
Cross-reactivity between cockroach and ladybug using the radioallergosorbent test

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Background: Home infestations from *Harmonia axyridis* (ladybug) occur throughout the United States. IgE-mediated sensitization with allergic disease has been reported. The prevalence of ladybug sensitization has been studied by questionnaire and allergy testing in patients diagnosed as having allergic disease. Cross-reactivity with cockroach exists.

Objectives: To determine the prevalence of ladybug specific IgE in the general population by specific IgE immunoassay and to examine cross-reactivity to cockroach.

Methods: An experimental solid phase for use in immunoassay was prepared using a ladybug extract, and performance characteristics were determined. Serum samples from 100 adult blood bank donors were tested using the ladybug specific IgE immunoassay. Known ladybug IgE-positive serum samples obtained from symptomatic patients were used to study cross-reactivity with German cockroach by specific IgE immunoassay inhibition.

Results: The mean background response of the assay solid phase was 51 fluorescent units with an analytical cutoff of 59 fluorescent units. It was estimated that a response of 88 fluorescent units corresponds to a specific IgE concentration of 0.1 kUa/L. The extinction dilution curve was linear to 0.1 kUa/L. The assay cutoff was set at 0.1 kUa/L. Of the 100 blood donor serum samples, 10 were positive for ladybug specific IgE. Inhibition assays revealed partial cross-reactivity with German cockroach.

Conclusion: Although an immunoassay solid phase prepared with ladybug whole body extract will identify persons in a general population sensitized to epitopes found in the extract, clinicians performing allergy testing using whole body ladybug extract should be aware that a positive result may or may not indicate that exposure to actual ladybug allergens is causing sensitization.

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INTRODUCTION

The ladybug (*Harmonia axyridis*) was introduced into the United States as a biological control agent. This member of the Coccinellidae family of beetles is native to Asia. Currently, ladybugs have been observed in almost every state in the United States.¹

The ladybug has emerged as a unique seasonal aeroallergen, causing symptoms between September and March. In the fall, swarms occur as the ladybugs gather together to search for warm overwintering sites.² These infestations can be severe and bothersome. In a survey of Ohio homeowners with ladybug infestation, 70% reported that the ladybugs emanated a foul odor and orange stains and 40% reported that the ladybugs bit them.³

Allergic diseases reported as being triggered by ladybug exposure include rhinitis, conjunctivitis, facial angioedema,

urticaria, asthma, and chronic cough. To our knowledge, no commercial extract is currently available for diagnosis or treatment. Har a 1 and Har a 2 allergens have been purified.⁴

Yarbrough et al⁵ published the initial report of ladybug allergic disease in humans in 1999. They observed 2 cases of allergic symptoms associated with ladybug exposure. Whole body extract was produced from ladybugs taken from the patients' homes, and both patients were skin test positive to the whole body extract. Subsequent eradication of ladybugs from the patients' homes resulted in symptomatic improvement.⁵

In a retrospective review of 1,400 West Virginia allergy patients, Goetz⁶ demonstrated a prevalence of 21% ladybug sensitization by skin prick testing with a whole body ladybug extract. The sensitization rate in rural areas was 30%; and in urban areas, 16%. Cockroach and ladybug showed a high degree of skin test concordance.⁶

A group of patients with ladybug-infested homes in Kentucky were queried via retrospective survey. In this population, 50% self-reported ladybug allergy, 19% self-reported allergy on direct contact, and 31% needed extra allergy medications during the times of ladybug infestation. Also, the timing of the worsened allergy symptoms correlated with the timing of ladybug infestation.⁷

The objectives of this study were as follows: (1) to determine the prevalence of ladybug specific IgE in the general population by specific IgE immunoassay and (2) to examine

