
Comparison of allergenic components between German cockroach whole body and fecal extracts

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Background: Cockroaches have been demonstrated to be an etiologic factor in allergic diseases. Further, sensitivity to cockroach places patients with asthma at risk for exacerbations that require emergency medical care.

Objective: This study compared the differences in allergenic components between German cockroach whole body and German cockroach fecal extracts (GWBE and GFE).

Methods: Patients with asthma and/or allergic rhinitis were skin prick tested with German cockroach extract (Bayer Corporation, West Haven, CT). Serum specimens from these patients, 25 with positive skin tests and 8 with negative tests, were used for the ELISA and immunoblot experiments.

Results: By ELISA, 72% (18 of 25) and 60% (15 of 25) of positive responders' sera showed IgE antibodies to GWBE and GFE, respectively, and the IgE levels to GWBE were highly correlated with those to GFE ($r = .84$, $P < .01$). In inhibition ELISA experiments, extensive cross-reactivity was observed between GWBE and GFE, slight cross-reactivity between GWBE and *Dermatophagoides farinae*, and no cross-reactivity between GFE and *D. farinae*. The two-site monoclonal antibody ELISA detected more of the German cockroach major allergens in GFE compared with GWBE; 6.2 times (2420 vs 390 U/mL) for Bla g 1 and 3 times (15.32 vs 5.07 $\mu\text{g/mL}$) for Bla g 2. In the immunoblot comparison of patients' sera, the IgE antibodies binding to GWBE were apparently different from those binding to GFE in all the positive responders' sera; eg, 50% or more of the 25 positive responders' sera reacted to 43- to 67-kDa proteins in GWBE and to 28- to 30-kDa proteins in GFE, respectively. No IgE antibodies bound to components in GWBE and GFE in the 8 negative responders' sera.

Conclusions: There are major differences between the allergenic components of GWBE and GFE. Based on the amounts of major allergens (Bla g 1, Bla g 2), German cockroach feces are a more important source of allergen than the whole body in respiratory allergic diseases.

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INTRODUCTION

Cockroach allergens are important sensitizing agents, particularly in patients with asthma who live in large, economically deprived, urban areas.¹⁻³

During infestation, cockroaches can produce a variety of materials that may contain significant allergenic activity. Potential sources of relevant cockroach allergens in house dust include whole bodies, cast skins, secretions, egg castings, and feces. Of these, major allergens have been known to be present in both whole body and feces.⁴ The sensitization route to cockroach has not

yet been well elucidated. Cockroach allergens, however, are believed to be inhaled; therefore, cockroach feces are likely to be the source of relevant cockroach allergens because desiccated feces particles probably disintegrate to become incorporated into house dust more readily than chitinous body parts and encased internal organs. In this respect, the importance of cockroach feces in respiratory allergy needs emphasis. This study compared the differences in allergenic components between German cockroach whole body and fecal extracts (GWBE and GFE) and measured the levels of German cockroach major allergens in GWBE and GFE.

METHODS

Patients

Patients with asthma and/or allergic rhinitis were skin prick-tested with 50 common inhalant allergens including German cockroach extract (Bayer Corporation, West Haven, CT) at our allergy clinic sequentially during the preceding year. From this group, 25 positive and 8 negative responders to German cockroach extract were recruited into this study. Their serum samples were collected with informed consent and stored at -20°C for use in the ELISA and immunoblot experiments. Skin reactivity was expressed as the ratio of wheal sizes of allergen and histamine according to the criteria of the Scandinavian Society of Allergy.⁵ A positive responder was defined as a subject who exhibited a response of 1+ or more to German cockroach extract in the skin prick test.

