

SENSITIZATION TO COCKROACH ALLERGENS OF ASTHMA PATIENTS IN JAPAN

Masahiro Sakaguchi¹⁾, Sakae Inouye¹⁾, Hiroshi Miyazawa²⁾, Toshinari Okabe³⁾, Hiroshi Yasueda⁴⁾,
Atsuhiko Muto⁵⁾, Ikuo Tanaka⁵⁾, Toru Akasaka⁶⁾, Chii-Huei Wu⁷⁾,
Martin D. Chapman⁸⁾ and Carsten Schou⁹⁾

¹⁾Department of Epidemiology, National Institute of Health, Shinjuku-ku, Tokyo, ²⁾Department of Medical Technology, Kyorin University, Hachioji, Tokyo, ³⁾Department of Pediatrics, Nagayama Hospital, Nippon Medical School, Tama, Tokyo, ⁴⁾Clinical Resesarch Center for Allergy and Rheumatology, National Sagamihara Hospital, Sagamihara, Kanagawa, ⁵⁾Department of Environmental Biology, Japan Environmental Sanitation Center, Kawasaki, Kanagawa, ⁶⁾Department of Clinical Research Center, Morioka National Hospital, Morioka, Iwate, ⁷⁾Department of Medical Research, Taichung Veterans General Hospital, Taichung, Republic of China, ⁸⁾Division of Allergy and Clinical Immunology, University of Virginia, Charlottesville, Virginia, ⁹⁾ALK Research, Hørsholm, Denmark

To evaluate the role of allergens from *Periplaneta fuliginosa*, which is the most predominant cockroach species in homes in Tokyo areas, for asthma sensitization, we measured specific IgE antibodies to two cockroaches, *P. fuliginosa* and *Blattella germanica*, and to a mite, *Dermatophagoides farinae*, in 171 sera from children with asthma by Pharmacia's CAP system. We found that 16% of the sera had anti-*P. fuliginosa* IgE, whereas 9.9% had anti-*B. germanica* and 85% anti-*D. farinae* IgE. Further, we measured the levels of *Per f* I (*Per a* I equivalent) allergen in the house dust from living room, kitchen and bedding. We detected the allergen in eight of ten homes. The *Per a* I equivalent levels in kitchen were higher than in other sites, but they were much lower than *Der* I and *Der* II as *Dermatophagoides* allergens.

Key words: Allergen — cockroach — IgE — mite

INTRODUCTION

It has been established that cockroach is an important source of indoor allergens in the USA¹⁾²⁾, as well as in other parts of the world^{3)–6)}. In the USA, the most common cockroaches are *Blattella germanica* and *Periplaneta americana*²⁾. Purification of two major allergens from *B. germanica*, *Bla g* I⁷⁾ and *Bla g* II⁸⁾ was reported. Also two major allergens, *Per a* I⁷⁾ and Cr-PI⁹⁾ were isolated from *P. americana*.

In Tokyo area, *Periplaneta fuliginosa* is the most common cockroach in homes, with *Blattella germanica* being a minor cockroach¹⁰⁾. However, there have been few studies on cockroach

allergies. In 1973, Nemoto et al. first reported a case of cockroach asthma¹¹⁾. Kawakami et al. reported that, in their preliminary study, several asthmatic patients had IgE antibodies to *B. germanica*¹²⁾. However, there has been no report about the prevalence of IgE antibody to *P. fuliginosa*, that is a major cockroach in Japan. Furthermore, there is no report about the level of cockroach allergens in the dust of Japanese homes.

In this study, to investigate the prevalence of sensitivity to cockroach, we measured specific IgE antibodies to *P. fuliginosa* among children with asthma. To investigate the allergen burden of cockroaches in homes of Japan, the levels of cockroach allergens were measured in dust from living room, kitchen and bedding, and compared with those of mite allergens.

