379).

Discussion

Allergen-specific mAbs can be used to purify and identify allergenic components in the crude extracts. Relevant pure allergens can then be standardized for immunodiagnosis and be used in the analysis of allergenic structure and antigenic/allergenic determinants. In addition, mAb-based assays for specific allergens will play important roles in epidemiological studies of allergic asthma, assessing patients' exposure to allergens, and the effects of avoidance regimens.

In this study, the production and preliminary characterization of seven mAbs against major allergens of American cockroach is described. The success in the production of Cr-PI MAbs was based on the use of spleen cells from mice immunized with a partially purified Cr-PI preparation. It is interesting that all mAbs bound strongly to 72 kD but weakly to 78 kD of Cr-PI, as demonstrated by immunoblot analysis (Fig. 1), although the concentration of both proteins was similar, as estimated by the naked eye with Coomassie brilliant blue R 250 staining (Fig. 3, strip e). It is possible that both 72 and 78 kD allergens may share some of the common determinant groups.

Using an ELISA based on the consideration that there is inhibition of antigen binding to the mAb₁ by the mAb₂, A-2, D-3, F-4, F-6, G-1 and H-1 were found to have similar epitope specificities. Thus, it appears that all of these six monoclonal mAbs recognize identical antigenic structures or bind to overlapping structures. As E-4 did not inhibit the binding of Cr-PI to A-2 (Fig. 2) it is apparent that they have different epitope specificities.

Both species-specific and shared interspecies allergens in cockroaches have been demonstrated by skin test and RAST [12], RAST inhibition and immunoblot analysis using human and rabbit antibodies [36]. Polyclonal rabbit anti-Cr-PI reacted with many different components of *B. orientalis*, *B. germanica*, *P. americana*, and Cr-PI (Fig. 4, strips a, b, c and d). However, all seven mAbs failed to react with crude *B. orientalis*, *B. germanica* and Cr-PII as demonstrated by ELISA (Table 1) which was further supported by immunoblot analysis (Fig. 4, strips f, g, j, k, l and o) and mAb-based double-antibody sandwich ELISA (Fig. 5).

mAbs, A-2 and E-4, reacted with many principal allergens [11] of crude *P. americana* (Fig. 4, strips h and n). It appears that these principal allergens of American cockroach may share some of the common determinant groups. The high activities of major allergens (*Amb a I* and *Amb a II*) may be due to the possession of determinant groups common to other proteins, as postulated for ragweed pollen by King *et al.* [37]. Shen *et al.* [20] reported that most of the major proteins of crude short ragweed

extract were recognized by mAbs which reacted with both *Amb a I* and *Amb a II* allergens. Specific mAbs reacting with multiple components of crude ryegrass (*Lolium perenne*) extract [21] or crude rye pollen (*Secale cereale*) extract [38] have been reported. It seems reasonable to observe that the presence of a certain allergenic determinant in several proteins is a common feature in other allergenic extracts as well as in American cockroach.

In summary, hybridomas were produced after immunization with Cr-PI. Specific anti-Cr-PI activity was detected in ELISA and immunoblotting. Seven stable subclones were obtained, and all mAbs reacted strongly with 72 kD and weakly with 78 kD allergens of American cockroach. mAbs, A-2, D-3, F-4, F-6, G-1 and H-8 recognize similar epitopes, and E-4 was found to have different epitope specificities with others. None of the mAbs recognized any common epitopes of Oriental and German cockroaches. Production of additional relevant mAbs, purification, and further characterization are currently in progress.

Acknowledgments

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References

- 1 Marchand AM. Allergy to cockroach. Bol Assoc Med Puerto Rico 1966; 58:49-53.
- 2 Bernton HS, Brown H. Cockroach. II. The relation of infestation to sensitization. South Med J 1967; 60:852-5.
- 3 Twarog RJ, Picone FJ, Strunk RS, So J, Colten HR. Immediate hypersensitivity to cockroach. J Allergy Clin Immunol. 1977; 59:154-60.
- 4 Kang B, Sulit N. A comparative study of prevalence of skin hypersensitivity to cockroach and house dust antigens. Ann Allergy 1978; 41:333-6.
- 5 Lan JL, Lee DT, Wu CH, Chang CP, Yeh CL. Cockroach hypersensitivity: preliminary study of allergic cockroach asthma in Taiwan. J Allergy Clin Immunol 1988; 82:736-40.
- 6 Kang B, Vellody D, Homburger H, Yunginer JW. Cockroach cause of allergic asthma. Its specificity and immunologic profile. J Allergy Clin Immunol 1979; 63:80-6.
- 7 Bernton HS, McMahon TF, Brown H. Cockroach asthma. Br J Dis Chest 1972; 66:61-6.
- 8 Kang B. Study on cockroach antigens as probable causative agent in bronchial asthma. J Allergy Clin Immunol 1976; 58:357-65.