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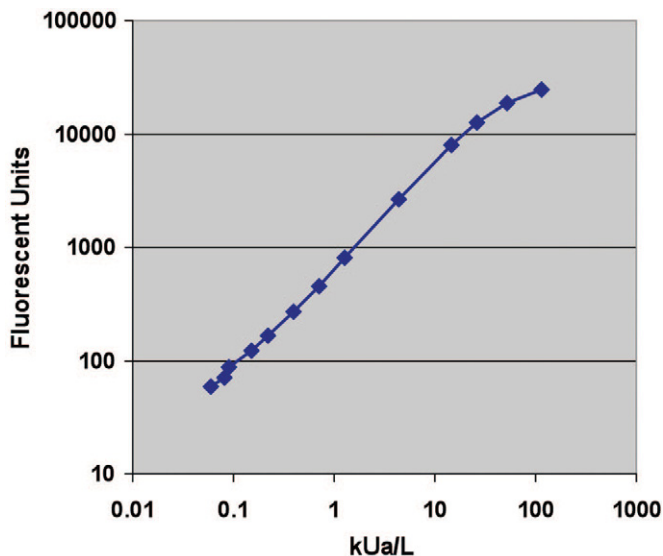


Figure 1. Performance characteristics of the ladybug immunoassay (ImmunoCAP). The extinction-dilution curve is linear to 0.1 kUa/L (single patient data).

cross-reactivity between the ladybug and the German cockroach (*Blattella germanica*).

METHODS

Approval for this study was obtained from the local Human Assurance Committee. Written patient informed consent was obtained. An experimental whole body ladybug extract (Greer Laboratories, Lenoir, North Carolina) was used to produce a solid-phase specific IgE blood test (ImmunoCAP; Phadia, Uppsala, Sweden) for use in an allergy testing system (UniCAP; Phadia). Performance characteristics of the solid phase were determined as previously described.⁸ To estimate

Table 1. Positive Ladybug Specific IgE Donors, With Corresponding Cockroach Specific IgE Levels

Ladybug specific IgE, kUa/L	Age, y/sex	Cockroach specific IgE, kUa/L
2.41	43/M	1.83
1.85	68/M	2.45
1.12	70/M	1.61
1.06	58/M	8.68
0.57	25/M	0.71
0.20	27/M	ND
0.14	19/F	0.80
0.14	37/M	0.15
0.12	33/M	4.06
0.10	20/M	1.85

Abbreviation: ND, no data.

the prevalence of sensitization in this community, randomly obtained serum samples from 100 adult blood bank donors were tested for ladybug specific IgE using the experimental ladybug solid phase. A χ^2 test was used to examine the null hypothesis that there is no difference in the prevalence of sensitization between males and females.

Specific IgE inhibition was performed to test for cross-reactivity. Serum samples from 3 ladybug-allergic patients were pooled. These patients had reported allergic symptoms on exposure to ladybug and had positive skin prick test results to a whole body ladybug extract. The serum pool was then incubated with a German cockroach extract (Greer Laboratories) at increasing concentrations. Then, ladybug specific IgE assays were performed. The percentage inhibition of the ladybug solid phase by the German cockroach extract inhibitor was calculated. Second, serum samples from 3 German cockroach-allergic patients were pooled. These serum samples were obtained from patients with allergic symptoms on exposure to cockroach and positive skin prick test results to

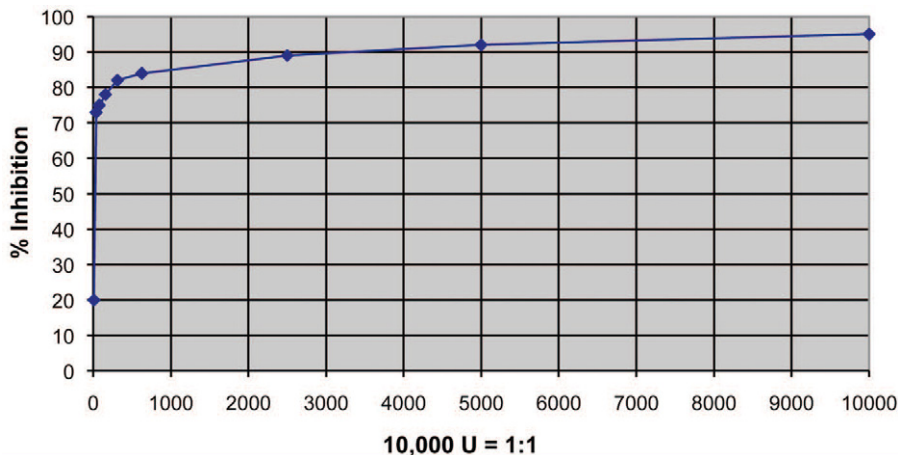


Figure 2. Ladybug solid-phase inhibition with German cockroach extract. U (units) = 1:1 indicates equal parts of neat extract (German cockroach extract 1:20 wt/vol) and positive ladybug serum pool.

