

Allergenicity and Cross-Reactivity of Booklice (*Liposcelis bostrichophila*): A Common Household Insect Pest in Japan

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Key Words

Asthma · Booklice · Cross-reactivity · Environmental allergen · Insect allergen · Respiratory allergy

Abstract

Background: Booklice (*Liposcelis bostrichophila*) are a common household insect pest distributed worldwide. Particularly in Japan, they infest 'tatami' mats and are the most frequently detected insect among all detectable insects, present at a frequency of about 90% in dust samples. Although it has been hypothesized that they are an important indoor allergen, studies on their allergenicity have been limited. **Methods:** To clarify the allergenicity of booklice and the cross-reactivity of this insect allergen with allergens of other insects, patients sensitized to booklice were identified from 185 Japanese adults with allergic asthma using skin tests and IgE-ELISA. IgE-inhibition analysis, immunoblotting and immunoblotting-inhibition analysis were performed using sera from these patients. Allergenic proteins contributing to specific sensitization to booklice were identified by two-dimensional electrophoresis and two-dimensional immunoblotting. **Results:** The booklouse-specific IgE antibody was

detected in sera from 41 patients (22% of studied patients). IgE inhibition analysis revealed that IgE reactivity to the booklouse allergen in the sera from one third of booklouse-sensitized patients was not inhibited by preincubation with extracts from any other environmental insects in this study. Immunoblotting identified a 26-kD protein from booklouse extract as the allergenic protein contributing to specific sensitization to booklice. The amino acid sequence of peptide fragments of this protein showed no homology to those of previously described allergenic proteins, indicating that this protein is a new allergen. **Conclusions:** Sensitization to booklice was relatively common and specific sensitization to this insect not related to insect panallergy was indicated in this population.

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Introduction

Airborne insect-derived particles have been implicated as a cause of respiratory allergy [1–5]. In particular, the significance of insects as occupational allergens is well established [1, 6, 7]. Considering that a large percentage



Fig. 1. *L. bostrichophila* (booklouse, banded psocid) (photo: Y. Kawakami).

of the general allergic asthma population is sensitized to any insect allergen, insect allergens are also presumed as important environmental allergens [8–13]. However, reports showing the significance of insect allergens other than cockroach allergens as indoor allergens have been limited. In particular, booklice are one of the most common household insect pests in tropical and subtropical countries and have been presumed as an important indoor environmental allergen. However, studies on the allergenicity of this insect have been quite limited.

Booklice are a common small household insect pest belonging to the Psocoptera order, members of which are distributed in all geographical regions, from the tropical to subarctic zones. Particularly in Japan, entomologic research on house dust samples has shown that booklice are the most frequently detected insect among all detectable insects, present at a frequency of about 90% in dust samples [14]. More than 3,000 species of booklice have been reported worldwide; more than 90 of these species have been reported in Japan [15]. Although most of them live outdoors, a few wingless species invade homes, factories and grain storage facilities. *Liposcelis bostrichophila* (booklouse, banded psocid) is one of the most common home-inhabiting species of booklice, which are brownish, soft-bodied and 1.0–1.3 mm long when fully grown (see fig. 1) [15, 16]. The primary food source for booklice is molds. Therefore, booklice prefer warm, damp conditions and are usually abundant in homes in summer and autumn [17]. They are called booklice because they infest old books, sheets of paper and stored food products in homes. They are also known to infest ‘tatami’, Japanese

traditional flooring mats. Despite being called booklice, they are not lice and do not feed on living animals.

In an earlier study in 1975 in Japan, serological IgE reactivity to booklice has been examined [18]. This study implies that the allergenicity of booklice is different from that of house dust mites. A study from India has shown that about 20% of Indian respiratory allergic patients are sensitized to booklice [19]. Immunoblotting analysis using their sera identified 67-, 59-, 43- and 27-kD booklouse proteins as the major allergens of booklice. However, although many previous studies have consistently shown cross-reactivity among arthropod allergens [20–22], the cross-reactivity between the booklouse allergen and other insect allergens has not been studied. Therefore, in this study, we assessed the cross-reactivity between the booklouse allergen and other arthropod allergens to clarify the genuine allergenicity of booklice.

The aim of our study was to elucidate the prevalence of sensitization to booklice (*L. bostrichophila*) and the allergenicity of booklice in the Japanese allergic asthma population. To exclude possible cross-reactivity between the booklouse allergen and other insect allergens, we assessed the sensitization profiles for all the insects that have been considered important in the literature and are frequently detected in indoor environments in Japan, and performed inhibition analysis. Immunoblotting and two-dimensional electrophoresis were also performed to identify the allergenic protein that contributes to specific sensitization to this insect.