Received: July 7, 1994

Accepted: October 13, 1994

Abbreviations: BSA bovine serum albumin; ELISA enzyme-linked immunosorbent assay; PBS phosphate-buffered saline; T Tween 20; SD standard deviation

MATERIALS AND METHODS

Patients' sera

Sera were obtained from 171 children with asthma at Department of Pediatrics, Saitama Medical School, which is located in the suburbs of Tokyo. The average of their age was 7.4 ± 3.9 [mean $\pm$ standard deviation (SD)] years, with a range of 1 to 15 years.

Specific IgE to allergens

Specific IgE antibodies to *P. fuliginosa*, *B. germanica* and *Dermatophagoides farinae* in their sera were measured by the CAP system (Pharmacia). Results were expressed as class score (0–6) with score >2 as antibody negative and the cutoff (positive-negative) point is class score 2^{13} .

Extracts of cockroaches

The cockroaches were chopped in a hand-held onion chopper before extraction. Ten percent (wt/vol) extracts were made in borate-buffered saline for 20 hr at 4°C. The extracts were centrifuged for 60 min at 4°C at $27,000 \times g$. The supernatants were filtrated with 0.45 μ m filter unit (Millex-HV, Millipore).

Allergens from floor and bedding dust

Dust samples were obtained from ten families around Hachioji in the suburbs of Tokyo. Nine of ten families had been living in wooden houses and another in a concrete-built apartment. The average of their age was 14.1 ± 5.4 (mean $\pm$ SD) years. Dust was collected from a 4 m² area each of floor and the surface of Japanese bedding (futon) with a vacuum cleaner (HA-100, Panasonic) for 5 min. The collected dust was mixed and shaken with glass beads, then sieved through a 0.3 mm mesh screen to obtain fine dust, which was then mixed with 1,000 times as much weight of phosphate-buffered saline (PBS) containing 0.05% Tween 20 (T) for 2 hr with constant rotation¹⁴⁾¹⁵⁾.

Allergen specific antibodies

Preparation of rabbit antisera to *Per a*¹⁷⁾, *Der p* I, *Der f* I and *Der II*¹⁶⁾, mouse monoclonal antibodies to Cr-PI¹⁷⁾ have previously been described.

Quantitation of Allergens

Cockroach (*Per a* I) and mite (*Der p* I, *Der f* I and *Der II*) allergens were quantified by a fluorometric sandwich ELISA¹⁸⁾¹⁹⁾ with slight modifications. A microplate (Microfluor W plate, Dynatech Laboratories) was coated with 1 μ g/ml of either rabbit anti-*Per a* I, *Der f* I, *Der p* I, or *Der II* IgG for 3 hr at 37°C. The plate was postcoated with 1% bovine serum albumin (BSA)-PBS for 1 hr at 37°C. After washing, several concentrations of standard allergens or diluted samples were added to the wells and the plate was incubated overnight at 4°C. The plate was washed, and then 0.2 μ g of biotinylated corresponding IgG antibodies as detector antibodies were added. The plate was incubated for 1 hr at room temperature. After washing, β -D-galactosidase conjugated streptavidin (Zymed Laboratories) was added. The plate was incubated for 1 hr at room temperature. After final washing, 0.1 mM 4-methylumbelliferyl- β -D-galactoside (Sigma) was added. The plate was incubated for 2 hr at 37°C. The enzyme reaction was stopped and the fluorescence intensity was read as fluorescence units on a fluorometric microplate reader.

A cockroach allergen (*Bla g* II) was quantified by a sandwich ELISA⁸⁾²⁰⁾ in the same way as above except that monoclonal anti-*Bla g* II IgG, polyclonal rabbit anti-cockroach antiserum and β -D-galactosidase conjugated anti-rabbit IgG (Zymed Laboratories) were used.

Reaction of monoclonal anti-Cr-PI antibodies with P. americana and P. fuliginosa extracts

A microplate (Immulon 2, Dynatech Laboratories) was coated with the crude extract of *P. americana* and *P. fuliginosa* (diluted 1/2,000) overnight at 4°C. The plate was postcoated with 1% BSA-PBS for 1 hr at 37°C. After washing, 1

$\mu\text{g/ml}$ each of two monoclonal anti-Cr-PI IgG was added to the wells and the plate was incubated for 1 hr at room temperature. The plate was washed, then $0.25\mu\text{g/ml}$ of biotinylated anti-mouse IgG (Zymed Laboratories) were added. The plate was incubated for 1 hr at room temperature. The following procedures were the same as the fluorometric sandwich ELISA method mentioned above.

