

Letter to the Editor

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

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 4084 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 (see Table E1 in this article's Online Repository at www.jacionline.org).³ In the 1990s, 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 1 patient.⁵⁻⁷ The uniqueness of the predicted cNP sequence⁸ 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 the *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.

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 resulting in an itchy raised bump within the past year. 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.

The *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 it in liquid nitrogen and then grinding it to a powder by using

mortar and pestle. The powder was incubated for 2 hours in 600 μ L of 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 *Escherichia 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) by using EZ-Link Sulfo-NHS-LC-Biotin (Pierce, Rockford, Ill) 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, Mich).⁹ Serum from the subjects was then incubated separately with *C. lectularius* and cNP ImmunoCAPs for 30 minutes at 37°C. IgE concentrations were measured by using standard ImmunoCAP methods.⁹ Optimum *C. lectularius* and cNP coating dilutions were determined to be 67 and 264 μ g/mL, respectively, on the basis of maximal IgE binding experiments with serum samples identified as positive in our initial screening. IgE levels against cockroach and dust mite, 2 major allergens in NYC, and the total IgE level were also measured to assess seroatopy. Inhibition of IgE binding to *C. lectularius* experiments were conducted with dust mite and cockroach allergen extracts (see the Methods section in this article's Online Repository at www.jacionline.org).

Seventeen (57%) subjects had detectable IgE levels (≥ 0.1 IU/mL) against the *C. lectularius* extract (Fig 1). Of these 17, 9 subjects had detectable IgE level against cNP, while 8 did not. All subjects with IgE against cNP had measureable levels of IgE against the *C. lectularius* extract. The presence of cNP in the *C. lectularius* extract was confirmed by Western blot (Fig 2; see the Methods section in this article's Online Repository at www.jacionline.org). While there was a correlation between IgE to *C. lectularius* and cNP ($R = 0.64$, $P = .061$) among those with measurable IgE levels to both, there was a wide range in the anti-cNP/IgE/anti-*C. lectularius* IgE ratios (0.05-3.2). The mean total IgE level was higher among subjects with than among subjects without IgE against the *C. lectularius* extract (117 vs 28.8 IU/mL; $P = .002$) and cNP (125 vs 47.6 IU/mL; $P = .057$). The prevalence of IgE against cockroach was more common among subjects with than among subjects without IgE against the *C. lectularius* extract (65% vs 35%; $P = .024$). Among the 8 subjects with measurable IgE levels against the *C. lectularius* extract but not cNP, most had IgE against dust mite (6 of 8 subjects) or cockroach (7 of 8 subjects). Among the subjects with IgE to dust mite

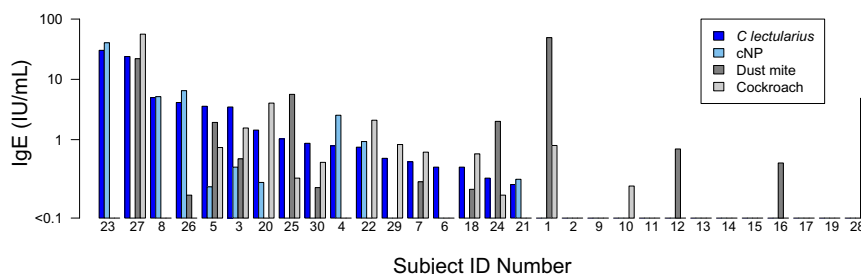


FIG 1. Concentrations of IgE antibodies against *C. lectularius* extract, *C. lectularius* nitrophorin recombinant protein, *Dermaphagoides farinae*, and German cockroach among the 30 adults reporting bed bug bites in the past year. Absence of a bar indicates that the subject had undetectable IgE levels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

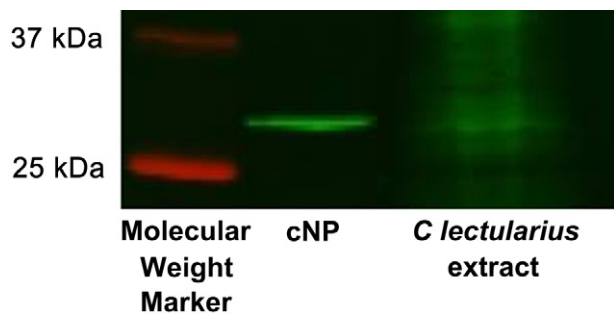


FIG 2. Western blot of human (subject 23) IgE binding to cNP and *C lectularius* extract. cNP is a 32-kDa protein. A similar sized band can be seen in the extract. The slight difference in size likely is due to posttranslational modifications of the cNP.

and cockroach, dust mite and cockroach allergen partially (and in 1 case, completely) inhibited the IgE response to *C lectularius*, while inhibition was minimal among the subjects without IgE to cockroach or dust mite (Table I). Reports of symptoms of wheeze, rhinitis, or cough on the day of or the day after the bite were similar among those without (≥ 0.1 IU/mL) than among those with IgE to either the *C lectularius* extract (54% vs 41%; $P = .49$) or cNP (52% vs 33%; $P = .34$).