Methods

Patient Selection

The patients were recruited from consecutive outpatients with asthma who first visited the allergy department of Sagami National Hospital between June 2007 and May 2009. The inclusion criteria for this study were (1) asthma patients meeting the asthma criteria of the American Thoracic Society and (2) patients who are considered to have allergic asthma. Allergic asthma was defined as showing positive results to one or more respiratory allergens by the screening skin intradermal test (the list of tested allergens is shown in online suppl. fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000329853). Finally, 185 allergic asthma patients met the study criteria and were enrolled in the study. This study was approved by the Ethics Committee of Sagami National Hospital, and all the patients provided their written informed consent.

Preparation of Extracts from Booklice, Other Insects and House Dust Mites

Booklice (*L. bostrichophila*) were reared on a diet of a mixture of 30 g of dried yeast (Asahi Food and Healthcare Ltd.) and 0.5 g of powdered rodent diet CE-2 (CLEA Japan Inc.). Booklice that

had been bred for 45 days at 25°C were used as the material. Afterwards, adult individuals that had been reared for successive generations were used for experiments. The booklice were carefully picked up one by one and gathered. The booklice were frozen, lyophilized, and defatted with ether. A small part of them were extracted using Coca's solution (0.5% NaCl, 0.275% NaHCO₃, 0.4% phenol) at 1:1,000 dilution (w/v) and used for the skin intradermal test. The remaining part was extracted in 0.125 M ammonium bicarbonate for 24 h at 4°C. After centrifugation (10,000 g), the supernatant was dialyzed against 5 mM ammonium bicarbonate and lyophilized.

Extracts of house dust mites and insects other than booklice that are considered important in the literature and are frequently detected in indoor environments in Japan were also prepared. Indian meal moths (*Plodia interpunctella*, IM), cigarette beetles (*Lasioderma serricorne*, CB) and house dust mites (*Dermatophagoides pteronyssinus*, DP) were also cultivated under the conditions appropriate for them. German cockroach (*Blattella germanica*, GC), midges (*Chironomus yoshimatsui*, MI), mosquitoes (*Cullidae* sp., MO), houseflies (*Musca domestica*, HF) and the wings of a silkworm (*Bombyx mori*, SM) were purchased from commercial suppliers. Their extracts were prepared as described for booklice.

Measurement of Level of Serum-Specific IgE Antibody

Colorimetric ELISA was carried out to determine the level of serum-specific IgE antibodies to these allergens as previously described [23]. Cyanogen bromide-activated paper disks were coupled with each preparation of allergen extract at 100 µg dry weight/disk. Allergen disks were incubated with 50 µl of patient serum for 3 h at room temperature. The disks were then washed and 50 µl of β-galactosidase-conjugated anti-human IgE (CAP System; Phadia AB) was added to them. After overnight incubation, the bound-enzyme activity was measured. The assay was calibrated using a control curve obtained from disks coupled with Japanese cedar pollen extracts and serial dilutions of pooled serum from patients with Japanese cedar pollen allergy; the level of the IgE antibody in these patients was measured using the CAP system (Phadia AB) in advance. The results were extrapolated from the control curve and expressed as units/ml. An IgE antibody level of ≥0.35 units/ml was regarded as positive.

Clinical and Environmental Risk Factors Associated with Sensitization to Allergens

The patients were also asked to complete a structured questionnaire focusing on possible environmental factors associated with allergen sensitization on the same day of the screening skin intradermal test. This questionnaire included questions about the age of the building they are living in, housing type (apartment or detached), type of flooring in the living room and bedroom (tatami mats, carpet or none) and amount of sunlight in their house (sufficient or insufficient). Multivariate logistic regression analysis was performed to identify risk factors for sensitization to each allergen (level of specific IgE antibody ≥0.35 units/ml).

IgE Inhibition Analysis

A pooled serum from booklouse-sensitized patients was prepared by mixing equivalent amounts of sera from the patients (n = 33) with the IgE antibody to booklice at ≥0.70 units/ml. IgE inhibition analyses using booklouse extracts as the solid phase

and extracts from CB, IM, GC, MI, SM, MO, HF, DP and booklice as inhibitors were performed using this pooled serum. First, the booklice extract was coated on a polystyrene microtiter plate with 96 wells (1 µg dry weight/well), and diluted serum was preincubated with a serial dilution (1:10) of an inhibitor extract (from 0.1 µg/ml to 1 mg/ml) and applied to booklouse extract-coated wells. IgE reactivity in this inhibition analysis was measured by fluorometric ELISA as previously described [24]. The results were calculated as a percentage of inhibition.

To examine individual patterns of cross-reactivity, IgE inhibition analyses using booklouse extracts as the solid phase and extracts from CB, IM, GC, SM, HF, DP and booklice as inhibitors (the concentrations of inhibitors were 0.064, 0.32, 1.6, 8, 40 and 200 µg dry weight/ml, respectively) were also performed using sera from the booklouse-sensitized patients.

