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IgE against bed bug (*Cimex lectularius*) allergens are common among adults bitten by bed bugs

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To the Editor

Cimex lectularius, the bed bug, is found worldwide and likely has been sharing human dwellings dating back to prehistoric times. With increased use of pesticides after World War II, the reports of bed bugs in developed countries decreased significantly.(1) Recently *C. lectularius* has made a resurgence, including in New York City (NYC) where housing violations for bed bugs rose from 82 to 4,084 between 2005 and 2009. (2) The potential health implications of this increase in domestic exposure are not known; however, there is some evidence that exposed individuals can make a type-1 allergic response to *C. lectularius* bites (Table e1 in the article's Online Repository at www.jacionline.org).(3) In the 1990's, researchers in Egypt reported skin test positivity to crude *C. lectularius* extract among asthmatic patients.(4) Another case report identified *C. lectularius* nitrophorin protein (cNP), a nitric oxide carrying protein found in the injected saliva, that elicited an IgE antibody response in one patient.(5)(6, 7) The uniqueness of the predicted cNP sequence,(8) suggests that IgE to cNP would indicate a hypersensitivity response that is specific to *C. lectularius*. Aside from the single case described above, (5) the development of IgE antibodies to cNP among individuals bitten by bed bugs has not been reported. Our goal was to develop assays for measuring IgE antibodies against both *C. lectularius* extract and the cNP protein in order to determine the prevalence of sensitization to bed bug allergens among adults with reported bed bug bites.

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Thirty NYC residents who reported being bitten by bed bugs were recruited through web advertisement (www.craigslist.org), fliers and physician referral. Entry criteria for the study included report of having been bitten by a bed bug within the past year resulting in an itchy raised bump. Qualifying consenting participants donated serum and were queried about bed bug exposure and respiratory and allergic symptoms (wheezing, coughing, pruritic rash, itchy eyes, or runny nose) at the time of being bitten. Columbia University's Institution Review Board approved this study.

C. lectularius extract was prepared from 160 mg of dried *C. lectularius* (generously donated by Louis Sorkin, Museum of Natural History and George Keeney, Ohio State University) by freezing in liquid nitrogen and grinding them to powder using mortar and pestle. The powder was incubated for 2 hours in 600 μ l of phosphate buffered saline (PBS) with Tween 20 at 30 °C. After 10 minutes of centrifugation, the supernatant was dialyzed against PBS to remove Tween 20. Recombinant cNP (prepared from *E. coli* as described previously)(7) was dialyzed in PBS and diluted to 1.7 μ g/ml. Both recombinant cNP and the *C. lectularius* extracts were biotinylated (separately) using EZ-Link Sulfo-NHS-LC-Biotin (Pierce, Rockford, IL) in a 10 mM solution of the Sulfo-NHS-LC-Biotin reagent. The biotinylated cNP and *C. lectularius* extracts were bound separately to streptavidin ImmunoCAPs as described previously (Phadia, Portage, MI).(9) Serum from the subjects was then incubated separately with the *C. lectularius* and cNP ImmunoCAPs for 30 minutes at 37 °C. IgE concentrations were measured using standard ImmunoCAP methods.(9) Optimum *C. lectularius* and cNP coating dilutions were determined to be 67 μ g/ml and 264 μ g/ml, respectively, based on maximal IgE binding experiments with serum samples identified as positive in our initial screening. IgE against cockroach and dust mite, two major allergens in NYC, and total IgE were also measured to assess seroatopy. Inhibition of IgE binding to *C. lectularius* experiments were conducted with dust mite and cockroach allergen extracts (methods in Online Repository).

