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Cockroach Allergen Detection and Cockroach Allergens of Allergic Thai Patients

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SUMMARY **Hybridomas secreting monoclonal antibodies (MAb) specific to American cockroach (*Periplaneta americana*) were produced through a fusion of immune splenocytes of a BALB/c mouse immunized with crude cockroach (CR) extract and mouse myeloma cells. Two hybridomas namely 38G6 and 3C2 were established. These specific hybridomas secreted IgG1 monoclonal immunoglobulins with antigenic specificities to CR protein components of over 207 to 72 kDa and 45 to 40 kDa, respectively. The monoclonal antibodies were applied to select their specific epitopes out of the crude CR extract using affinity chromatography. A Prausnitz-Kustner test revealed that these epitopes were allergens which caused wheals and flares of the skin of a guinea-pig previously sensitized with a pool of serum samples from CR allergic patients. The monoclonal antibodies were also used in a capture ELISA to detect specific IgE in serum samples of allergic Thai patients. It was found that 72% and 76% of the patients had IgE antibodies to the epitopes of MAb 38G6 and MAb 3C2, respectively, indicating that the two epitopes are major CR allergens among the CR allergic Thai patients. An antibody-sandwich ELISA was developed for quantitative detection of CR allergens using the two monoclonal antibodies as a capture reagent and rabbit polyclonal antibodies to crude CR extract as a detection reagent. The assay could detect allergenic epitopes contained in as little as 122 pg of crude cockroach extract, and has high potential for direct measurement of the marker allergens in extracts of environmental samples.**

As one of the countries located in a tropical and humid climate, Thailand serves as home for several genera and species of cockroaches (CR). Products of CR are regarded as one of the most important indoor allergens worldwide, second only to the house dust mite allergens.[1-3] There has been an increasing trend of CR allergy among the Thai population. Studies in 1997 revealed that CR allergy represented 44-61% of the overall allergy rate in allergic Thai patients.[3,4] Among the CR allergic patients, a significant proportion, especially young children, suffer frequent asthmatic attacks that need immediate hospitalization. Treatment, however, is mainly symptomatic. A decreased exposure to the CR allergens is the main practical guide to reduce the attack rates, as there is a strong quantitative correlation between the hypersensitivity and the degree of CR allergen exposure.[5-7]

In spite of the high incidence of the CR allergy in the population, basic information and research data about CR allergens are lacking in Thailand. Allergenic components of the CR have never been studied. Monitoring of a patient's allergic status is usually performed using crude CR extract with skin prick testing.[8-10] The allergenic quality of the extract is subject to great variations. Currently detection of CR allergens in the homes of patients, which is a major tool for determining the degree of a patient's

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exposure to the CR allergens and for choosing the right intervention, is done with imported diagnostic test kits. Not only are these test kits expensive, they are also not really effective for Thai patients, as there are differences in host genetics, environmental factors, allergen nature and many other attributes between Thai patients and those of other areas of the world. Therefore, local cockroach allergens needed to be studied in more detail.

In this article, we report the production of specific monoclonal antibodies (MAb) to allergenic components of the American cockroach (*Periplaneta americana*) and the use of these antibodies in a sandwich ELISA to measure CR allergens. Major CR allergens among allergic Thai patients were also studied.

MATERIALS AND METHODS

Serum samples

Serum samples were collected from 25 pediatric patients who visited the Pediatric Clinic, De-partment of Pediatrics, Siriraj Hospital, Bangkok, and whose skin prick test was positive against crude CR extract. Informed consents were obtained from their parents or their legal representatives before taking the blood samples.

