Multicolored Asian lady beetle hypersensitivity: a case series and allergist survey

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Background: Multicolored Asian lady beetles (*Harmonia axyridis*) have been used as a biological control agent against crop-destroying aphids in the United States. Outside their natural habitat, *H axyridis* seeks refuge in homes during fall and winter, leading to patient complaints and symptoms of rhinitis, wheezing, and urticaria on exposure to the beetles.

Objective: To gain a better understanding of the character and spectrum of allergic disease provoked by exposure to home-infesting lady beetles.

Methods: Eight patients with allergic symptoms suspected of being caused by *H axyridis* and consistent with an IgE-mediated process were identified and interviewed. A whole-body extract from *H axyridis* was prepared. Western blots using the patients' serum identified specific IgE antibodies in the extract. Through a novel technique, immunohistochemical analysis using beetle sections overlayed with patient serum was performed. A random survey of allergists from across the United States was also performed to evaluate experience with cases of lady beetle allergy.

Results: Western blots revealed IgE binding to 5 proteins with molecular weights of approximately 8.6, 21, 28, 31, and 75 kDa. Specific IgE bound to proteins localized in the beetle's mouth and leg areas. The allergist survey revealed positive responses in North Central, Mid-Atlantic and New England states.

Conclusion: In 8 patients with allergic symptoms on exposure to high levels of lady beetles, specific IgE bound to proteins from *H axyridis*. There was also an increased frequency of suspected cases of lady beetle allergy in endemic areas.

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INTRODUCTION

Lady beetles have long been respected in agriculture for their ability to consume aphids and other small insects detrimental to the farming industry. *Harmonia axyridis* (class: Insecta; order: Coleoptera; family: Coccinellidae) (Fig 1), a native species of Japan and Asia, was intentionally imported and released in the United States on several occasions between 1916 and 1981 as a biological control agent against crop-damaging aphids. After introductions in various locations, the first established *H axyridis* population was documented in southeastern Louisiana in 1988.[1] Since then, they have appeared across most of North America, except for Montana, Wyoming, and parts of the southwestern United States.[2] In many areas, they have dominated and displaced native lady beetle species.[2,3] *Harmonia axyridis*, more commonly known as multicolored Asian lady beetles (MALBs), have also been released in Europe, with established populations reported in France, Germany, the Netherlands, and, most recently, Britain.

Unfortunately, MALBs are gaining increasing notoriety as nuisance pests. Their once highly regarded reputation is overshadowed by their overwintering and migration habits that occur in the fall. With freezing temperatures and shortened photoperiods, MALBs innately seek sanctuary in crevices of cliffs and rock outcroppings and may return annually to the same sites. In many flat farming landscapes where tall rock formations are uncommon, the preferred overwintering site has become individual homes.[3] In 2003, distressed homeowners filed manifold complaints about masses of beetles invading their homes and swarming around their windows and doorways (North Central Region Integrated Pest Management Center, unpublished data, 2003). A survey of such homeowners in Ohio[3] showed that 70% of 1,148 respondents reported that the beetles emanated foul odors and emitted orange stains. More than 40% of the respondents reported that the beetles bit them. In homes infested with "millions of beetles, too numerous to count," 25% of survey respondents reported "allergic reactions" to the beetles. The purposes of this study were to further investigate "lady bug" allergy in patients complaining of symptoms on exposure to MALBs and to gain a better understanding of the significance and impact of MALBs as a cause of allergic disease in the United States.

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Figure 1. The multicolored Asian lady beetle ranges in length from 4.4 to 7.5 mm and may be orange, red, yellow, or black, with many, few, or no spots. The beetle may display a characteristic "M" pattern on its pronotum. Photograph by Scott Bauer, US Department of Agriculture, Agricultural Research Service, Beltsville, MD.