Seventeen (57%) subjects had detectable IgE (≥ 0.1 IU/ml) against the *C. lectularius* extract (Figure 1). Of those seventeen, nine subjects had detectable IgE against cNP, while eight did not. All subjects with IgE against cNP had measureable IgE against *C. lectularius* extract. The presence of cNP in the *C. lectularius* extract was confirmed by Western blot (Figure 2; methods in Online Repository). While there was a correlation between IgE to *C. lectularius* and cNP ($R=0.64$, $P=0.061$) among those with measurable IgE to both, there was a wide range in ratio of anti-cNP IgE:anti- *C. lectularius* IgE (0.05 to 3.2). Mean total IgE was higher among subjects with, as compared to subjects without IgE against *C. lectularius* extract (117 vs. 28.8 IU/ml, $P=0.002$) and cNP (125 vs. 47.6 IU/ml, $P=0.057$). The prevalence of IgE against cockroach was more common among subjects with, compared to without IgE against *C. lectularius* extract (65% vs. 35%, $P=0.024$). Among the 8 subjects with measurable IgE against *C. lectularius* extract but not cNP, most had IgE against dust mite (6 of 8 subjects) or cockroach (7 of 8). Among the subjects with IgE to dust mite and cockroach, dust mite and cockroach allergen partially (and in one case, completely) inhibited the IgE response to *C. lectularius*, while inhibition was minimal among the subjects without IgE to cockroach or dust mite (Table 1). Report of symptoms of wheeze, rhinitis or cough on the day of or day after the bite were similar among those without (≥ 0.1 IU/ml) as compared to those with IgE to either *C. lectularius* extract (54% vs. 41%, $P=0.49$) or cNP (52% vs. 33%, $P=0.34$).

In summary, we have developed assays for measuring IgE antibodies to both *C. lectularius* extract and a unique protein from *C. lectularius* (cNP). IgE antibodies to *C. lectularius* and cNP were common among adults who reported being bitten and having a visible response to bed bugs. That many (30%) individuals had IgE to cNP, which has limited homology to proteins from other species, suggests a response specific to bed bug exposure. That there

was variability in the correlation between concentrations of IgE to cNP and *C. lectularius* extract (including individuals with measurable IgE to *C. lectularius* extract, but not cNP) suggests that sensitized individuals were exposed to other allergens found in the *C. lectularius* extract in addition to cNP. Many subjects who had IgE to the *C. lectularius* extract also had IgE to dust mites and/or cockroaches and among these individuals, partial inhibition of binding to *C. lectularius* was observed with dust mite and cockroach extracts. This indicates the presence of cross reactivity with allergens from cockroaches and/or dust mites. While we were unable to demonstrate an association between allergic symptoms and IgE to either *C. lectularius* extract or cNP, this may be a function of our study's relatively small sample size and the limitations of retrospective reporting of symptoms. Given the large increase in human exposure to bed bugs in NYC and elsewhere and the demonstrated IgE response to allergens from *C. lectularius* it is clear that future studies need to examine the clinical relevance of IgE responses to bed bug allergens on allergic symptoms.

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Figure 1. Concentrations of IgE antibodies against *C. lectularius* (crude extract), *C. lectularius* nitrophorin (cNP) recombinant protein, *D. farinae* and German cockroach among the 30 adults reporting bed bug bites in the past year
 Absence of a bar indicates that the subject had undetectable IgE levels (i.e., <0.1 IU/ml).

Figure 2. Western blot of human (subject #23) IgE binding to cNP and *C. lectularius* extract
cNP is a 32 kD protein. A similar sized band can be seen in the extract. The slight difference in size likely is due to post-translational modifications.

Table 1

Percent inhibition[&] of IgE binding with (A.) dust mite and (B.) cockroach extracts.

A. Inhibition with dust mite extract

Subject	Co-sensitized to dust mite	Inhibition of IgE against*	
		Dust mite	<i>C. lectularius</i>
25	Yes	97%	>77% [#]
27	Yes	95%	88%
8	No	-	14%
23	No	-	4%

B. Inhibition with cockroach extract

Subject	Co-sensitized to dust mite	Inhibition of IgE against*	
		Cockroach	<i>C. lectularius</i>
3	Yes	89%	79%
27	Yes	93%	58%
8	No	-	14%
23	No	-	4%

[&]Percent inhibition was calculated by subtracting the IgE measured after pre-incubation with allergen extract from IgE measured after incubation with phosphate buffered saline and dividing by the latter.

* IgE against dust mite, *C. lectularius* or cockroach measured after inhibition.

[#] After incubation with dust mite extract, IgE to *C. lectularius* was below the limit of detection (<0.1 IU/ml); however, the uninhibited sample was also low (0.43 IU/ml).