Preparation of cockroach extract (CR extract)

Adult American cockroaches (*Periplaneta americana*) caught from houses in Bangkok were entomologically identified and kept at –70°C until use. The frozen CR were ground to fine pieces in liquid nitrogen, and 0.15 M phosphate buffered saline, pH 7.4, (PBS) containing protease inhibitors [10 mM ethylenediamine tetraacetic acid, 0.1 mM phenylethylsulphonyl fluoride, 1 μM epoxysuccinyl-leucylamino-(4-guanidino)-butane] was added to a ratio of 1:10 (w/v). The preparation was subjected to sonication at 20 kHz, 4°C, 3 minutes for three times and then centrifuged at 5,000 × *g* for 15 minutes. The supernatant was dialysed against distilled water at 4°C overnight. Dry weight and protein content of the preparation were determined.[11]

Preparation of heterologous antigens

Extracts were also prepared from other species of cockroaches, i.e. German cockroaches (*Blatella germanica*) and Harlequin cockroaches (*Neostylopyoga rhombifolia*), as well as from house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*). Additionally, whole cell homogenates were prepared from the bacteria listed in Table 1. A loopful of bacteria taken from an overnight trypticase soy agar plate was inoculated into 50 ml of LB broth and incubated at 37°C with shaking aeration overnight. The bacterial pellet was collected by centrifugation at 10,000 × *g*, 4°C, for 20 minutes, and resuspended in small volume of PBS. The preparation was sonicated at 20 kHz, 4°C, for 3 minutes, five times. The protein content of the preparation was then determined. The *Entamoeba histolytica* extract was a kind gift from Professor Dr. Wanchai Maleewong, Department of Parasitology, Faculty of Medicine, Khon Kaen University, Thailand.

Hybridoma production

Young adult BALB/c mice (6 to 7 weeks old at the start of the immunization) were kindly supplied by the Armed Forces Research Institute of Medical Sciences (AFRIMS), US Component, Bangkok, Thailand. Three mice were bled individually via the retro-orbital plexi, and the sera were collected, pooled and used as negative control. After bleeding, each mouse was intraperitoneally injected with 0.2 ml of a mixture containing 50 μg of the American CR extract and an equal volume of Freund's complete adjuvant. The mice were reimmunized five more times at 2 week intervals using the same route and the same immunogen, but composed of 100 μg of the CR extract in Freund's incomplete adjuvant. Fourteen days after the last booster dose each mouse was bled and their sera were assessed for antibody titers against the homologous antigen using an indirect ELISA. The immune mouse with the highest titer was used as a spleen cell donor for hybridoma production, while the others were bled and their sera were pooled and subsequently used as positive control serum (PS). Three days before the cell fusion, the selected immune mouse was given an intravenous injection of 50 μg of the immunogen in 0.2 ml of normal saline solution (NSS).

Three days after the intravenous booster, the immune mouse was bled and the serum was subsequently used as immune serum (IS). The animal was then sacrificed by cervical dislocation. Spleen cells were fused with P3x-63-Ag8.653 myeloma cells using polyethylene glycol 4,000 as fusogen at a spleen cell/myeloma cell ratio of about 10:1 for the production of hybridomas as previously described.[12,13] Culture fluids were collected and screened

for antibodies against the homologous antigen. Cells from the antibody positive-wells were subjected to cloning by the limiting dilution method using spleen cells of the non-immune BALB/c mouse as feeder cells. Culture fluids from these clones (hybridomas) were retested against the homologous antigen as well as against the heterologous antigens (Table 1) for cross-reactivity using an indirect ELISA. Antigenic specificities of the monoclonal antibodies (MAbs) secreted by the individual clones were determined by Western blotting (WB) against the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) separated-homologous antigen. The isotypes of the MAbs were determined using the Mouse Typer kit (Bio-Rad, USA).

Preparation of rabbit polyclonal antibodies (PAb) to American CR extract

A young adult New Zealand White rabbit, weighing about two kilograms at the beginning of the immunization, was injected subcutaneously five times at two week intervals with the American CR extract. The first dose consisted of 1 mg dry weight of the antigen dissolved in Freund's complete adjuvant (1:1 v/v), followed by four consecutive booster doses of 0.5 mg consisting of the same antigen in Freund's incomplete adjuvant. The rabbit was bled 14 days after the last immunization and the serum

Table 1 Cross-reactivity testing of the monoclonal antibodies produced by the selected specific monoclones (hybridomas)