SDS-PAGE, Isoelectric Focusing Gel Analysis and Two-Dimensional Electrophoresis

SDS-PAGE was carried out using 14% polyacrylamide gel (TEFCO) under reducing condition and proteins were stained with Coomassie Brilliant Blue R-250. Isoelectric focusing gel electrophoresis was performed using ampholine polyacrylamide gel, pH 3–10 (TEFCO), in accordance with the manufacturer's instructions. For two-dimensional electrophoresis, the extract was purified by ammonium sulfate precipitation, and the resulting precipitate was suspended in rehydration buffer. Then, 100 µg of purified booklouse extract was applied to an immobilized pH 3–5.6 gradient strip (GE Healthcare) for rehydration. Strips were equilibrated and then loaded onto 14% SDS-polyacrylamide gel. The electrophoresed isoelectric focusing and two-dimensional SDS gels were also stained with Coomassie Brilliant Blue.

Immunoblotting and Immunoblotting-Inhibition Analysis

Protein bands separated by SDS-PAGE were transferred onto nitrocellulose membranes using a Model STB-88 Safety Blotting unit (TEFCO) in accordance with the manufacturer's instructions. After incubation overnight with a blocking buffer (PBS containing 3% bovine serum albumin and 0.1% Tween 20), the membranes were incubated with the patients' sera diluted at 33% for 1 h at room temperature, followed by horseradish-peroxidase-conjugated goat anti-human IgE (Biosource International). The antibody-reactive bands were detected by the enhanced chemiluminescence system (ECL Western blotting analysis system; GE Healthcare) in accordance with the manufacturer's instructions. When immunoblotting inhibition was carried out, patient sera (diluted at 20%) were preincubated with an excessive dose of inhibitor allergen extracts for 1 h at room temperature, and immunoblotting was performed as described above.

Enzymatic Digestion, Isolation of Peptide Fragments and Amino Acid Sequencing

Protein spots of interest were obtained from two-dimensional gels, and in-gel trypsin digestion was performed. Peptide fragments were separated by reverse-phase HPLC, and peptide peaks were monitored at 210 nm using a UV detector. Amino acid sequencing was performed on selected peaks using a protein sequencer (Procise 494 HT; Applied Biosystems). The sequence obtained was compared with protein sequences in the BLAST and UniProt databases.

Statistical Analyses

Spearman's rank correlation test was performed to determine the correlations between the levels of the IgE antibody to two allergens, using sera from patients in whom IgE antibody to either of these two allergens could be detected. For categorical variables, Fisher's exact test was performed for significance testing. Data were analyzed using SPSS for Windows version 11.0 (SPSS Inc.).

Results

Prevalence of Sensitization to Booklice

The prevalence of positive skin intradermal test results for the booklouse allergen was 42%, which was the third most frequently detected allergen among the screened allergens (see online suppl. fig. 1). However, the prevalence of the IgE antibody to booklice was 22% (see fig. 2), which was the third highest prevalence among the studied insects.

The prevalence of the IgE antibody to DP was markedly high (73%). That to SM was 35%, which was the highest among the studied insect allergens, whereas that to GC was relatively low (16%). These sensitization profiles for arthropod allergens of this study population were compatible with those in the previous studies in Japan [25–27].

Clinical and Environmental Factors Associated with Sensitization to Studied Allergens

Multivariate logistic regression analysis revealed clinical and environmental factors associated with the presence of the IgE antibody to studied arthropod allergens (see online suppl. table 1). Generally, male gender and current smoking were associated with sensitization to booklice and other studied arthropod allergens. On the other hand, using tatami mats and/or carpets in bedrooms was a significant risk factor for sensitization only to booklice with an odds ratio of 5.9 (95% confidence interval, 1.2–29.0), whereas this was not significantly associated with sensitization to other arthropod allergens.

Cross-Reactivity between Booklouse Allergen and Other Arthropod Allergens

To elucidate a possible cross-reactivity between the booklouse allergen and other arthropod allergens, the levels of IgE antibody to the insect allergens were compared. Although there were a few exceptions, the correlations between the levels of the IgE antibody to insect allergens were generally moderate to strong (data not shown). In particular, strong correlations were observed between the levels of IgE antibody to SM and HF, those

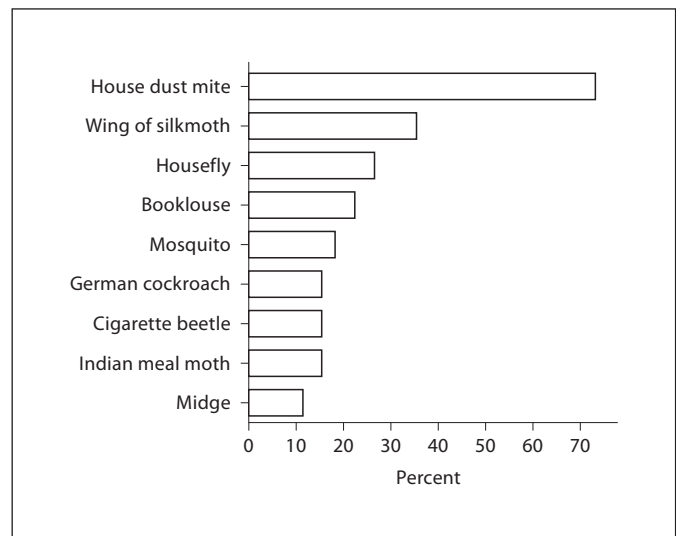


Fig. 2. Prevalence of IgE antibody to environment arthropod allergens among studied allergic asthma patients (n = 185).