RESULTS

Prevalence of specific IgE antibodies to cockroaches among children with asthma

Cockroach-specific IgE antibodies in 171 patients with asthma were measured by the CAP system (Table 1). Of 171 patients, 27 (16%) had anti-*P. fuliginosa* IgE and 17 (9.9%) anti-*B. germanica* IgE antibodies. Of the 171 patients with asthma, 145 (85%) had specific IgE antibodies to *D. farinae*. Mean positive CAP score (5.0) to *D. farinae* was higher than that (2.1) to *P. fuliginosa*.

Determination of Per f I allergen levels in house dust

Since we do not have an assay system for *P. fuliginosa* allergens, we used a *Per a* I assay method. First, cross-reactivity between *Per a* I and *Per f* I was investigated; the levels of *Per a* I in the extract of *P. americana* and *P. fuliginosa* were measured by the sandwich ELISA with rabbit antibodies against *Per a* I. The two extracts showed exactly identical dose-response curves in the *Per a* I assay (Fig. 1). Further, the reaction curves were parallel to the standard curve of *Per a* I. Therefore, we thought that *Per f* I levels in the dust can be expressed by *Per a* I levels.

Reaction of monoclonal anti-Cr-PI antibodies with crude extracts of P. americana and P. fuliginosa

We investigated whether an equivalent protein of Cr-PI (the second major allergen of *P.*

Table 1 Positive rate of anti-cockroach and mite IgE antibodies in 171 Japanese children with asthma measured by the CAP system

Allergen	Positive %	CAP score*
<i>Periplaneta fuliginosa</i>	16.0 (27/271)	2.1 ± 0.27
<i>Blattella germanica</i>	9.9 (17/171)	2.2 ± 0.48
<i>Dermatophagoides farinae</i>	85.0 (145/171)	5.0 ± 0.90

*Each value is the mean $\pm$ SD.

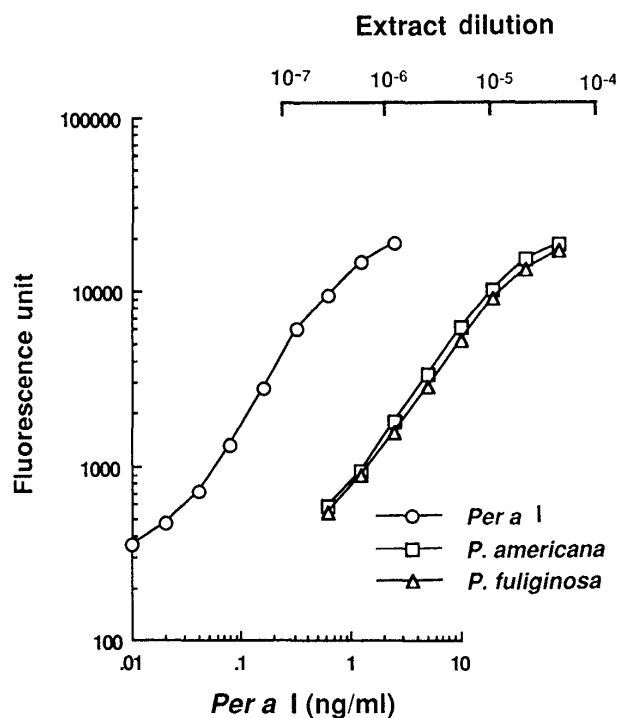


Fig. 1. Comparison of *Per a* I standard curve with the dilution curves of *P. americana* and *P. fuliginosa*.

americana) can be detected in extract of *P. fuliginosa* by use of two monoclonal antibodies to Cr-PI. We found that the two antibodies (A-2 and E-4) had strong reaction to *P. americana* but weak reaction to *P. fuliginosa* (Fig. 2). Thus, the Cr-PI equivalent allergen in extract of *P. fuliginosa* could not be measured by these monoclonal antibodies.

