Hexamerin-2 Protein as a Novel Allergen in Occupational Locust Allergy

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Abstract

Background: Locusts as model systems are widely used in many biological laboratories worldwide. Occupational exposure to locusts induces a high prevalence of allergic sensitization. However, knowledge on occupational locust allergens remains unclear. This study aimed to identify a novel allergen from locusts that causes occupational allergies. Methods: We conducted a 20-year retrospective survey of 94 persons using questionnaires and a cross-sectional survey of 57 persons using questionnaires and immunological tests for occupational allergies in long-term locust laboratories. We identified the major allergens by immunoblotting and analysed them by LC-MS/MS. The allergenicity of the major allergen proteins was assessed by specific IgE (sIgE) detection, immunoblotting and ELISA inhibition assays. Results: The retrospective survey indicated that the frequency of occupational allergies was relatively low (13.8%), while the cross-sectional survey showed a higher frequency (40.4%). The symptoms in most allergic males were allergic rhinitis and asthma, while females showed higher prevalence of atopic dermatitis. Occupational exposure for 2-3 h per day or continuing one and half years obviously increased the allergy risk. We identified the hexamerin-2 protein as a major allergen in locusts. Purified hexamerin-2 protein achieved approximately 60% serum IgE reactivity with locust protein extract. The potential for cross-reactivity with cockroaches was indicated by sequence alignment of the hexamerin-2 protein and allergens of cockroaches. Conclusion: Occupational exposure is an important risk factor for locust allergy. The hexamerin-2 protein of locusts as a major allergen in occupational allergy was identified for the first time.

Original Article

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Abstract

Background: Locusts as model systems are widely used in many biological laboratories worldwide. Occupational exposure to locusts induces a high prevalence of allergic sensitization. However, knowledge on occupational locust allergens remains unclear. This study aimed to identify a novel allergen from locusts that causes occupational allergies.

Methods: We conducted a 20-year retrospective survey of 94 persons using questionnaires and a cross-sectional survey of 57 persons using questionnaires and immunological tests for occupational allergies in long-term locust laboratories. We identified the major allergens by immunoblotting and analysed them by LC-MS/MS. The allergenicity of the major allergen proteins was assessed by specific IgE (sIgE) detection, immunoblotting and ELISA inhibition assays.

Results: The retrospective survey indicated that the frequency of occupational allergies was relatively low (13.8%), while the cross-sectional survey showed a higher frequency (40.4%). The symptoms in most allergic males were allergic rhinitis and asthma, while females showed higher prevalence of atopic dermatitis. Occupational exposure for 2-3 h per day or continuing one and half years obviously increased the allergy risk. We identified the hexamerin-2 protein as a major allergen in locusts. Purified hexamerin-2 protein achieved approximately 60% serum IgE reactivity with locust protein extract. The potential for cross-reactivity with cockroaches was indicated by sequence alignment of the hexamerin-2 protein and allergens of cockroaches.

Conclusion: Occupational exposure is an important risk factor for locust allergy. The hexamerin-2 protein of locusts as a major allergen in occupational allergy was identified for the first time.

KEYWORDS

Locust, *Locusta migratoria* , Occupational allergy, Hexamerin-2 protein, Immunoblotting

1l INTRODUCTION

Allergy may be defined as a hypersensitive reaction of the immune system to a definite stimulus, termed allergen, at a dose tolerated by normal subjects.[1] Occupational respiratory allergy is a global health problem; occupational allergic sensitization has exceeded 25%, and approximately 10% of exposed individuals develop asthma.[2,3] Occupational exposure to insects induces a high prevalence of allergic sensitization, which can lead to clinical inhalant allergies and contact allergies.[4,5] Insect species of at least 12 different orders have been implicated in the promotion of inhalant allergies in humans, including locusts, cockroaches, and honeybees.[6,7]Orthopteran insects, including grasshoppers, locusts, crickets, and cockroaches, are important occupational hazards because of the severe symptoms caused by insect allergens.[8,9]