to SM and MI, and those to HF and MI (correlation coefficients: 0.63, 0.72, and 0.74, respectively). However, as shown in figure 3a, the correlations between the levels of the IgE antibody to booklice and other insects were weak to moderate and that between those to booklice and DP was weak.

IgE inhibition analysis was also performed on the pooled serum from 33 booklouse-sensitized patients with the booklouse-specific IgE antibody at ≥ 0.70 units/ml. As shown in figure 3b, none of the other arthropod allergen extracts could fully inhibit the IgE reactivity to the booklouse allergen, indicating the existence of allergenic proteins that have allergenicity independent of that of other arthropod allergens in the booklice extract. Inhibition by shrimp (*Penaeus japonicus*) tropomyosin, Pen j 1 (concentrations ranging from 3.2 ng/ml to 10 μ g/ml), was also examined (data not shown), but no inhibition was observed (maximal inhibition, 0%).

To explore individual patterns of IgE cross-reactivity between the booklouse allergen and other arthropod allergens for each patient, inhibition analyses were separately repeated on 33 booklouse-sensitized patients (table 1). We classified the patients into two groups according to the results of these inhibition analyses: 22 patients whose IgE reactivity to booklice was inhibited by more than 50% by any other studied arthropod allergens were considered as patients with a cross-reactive sensitization pattern (group C). However, specific sensitization to the booklouse aller-