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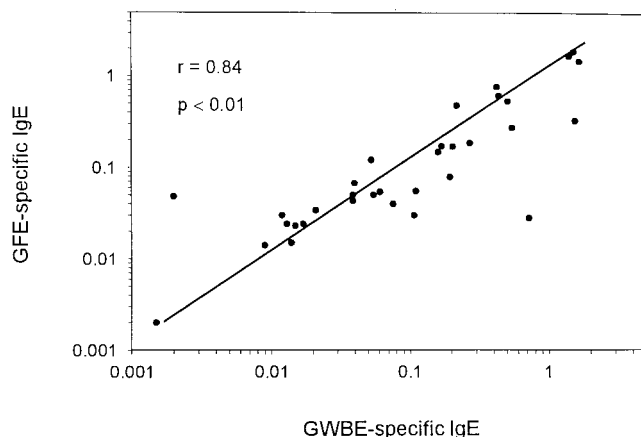


Figure 1. Correlation between GWBE- and GFE-specific IgE levels in serum specimens from 25 skin test-positive patients and 8 skin test-negative patients. ELISA used microtiter plates coated with GWBE or GFE and tested 50 μ L of each patient's serum.

Preparation of German Cockroach Extracts

Fifty adult German cockroaches were donated from the Department of Parasitology, Yonsei University College of Medicine, and were fed for 7 days (168 hours) with artificial bait. The artificial food (manufactured by Samyang Co. Ltd., Seoul, Korea) was made using the following raw materials: corn, wheat, soybeans, fish powder, soybean oil, limestone, salt, potassium chloride, vitamins, and minerals. The main components are 23.2% crude protein, 4.0% crude fat, 6.0% crude fiber, 10% crude ash, 0.6% calcium, and 0.4% phosphate. The cockroaches did not starve before they were killed. To prepare the GWBE, anesthetized cockroach whole bodies were collected separately from feces, lyophilized with liquefied nitrogen, and reduced to powder. The powder was defatted with diethyl ether and extracted with stirring in phosphate buffered saline (1:20 wt/vol) with protease inhibitor cocktail⁶ for 72 hours at 4° C. The resulting extract was centrifuged at 18,000 rpm for 30 minutes, and the supernatant was dialyzed against distilled water. This dialyzed supernatant was centrifuged again to remove insoluble materials and lyophilized.

The total dry mass of feces was measured to calculate the daily amount of feces defecated per German cock-

roach. To prepare the GFE, the same procedure was followed as that used for the GWBE without adding protease inhibitor cocktail. The protein contents of the GWBE and GFE, measured by a BioRad method,⁷ were 570 μ g/mL and 1010 μ g/mL, respectively. The *Dermatophagoides farinae* extract, which was used for the inhibition ELISA, was prepared in the same manner as the GWBE.

ELISA for German Cockroach-Specific IgE/Inhibition ELISA

GWBE or GFE was coated onto a 96-well polyvinyl microtiter plate (Costar, Cambridge, MA) overnight at 4° C by adding 50 μ L of 0.1 mol/L carbonate buffer (pH 9.6) containing 10 μ g/mL of the crude protein. After blocking with 1% bovine serum albumin, the