Organism	Homologous and heterologous antigens	38G6	3C2	6G2
CR	*Periplaneta americana*	+	+	+
	Blatella germanica	-	-	+
	Neostylopyoga rhombifolia	-	-	+
House dust mites	*Dermatophagoides farinae*	-	-	-
	D. pteronyssinus	-	-	-
Bacteria	*Citrobacter diversus*	-	-	+
	Enterobacter aerogenes	-	-	-
	E. cloacae	-	-	+
	Escherichia coli O125			
	E. coli O186	-	-	-
	Klebsiella pneumoniae	-	-	+
	Proteus mirabilis	-	-	-
	P. vulgaris	-	-	+
	Pseudomonas aeruginosa	-	-	+
	Salmonella Typhi	-	-	+
	S. Typhimurium	-	-	-
	Shigella boydii 9	-	-	+
	S. dysenteriae 4	-	-	+
	S. flexneri 1a	-	-	-
	S. sonnei phase I	-	-	-
	Staphylococcus aureus	-	-	+
	S. epidermidis	-	-	-
	Streptococcus faecalis	-	-	-
	Yersinia enterocolitica	-	-	+
Protozoa	*Entamoeba histolytica*	-	-	-

antibody titer was assessed by an indirect ELISA.

ELISAs

An indirect ELISA was used for determining antibody titers in sera of immunized mice and an immunized rabbit as well as for detecting antibodies in the cell culture fluids during the screening process for positive hybrids. This technique was also used for determining specificity and cross-reactivity of the MAbs. The microtiter plates (Costar, USA) were coated with 100 µl per well of the appropriate antigen (20 µg of antigen per ml of carbonate-bicarbonate buffer, pH 9.6). The antigen-sensitized plates were incubated at 37°C overnight. The unbound antigens were washed off with PBS, pH 7.4, containing 0.5% Tween-20 (PBST). The unoccupied sites on the plates were blocked with 1% bovine serum albumin (BSA) in PBS (200 µl per well) and incubated at 37°C for one hour. The excess BSA was washed off, and 100 µl of antibody preparation (serially diluted individual mouse immune sera, serially diluted rabbit antiserum, negative control serum, PS, IS, diluted or undiluted culture fluids of hybrid cells) as well as fresh culture medium or culture fluid of myeloma cells, which served as a negative control or blank, were added to the appropriate wells. The antigen-antibody reaction was allowed to take place for one hour at 37°C. After washing thoroughly with PBST, 100 µl of a 1:1,000 dilution of rabbit anti-mouse Ig-horseradish peroxidase (Dakopatts, Glostrup, Denmark) or goat anti-rabbit Ig-horseradish peroxidase (Bio-Rad, USA) in PBS containing 0.2% BSA and 0.2% gelatin was added to each well and incubated as above for one hour. The unbound conjugate was removed by washing with PBST. The enzyme-substrate was added to all wells (100 µl per well). The reaction was allowed to take place in the dark for 30 minutes and was then stopped by adding 50 µl of 1N NaOH per well. The optical density of each well was measured at 492 nm using an ELISA reader (Multiscan *EX*; Labsystems, Helsinki, Finland). The ELISA titer of the antibody preparation was the highest dilution of the antibody that gave an optical density of ≥ 0.05. One indirect ELISA unit was defined as the smallest amount of antibody which gave a positive ELISA reaction.

A capture ELISA was used for detecting IgE to CR allergenic components in serum samples of CR allergic patients. The wells of the microtiter ELISA plates were coated with individual MAbs to CR allergens (40 µg protein per ml or 128 indirect ELISA units of MAb 38G6 and 64 indirect ELISA units of MAb 3C2; 100 µl per well). The plates were incubated at 37°C overnight. All wells were washed three times with PBST and blocked appropriately according to the indirect ELISA procedure. After blocking and washing away the excess blocking reagent, crude CR extract was added to all wells (2 µg/well), incubated at 37°C for 1 hour and washed. The serum samples of the individual patients were then added to the appropriate wells and re-incubated as above. After the incubation, unbound material was removed by washing three times; rabbit anti-human IgE–biotin conjugate (Zymed Laboratories, USA) diluted 1:1,000 was added to each well (100 µl per well). After 20 minutes at 37°C, the wells were washed and streptavidin-HRP (Dakopatts; diluted 1:2,000) was added, incubated and washed, followed by the addition of a substrate solution for 30 minutes. The reaction was stopped by adding 50 µl aliquots of 1 N NaOH to the individual wells. The optical density of the content in each well was determined at 490 nm against a blank (a well with no serum sample). A positive result (the presence of specific IgE to the CR allergic component) was defined as giving an OD of 0.05 or higher.