METHODS

Eight patients with allergic symptoms suspected of being caused by lady beetles were identified and interviewed by telephone after informed consent for participation in the study was obtained by the primary allergist. Parents were interviewed for patients younger than 18 years. Skin prick testing (SPT) was performed at the discretion of the primary allergist using a panel of aeroallergen extracts that included a lady beetle extract and positive (histamine, 1 mg/mL) and negative (sodium chloride solution, human serum albumin, and phenol) controls. Skin test reactions were scored on a scale from 0 to 4 (0 = no reaction, 1 = flare, 2 = wheal measuring <3 mm greater than the negative control, 3 = wheal measuring 3 mm greater than the negative control with a surrounding flare, and 4 = wheal measuring 3 mm greater than the negative control with flare and pseudopod). The nonirritant nature of the lady beetle extract used by the primary allergist (D.J.-W.) was verified in an equal number of patients with negative results.

Lady Beetle Extract

Lady beetles, collected from an infested home in the patients' regional area, were ground to a coarse powder using a mortar and pestle. One well-preserved beetle was sent to an entomologist to verify the identity of the beetles species as *H axyridis* (Pallas). Protein extraction was performed by stirring 5.6 g of ground beetles in a solution of 0.125M ammonium hydrogen carbonate at 4°C overnight. The solution was centrifuged at 2,000 rpm for 20 minutes. The supernatant was passed sequentially through 8-, 3-, 1.2-, 0.8-, and 0.45-μm filters. The filtrate was dialyzed against distilled water for approximately 24 hours at 4°C in 3,500-Da dialysis tubing. A cloudy precipitate formed in the dialysis tubing 6 hours after the second exchange of distilled water. On stirring, this precipitate emulsified. This solution was filtered a second time sequentially through 3-, 0.45-, and 0.2-μm filters. The resulting clear amber extract was frozen, lyophilized, and then stored at 4°C.

Using the technique of Lowry et al,[4] the extract was found to contain 572 μg of protein per 1 mg of extract. The extract (10 μg of lyophilized lady beetle extract/20 lambda of sample diluent) was electrophoresed on PAGEr Gold TRIS-glycine (Cambrex, Rockland, ME) 10% to 20% gradient precast gels using TRIS-glycine sodium dodecyl sulfate running buffer. ProSieve protein markers (Cambrex) were used for molecular weight controls, which provided 10 calibration points from 5 to 225 kDa.

Immunoblots

Beetle proteins from sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels were transferred to 0.45-μm nitrocellulose membranes. The membranes were washed, blocked with 20% fetal bovine serum or phosphate-buffered saline, and incubated overnight with 1:10 dilutions of each of the 8 patients' serum in 10% fetal bovine serum. Cord blood serum (diluted 1:10) was used as the negative control. The membranes were then washed, incubated with mouse anti–human IgE monoclonal antibody (Sigma-Aldrich Corp, St Louis, MO), washed again, and then incubated overnight with alkaline phosphatase–conjugated goat anti–mouse IgG (Chemicon International, Temecula, CA). After development with substrate, the molecular weight of each protein bound by IgE was determined from the molecular weight standards.

Immunohistochemical Analysis

Dead lady beetles were processed, embedded in paraffin, sectioned, placed on frosted positively charged slides, heat fixed at 60°C, and then deparaffinized using 3 changes of xylene for 3 minutes each and 4 changes of absolute ethanol for 2 minutes each. Slides were rinsed for 2 minutes, placed in Peroxidazed solution (Biocare Medical, Concord, CA) for 5 minutes to block endogenous peroxidase activity, and then rinsed in 2 changes of deionized water. Background components were blocked using Sniper (Biocare) for 5 minutes, according to the manufacturer's directions. Slides were rinsed, placed in Reveal solution (Biocare), and then put into the decloaking chamber. Tissue sections were overlaid with human serum containing lady beetle specific IgE (1:100 dilution) for 30 minutes, washed with 3 changes of Tris-buffered saline (TBS) (pH 6.8), covered with polyclonal horseradish peroxidase–conjugated rabbit anti–human IgEα at 1:50 dilution (DakoCytomation, Glostrup, Denmark) for 30 minutes, and then rinsed with 3 changes of TBS. Slides were covered with Secondary Link (Biocare) for 10 minutes, rinsed in 3 changes of TBS, treated with horseradish peroxidase label for 10 minutes, and rinsed again in 3 changes of TBS. Sections were developed using Romulin Red chromogen (Biocare) according to the manufacturer's directions, rinsed in deionized water for 2 minutes, counterstained with Gills III hematoxylin for 2 minutes, washed in water for 2