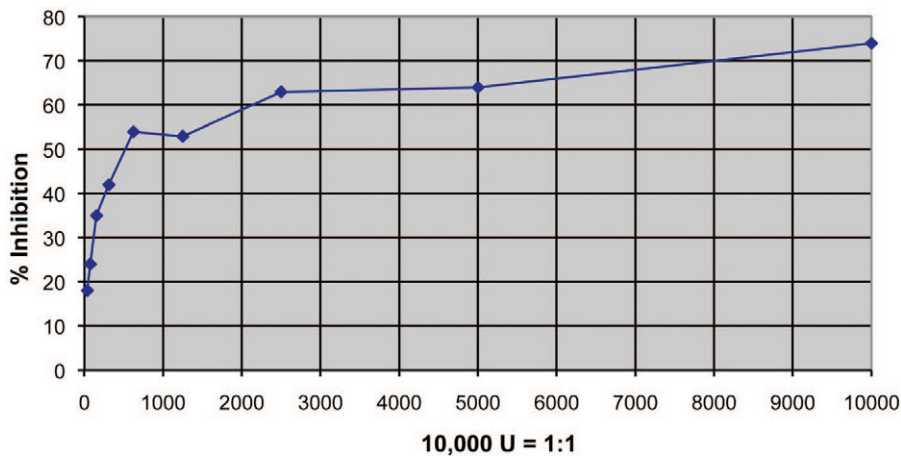


Figure 3. German cockroach solid-phase inhibition with ladybug extract. U (units) = 1:1 indicates equal parts of neat extract (ladybug extract 1:20 wt/vol) and positive German cockroach serum pool.

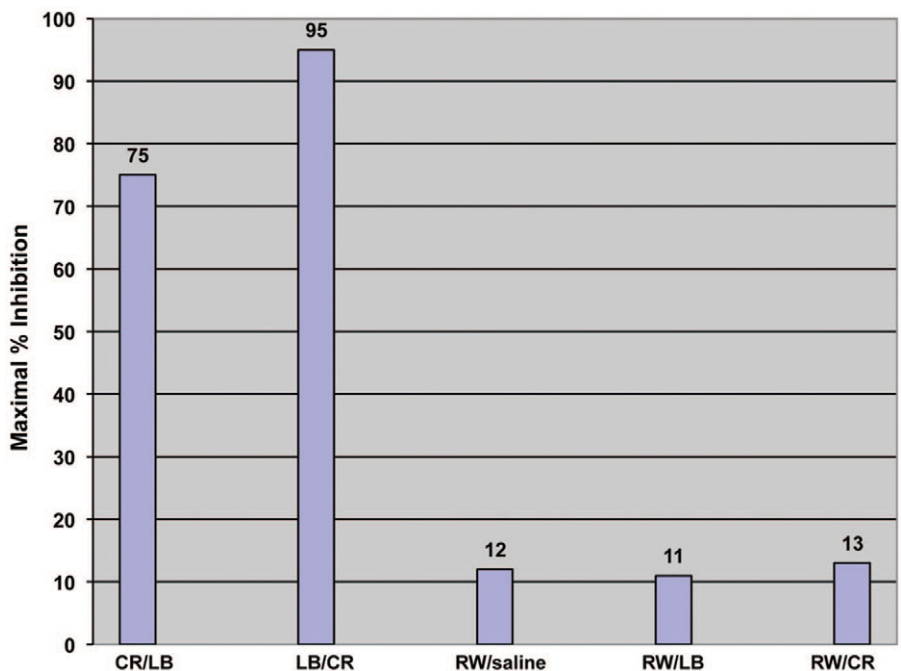


Figure 4. Maximal inhibitions compared with ragweed controls. CR/LB indicates German cockroach solid-phase maximal inhibition by ladybug extract; LB/CR, ladybug solid-phase maximal inhibition by German cockroach extract; RW/CR, ragweed solid-phase maximal inhibition by German cockroach extract; RW/LB, ragweed solid-phase maximal inhibition by ladybug extract; RW/saline, ragweed solid-phase maximal inhibition by saline.

commercially available German cockroach extract. This serum pool was combined with whole body ladybug extract at increasing dilutions. Then, German cockroach specific IgE assays were performed. The percentage inhibition of the cockroach solid phase by the ladybug extract inhibitor was calculated. For controls, the maximal specific inhibition at a 1:1 mixture of extract and inhibitor was performed using a short ragweed solid phase and known short ragweed positive serum samples inhibited by ladybug whole body extract,

German cockroach extract, and diluent (50% glycerol). Results are reported descriptively.