SDS-PAGE and Western blot analysis (WB)

SDS-PAGE was carried out in a vertical slab gel apparatus (Mini Protean cell, Bio-Rad, USA) according to the method of Laemmli.[14] A 4% stacking gel and 12% separating gel were used in the procedure. WB was performed by transblotting the SDS-PAGE separated-antigen from the gel to a nitrocellulose membrane (NC).[13,15] The empty sites on the NC were blocked by soaking the NC in a blocking buffer (3% BSA, 0.5% gelatin in PBS, pH 7.4) at room temperature with gentle rocking for 1 hour. After washing thoroughly, the NC was treated with an antibody preparation (at an appropriate dilution of IS or selected MAbs or undiluted culture fluids of the hybridomas) at room temperature for 1 hour. After washing as above, the membrane was put in a solution of rabbit anti-mouse Ig-horseradish peroxidase conjugate (Dakopatts) at a dilution of 1:1,000 in PBS, pH 7.4 containing 0.2% BSA, 0.2% gelatin for 30 minutes at room temperature. The NC was washed with 1/15 M phosphate buffer, pH 7.6, before being placed in a substrate solution for 5 minutes, washed with distilled water and air-dried.

Affinity purification of individual CR allergic components

Hybridomas (clones 38G6 and 3C2) were grown in a serum free-medium. The MAb in the culture fluids were concentrated using Amicon PM10 ultrafiltration (Amicon, USA). The MAb IgG were coupled to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions. The affinity gels were used as immunoadsorbances for recovering the respective epitopes from the crude CR extract. The crude CR extract was mixed with the MAb-coupled Sepharose gel and the mixture was rotated at 4°C overnight. The gel was washed several times with 0.01 M phosphate buffer, pH 7.4, by low speed (250 × *g*) centrifugation and then packed into a 1 × 10 cm column; the column was washed with the buffer until the effluent contained no detectable OD at 280 nm. The gel bound CR component was eluted out in 1 ml fractions with 0.1 M glycine-HCl, pH 3.0 and immediately neutralized by adding two drops of 1 M Tris-HCl, pH 8.5 to each ml of the eluate. Fractions with an OD at 280 nm were pooled, dialysed against distilled water and lyophilized. These purified CR epitopes of MAb 38G6 and MAb 3C2 were subsequently used in a Prausnitz-Kustner (P-K) test in order to determine their allergenicity.

Determination of allergenicity of the affinity purified-CR components

A Prausnitz-Kustner (P-K) test was used to determine the allergenicity of the affinity purified-CR components specific to MAb 38G6 and MAb 3C2. A pool of CR allergic patients' serum samples was used to sensitize the skin of a guinea-pig. The serum samples were obtained from a cohort of patients of the Pediatric Clinic, Siriraj Hospital, Bangkok. They contained specific IgE to crude CR extract as detected previously by ELISA. Fifty microliter aliquots of the serum pool were injected intradermally into the shaved skin of a guinea-pig. After 24 hours, the same spots were individually injected with the affinity purified-CR components specific to the MAb 38G6 and MAb 3C2. PBS with a pH of 7.4 was used as a negative control. Hypersensitive reactions, i.e. wheals and flares, were observed 15 minutes after the injection of the CR components, but not at the negative control.

Cockroach allergen detection

A sandwich ELISA was developed for the detection of CR allergens. One hundred microliter aliquots of a mixture of MAb 38G6 and MAb 3C2 (containing 40 μg protein per ml carbonate-bicarbonate buffer, pH 9.6, and 128 indirect ELISA units [EU] of MAb 38G6 and 64 EU of MAb 3C2) were added to the wells of a microtiter ELISA plate (Costar, USA). The plate was incubated at 37°C until dry. The wells were washed three times with PBST and blocked and washed as for the indirect ELISA. Then serial falling concentrations of crude CR extract in 100 μl PBS, pH 7.4, were added to the appropriate wells. The plates were incubated at 37°C for 1 hour, washed thoroughly with PBST, after which rabbit PAb to CR extract was added as a detection reagent (32 EU per ml), incubated as above and washed. Subsequently 1:2,000 goat anti-rabbit Ig-horseradish peroxidase (Bio-Rad, USA) was added to all wells (100 μl per well). After incubation at 37°C for 1 hour and extensive washing with