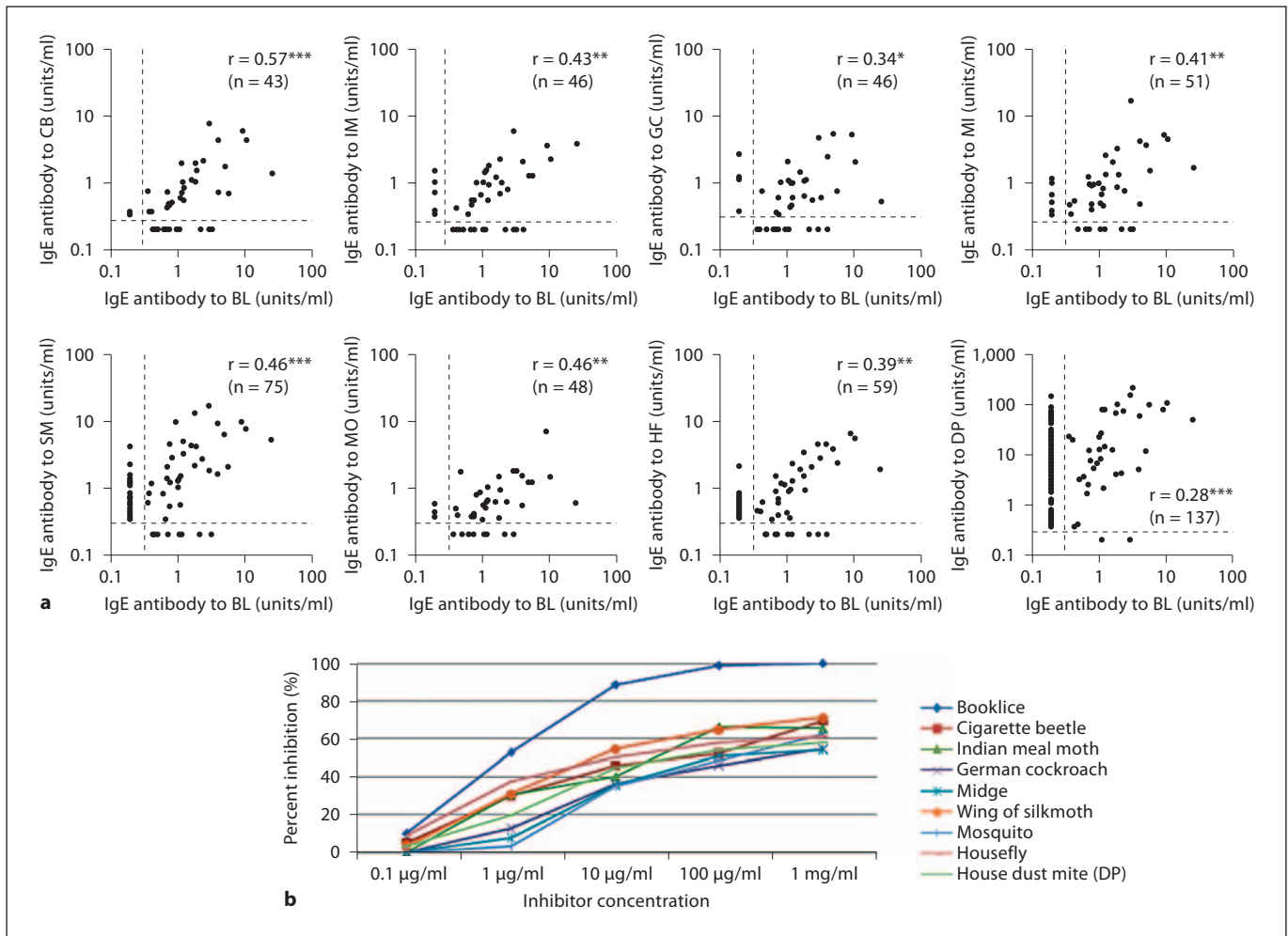


Fig. 3. Cross-reactivity between booklouse allergen and other arthropod allergens. Correlations between levels of IgE antibody to booklice and those to other arthropods (a). IgE inhibition analysis in pooled serum from 33 booklouse-sensitized patients (b). BL = Booklice; CB = cigarette beetle; IM = Indian meal moth; GC = German cockroach; MI = midge; SM = wing of silkworm; MO = mosquito; HF = housefly; DP = *Dermaphagoides pteronyssinus*. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

gen, not inhibited by any other arthropod allergens, was observed in 11 patients, and they were considered as patients with a specific sensitization pattern (group S).

Immunoblotting and Immunoblotting Inhibition

To elucidate the allergenic protein contributing to the specific sensitization to booklice, the results of immunoblotting of booklice extract were compared between groups S and C. The patterns of SDS-PAGE and immunoblotting of the booklice extracts are shown in figure 4a. The immunoblotting detection of the booklice extracts showed positive in 25 (76%) of the 33 studied patients with the booklouse IgE antibody >0.70 units/ml.

Eight allergenic bands were detected; that is, 100, 85, 43, 40, 35, 31, 26 and 16-kD protein bands. The prevalences of detection of these bands were 21, 52, 15, 24, 27, 18, 36 and 9%, respectively. Therefore, the 85- and 26-kD protein bands were considered to correspond to the major allergens for booklouse allergy.

Group S patients were significantly more likely to be sensitized to the 26-kD protein than group C patients (prevalences of sensitization: 64% for group S and 23% for group C; $p = 0.02$). Moreover, for 5 of 11 patients of group S (patients No. 4, 7, 9, 14 and 24), the only protein detected in the booklouse extract was the 26-kD protein. Immunoblotting inhibition analysis was performed on two

Table 1. Sensitization profiles and results of IgE inhibition analyses of sera from booklouse-sensitized patients

Patient No.	Group S/C ^a	Level of serum-specific IgE antibody (class ^b)										IgE inhibition analysis (maximum inhibition)						
		BL	CB	IM	GC	MI	SM	MO	HF	DP	CB	IM	GC	SM	HF	DP	BL	
<i>Patients with specific sensitization pattern</i>																		
1	S	3	2	2	2	2	2	2	2	5	0	0	0	6	12	3	97	
2	S	4	2	3	1	2	3	1	2	4	0	33	0	40	4	3	100	
3	S	2	0	0	1	0	0	2	2	6	0	7	0	7	0	4	98	
4	S	2	0	0	0	0	0	0	0	3	0	0	0	2	0	4	97	
5	S	3	2	0	0	1	2	1	0	5	22	24	9	35	34	29	94	
6	S	2	0	2	2	0	2	0	1	4	20	43	34	0	0	1	93	
7	S	2	0	0	2	1	2	1	1	3	10	18	11	7	21	18	71	
8	S	2	0	0	0	1	1	1	2	6	15	27	9	18	13	16	93	
9	S	2	1	0	0	0	0	0	0	3	0	0	4	7	6	3	86	
10	S	2	0	0	0	0	0	0	0	4	6	27	0	23	21	26	94	
11	S	2	0	1	0	2	3	2	2	3	28	21	0	18	7	31	93	
<i>Patients with cross-reactive sensitization pattern</i>																		
12	C	3	3	3	3	3	3	3	3	5	86	81	80	83	83	86	99	
13	C	2	2	0	1	2	2	1	2	5	95	94	56	91	95	93	99	
14	C	3	3	2	2	3	3	2	3	6	99	96	95	99	99	98	100	
15	C	3	2	2	3	3	3	2	3	3	71	67	62	80	81	70	97	
16	C	2	2	2	1	2	2	1	2	5	97	91	93	95	97	88	98	
17	C	2	0	0	0	0	2	0	0	0	57	1	19	35	22	43	97	
18	C	2	2	1	1	0	0	0	0	2	98	86	96	43	20	5	88	
19	C	2	2	2	2	2	3	1	2	3	92	92	80	94	92	92	96	
20	C	2	2	2	2	2	3	2	2	6	96	93	79	95	96	91	99	
21	C	2	3	3	3	3	3	2	3	6	62	66	73	78	95	56	87	
22	C	2	1	2	2	2	2	2	2	3	94	86	84	69	50	35	80	
23	C	2	2	2	2	1	1	1	1	0	97	93	94	74	50	20	83	
24	C	2	1	2	1	2	2	1	2	5	80	93	17	94	95	93	96	
25	C	2	1	2	0	1	2	0	2	3	88	87	29	91	89	87	94	
26	C	2	2	2	2	2	3	2	2	3	83	90	87	91	91	68	90	
27	C	2	0	0	0	2	3	1	1	3	9	18	12	34	19	66	97	
28	C	2	2	2	2	2	3	2	2	5	99	99	89	100	100	99	100	
29	C	2	0	2	2	0	2	0	1	2	95	96	40	97	97	97	97	
30	C	2	2	2	1	2	2	1	2	3	100	98	98	99	100	96	99	
31	C	2	1	1	1	1	2	0	1	3	78	84	79	56	37	54	78	
32	C	2	2	1	1	2	2	0	2	2	92	90	45	96	97	97	93	
33	C	3	3	2	2	3	3	2	3	3	98	97	82	98	98	95	99	