minutes, placed in ammonia water for 5 seconds, and washed again for 2 minutes. After slide dehydration and mounting, specimens were examined using light microscopy. Lady beetle tissue sections treated with universal negative control sera (Biocare) served as the negative control, and preexisting fetal villous stromal specimen sections served as the positive control.[5]

Last, questionnaires designed to evaluate the prevalence of practicing allergists' experiences with cases of lady beetle allergy were mailed to 200 randomly selected allergists across the continental United States. The survey consisted of 5 questions that evaluated the regional specificity of lady beetle allergy diagnoses, allergists' use of lady beetle extract for diagnosis or therapy, and allergists' interest in obtaining a commercialized lady beetle extract. Investigative protocols for the case interviews, the allergist survey, and the immunoblot and immunohistochemical procedures were approved by the Wilford Hall Medical Center institutional review board.

RESULTS

Patient Interviews

Eight unrelated patients, all females aged 10 to 50 years, were enrolled. All the patients remarked that their allergy symptoms seemed to develop or worsen during the fall, temporally coinciding with massive lady beetle invasions of their homes. The annual fall infestations began as early as September, shortly after crop harvesting. Patients estimated that they removed ½ to 8 cups of lady beetles from their homes daily during peak infestation in October and November. Patients reported that their allergy symptoms improved and often resolved in the spring with subsequent lady beetle clearance from their homes.

Seven patients complained of symptoms of worsening rhinitis, sneezing, congestion, or nasal itching beginning in October (Table 1). Five patients complained of watery, itchy, or red eyes when cleaning up lady beetles. One patient described recurrent symptoms consistent with scleral chemosis whenever she worked inside a barn heavily infested with beetles.

Five of 8 patients also complained of chest tightness, cough, wheezing, or shortness of breath during the fall and winter months, with acute worsening when vacuuming, sweeping, or killing beetles. Four of these 5 patients had a history of mild intermittent or exercise-induced asthma. These patients reported marked increases in albuterol use during fall and winter, prompting initiation of daily inhaled corticosteroid therapy during this time.

Three patients reported developing pruritic red welts on their skin several minutes after contacting or receiving a bite from lady beetles. The wheals ranged from 4 to 12 mm, appeared 10 minutes after the beetle bite, and often persisted for 2 to 24 hours before resolving. Patient 2, who reported recurrent symptoms of hives and rhinitis on exposure to lady beetles, also described the acute onset of 2 episodes of lip and eye angioedema, throat tightness, voice hoarseness, and shortness of breath during the time of peak lady beetle infestation of her home. She received emergency care on both occasions. During her first episode, her tryptase level measured 6 hours after the onset of symptoms was elevated at 13.4 ng/mL (reference range, $<$11 ng/mL). Other potential causes for her anaphylaxis were investigated, but no source was found. All of her symptoms resolved with the exodus of beetles from her home in the spring and have not since recurred.

Characteristics of the patients' homes were also considered in this study. Homes ranged in age from 5 to 75 years. More than half of the homes were abutted by heavily wooded areas. Six patients lived adjacent to farmland. Some owners noted that seasonal beetle invasions seemed heavier in years when soybean crops were harvested. More than 50% of the homes had high, arched, or cathedral ceilings. Some owners reported that the degree of infestation of their homes amplified annually until home renovations were undertaken, such as roof or chimney repairs, sealing of structural cracks and crevices, and removal of a nearby heavily infested barn. One patient, who experienced exacerbations of symptoms each fall for 4 years, appreciated the resolution of her symptoms on moving to a new home uninhabited by beetles.