Table 2 List of the established hybridomas

Parental clone	Established hybridomas
CR38G6	✓CR38G6E7*
	CR38G6F7
	CR38G6G5
	CR38G6F10
	CR38G6G10
	CR38G6G9
	CR38G6F5
	CR38G6F2
CR3C2	✓CR3C2G6*
	CR3C2D7
CR6G2	CR6G2F11*
	CR6G2F12
	CR6G2C8
	CR6G2E7

✓ Hybridomas which their secreted MAbs were further used.
*Renamed as 38G6, 3C2 and 6G2, respectively.

PBST, freshly prepared peroxidase substrate was added for color development. The reaction was stopped after a 30 minute incubation by adding 50 µl of 4 N H_2SO_4 to each well. The OD of the content of each well was measured at 492 nm with an ELISA reader (Multiscan *EX*; Labsystems, Helsinki, Finland) against a blank (PBS, pH 7.4, without CR extract). The lowest limit of the allergen detection assay was the amount of CR extract which gave an OD ≥ 0.05.

RESULTS

A total of 2.82×10^8 spleen cells were obtained from a selected immunized mouse which had an indirect ELISA titer against the crude CR extract of 1:25,600; the cells were mixed with about 2.8×10^7 myeloma cells in the cell fusion procedure. The culture fluids from 496 wells which revealed growing cells were tested for antibodies against the homologous antigens, and the fluids of 90 wells (19.39 %) were positive. The positive culture fluids were then subjected to WB analysis against the SDS-PAGE separated-CR extract. Cells from 7 culture wells of which the culture fluids showed different patterns in the WB analysis were subsequently cloned by the limiting dilution method and 14 hybridomas were established (Table 2). The monoclonal antibodies (MAb) secreted by these clones gave three different patterns in WB analysis against the SDS-PAGE separated-crude CR extract, i.e. MAb of the pattern 1 revealed bands at > 207 to 72 kDa, MAb of pattern 2 reacted with the antigens located between 88 to 40 kDa while MAb of pattern 3 were reactive to components from 45 to 40 kDa (Fig. 1). The MAb showing patterns 1 and 3 did not cross-react to any of the heterologous antigens when tested by the indirect ELISA; however, MAb of pattern 2 reacted not only to the homologous antigen but also to several heterologous antigens listed in Table 1 which included *B. germanica*, *N. rhombifolia*, *Citrobacter diversus*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella* Typhi, *Shigella boydii*, *S. dysenteriae*, *Staphylococcus aureus* and *Yersinia enterocolitica*. Clones 38G6B7 and 3C2G6 (renamed 38G6 and 3C2, respectively) were selected as representatives of the hybridomas secreting MAb of patterns 1 and 3, respectively. Both clones secreted IgG1-kappa immunoglobulins. At the stationary phase of their *in vitro* growth, their culture fluids gave indirect ELISA titers of 1:128 and 1:64, respectively.

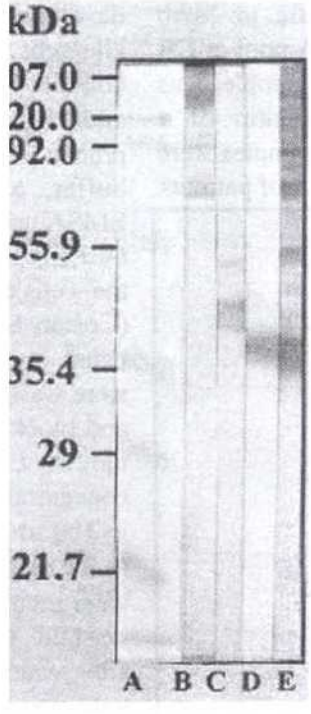

Fig. 1 Western blot analysis (WB) patterns of monoclonal antibodies in spent culture media of hybridomas 38G6 (lane B), 6G2 (lane C), 3C2 (lane D) and mouse immune serum (IS) (lane E) against the SDS-PAGE-separated American cockroach antigens Lanes A, Molecular mass markers; numbers at the left are molecular masses in kDa.