RESULTS

The performance characteristics of the ladybug solid phase are shown in Figure 1. The mean background response was 51 fluorescent units, with a coefficient of variation of 4.9%. The analytical cutoff (background plus 3 SDs) was 59 fluorescent units. Eighty-eight fluorescent units corresponded to a

ladybug specific IgE level of 0.1 kUa/L. The extinction-dilution curve was linear to 0.1 kUa/L, and the assay cutoff was set at 0.1 kUa/L.

Of the 100 randomly obtained serum samples from a local blood bank, 10 were positive at relatively low levels for ladybug specific IgE (Table 1). The range for ladybug specific IgE was 0.10 to 2.41 kUa/L. The age range for these individuals with positive test results was 19 to 70 years. Of the 100 donors, 39 were female, but 9 of the 10 sensitized individuals with positive test results were male, a significant difference (χ^2 test, $P < .001$).

The German cockroach extract inhibited the ladybug solid phase, with a maximal inhibition of 95% at a 1:1 mixture of extract and serum pool (Fig 2). The ladybug whole body extract inhibited the German cockroach solid phase, with a maximal inhibition of 75% at a 1:1 mixture of extract and serum pool (Fig 3). The short ragweed solid phase used as a control was maximally inhibited 12%, 11%, and 13% by saline, ladybug extract, and cockroach extract, respectively (Fig 4).

DISCUSSION

In this study, an experimental ladybug solid phase identified 10% of a local population as having IgE antibody that binds to epitopes present on the solid phase. This finding could reflect primary sensitization to epitopes of ladybug, cockroach, or other allergens. Specific IgE inhibition assays revealed partial cross-reactivity between *Harmonia axyridis* and *B germanica*. Thus, it would be difficult to define the initial sensitizing allergen in these patients. The high proportion of males with evidence of ladybug sensitization has not been previously reported and cannot be explained at this time.

A prior study of cross-reactivity investigated the demographics of patients with positive ladybug IgE. In one group, serum samples from 15 ladybug IgE-positive patients were pooled. These 15 outpatients all had allergy symptoms and ladybug-infested homes. All were white, and all lived in a rural or suburban setting. Immunoinhibition assays of the pooled serum samples suggested limited cross-reactivity to German cockroach. In another group, serum samples from 18 ladybug IgE-positive patients were pooled. These 18 urban emergency department patients were all being treated for asthma exacerbations, and 17 were African American. Immunoinhibition assays of the pooled serum samples revealed extensive cross-reactivity to German cockroach.⁴ Perhaps the patient demographics, with resultant known variation in allergen exposures, can provide valuable assistance in interpreting ladybug allergy testing results.

Ladybug washings have also been used to skin test for ladybug sensitization. These washings were effective and gave the same results as ladybug whole body extract.⁹ Also, the ladybug hemolymph exuded from the mouth and leg parts has been shown to bind ladybug IgE from serum samples of

ladybug-allergic patients.³ Molecularly based studies of allergens from German cockroach and allergens from ladybug washings and ladybug whole body extract might shed light on the source of the cross-reacting epitopes, and result in testing materials with more specificity for ladybug.

Ladybug sensitization should be considered in the evaluation of patients with allergic disease, especially when symptoms occur from September to March. Clinicians should ask about ladybug exposure and ladybug home infestation. A commercially available ladybug extract with a high specificity for ladybug allergens would be a useful addition to currently available extracts. The use of species-specific, clinically relevant allergens for ladybug would permit a more accurate determination of the nature of sensitization. Our experience demonstrates that assays such as ours may not be useful to distinguish between ladybug and cockroach sensitization.

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