- 9 Schulaner FA. Sensitivity to the cockroach in three groups of allergic children. *Pediatrics* 1970; 45:465-6.
- 10 Mendoza J, Synder FD. Cockroach sensitivity in children with bronchial asthma. *Ann Allergy* 1970; 28:159-63.
- 11 Wu CH, Lan JL. Cockroach hypersensitivity: isolation and partial characterization of major allergens. *J Allergy Clin Immunol* 1988; 82:727-35.
- 12 Stankus RP, O'Neil CE. Antigenic/allergenic characterization of American and German cockroach extracts. *J Allergy Clin Immunol* 1988; 81:563-70.
- 13 Bear H, Godfrey, Maloney CJ, Norman PS, Lichtenstein LM. The potency and antigen E content of commercially prepared ragweed extracts. *J Allergy* 1970; 45:347-54.
- 14 Foucard T, Johanson SGO, Bennich H, Berg T. *In vitro* estimation of allergens by a radioimmune antiglobulin technique using human IgE antibodies. *Int Arch Allergy Appl Immunol* 1972; 43:360-70.
- 15 Aas K. Clinical and experimental aspects of standardization and purification of allergens. *Int Arch Allergy Appl Immunol* 1975; 49:44-54.
- 16 Lichtenstein LM. Standardization and efficacy of allergen extracts. *N Engl J Med* 1976; 295:1195-6.
- 17 Baldo BA, Uhlenbruck G. Allergen standardization using lectins. *Lancet* 1977; i:802.
- 18 Krilis S, Baldo BA, Basten A. Standardization of allergens: lectins and monoclonal antibodies as primary standards for house dust mite allergens. *Int Arch Allergy Appl Immunol* 1981; 66 (suppl 1):252-8.
- 19 Krilis S, Baldo BA, Raison RL, Callard RE, Basten A. Standardization of antigen E in ragweed pollen extracts using a monoclonal antibody based immunoassay. *J Allergy Clin Immunol* 1983; 71:261-5.
- 20 Chapman MD. Allergen specific monoclonal antibodies: new tools for the management of allergic disease. *Allergy* 1988; 43 (suppl 5):7-14.
- 21 Shen HD, Chang LY, Su SN, Han SH. Characteristics of five monoclonal antibodies to major allergens of the short ragweed pollen. *Int Arch Allergy Appl Immunol* 1988; 85:167-73.
- 22 Smart IR, Heddle J, Zqla H, Bradley J. Development of monoclonal mouse antibodies specific for allergenic components in rye grass (*Lolium perenne*) pollen. *Int Arch Allergy Appl Immunol* 1983; 72:243-8.
- 23 Kahn C, Marsh DG. Analysis of grass group I allergens using monoclonal antibodies. *J Allergy Clin Immunol* 1983; 71:95.
- 24 Theobald K, Bohn A, Thiel H, Rasche B, Ulmer WT, Konig W. Production of monoclonal antibodies against wheat flour components. *Int Arch Allergy Appl Immunol* 1983; 72:84-6.
- 25 Agarwal K, Helm RM, Hisamatsu K, Gleich GJ, Yuninger JW. Monoclonal antibody to ALT-1 and its use in affinity chromatography. *J Allergy Clin Immunol* 1983; 71:160.
- 26 Heymann PW, Chapman MD, Platts-Mills TAE. Antigen Der f1 from the dust mite *Dermatophagoides farinae*: structural comparison with Der p1 from *D. pteronyssinus* and epitope specificity of murine IgG and human IgE antibodies. *J Immunol* 1986; 137:2841-7.
- 27 Chapman MD, Sutherland WM, Platts-Mills TAE. Recognition of two *Dermatophagoides pteronyssinus*-specific epitopes on antigen PI by using monoclonal antibodies: binding to each epitope can be inhibited by sera from dust mite-allergic patients. *J Immunol* 1984; 133:2488-95.
- 28 Chapman MD, Heymann PW, Sutherland WM, Platts-Mills TAE. Recognition of species-specific and cross-reacting antigenic determinants on house dust mite (*Dermatophagoides*) allergens using monoclonal antibodies. *Int Arch Allergy Appl Immunol* 1985; 77:166-8.
- 29 Galfre G, Milstein C. Preparation of monoclonal antibodies: Strategies and procedures. *Meth Enzymol* 1981; 73:3-46.
- 30 Alsenz J, Loos M. A rapid and simple ELISA for the determination of duplicate monoclonal antibodies during epitope analysis of antigens and its application to the study of C1-INH. *J Immunol Methods* 1988; 75-84.
- 31 Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 227:680-5.
- 32 Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc Natl Acad Sci U.S.A.* 1979; 76:4350-4.
- 33 Ford SF, Tovey ER, Baldo BA. Identification of orchard grass (*Dactylis glomerata*) pollen allergens following electrophoretic transfer to nitrocellulose. *Int Arch Allergy Appl Immunol* 1985; 78:15-21.
- 34 Avrameas S, Ternynck T, Guesdon JL. Coupling of enzymes to antibodies and antigens. *Scand J Immunol* 1978; 8 (suppl 7):7-23.
- 35 Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72:248-54.
- 36 Helm RM, Bandele RD, Swanson MC, Campbell AR, Wynn SR. Identification of a German cockroach-specific allergen by human IgE and rabbit IgG. *J Allergy Clin Immunol* 1988; 87:230-8.
- 37 King TP, Norman PS, Lichtenstein LM. Studies on ragweed pollen allergens. *Ann Allergy* 1967; 25:541-53.
- 38 Westphal W, Becker WM, Schlaak M. Analysis of rye pollen (*Secale cereale*) allergens using patients' IgE, immunoprint, western blot and monoclonal antibodies. *Int Arch Allergy Appl Immunol* 1988; 86:69-75.

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