The two hybridomas were separately grown in a serum free-culture medium. The secreted MAb were coupled to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) and these affinity resins were used to recover the respective epitopes of the MAb from the crude CR extract. The recovered components when used in the P-K test elicited wheals and flares, i.e. positive P-K test, in the skin of a guinea-pig sensitized with human IgE from serum samples of CR aller-

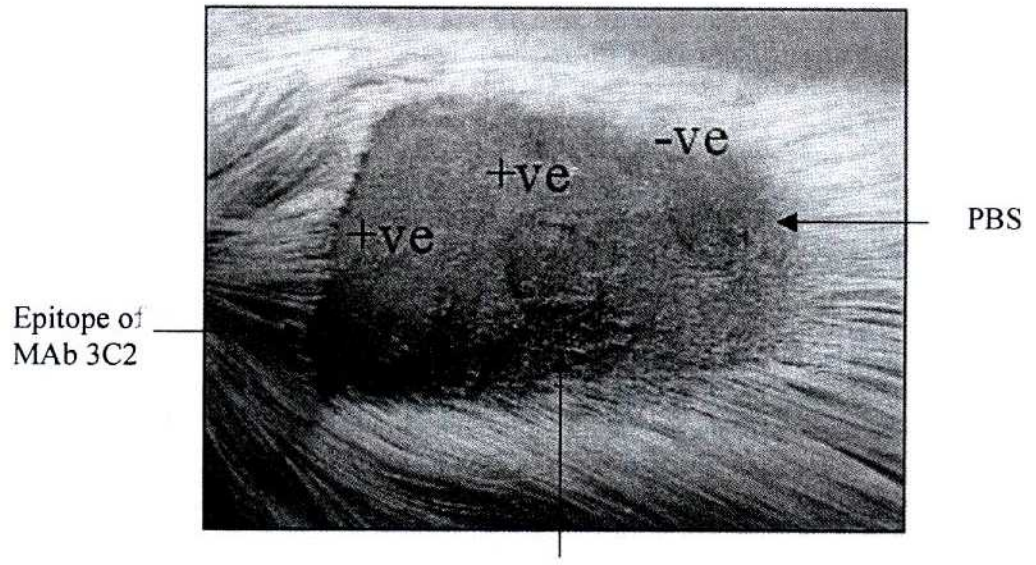

Fig. 2 Results of P-K test of affinity purified CR components which were specific epitopes of MAb 38G6 and MAb 3C2 and PBS (negative control) on skin of a guinea pig previously injected with a pool of sera of CR allergic Thai patients.

gic patients (Fig. 2), indicating that the MAb specific epitopes are CR allergic components of the Thai patients. SDS-PAGE separated-epitopes stained by Coomassie Brilliant Blue stain revealed components from > 207 to 72 and 45 to 40 kDa (data not shown) which conformed with the results of the WB analysis of Fig. 1 demonstrating that the MAb 38G6 and MAb 3C2 reacted to these components of the crude CR extract.

A sandwich ELISA using a mixture of MAb 38G6 and MAb 3C2 as a capture reagent and the PAb IgG anti-CR extract as a detection reagent revealed that the test could detect the epitopes contained in as little as 122 pg of crude American CR extract.

The MAb 38G6 and MAb 3C2 were deliberately used in a capture ELISA to detect IgE in serum samples of 25 pediatric patients who were skin prick test positive to crude CR extract. It was found that 18 of 25 patients (72%) and 19 of 25 patients (76%) gave positive capture ELISA results, implying that the serum samples tested contained IgE to the specific epitopes of MAb 38G6 and MAb 3C2, respectively.