BL = Booklice; CB = cigarette beetle; IM = Indian meal moth; GC = German cockroach; MI = midge; SM = wing of silkmoth; MO = mosquito; HF = housefly; DP = *Dermatophagoides pteronyssinus*.

^a Patients were divided into two groups according to results of inhibition analyses. Group S, patients with specific sensitization

pattern; group C, patients with cross-reactive sensitization pattern.

^b Levels of the specific IgE antibody were classified as follows: class 0, <0.34 units/ml; class 1, 0.35–0.69 units/ml; class 2, 0.70–3.49 units/ml; class 3, 3.50–17.49 units/ml; class 4, 17.50–49.99 units/ml; class 5, 50.00–99.99 units/ml; class 6, ≥100 units/ml.

serum samples from one representative patient of group S (patient No. 2) and one representative patient of group C (patient No. 14) (fig. 4b). For both serum samples, IgE reactivity to the 26-kD protein was not inhibited by preincubation of the serum samples with the SM, DP or Pen j 1 extract. These findings indicate that this 26-kD protein is associated with specific sensitization to booklice.

Immunoblotting of Two-Dimensionally Separated Proteins

To identify this 26-kD protein, immunoblotting detection was performed on two-dimensionally separated booklice proteins. As shown in figure 5a, most of the booklice protein bands were located in the low PI range. Immunoblotting using the pooled serum from three pa-