Table 1. Summary of Symptoms, Skin Prick Testing, and IgE Immunoblots to MALB

Patient No./ sex/age, y	Asthma	Rhinitis	Conjunctivitis	Urticaria	MALB skin prick test result*	IgE blot protein weights, kDa
1/F/33	X	X		X	4	8.6, 28
2/F/30		X	X	X	0	75
3/F/35	X	X	X	X	4	8.6, 28, 75
4/F/33	X	X	X		4	8.6, 75
5/F/30	X	X	X		4	8.6
6/F/50			X		4	8.6
7/F/12	X	X			4	8.6, 31
8/F/10		X			4	8.6, 21, 28

Abbreviation: MALB, multicolored Asian lady beetle.

* See the "Methods" section for an explanation of the scoring system.

SPT and IgE Immunoblots

Results of SPT to lady beetle extract and the immunoblots are summarized in Table 1. Seven of the 8 patients had positive SPT reactions to the lady beetle extract, including 1 patient (patient 8) who developed a wheal measuring more than twice the size of the histamine control. One patient who had a positive SPT reaction to lady beetle extract had negative SPT and intradermal test results to an aeroallergen panel. Two patients had positive SPT reactions to dust mite, and none of the patients in this study demonstrated a positive reaction to a cockroach mix. In patient 2, SPT results to a lady beetle extract were negative. However, her immunoblot showed IgE binding to a protein at 75 kDa, shared with 2 other patients (Fig 2). In 7 patients, immunoblots showed serum specific IgE binding to a protein measuring approximately 8.6 kDa (Fig 2). Bound IgE was also demonstrated to proteins at 28 kDa in 3 patients, at 31 kDa in 1 patient, and at 21 kDa in 1 patient (Fig 2).

Immunohistochemical Analysis

Areas of red staining on the specimens revealed areas of high concentrations of lady beetle allergen within the beetle, whereas brown staining was indicative of chitin. Red staining was identified in the mouthparts of the lady beetle and in areas of the abdomen adjacent to the legs (Figs 3 and 4). No staining was noted in muscle, nervous, or reproductive tissues of the beetle. Slight nonspecific staining was noted in the negative control, but the staining intensity and tissue area involved were minimal compared with the study beetle and the positive control (figure not shown).

Results of the Allergist Survey

A survey was sent to approximately 3 to 5 randomly selected allergists in each state across the continental United States (N = 200 surveys). The response rate was 40%. Twenty-eight percent of responding allergists suspected the diagnosis of lady beetle allergy in a few of their patients. Few allergists performed SPT with a lady beetle extract. One allergist used lady beetle extract for SPT and for immunotherapy.

Most allergists who either diagnosed or suspected the diagnosis of lady beetle allergy in their patients practiced in the North Central, Northeast, and Southeast regions of the United States. More than 1 allergist in each of the states of North Carolina, Pennsylvania, Maryland, Ohio, Indiana, Kentucky, and Wisconsin reported suspecting the diagnosis of lady beetle allergy in 1 or more of their patients. Additional states with allergists reporting suspected lady beetle allergy included Minnesota, Illinois, Missouri, West Virginia, Virginia, New Jersey, South Carolina, Vermont, and New Hampshire.

DISCUSSION

The MALB has been identified as a possible trigger for allergic rhinoconjunctivitis and asthma in a few case reports.[6–10] Previously published reports have demonstrated evidence of IgE binding to lady beetle proteins at molecular weights of 16.6, 28.2, and 30 kDa and additional proteins