Cockroach allergen levels in April and September

The levels of *Per a* I equivalent (*Per a* I eq) in the floor dust of kitchen in seven homes were measured in April and September (Fig. 3). The

Sensitization to Cockroach Allergens

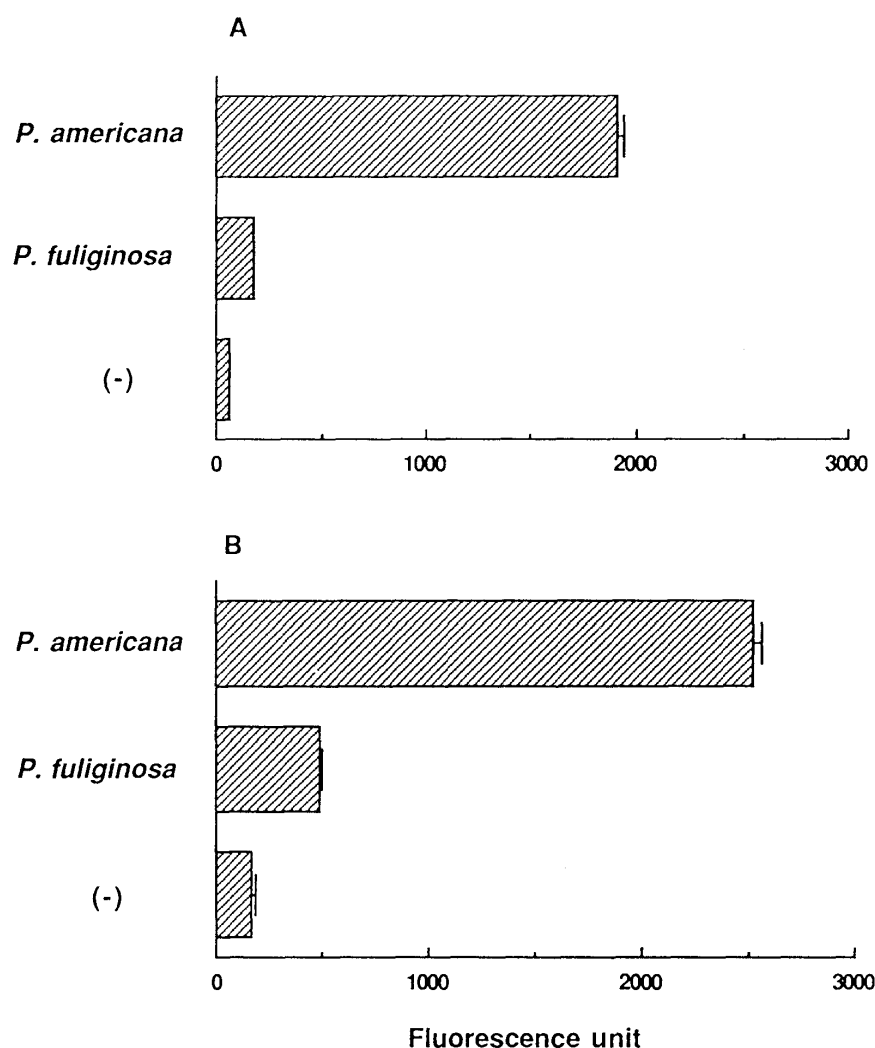


Fig. 2. Reactivity of anti-Cr-PI monoclonal antibodies to *P. americana* and *P. fuliginosa*. A: monoclonal antibody (A-2), B: monoclonal antibody (E-4).