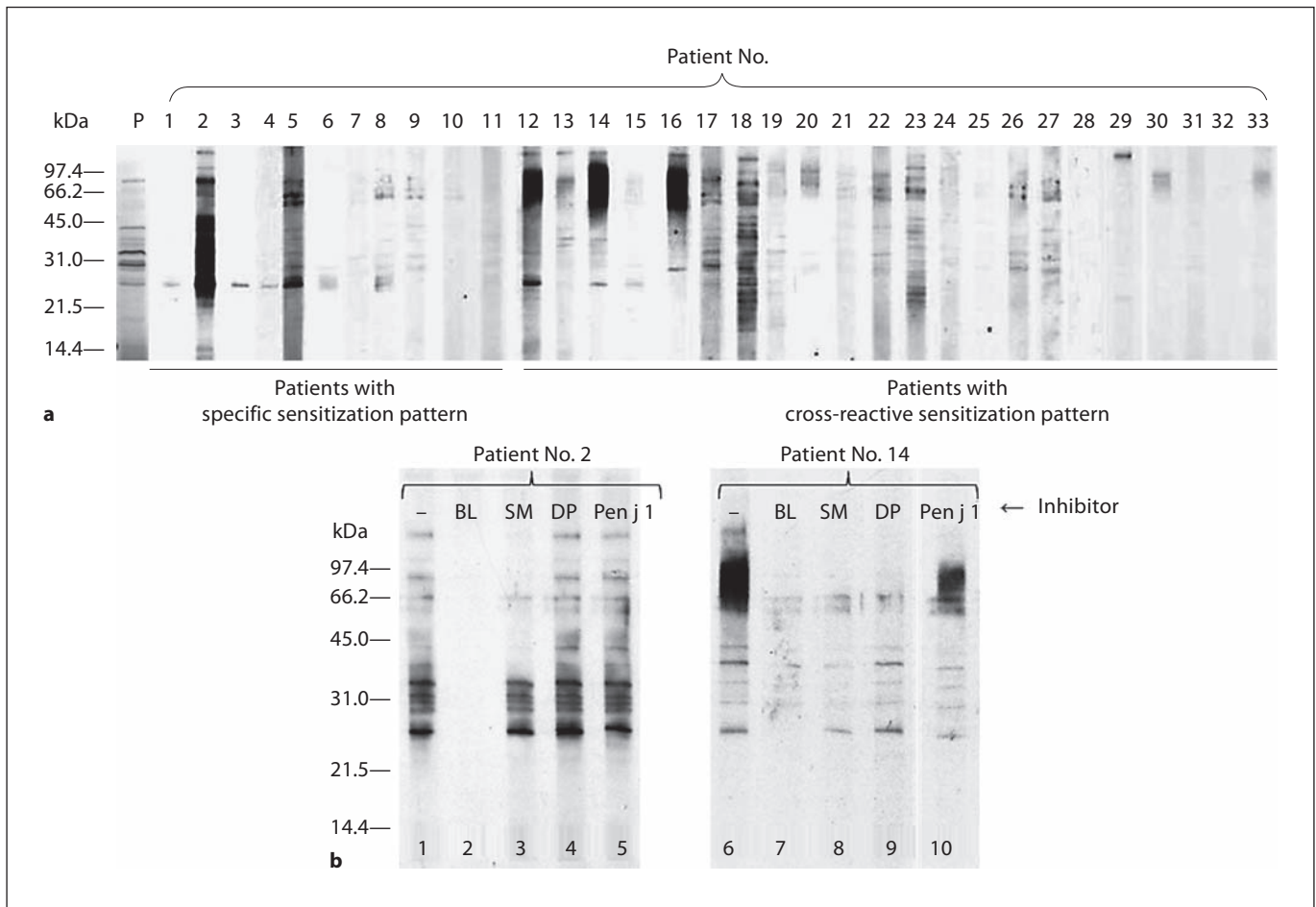


Fig. 4. Immunoblotting for booklouse-sensitized patients (**a**) and immunoblotting inhibition for two selected patients (**b**) of IgE reactivities to booklice extracts. P = SDS-PAGE of total booklouse protein (40 µg dry weight/lane); - = without inhibitor. Concentrations of inhibitors were as follows: booklice (BL), 2 mg/ml; wing of silkworm (SM), 2 mg/ml; *Dermatophagoides pteronyssinus* (DP), 2 mg/ml; Pen j 1, 40 µg/ml.

tients of group S (patients No. 1, 3 and 4) revealed intense IgE binding to a 26-kD protein spot at an isoelectric point of about 3.7 (fig. 5b, c). The nonatopic control serum did not show any immunodetection (data not shown). Peptide fragments produced upon digestion of the 26-kD protein spot from a two-dimensional gel were isolated by reverse-phase HPLC, and two of them were randomly selected and sequenced (online suppl. fig. 2). These sequence data will appear in the UniProt knowledgebase under the accession number P86712. The identified amino acid sequence of these fragments showed no homology to previously described allergenic proteins. This allergenic protein was named Lip b 1.0101 by the International Allergen Nomenclature Committee.

Discussion

This study revealed that sensitization to booklice is relatively common among Japanese patients with allergic asthma, and some of the sensitized patients show specific sensitization to booklice not explained by cross-reactivity with other insect allergens. Furthermore, we identified the novel 26-kD booklouse protein named Lip b 1.0101 as the protein contributing to the specific sensitization to booklice. These findings suggest that booklice should be recognized as an important household respiratory allergen for patients with allergic asthma. Booklouse-specific treatments including pest control and immunotherapy may be preferable for the sensitized patients.