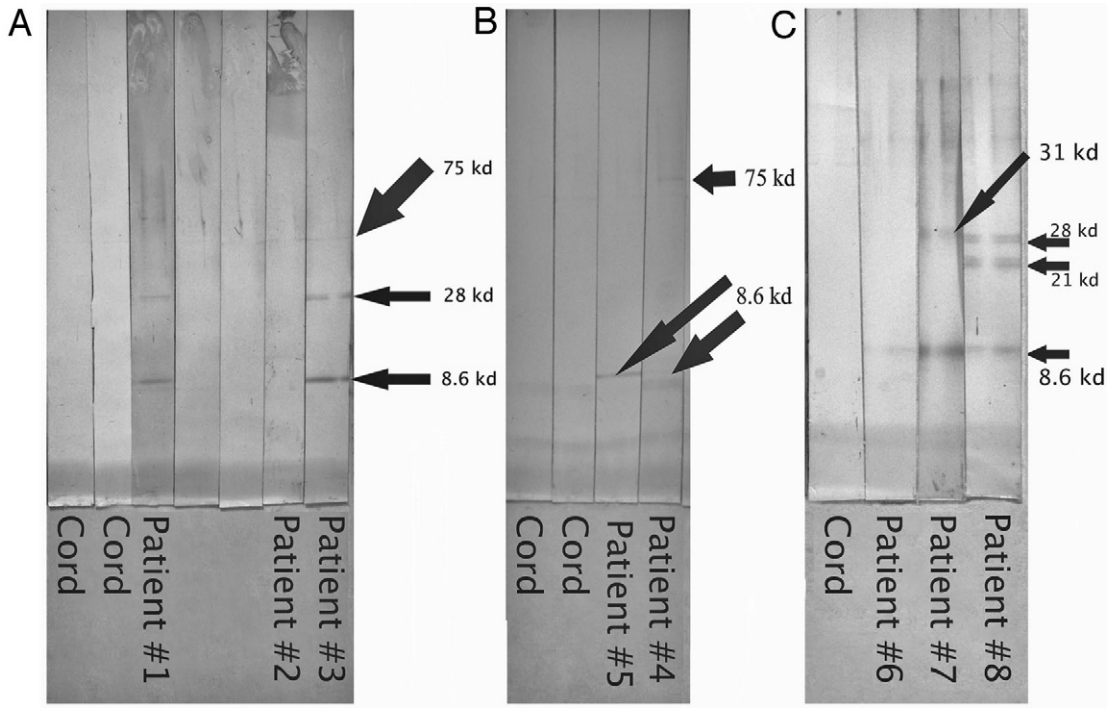

Figure 2. Immunoblots showing serum IgE binding to beetle proteins measuring approximately 8.6 kDa in patients 1, 3, 4, 5, 6, 7, and 8; 28 kDa in patients 1, 3, and 8; and 75 kDa in patients 2, 3, and 4. Patients 7 and 8 also show IgE binding to protein bands at 31 and 21 kDa, respectively.