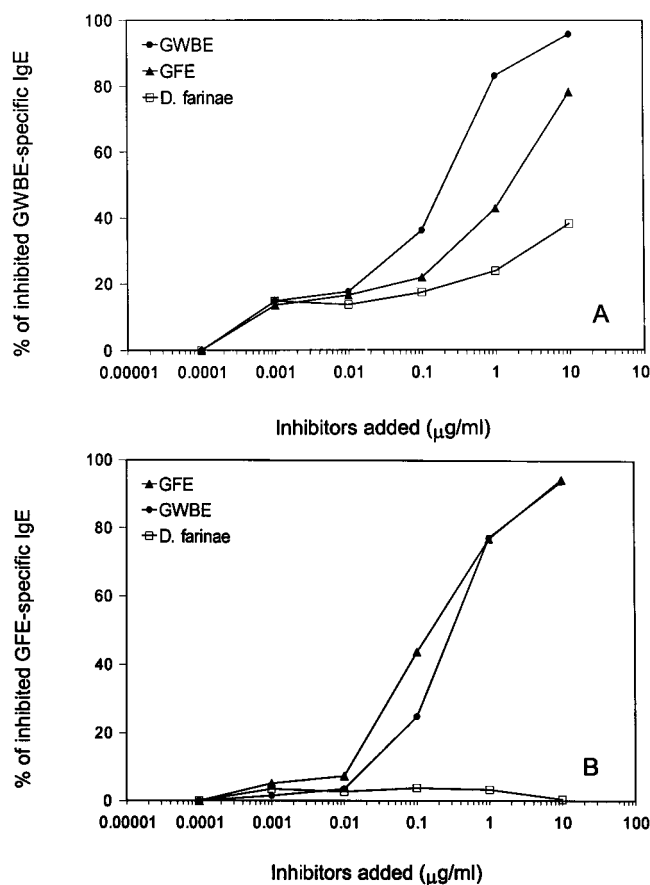


Figure 2. Cross-reactivity among specific IgE to GWBE, GFE, and *D. farinae* extracts by inhibition ELISA. A shows plates coated with GWBE and B shows plates coated with GFE. Inhibition with the three extracts was detected with a serum pool from 12 patients who were skin test-positive to German cockroach extract.

antigen-coated immunoplate was incubated for 1 hour at room temperature with 50 μ L of undiluted sera from the patients who showed positive skin reactions to GWBE or GFE. After washing with phosphate-buffered saline containing 0.1% Tween 20 (PBST), the immunoplate was incubated with 1:500 biotinylated polyclonal anti-human IgE (Vector, Burlingame, CA) for 1 hour followed by incubation with 1:500 streptavidin-peroxidase (Sigma, St. Louis, MO) for 30 minutes at room temperature. After washing with PBST, 100 μ L of ABTS solution [25 mg of 2,2'-azino-bis-ethylbenzthiazoline-sulfonic acid in 50 mL of 50 mmol/L citrate buffer (pH 4.2) containing 50 μ L of 0.03% hydrogen peroxide] was added to each well, and after 5 minutes, 100 μ L of 2 mmol/L NaN_3 was added to stop the reaction. The colorimetric reaction was monitored by absorbance at 405 nm on an ELISA reader (Dynatec, Alexandria, CA).

For the inhibition ELISA, serum specimens from 12 patients who tested positive to German cockroach antigen and also showed absorbencies of 0.1 or more to both GWBE and GFE were pooled. Aliquots from this serum pool were preincubated with GWBE or GFE or *D. farinae* extract overnight at 4° C at several extract concentrations (10^{-3} , 10^{-2} , 10^{-1} , 1, and 10 μ g/mL, respectively), before adding the pooled serum to the GWBE- or GFE-coated immunoplates. The remaining steps in the ELISA were performed as described above.

Measurement of Major Allergens (Bla g 1, Bla g 2) in German Cockroach Extracts

The monoclonal antibodies (10 μ g) to the German cockroach major allergens, Bla g 1 and Bla g 2 (Indoor Biotechnologies, Cardiff, Wales), were mixed with 50 μ L of 0.1 mol/L carbonate buffer (pH 9.6), and then coated onto their respective 96-well polyvinyl microtiter plate (Costar) overnight at 4° C. After blocking with 1% bovine serum albumin, the antibody-coated immunoplates were incubated with ei-

ther 50 μ L of GWBE or GFE containing 1 mg/mL crude protein and reference allergens, respectively, for 1 hour at room temperature. After washing with PBST, the immunoplates were incubated with 50 μ L of rabbit anti-cockroach polyclonal antibody (Indoor Biotechnologies) diluted 1:200 in PBST for 1 hour at room temperature. After washing with PBST, the immunoplates were incubated again with 50 μ L of peroxidase-conjugated goat anti-rabbit IgG (Sigma) diluted 1:1000 in PBST for 1 hour at room temperature. After washing with PBST, the remaining steps were performed as described previously.