Locusts are not only one of the world's most destructive agricultural pests but also model systems in many laboratories for studying behaviour, physiology, neuroscience, and phenotypic plasticity.[10,11] Several cases

of occupational allergies have been described as allergies to the migratory locust, American locusts and grasshoppers, with symptoms of asthma, dermatitis, rhinitis and even shock.[12] In 1953, a survey of locust sensitization of the personnel of several institutions for locust research indicated that out of 34 workers in two locust research stations, four individuals showed allergic symptoms, and 14 others showed atopy.[13] Moreover, a survey of the work-related symptoms indicated wheezing or breathlessness accounted for 26% of the symptoms, followed by rhinitis, asthma and urticaria.[14] Questionnaires, skin prick tests (SPTs) and specific IgE (sIgE) measurements showed that 25%-34% of individuals working in a research centre were allergic to locusts.[8,9] Even dead locusts can induce severe contact urticaria in zookeepers during feeding of reptiles with the migratory locusts.[15] Therefore, identification of the major allergens from locusts is urgently needed to diagnose locust-specific allergies and to assess the environmental conditions in work environments.

Research on the identification of allergens from the migratory locusts is highly preliminary, although many proteins are considered possible allergens to humans. Among the proposed locust protein spectra, the most likely allergens identified by immunoblotting were approximately 68, 43, 37, 29, and 18 kDa in molecular weight.[9] However, the allergens related to IgE from 10 locust laboratory workers were reported to be 70, 55, 35 and 30 kDa in molecular weight.[7] In one recent study, isolation of proteins from patients allergic to locusts showed that the allergens were approximately 130, 70, 38, 29 and 19 kDa in molecular weight.[16] The above inconsistent findings suggested that the major allergens of locusts and their molecular nature remain unclear.

In our laboratory, the migratory locust (*Locusta migratoria*) has been studied as a model system for 30 years. The students and staff in our team have exposure history from rearing and breeding locusts, providing us the opportunity to investigate occupational locust allergy. Our research focused on the factors determining the incidence of allergy in specific exposure settings and the patterns of immunological responses.

2 | METHODS

2.1 | Subjects

The occupational allergic questionnaire surveys covered two parts. One was a retrospective survey of locust allergy in cohort from a locust laboratory from 2000 to 2019. In this survey, we investigated 94 students and staff (49 males, 45 females) who worked on locust biology, rearing and breeding locusts at the Institute of Zoology, Chinese Academy of Sciences (Beijing, China). The other was a cross-sectional survey of 57 scientists and students (24 males, 33 females) in 2020 in our two laboratories at the Institute of Zoology, Chinese Academy of Sciences (Beijing, China) and College of Life Science, Hebei University (Baoding, Hebei Province, China) (Table 1).

Serum samples were collected from 57 subjects with history of exposure to locusts and 10 healthy controls without exposure history and allergy symptoms from Beijing Tongren Hospital (Table 1). Total IgE levels and sIgE levels for common inhalant allergens were measured by the UniCAP allergen detection system (Pharmacia Diagnostics, Uppsala, Sweden). Written informed consent was obtained from all participants.

2.2 | Preparation of locust protein extracts

Migratory locusts reared in cages (30 cm × 30 cm × 32 cm) with a diet of fresh wheat seedlings were obtained from colonies maintained in the College of Life Science, Hebei University, Hebei Province, China. Samples of frozen migratory locust were ground to a powder in a mortar and pestle using liquid nitrogen. The samples were solubilized in PBS (0.01 M, pH 7.2), the mixture was centrifuged at 15,000 rpm for 15 min at 4°C, and the supernatant was collected. The total protein content was determined by the BCA method.

2.3 | Skin prick test

For the SPT, participants did not take any medications (antihistamines, steroids, and other drugs) for at least 2 weeks. SPT against crude protein extracts (2.0 mg/ml) of locusts and commercial extracts of*Blattella germanica* (produced by Beijing Union Hospital) was conducted in the Beijing Tongren Hospital, Beijing, China. Histamine was used as the positive control, and 0.9% saline was used as the negative control. The

SPT results were recorded 15 min later. The wheal size was calculated as the mean of the longest diameter and the length of the perpendicular line through its middle. The ratio of the allergen wheal size to histamine wheal size was the skin index. The skin index was expressed as 2+ when the wheal diameter was half that of the positive control and 3+ when the mean wheal diameter was equal to that of the positive control. Allergy to locust was defined as skin index [?] 2+ combined with allergic symptoms. Atopy to locust was defined as skin index [?] 2+ but without allergic symptoms after locust exposure. Non-atopy was defined as skin index $<$ 2+ and no allergic symptoms after locust exposure.