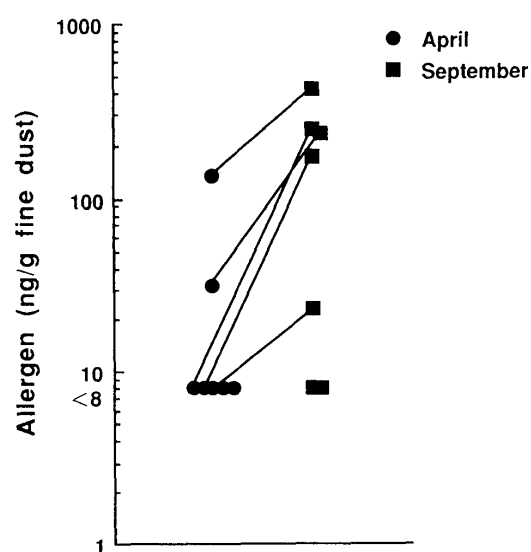


Fig. 3. Seasonal change of the level of *Per a* I eq in dust from kitchen.

allergen in the floor dust was detected in five of seven homes in September but in only two of seven homes in April. The geometric mean (67.2 ng/g fine dust) of *Per a* I eq in September is higher than that (14.6 ng/g fine dust) in April. There is a significant seasonal change in the level of *Per a* I eq ($p > 0.05$).

Comparison of allergen levels in dust of homes

To evaluate the distribution of cockroach allergens in homes, the levels of *Per a* I eq were measured in dust from living room, kitchen and bedding in September (Fig. 4). *Per a* I eq allergen in dust of kitchen was detected in eight of ten homes (geometric means values, 88.6 ng/g fine dust) (Fig. 4A). The level of *Per a* I eq in

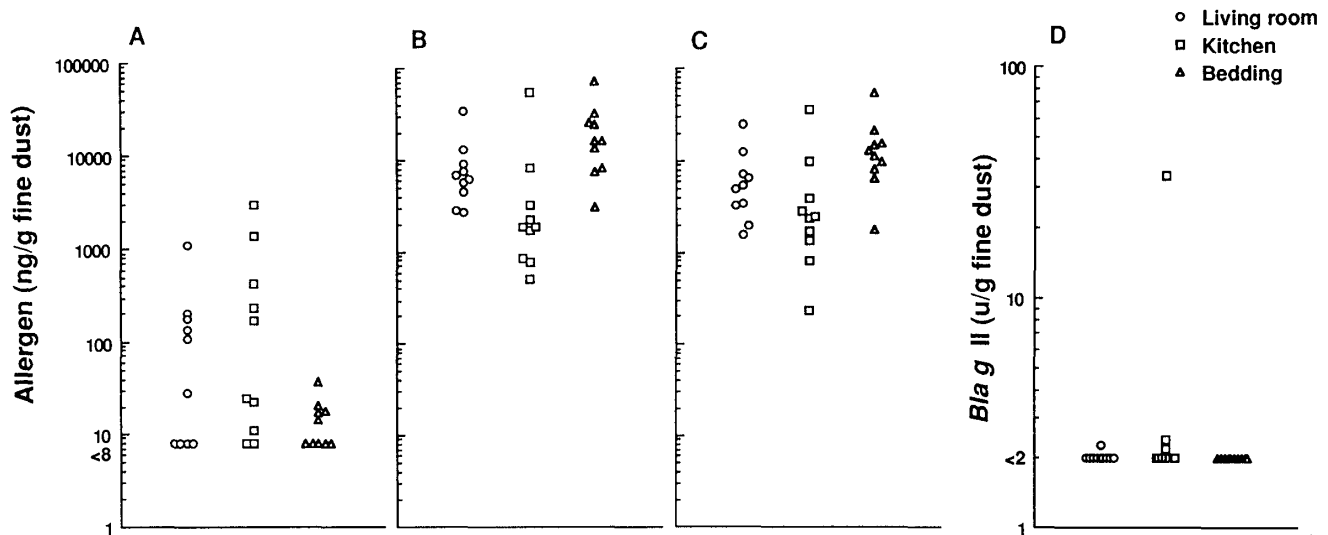


Fig. 4. Allergen levels in dust from living room, kitchen and bedding.
A: *Per a* I eq, B: *Der* I, C: *Der* II, D: *Bla g* II.

dust of bedding was very low (geometric means value, 12.7 ng/g fine dust) ($p < 0.05$). *Bla g* II allergen in dust of kitchen were detected in only three of ten homes (Fig. 4D). To compare the level of cockroach allergens with that of mite allergens in these sites, we measured mite allergens in dust from them. The levels of *Der* I and *Der* II in dust of these sites were very high (Fig. 4B, 4C). The mean level of *Der* I and *Der* II in kitchen were 2,420 and 2,610 ng/g fine dust, respectively, which were higher than that of *Per a* I eq allergens in kitchen.