SDS-PAGE and IgE immunoblot

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli.⁸ Briefly, 10 μ g of GWBE or GFE crude protein per lane, or 100 to 150 μ g per gel, was mixed with sample buffer containing 2% sodium do-

decyl sulfate, 14.4 mmol/L 2-mercaptoethanol, 60 mmol/L Tris-HCl, 25% glycerol, and 0.1% bromphenol blue, boiled for 5 minutes, and electrophoresed (Hoeffer Mighty Small, San Francisco, CA) in 13.5% polyacrylamide separation gel with 4% stacking gel. The separated proteins were stained with Coomassie brilliant blue or transferred (Transpor; Hoeffer, San Francisco, CA) to nitrocellulose membrane (pore size 0.45 μ m; Amersham, Buckinghamshire, UK). Blotted membrane strips were stained using Ponceau's solution or blocked with 5% defatted milk in PBST for 1 hour. Serum specimens from the patients with positive and negative skin tests were diluted 1:4 in PBST, incubated with the blotted membrane strips overnight at room temperature, and washed three times with PBST. Bound IgE was detected after incubation for 1 hour with goat polyclonal anti-human IgE conjugated with alkaline phosphatase (Sigma) diluted 1:2000 in

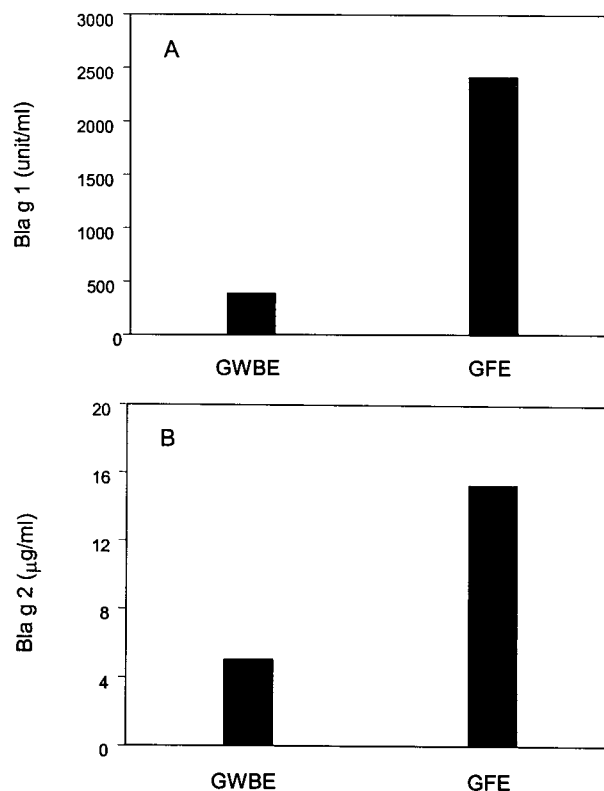


Figure 3. Levels of the German cockroach major allergens in GWBE and GFE by two-site monoclonal antibody ELISA. A shows the levels for Bla g 1 and B shows the levels for Bla g 2.

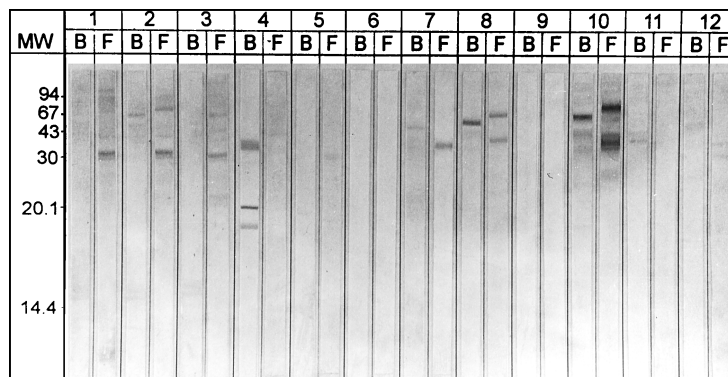


Figure 4. Comparison of IgE bound to GWBE and GFE by immunoblot. Lanes 1 through 25 show the sera from positive skin responders to German cockroach extract and lanes 26 through 33 show the sera from negative skin responders to German cockroach extract. B, GWBE-blotted; F, GFE-blotted membrane strips; MW, molecular weight.