DISCUSSION

Allergic reactions to cockroaches (CR) were first described by Bernton and Brown in 1964. Thereafter, CR has been known and accepted worldwide as one of the significant indoor aeroallergens.[10,16,17] There has been a long list of reports on a direct link of CR allergen exposure and the main clinical manifestation, i.e. asthma, among sensitized individuals.[18] Asthmatic subjects with CR allergy had a longer duration of asthma and showed a higher proportion of steroid dependency, suggesting more severe disease, compared to patients allergic to ragweed.[19] Avoidance measures including CR elimination and environmental control can be effective tools in the management of asthmatic patients.[18,20,21]

Despite high incidences of CR hypersensitivity among the Thai population,[4] systematic identification and characterization of the CR allergic components causing allergic

problems among the Thais were never carried out. Studies elsewhere have indicated that both bodies and feces of American and German cockroaches are important sensitizing agents in atopic asthma.[22] Major CR allergenic components varied geographically, demographically and terminologically. Allergenic components have been reported to range in molecular weights from 6 to 200 kDa.[23-26]

In this study, two hybridomas secreting specific monoclonal antibodies to American cockroaches, namely clones 38G6 and 3C2, were produced. The MAb secreted by the two clones reacted to components located at >207 to 72 kDa and at 45 to 40 kDa in the SDS-PAGE separated- crude extract of the American CR. The two epitopes obtained from MAb-based affinity column chromatography of the American CR crude extract were reactive to 72% and 76% of serum samples of the Thai CR allergic patients, indicating that they are major allergens among the CR allergic Thai patients. This finding is the first report on allergenic cockroach components causing allergic problem among Thais.

Significant allergens of American cockroaches ranging from 6 to 120 kDa have been identified elsewhere by various immunological methods in different laboratories.[23-27] Our findings that the allergenic components >207 to 72 kDa and 45 to 40 kDa were the major allergens among the Thai patients conformed with those of earlier reports from other countries. For example, Wu *et al.*[27] fractionated crude extract of the American CR by Sephadex G-150 and found that 73.0% and 93% of the allergic Taiwanese patients, who were skin prick test positive to the crude American CR extract, were reactive to components with 78 and 72 kDa (named CR-PI fraction) and 12 to 45 kDa (named CR-PII fraction), respectively. Schou *et al.*[26] proposed a name Per a I for the major American CR allergenic fraction eluted from Sephadex G-75 with a molecular size of 37 to 33 kDa which bound IgE of 100% of American patients whose skin prick test was positive to CR antigen. The Per a I had a size of 28 kDa in a high-performance liquid chromatography gel filtration and in a SDS-PAGE it revealed a minor band at 25 kDa with most of the protein located at 6 kDa. Helm *et al.*[28] reported a protein band of 36 kDa and several bands below 33 kDa of an SDS-PAGE separated- extract of American CR that bound IgE of CR allergic American patients. Obviously, there exists a considerable variation regarding the characteristics of American CR allergic components reported from various laboratories. The discrepancies may be due to differences in the methods of antigen extraction and characterization or to the degree of protein denaturation, degradation, polymerization or aggregation as has been suggested by Schou *et al.*[26]

The monoclonal antibodies produced in this study when used in a sandwich ELISA could detect the allergenic components in as little as 122 pg of the CR extract. The detection test so-developed, therefore, has a high potential in quantifying the CR allergens in homes of allergic patients and environmental dust samples. The purified epitopes of the two MAbs may serve as standardized marker allergens for the detection and quantification of IgE in serum samples of allergic patients.

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REFERENCES

1. Bernton HS, Brown H. Cockroach II: The relation of infestation to sensitization. South Med J 1967; 60: 852-5.
2. Helm RM, Cockrell G, Stanley JS, Brenner R, Burks AW, Bannon GA. A major allergen involved in IgE mediated cockroach hypersensitivity is a 90 kD protein with multiple IgE binding domains. Adv Exp Med Biol 1996; 409: 267-8.
3. Kongpanichkul A, Vichyanond P, Tuchinda M. Allergen skin test reactivities among asthmatic Thai children. J Med Assoc Thai 1997 Feb; 80 (2): 69-75.
4. Pumhirun P, Towiwat P, Mahakit P. Aeroallergen sensitivity of Thai patients with allergic rhinitis. Asian Pacific J Allerg Immunol 1997; 15: 183-5.
5. Gelber LE, Seltzer L, Bouzoukis JK, Pollart SM, Chapman MD, Platts-Mills TAE. Sensitization and exposure to indoor allergens (dust mite, cat and cockroach) as risk factors for asthma among patients presenting to hospital. Am Rev Respir Dis 1993; 147: 573-8.
6. Arruda LK, Vailes LD, Ferriani VP, Santos AB, Pomes A, Chapman MD. Cockroach allergens and asthma. J Allergy Clin Immunol 2001; 107: 419-28.
7. Litonjua AA, Carey VJ, Burge HA, Weiss ST, Gold DR. Exposure to cockroach allergen in the home is associated with incident doctor-diagnosed asthma and recurrent wheezing. J Allergy Clin Immunol 2001; 107: 41-7.
8. Bernton HS, Brown H. Insect allergy-preliminary studies of the cockroach. J Allergy 1964; 35: 506-13.
9. Kang B. Study on cockroach antigen as a probably causative agent in bronchial asthma. J Allergy Clin Immunol 1976; 58: 357-65.