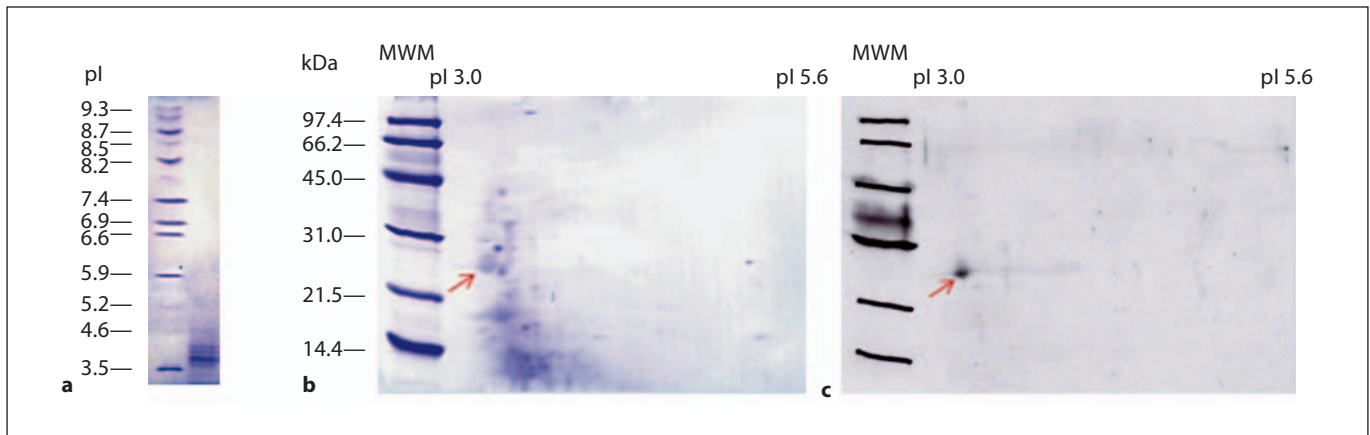


Fig. 5. Isoelectric focusing PAGE (a), two-dimensional PAGE (b) and two-dimensional IgE immunoblotting (c) of booklice extract. Immunoblotting on two-dimensionally separated proteins with pooled serum from three representative patients of group S identified a 26-kD allergenic protein spot at pI of about 3.7 (c). This spot is also marked by a red arrow in the two-dimensional proteomics map (b).

In our study, the immunoblotting of sera from booklouse-sensitized patients revealed eight allergenic bands. Of them, the prevalences of immunodetection of the 85- and 26-kD protein bands were high. Thus, they were considered to be important allergenic proteins for booklouse allergy. Our findings were partially different from those of Patil et al. [19]. They have shown that 67-, 59-, 43- and 27-kD proteins are the major booklouse allergens in Indian allergic patients [19]. One possible reason for the discrepancy between our findings and those by Patil et al. [19] may be related to the difference in predominant environmental insect allergen source between the studied communities, which possess allergenic proteins potentially cross-reactive with booklouse allergens. In fact, reports have shown that the prevalence of sensitization to an environmental insect allergen has a large variation between communities [12, 28, 29]. There is the possibility that sensitization to some of the booklice protein bands is simply the result of cross-reactivity with proteins from other dominant insect allergen sources in communities, and they may be different between the studied populations. Patil et al. [19] did not show the cross-reactivity of the booklouse allergen with other insect allergens. However, in our study, we found that the 26-kD protein, Lip b 1.0101, was specific to booklouse sensitization, after considering the results of inhibition analysis using other insect extracts.

The allergenic proteins that contribute to cross-reactivity between the booklouse allergen and other insect allergens were unknown. In our study, shrimp tropomy-

osin, Pen j 1, did not inhibit IgE reactivity to the booklouse allergen in pooled serum from booklouse-sensitized patients. Therefore, tropomyosin does not have a major impact on cross-reactivity between the booklouse allergen and other arthropod allergens. Our findings showed the possible contribution of the 85-kD booklouse protein to cross-reactivity between the booklouse and SM allergens. However, we do not have any information about the characteristics of this protein.

Many studies have shown that the amount of an allergen in house dust is a risk factor for sensitization to this allergen [30–34]. Therefore, it is hypothesized that, because booklice infest tatami mats and/or carpets, using such flooring in the house may be a risk factor for exposure and sensitization to the booklouse allergen. In this study, we identified using tatami mats and/or carpets in the bedroom as a strong risk factor for sensitization to booklice among allergic asthma patients using multivariate analysis of responses to the structured environmental questionnaire. Nevertheless, this analysis has a limitation, namely the assessment of exposure to the booklouse allergen was not confirmed by environmental sampling. A population-based survey with environmental sampling is required to establish the relationship between exposure and sensitization to booklice.

We could not show the clinical relationship between exposure to booklice and asthma exacerbation in this study. A preliminary bronchial provocation test using booklouse extract showed positive in some booklouse-sensitized patients (data not shown). There were no pa-

tients who reported by themselves the worsening of their asthma after exposure to booklice. In general, because booklice are very small, most people are not aware of their presence in their house unless a massive proliferation has occurred. Furthermore, this insect usually coexists with house dust mites in the indoor environment; therefore, it is difficult to discriminate patients clinically allergic to booklice from those allergic to house dust mites on the basis of case history. Conversely, unless we perform a skin test or measure the level of IgE antibody to booklice, we cannot identify patients who are sensitized to booklice on the basis of only case history.

In conclusion, in this study, we identified the unique allergenicity of booklice and Lip b 1.0101 as the allergenic protein that contributes to specific sensitization to booklice in the allergic asthma population. More than 20% of our Japanese allergic asthma patients were sensitized to this insect, and one third of them showed specific sensitization to booklice not explained by cross-reactivity with other insect allergens. Therefore, these findings indicate that booklice should also be considered as a possible causal allergen for patients presenting with house dust hypersensitivity. Because booklice prefer warm and damp conditions as mites do, the worldwide tendency to

highly airtight dwellings may promote increases in the number of booklice in the house and that of patients with booklouse allergy. Considering the worldwide distribution of this insect, sensitization to this insect is considered to be also common among populations other than Japanese. More research is required to confirm the sensitization to this insect in other populations.

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Disclosure Statement

The authors declare no conflicts of interest.

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