PBST followed by 0.66% 4-nitroblue tetrazolium and 0.33% 5-bromo-4-chloro-3-indolyl phosphate, used as substrate (Promega, Madison, WI).

RESULTS

Detection of German Cockroach-Specific IgE by ELISA

ELISA showed elevated IgE antibody levels to GWBE in 72% (18 of 25) of the serum specimens from the skin test-positive patients; likewise, 60% (15 of 25) showed elevated IgE antibody levels to GFE. The cut-off level for a significant response to GWBE and GFE was determined by an absorbance of 0.1 or more. The IgE reactivity to GWBE was highly correlated with that to GFE for all 33 patients ($r = 0.84$, $P < 0.01$, Fig 1).

Cross-reactivity among GWBE, GFE, and *D. farinae*

In the inhibition ELISA, the IgE bound to either GWBE or GFE was inhibited in a concentration-dependent manner by both GWBE and GFE (Fig 2, A and B). For example, when the concentration of inhibitor GWBE or GFE was 10 $\mu\text{g/mL}$, the IgE bound to GWBE or GFE was inhibited by GFE or GWBE by 78% and 94%, respectively. *D. farinae* extract did not inhibit the IgE bound to GFE and slightly inhibited the IgE bound to GWBE (by 38% at 10 $\mu\text{g/mL}$). Thus, there is evidence for extensive cross-reactivity of IgE antibodies between GWBE and GFE.

Levels of German Cockroach Major Allergens, Bla g 1 and Bla g 2

The two-site monoclonal antibody ELISA evaluated the levels of the two German cockroach major allergens (Bla g 1, Bla g 2) in GWBE and GFE. The level of Bla g 1 in GFE was 6.2 times (2420 U/mL vs 390 U/mL) the level in GWBE and the level of Bla g 2 was 3 times (15.32 $\mu\text{g/mL}$ vs 5.07 $\mu\text{g/mL}$) the level in GWBE (Fig 3, A and B).

IgE-Binding Properties by Immunoblot

SDS-PAGE of GWBE and GFE showed at least 15 protein bands ranging from 15 to 100 kDa (data not shown). In Western blot analyses, the

IgE in all the positive responders' sera bound to certain components in the GWBE that were apparently different from those in the GFE (Fig 4). In the GFE immunoblot, 28- to 30-kDa (56%, 14 of 25) and 67- to 94-kDa (48%, 12 of 25) proteins showed marked IgE reactivity, whereas in the GWBE immunoblot, 43- to 67-kDa (52%, 13 of 25) and 15- to 16-kDa (44%, 11 of 25) proteins had marked IgE reactivity (Fig 5). Other allergenic components in GFE showing moderate IgE reactivity were 38 to 40 kDa (40%, 10 of 25) and 43 to 67 kDa (36%, 9 of 25), whereas those in GWBE were 67 to 94 kDa (28%, 7 of 25). Interestingly, there was no IgE reactivity at all to 15- to 16-kDa and 100-kDa or greater proteins in the GFE immunoblot in contrast to the GWBE immunoblot (Figs 4 and 5).

DISCUSSION

Both cockroach allergens and mite allergens are considered important and relevant allergenic components in house dust. We determined that one German cockroach defecated approximately 2.99 μg dry weight of feces daily (24 hours; Fig 6); this is a considerable amount because cockroaches reproduce rapidly and are difficult to exterminate. One survey of house dust samples found that approximately 20% of the homes tested had detectable levels of cockroach allergens, although