DISCUSSION

We investigated homes of Japan for existence of cockroach allergens. In this preliminary study, we found that *Per a* I eq allergen was detected in eight of ten homes. However, its level was lower than that reported in the Tempa area of south Florida¹⁸⁾. In the Tempa areas, of 73 dust samples, 71 contained 2 to 200,000 ng of *Per a* I eq ng/g fine dust; most samples (59/71) contained between 10 and 10,000 ng of *Per a* I¹⁸⁾. High level of *B. germanica* allergens was detected in the USA and the level of *Bla g* II in urban areas was higher than that in suburban areas²¹⁾. Cockroach allergy has been reported in studies

of patients with asthma in economically disadvantaged people in large urban areas in the USA, because cockroach infestation is heavy in their homes¹⁾²²⁾²³⁾. In this study, we measured the level of cockroach allergens in dust from homes in the suburban areas, because unlike the USA, there are so few economically disadvantaged people in the urban area of Tokyo.

In this study, we investigated the distribution of cockroach allergens. The highest level of *Per a* I eq was detected in dust from kitchen. The level of *Per a* I eq was very low in dust from bedding. Probably, cockroaches are in highest concentration in kitchens for their food. A previous study reported that dust from kitchen floors contained 50-fold more cockroach allergens than dust from bedroom²⁰⁾. In contrast, the levels of mite allergens (*Der* I and *Der* II) were highest in bedding, which reconfirms results of previous studies¹⁴⁾¹⁵⁾. The distribution of cockroach allergen was different from that of mite allergens. A suitable site in collecting dust might be kitchen for evaluation of the level of cockroach allergens in homes.

We found seasonal change of the level of *Per a* I eq in the floor dust of kitchens. Entomological studies reported that the cockroach is likely to be a year-round inhabitant of homes¹⁰⁾. However, growth of cockroach need sufficient tem-

perature. We think that preparation of dust samples for cockroach allergens should be carried out in summer season.

In this study, the prevalence of cockroach hypersensitivity in Tokyo was lower than that reported in the USA²²⁾²³⁾. It is likely that high prevalence of IgE in the USA to cockroach allergens depend on heavy cockroach infestation there.

It was reported that there is a cross-reactivity between *P. americana* and *B. germanica*, which do not cross-react with non-cockroach insects and mite¹⁸⁾. Despite *B. germanica* being a minor cockroach, about 10% of the patients with asthma had IgE antibodies to *B. germanica*. A previous study reported that 14% of patients with asthma had IgE antibody to *B. germanica* in Kanto area including Tokyo²⁴⁾. It seems that IgE antibodies to *Periplaneta* cockroaches react with *B. germanica*.

In this study, all patients with IgE antibody to cockroach had high levels of IgE antibody to mite. Furthermore, many patients (85%) were found to have IgE antibodies to mite, which also reconfirmed the results of previous studies in Japan²⁴⁾²⁵⁾. This higher prevalence of mite allergy is due to heavier infestation of mite than of cockroach in homes of Japan. From the prevalence of IgE antibodies and the amount of allergens in houses, it seems that cockroach is a not as important allergen as mite. However, there is a growing tendency to build airtight homes, which will supply more sufficient moisture and temperature in homes. Such an indoor environment might promote growing of mite and cockroach, with resulting increase of their allergies.

We thank Yoshie Yoshizawa for excellent technical assistance. We also thank Akira Tanaka and Hiroko Tomiyama, Kabi Pharmacia Diagnostics for measurement of specific IgE antibodies.