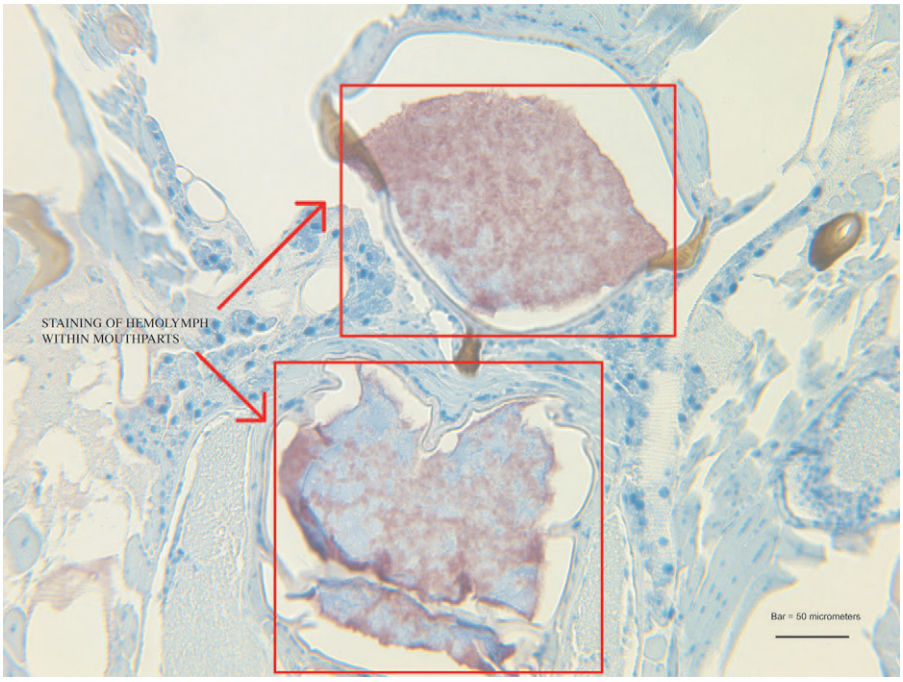

Figure 3. Immunohistochemical appearance of the lady beetle's mouthparts at high-power magnification. Red staining reveals areas of bound IgE.

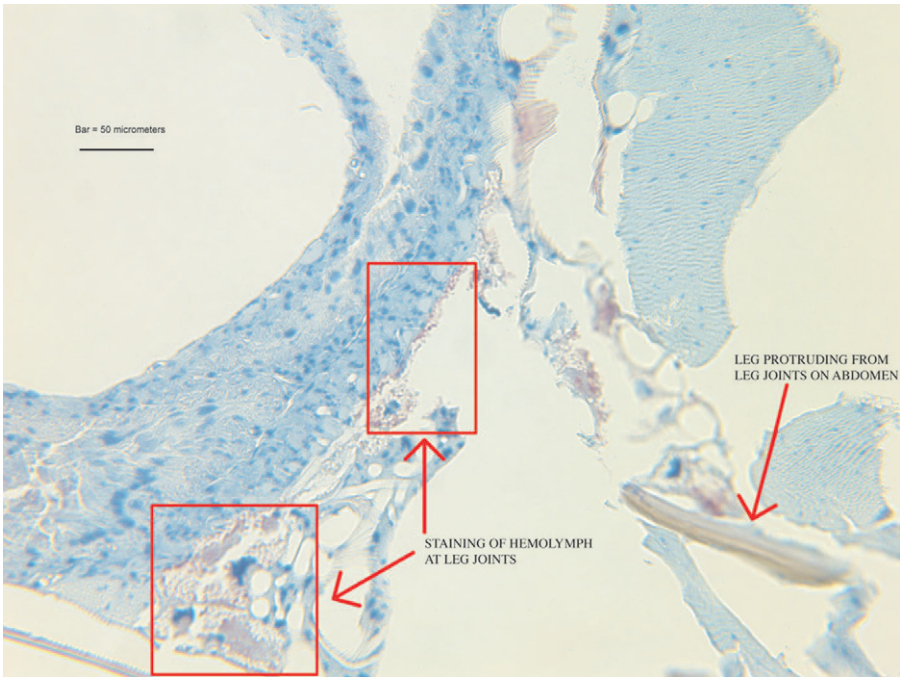

Figure 4. Immunohistochemical appearance of the lady beetle's leg protruding from the abdomen at high-power magnification. Red staining reveals areas of bound IgE.

between 40 and 80 kDa.[6–10] Seven of the 8 patients in the present study showed IgE binding to a protein measuring approximately 8.6 kDa that has not been previously reported. We speculate that this small approximate 8.6-kDa protein may dimerize to form the 16.6-kDa protein reported previously.[6–8]

Along with the demonstration of specific IgE to proteins in a whole-body lady beetle extract, the diagnosis of lady beetle allergy is further supported by the distinct seasonality and recurrence of symptoms on exposure paralleling the annual invasions of lady beetles in the patients' homes. Most homeowners reported that the beetle infestations of their homes had recurred for 1 to 3 years before the onset of their symptoms, suggesting a period of allergen sensitization. All the patients in this study reported that their symptoms improved or resolved during the spring months with the exodus of lady beetles from their homes. More than three fourths of the patients discontinued their maintenance asthma and allergy medications during this beetle-free period. Most of these patients, however, reported resuming their medication use in the fall owing to symptom relapse with the concomitant reappearance of beetles in their homes.