In summary, we have developed assays for measuring IgE antibodies to both the *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 the *C lectularius* extract (including individuals with measurable IgE levels to the *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 the *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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TABLE I. Percent inhibition* of IgE binding with dust mite and cockroach extracts

A. Inhibition with dust mite extract			
Subject	Cosensitized 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	Cosensitized 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 level measured after preincubation with allergen extract from the IgE level measured after incubation with PBS and dividing by the latter.

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

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

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METHODS

Inhibition experiments

From subjects with IgE to *C lectularius*, 2 subjects with IgE to dust mite, 2 subjects with IgE to cockroach, and 2 subjects without IgE to dust mite or cockroach were selected for inhibition experiments. Skin test extracts for *Der-matophagoides farina* (dust mite) and *Blatella germanica* (cockroach) (Greer, Lenoir, NC) were buffer exchanged with PBS in order to remove glycerin by using the Amicon Ultra-0.5 centrifugal filter unit with a molecular cutoff of 10 kDa (Millipore, Billerica, Mass). Serum (25 μ L) was incubated at 37°C with either cockroach or dust mite extract or PBS (25 μ L) for 2 hours. Samples were then assayed for IgE against the *C lectularius* extract, cockroach, or dust mite as described for the undiluted samples. Percent inhibition was calculated by subtracting the IgE level measured after incubation with allergen extract from the IgE level measured after incubation with PBS and dividing by the latter concentration.

Western blot

Approximately 25 μ g of cNP and 240 μ g of the *C lectularius* extract in sample buffer (6% glycerol [vol/vol]; 30 mM Tris, pH 6.8; 1.2 mM EDTA; 1.2% SDS [wt/vol]; and 8.64 mM β -mercaptoethanol) were loaded in different wells of a 12 + 2 well precast Tris-HCl gel (4%-15%) (Criterion; Bio-Rad Hercules, Calif). Gels were run at 50 mA for 1.5 hours and then protein was transferred onto a nitrocellulose membrane (500 mA for 1 hour). The membrane was blocked for 1 hour with Odyssey Blocking Buffer (Licor, Lincoln, Neb) before being incubated overnight at 4°C with subject 23's serum (1:10). Membranes were then incubated with a goat antihuman IgE epsilon DyLight 800 (1:1000, Antibodies Online, Atlanta, Ga) before being visualized by using the Odyssey imaging system (Licor).

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TABLE E1. Previous reports of clinical reactions to bed bugs

Author (year)	No. of patients	Primary clinical presentation	Secondary reaction
Kinnear (1948) ^{E1}	6	Erythematous bullae on calves	None reported
Parsons (1955) ^{E2}	1	Itchy lesions and mild neck swelling within 2 h after bites	Upper extremity, neck, and perioral edema, severe dyspnea within 3.5 h of bites; diagnosed as anaphylaxis
Sansom et al (1992) ^{E3}	2	Patient 1 developed diffuse intensely pruritic, papular lesions 60 h after bites; patient 2 developed nonpruritic, erythematous, papular lesions	Patient 1 developed florid, hemorrhagic, bullous rash with edema 6 d after bites
Tharakaram (1999) ^{E4}	1	Pruritic, erythematous rash that evolved into a vesiculobullous eruption over 3 wk	None reported
Fletcher et al (2002) ^{E5}	1	Extremely pruritic widespread bullous eruption; urticarial papules and plaques	None reported
Liebold et al (2003) ^{E6}	1	Papular pruritic rash	Second exposure 4 wk later developed diffuse pruritic, erythematous large tense bullae with mild pyrexia
Ter Poorten et al (2005) ^{E7}	1	Diffuse intensely pruritic erythematous macules and papules; few discrete urticarial papules	None reported
Leverkus et al (2006) ^{E8}	1	Multiple pruritic, erythematous papules, nodules, and blisters; several nodules with underlying erythema and moderate peripheral whealing	None reported
Scarupa and Economides (2006) ^{E9}	17	Papular urticaria–like dermatitis	None reported
Masetti and Bruschi (2007) ^{E10}	2	Patient 1 developed pruritic erythematous macules and papules; patient 2 developed severe diffuse pruritic bullous eruptions	None reported
Goddard and deShazo (2008) ^{E11}	1	Pruritic, erythematous “bumps” 1 d after bite	Two days after initial bites developed erythematous papules; 12 d after initial bites rash improved but developed worsening pruritis
Stucki and Ludwig (2008) ^{E12}	1	Multiple pruritic erythematous papules 1 d after bites	None reported
Mumcuoglu (2008) ^{E13}	1	Pruritic erythematous “spots”	None reported
Levy Bencheon et al (2011) ^{E14}	2	Patient 1 developed diffuse pruritic erythematous maculopapular lesions with purpuric centers; patient 2 developed pruritic rash of several months	None reported