REFERENCES

- 1) Solomon, W.R. and Mathews, K.P.: Aerobiology and Inhalant allergens. In "Allergy: Principle and Practices" (ed. by E.J. Middleton, et al), CV Mosby, St. Louis, pp. 312-372, 1988.
- 2) Chapman, M.D.: Dissecting cockroach allergens. *Clin. Exp. Allergy* **23**, 573-578, 1993.
- 3) Choovivathanavanich, P., Suwanprateep, P. and Kanthavichitra, N.: Cockroach sensitivity in allergic Thais. *Lancet* **2**, 1362-1363, 1970.
- 4) Throng, Y.H., Omar, A., Kok, A. and Robinson, M. J.: Skin reactivity to household aeroallergens in children with bronchial asthma. *Sing. Med. J.* **17**, 90-91, 1976.
- 5) Fraser, B.N.: Cockroaches in relation to bronchial asthma in the Durban area. *S. Afr. Med. J.* **55**, 637-639, 1979.
- 6) Lan, J.L., Lee, D.T., Wu, C.H., Chang, C.P. and Yeh, C.L.: Cockroach hypersensitivity; preliminary study of allergic cockroach asthma in Taiwan. *J. Allergy Clin. Immunol.* **82**, 736-740, 1988.
- 7) Schou, C., Lind, P., Fernandez-Caldas, E., Lockey, R. F. and Løwenstein, H.: Identification and purification of an important cross-reactive allergen from America (*Periplaneta americana*) and German (*Blattella germanica*) cockroach. *J. Allergy Clin. Immunol.* **86**, 935-946, 1990.
- 8) Pollart, S.M., Mullins, D.E., Vailes, L.D., Hayden, M.L., Platts-Mills, T.A.E., et al.: Identification, quantitation, and purification of cockroach allergens using monoclonal antibodies. *J. Allergy Clin. Immunol.* **87**, 511-521, 1991.
- 9) Wu, C.H. and Lan, J.L.: Cockroach hypersensitivity; isolation and partial characterization of major allergens. *J. Allergy Clin. Immunol.* **82**, 727-735, 1988.
- 10) Ogata, K., Tanaka, I. and Yasutomi, K.: Cockroach and their control. Japan Environmental Sanitation Center, Kawasaki, Kanagawa, pp. 14-48, 1989.
- 11) Nemoto, T., Aoki, H., Ike, A. and Kobayashi, T.: One case of cockroach asthma. *Jpn. J. Allergol.* **22**, 635-639, 1973 (in Japanese).
- 12) Kawakami, T., Suto, C., Yagura, T. and Kumada, N.: Studies on cockroach allergy. I. Allergenicity of common domestic cockroaches of Japan. *Jpn. J. Saint. Zool.* **33**, 233-238, 1982 (in Japanese).
- 13) Ewan, P.W. and Coote, D.: Evaluation of a capsulated hydrophilic carrier polymer (the Immuno CAP) for measurement of specific IgE antibodies. *Allergy* **45**, 22-29, 1990.
- 14) Sakaguchi, M., Inouye, S., Yasueda, H., Irie, T., Yoshizawa, S. and Shida, T.: Measurement of allergens associated with dust mite allergy. II. Concentrations of airborne mite allergens (*Der I* and *Der*