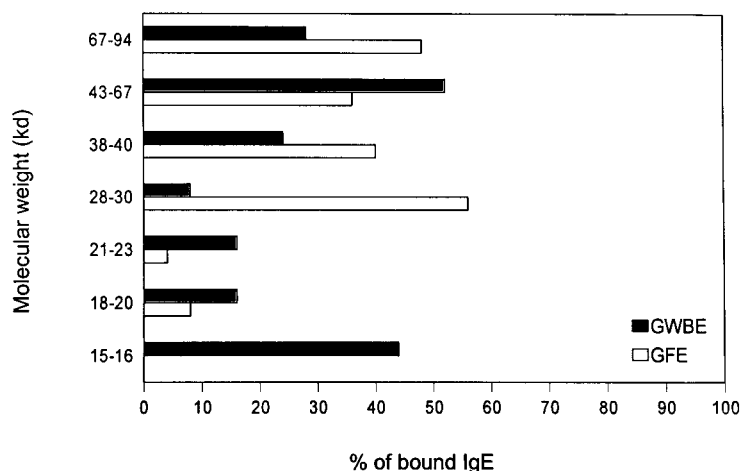


Figure 5. Comparison of the IgE reactivity to proteins in GWBE and GFE by immunoblot.



Figure 6. Photomicrograph showing feces of German cockroach ($\times 30$).

there was no obvious evidence of infestation.⁹ Further, dust from kitchen floors and cabinets contained 50-fold more cockroach allergen (mean, 10,755 U/g of dust) compared with dust from bedrooms and upholstered furniture (204 U/g). Another study showed that >80% of children with bedroom Bla g 1 or Bla g 2 of 1 U/gm dust or greater demonstrated skin sensitivity to cockroach allergen.¹⁰ Additionally, lower socioeconomic status, age >11 years, and African-American race were all independently associated with cockroach allergen sensitization.

Most previous studies established that the components in GWBE binding to IgE antibodies were very similar to those in GFE, and GWBE and GFE RAST results were highly and significantly correlated.^{4,11,12} Thus, cockroach feces might provide an important source of sensitizing antigen. Detailed reading of these previous studies, however, does show the discordant reactivity in Western blot analyses comparing GWBE with GFE, where some differences are reported in the percentage of sera reactive to either GWBE or GFE. Musmand et al⁴ insisted that this disparity was because of differences in the relative concentrations of allergens present in GWBE and GFE, the presence of unique allergens in GWBE or GFE, or cleaved fragments of larger GWBE allergens in GFE as a result of natural degradation caused by proteolytic enzymes present in the cockroach

alimentary tract. Although these factors were considered, Musmand et al⁴ concluded that the overall reactivities to GWBE and GFE were similar. However, the components in GWBE binding to IgE antibodies in our Western blot analysis were apparently different from those in GFE in the positive responders' sera. Further, we observed that the allergenic proteins showing the most marked IgE reactivity were 43 to 67 kDa for GWBE and 28 to 30 kDa for GFE compared with the 60-kDa proteins for GWBE and GFE in Musmand et al.⁴ Interestingly, 100-kDa or greater allergenic components were observed in only GWBE; this finding contradicts an earlier report of Musmand et al.⁴ Collectively, there may be distinctive differences in the allergenic components between GWBE and GFE.

We also measured the amount of German cockroach major allergens, Bla g 1 and Bla g 2, in GWBE and GFE with a two-site monoclonal antibody ELISA. The GFE contained 6.2 times the amount of Bla g 1 and 3 times the amount of Bla g 2 compared with GWBE, suggesting that German cockroach fecal material has much more allergenic activity than the whole body.