10. Kang B, Sulit N. A comparative study of skin hypersensitivity to cockroach and house dust antigens. Am Allergy 1978; 41:333-6.
11. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principal of protein-dye binding. Anal Biochem 1976; 72: 248-54.
12. Galfre G, Howe SC, Milstein C, Butcher GW and Howard JC. Antibodies to major histocompatibility antigens produced by hybrid cell lines. Nature 1977; 266: 550-2.
13. Chaicumpa W, Srimanote P, Sakolvaree Y *et al.* Rapid diagnosis of cholera caused by *Vibrio cholerae* O:139. J Clin Microbiol 1998; 36: 3595-600.
14. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680-5.
15. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedures and some applications. Proc Natl Acad Sci 1979; 76:4350-4.
16. Pollart SM, Smith TF, Morris EC, Gelber LE, Platts-Mills TAE, Chapman MD. Environmental exposure to cockroach allergens: analysis with monoclonal antibody-based enzyme immmunoassays. J Allergy Clin Immunol 1991; 87: 505-10.
17. Platts-Mills TAE, Wheatley LM, Aalberse RC. Indoor versus outdoor allergens in allergic respiratory disease. Curr Opin Immunol 1998; 10(6): 634-9.
18. Barnes C, Tuck J, Simon S, Pacheco F, Hu F, Portnoy J. Allergenic materials in the house dust of allergy clinic patients. Ann Allergy Asthma Immunol 2001; 86: 517-23.
19. Kang B, Wu CH, Johnson J. Characteristics and diagnoses of cockroach-sensitive bronchial asthma. Ann Allergy. 1992; 68(3): 237-44.
20. Platts-Mills TAE. How environment affects patients with allergic disease: indoor allergens and asthma. Ann Allergy. 1994; 72(4): 381-4.
21. Reed C, Swanson M, Yunginger J. Measurement of allergen concentration in the air as an aid in controlling exposure to aeroallergens. J Allergy Clin Immunol 1986; 78: 1028-1030.
22. Lehrer SHW, Menon P, Stankus RP. Comparison of cockroach allergenic activity in whole body and fecal extracts. J Allergy Clin Immunol 1991; 87: 505-10.
23. Twarog RJ, Picone FJ, Strunk RS *et al.* Immediate hypersensitivity to cockroach. J Allergy Clin Immunol 1977; 59: 154-60.
24. Wu CH, Lan JL. Cockroach hypersensitivity: isolation and partial characterization of major allergens. J Allergy Clin Immunol 1988; 82: 727-35.
25. Stankus RP, O'Neill CE. Antigenic/allergenic characterization of American and German cockroach extracts. J Allergy Clin Immunol 1988; 81: 563-70.
26. Schou C, Lind P, Fernandez-Caldas E, Lockey RF, Lowenstein H. Identification and purification of an important cross-reactive allergen from American *(Periplaneta americana)*, German *(Blatella germanica)* cockroaches. J Allergy Clin Immunol 1990; 86: 935-46.
27. Wu CH, Hsieh MJ, Huang JH, Luo SF. Identification of low molecular weight allergens of American cockroach and production of monoclonal antibodies. Ann Allergy Asthma Immunol 1996; 76: 195-203.
28. Helm RM, Burks AW, Williams LW, Milne DE, Brenner RJ. Identification of cockroach aeroallergens from living cultures of German or American cockroaches. Int Arch Allergy Immunol 1993; 101: 359-63