- II) in the houses. *Int. Arch. Allergy Appl. Immunol.* **90**, 190-193, 1989.
- 15) Sakaguchi, M., Inouye, S., Yasueda, H. and Shida, T.: Concentration of airborne mite allergens (*Der I* and *Der II*) during sleep. *Allergy* **47**, 55-57, 1992.
 - 16) Yasueda, H., Mita, H., Yui, Y. and Shida, T.: Measurement of allergens associated with dust mite allergy. I. Development of sensitive radioimmunoassays for the two groups of *Dermatophagoides* mite allergens, *Der I* and *Der II*. *Int. Arch. Allergy Appl. Immunol.* **90**, 182-189, 1989.
 - 17) Wu, C.H., Chiang, B.T., Fann, M.C. and Lan, J.L.: Production and characterization of monoclonal antibodies against major allergens of American cockroach. *Clin. Exp. Allergy* **20**, 675-681, 1990.
 - 18) Schou, C., Fernandez-Caldas, E., Lockey, R.F. and Løwenstein, H.: Environmental assay for cockroach allergens. *J. Allergy Clin. Immunol.* **87**, 828-834, 1991.
 - 19) Sakaguchi, M., Inouye, S., Irie, T., Miyazawa, H., Watanabe, H., et al.: Airborne cat (*Fel d I*), dog (*Can f I*), and mite (*Der I* and *Der II*) allergen levels in the homes of Japan. *J. Allergy Clin. Immunol.* **92**, 797-802, 1993.
 - 20) Pollart, S.M., Smith, T.F., Morris, E.C., Gelber, L.E., Platts-Mills, T.A.E. and Chapman, M.D.: Environmental exposure to cockroach allergens; analysis with monoclonal antibody-based enzyme immunoassays. *J. Allergy Clin. Immunol.* **87**, 505-510, 1991.
 - 21) Gelber, L.E., Seltzer, L.H., Bouzoukis, J.K., Pollart, S.M., Chapman, M.D. and Platts-Mills, T.A.E.: Sensitization and exposure to indoor allergens as risk factors asthma among patients presenting to hospital. *Am. Rev. Respir. Dis.* **147**, 573-578, 1993.
 - 22) Kang, B.C., Wu, C.W. and Johnson, J.: Characteristics and diagnoses of cockroach-sensitive bronchial asthma. *Ann. Allergy* **68**, 237-244, 1992.
 - 23) Kang, B.C., Johnson, J. and Veres-Thorner, C.: Atopic profile of intercity asthma with a comparative analysis on the cockroach-sensitive and ragweed-sensitive subgroups. *J. Allergy Clin. Immunol.* **92**, 802-811, 1993.
 - 24) Ito, K., Suko, M., Miyamoto, T., Kobayashi, S., Nakazawa, T., et al.: Study on the clinical usefulness of RAST using insect and mite allergen discs. *Jpn. J. Allergol.* **38**, 413-422, 1989 (in Japanese).
 - 25) Uchiyama, K., Akasaka, T., Obikawa, K., Okada, F., Suzuki, I., et al.: *Dermatophagoides farinae* and Japanese cedar (Sugi) pollen specific immunoglobulin E antibodies in asthmatic children measured by radioallergosorbent test and enzyme-linked immunosorbent assay. *Jpn. J. Allergol.* **38**, 451-458, 1989 (in Japanese).

日本の喘息児におけるゴキブリアレルゲンに対する感作状況

- 1) 国立予防衛生研究所感染症疫学部, 2) 杏林大学保健学部,
- 3) 日本医科大学付属永山病院, 4) 国立相模原病院臨床研究部,
- 5) 日本環境衛生センター環境生物部, 6) 国立療養所盛岡病院小児科,
- 7) *Department of Medical Research, Taichung Veterans General Hospital,*
- 8) *Department of Internal Medicine, University of Virginia,* 9) *ALK Research*

阪口雅弘¹⁾ 井上 栄¹⁾ 宮沢 博²⁾ 岡部俊成³⁾

安枝 浩⁴⁾ 赤坂 徹⁵⁾ 武藤敦彦⁶⁾ 田中生男⁶⁾

Chii-Huei Wu⁷⁾, Martin D. Chapman⁸⁾

Carsten Schou⁹⁾

クロゴキブリアレルゲンに対する感作状況を調べるため、喘息児171名のゴキブリに対するIgE抗体陽性率と家庭内のゴキブリ主要アレルゲン量を測定した。喘息児171名のクロゴキブリおよびチャバネゴキブリとダニに対するIgE抗体をCAP法で調べたところ、喘息児の特異IgE陽性率はクロゴキブリ16%、チャバネゴキブリ9.9%とダニ85%に比べ、低かった。ゴキブリアレルゲンは10軒中8軒にみられた。居間、台所、寝具のゴミ中のゴキブリアレルゲンは台所で最も高かったが、いずれの場所でもダニアレルゲンに比べ、低かった。