Another important aspect of our study concerned the cross-reactivity of German cockroach extracts with *D. farinae* extract. House dust mite and cockroach are known to be important components of indoor allergens. One

survey reported that most patients (17 of 19) with cockroach-specific IgE in their sera were also sensitized to *Dermatophagoides pteronyssinus* allergens.¹³ Another study of Turkish patients with asthma showed that among cockroach-sensitive patients, 70% also showed positive skin prick tests for mites.¹⁴ It is not clear whether the association between cockroach and *D. pteronyssinus* sensitization is determined by cross-reactivity between allergens or by their coexistence in the same indoor environments. Riario-Sforza et al¹⁵ reported that 113 (78.2%) of 170 patients with a positive skin response to cockroach extract were also positive for *D. pteronyssinus*. RAST inhibition showed cross-reactivity between cockroach and *D. pteronyssinus*, and isoelectric focusing detected a series of bands focusing at the same pI for both cockroach and *D. pteronyssinus* relevant allergens. In contrast, our study results revealed no cross-reactivity between the GFE and the *D. farinae* extract, and limited cross-reactivity between the GWBE and the *D. farinae* extract. Thus, cross-reactivity between German cockroach with mites needs further study.

CONCLUSION

This investigation, which used inhibition ELISA, two-site monoclonal antibody ELISA, and Western blot analyses found distinctive differences in the allergenic components between GWBE and GFE. Further, based upon the amount of major allergens in the feces, GFE are a more important and relevant allergen source than the whole body in respiratory allergic diseases. Our study also revealed that a cockroach fecal extract does not crossreact with a *D. farinae* extract, but further confirmatory data are needed.

REFERENCES

1. Kang B. Study on cockroach antigen as a probable causative agent in bronchial asthma. *J Allergy Clin Immunol* 1976;58:357-365.
2. Morris EC, Smith TF, Kelly LB. Cock-

- roach is a significant allergen for inner city children. *J Allergy Clin Immunol* 1986;77:206.
3. Stankus RP, O'Neil CE. Antigenic/allergenic characterization of American and German cockroach extracts. *J Allergy Clin Immunol* 1988;81:563–570.
 4. Musmand JJ, Horner WE, Lopez M, Lehrer SB. Identification of important allergens in German cockroach extracts by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blot analysis. *J Allergy Clin Immunol* 1995;95:877–885.
 5. Northern Society of Allergology. Proceedings Communications Gesellschaftsverhandlungen. *Acta Allergol* 1972;27:439–468.
 6. Bollag DM, Edelstein SJ. Preparation for protein isolation. In: *Protein Methods*. New York: Wiley-Liss, 1991: 1–25.
 7. Harfast B, van Hage-Hamsten M, Ansoategui IJ, et al. Monoclonal antibodies to *Lepidoglyphus destructor*: delineation of cross-reactivity between storage mites and house dust mites. *Clin Exp Allergy* 1992;22:1032–1037.
 8. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680–685.
 9. Pollart SM, Smith TF, Morris EC, et al. Environmental exposure to cockroach allergens: analysis with monoclonal antibody-based enzyme immunoassays. *J Allergy Clin Immunol* 1991;87:505–510.
 10. Sarpong SB, Hamilton RG, Eggleston PA, Adkinson NF Jr. Socioeconomic status and race as risk factors for cockroach allergen exposure and sensitization in children with asthma. *J Allergy Clin Immunol* 1996;97:1393–1401.
 11. Lehrer SB, Horner WE, Menon P, Stankus RP. Comparison of cockroach allergenic activity in whole body and fecal extracts. *J Allergy Clin Immunol* 1991;87:574–580.
 12. Horner WE, Kailas J, Stankus RP, Lehrer SB. Common German cockroach whole body and fecal allergens: immunoprint inhibition studies. *Int Arch Allergy Appl Immunol* 1990;93:256–262.
 13. Liccardi G, Russo M, D'Amato M, et al. Sensitization to cockroach allergens in a sample from the urban population living in Naples (southern Italy). *J Invest Allergol Clin Immunol* 1998;8:245–248.
 14. Mungan D, Celik G, Sin B, et al. Characteristic features of cockroach hypersensitivity in Turkish asthmatic patients. *Allergy* 1998;53:870–873.
 15. Riario-Sforza GG, Della Torre F, Antonicelli L, et al. Sensitization to cockroach in Italy: a multicentric study. *Allergy Asthma Proc* 1997;18:23–28.

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