The onset and duration of the patients' symptoms reliably coincide with the overwintering behavior of MALBs. With colder weather and a shortened photoperiod, many lady beetles enter a dormant state or die, instigating enormous cleanup efforts. Most patients in this study were primarily involved in the cleanup and removal of the beetles from their homes, likely leading to their sensitization. Some patients reported that their symptoms due to lady beetles increased in the spring. This coincides with the increased activity of dormant MALBs in the spring with the onset of warmer weather and the lengthened photoperiod.

Although some patients were sensitized to other allergens, the seasonality of their symptoms correlated only to exposure to lady beetles. Consideration of mold allergy as an etiology was abandoned because most patients had negative test results to a variety of mold extracts. In addition, no correlation between lady beetle sensitization and dust mite or cockroach sensitization was found on SPT. This suggests that clinically significant cross-reactivity among these 3 allergens was absent in the patients in this study.

One patient (patient 2) with symptoms of recurrent urticaria, rhinitis, and conjunctivitis on beetle exposure also described 2 distinct episodes consistent with anaphylaxis in a 2-month period during peak beetle infestation of her home. Although her immunoblot showed a band at 75 kDa, results of SPT to lady beetle, performed approximately 1 year after her anaphylactic episodes, were negative. In the interval between her anaphylaxis and SPT, she had no further beetle exposure, which may explain the loss of SPT reactivity. She has remained asymptomatic since termination of her exposure to lady beetles. Although a direct antigen challenge (ie, mucosal, bronchial, or direct environmental challenge) might confirm beetle-induced anaphylaxis, it was not performed in this study. Because we could not verify that lady beetles were the cause of her anaphylaxis, this reaction is not included in Table 1.

The route of exposure to lady beetle allergens was also considered in this study. When perturbed, MALBs secrete orange-colored hemolymph laden with alkaloids from femoral-tibial pores in their legs in a process called *reflexive bleeding*.[11] The alkaloids assist in chemical defense and contribute to the beetle's offensive odor. In a study of "blood extracts" from *H axyridis*, the secreted fluid was found to contain allergenic proteins.[12] Additional allergens may originate from excrement or beetle body parts. Regardless of the source, the respiratory symptoms experienced by patients suggest that proteins from MALBs easily become airborne and are then inhaled. Recent entomology reports concede that MALBs occasionally bite humans. Thus, bites from MALBs likely introduce allergenic proteins into the skin, leading to IgE-mediated urticaria. A scanning electron microscope image reveals the appearance of coccinellid mouthparts in detail (Fig 5).

In the immunohistochemistry portion of this study, high concentrations of IgE binding appeared in the mouthparts and near the leg joints of the MALB specimen. This is not surprising given that the allergen-containing hemolymph[12] is secreted by the beetle through its femoral-tibial pores. Further studies with more samples and improvement of the fixing and staining processes to increase preservation of the beetle structure are needed to confirm these findings.

In the final portion of this study, the regional locations of physicians suspecting lady beetle allergic diathesis reflected that of the North Central Region Integrated Pest Management Center's account of lady beetle home invasions across the United States in 2003 (North Central Region Integrated Pest Management Center, unpublished data, 2003). These data showed that most reported home infestations originated from states in the North Central, Northeast, and Southeast regions of the United States (data not shown). A weakness of the survey is that the questionnaires were sent to only a fraction of the practicing allergists in each state, which may not adequately reflect the true impact of lady beetle allergy in this

Figure 5. Scanning electron microscope image of the coccinellid mouthparts. Image obtained from the Rippel Electron Microscope Facility, Dartmouth College, Hanover, NH.

country. Reporting bias may have also influenced this survey; however, two thirds of the allergists reported no suspicion of lady beetle allergy in their patients.

Sixty percent of responding allergists expressed interest in obtaining a commercialized lady beetle extract. Incorporating lady beetle SPT in his practice in West Virginia, Goetz[13] reported a 21% prevalence of lady beetle sensitization in 1,900 SPTs compared with prevalences of 27% and 40% for cockroaches and dust mites, respectively. These findings and the outcomes of the present study suggest that the interest in use of more widespread SPT for the evaluation for lady beetle allergy is not unfounded.

CONCLUSION

The seasonal invasion of homes by lady beetles causes significant distress and annoyance for homeowners. In addition, proteins from MALBs can cause a variety of IgE-mediated hypersensitivity reactions. We described the largest series of patients to date, including the first 2 cases in children, with immediate hypersensitivity attributable to MALBs. Symptoms caused by MALBs, at the minimum, include rhinitis, conjunctivitis, wheezing, and urticaria. We demonstrate that affected patients' serum IgE bound to MALB proteins with molecular weights of approximately 8.6, 21, 28, 31, and 75 kDa. In addition, through a novel immunohistochemical technique using whole beetle bodies, we demonstrated the binding of specific IgE to proteins in the beetle's mouth and in parts adjacent to the legs. Findings from the cases and the allergist survey suggest that the MALB is a rapidly emerging allergenic pest with significant implications for susceptible patients and for allergists practicing in endemic areas.

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REFERENCES

1. Chapin JB, Brou VA. *Harmonia axyridis* Pallas, the third species of the genus to be found in the United States (Coleoptera: Coccinellidae). *Proc Entomol Soc Wash*. 1991;93:630–635.
2. Koch RL. The multicolored Asian lady beetle, *Harmonia axyridis*: a review of its biology, uses in biological control, and non-target impacts. *J Insect Sci*. 2003;3:32:1–16.
3. Huelsman MF, Kovach J, Jasinski J, Young C, Eisley B. Multicolored Asian lady beetle as a nuisance in households in Ohio. In: Jones SC, Zhai J, Robinson WH, eds. *Proceedings of the 4th International Conference on Urban Pests*. Blacksburg, VA: Pocahonta Press; 2002:243–250.
4. Lowry OH, Rosenbrough NJ, Farr L, Randau RL. Protein measurement with folin phenol reagent. *J Biol Chem*. 1951;193: 265–275.
5. Ekstrom ES, Nilsson C, Holmlund U, et al. IgE is expressed on, but not produced by, fetal cells in the human placenta irrespective of maternal atopy. *Clin Exp Immunol*. 2002;127:274–282.
6. Yarbrough JA, Armstrong JL, Blumberg MZ, Phillips AE, McGahee E, Dolen WK. Allergic rhinoconjunctivitis caused by *Harmonia axyridis* (Asian lady beetle, Japanese lady beetle, or lady bug.) *J Allergy Clin Immunol*. 1999;104:704–705.
7. Magnan EM, Sanchez H, Luskin AT, Bush RK. Multicolored Asian lady beetle (*Harmonia axyridis*) sensitivity [abstract]. *J Allergy Clin Immunol.* 2002;109:s80.
8. Armstrong JL, Dolen WK, Phillips AE, Blumberg MZ, Yarbrough JA. Lady bug allergy [abstract]. *J Allergy Clin Immunol*. 1999;103:s166.
9. Kagen SL, Muthiah R. Ladybugs: a new cause of allergy [abstract]. *Ann Allergy Asthma Immunol*. 2000;84:146.
10. Pence HL, Esch RE, Armstrong JL, Dolen WK. Lady bug induced asthma [abstract]. *Ann Allergy Asthma Immunol*. 2000; 84:117.
11. Glisan King A, Meinwald J. A review of the defensive chemistry of Coccinellids. *Chem Rev*. 1996;96:1105–1122.
12. Magnan EM, Sanchez H, Bush RK. Detection of multicolored Asian lady beetle *Harmonia axyridis* allergens in their blood [abstract]. *J Allergy Clin Immunol*. 2003;111:s245.
13. Goetz DW. *Harmonia axyridis* ladybug hypersensitivity in clinical allergy practice [abstract]. *J Allergy Clin Immunol*. 2006; 117:s29.

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