2.4 l ELISA of sIgE for locust protein extracts

The concentration of the crude protein extracts was adjusted to 500 μg/ml in 0.01 M PBS (pH 7.2), and the extracts were added to 96-well polystyrene microtiter plates (100 μl/well) (BBI, Sangon Biotech, China) and incubated overnight at 4°C. Then, the cells were blocked with skimmed milk in PBS (5%) at 37°C for 2 h and washed 3 times in TBS containing 0.05% Tween 20 (TBST). Wells were then emptied and coated for 2 h with 100 μl of sera from individuals and 10 healthy controls (1:30, diluted with 5% skimmed milk in PBS) at 37°C. The bound IgE was then reacted with 100 μl of horseradish peroxidase (HRP)-labelled goat anti-human IgE (1:3,000 dilution). 3,3',5,5'-Tetramethylbenzidine (TMB) was used as the substrate, and the reaction was stopped by 50 μl of 2 M H_2SO_4.

2.5 l SDS-polyacrylamide gel electrophoresis analysis and IgE immunoblotting

The crude protein extracts were separated in triplicate by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Samples (50 μg) were loaded onto a SDS-PAGE gel (10% separating gel and 4% stacking gel) at 20 μl/lane and electrically separated by the Mini-PROTEIN Tetra system (Bio-Rad; Hercules, CA, USA). The gels were stained with Coomassie Brilliant Blue R-250. For immunodetection of IgE-binding proteins, the separated proteins were electroblotted onto a PVDF membrane (Immobilon, Merck Millipore, Germany) by applying a constant current of 350 mA for 55 min at room temperature. Then, the membrane was blocked with 5% skimmed milk in TBST and incubated overnight at 4°C with a 1:30 dilution in 5% skimmed milk of pooled sera, the 9 individual sera and a negative control of pooled sera from 10 healthy individuals. Bound IgE antibodies were detected using HRP-conjugated goat anti-human IgE (Abnova, Taiwan) at a dilution of 1:10,000 in 5% skimmed milk. The membrane was incubated with Super Signal ECL Chemiluminescent Substrate (Thermo Scientific, USA). Finally, immunoreactive bands were visualized using Fusion Fx (Vilber Lourmat, France).

A band at approximately 70 kDa from the locust proteins, recognized by immunoblotting of the pooled sera of locust-allergic patients, was analysed by LC-MS/MS, and the peptide sequences obtained were aligned with the migratory locust proteome at Beijing Protein Innovation.

2.6 l Cloning, expression, and purification of the hexamerin-2 protein from locust

Total RNA was isolated from the whole body of migratory locusts by using TRIzol reagent (Invitrogen, Thermo, USA). RNA was reverse-transcribed to cDNA using a reverse transcription kit (Promega, USA). Complete open reading frames (ORFs) for the hexamerine-2 protein were amplified from the cDNA using the PCR primers HEXA-2-F (5'-GTGCTGCATCGCGAGGACAC-3') and HEXA-2-R (5'-GTCGAAATGCGCGTTGGGCA-3'). A construct was created by cloning the hexamerin-2 gene into the plasmid pET 30a vector and expressed in *Escherichia coli* BL21. Expression and purification of the hexamerin-2 protein were then conducted by Beijing Protein Innovation.

2.7 l Immunological tests of the hexamerin-2 protein in sera from patients with occupational locust allergy

For the ELISA, the concentration of the crude locust proteins was adjusted to 500 μg/ml in 0.01 M PBS (pH 7.2), applied to 96-well polystyrene microtiter plates (100 μl/well) (BBI, Sangon Biotech, China) and incubated overnight at 4°C. After this, the ELISA was continued as previously described.

For the immunoblotting assay, SDS-PAGE-resolved purified proteins were electro-transferred onto a PVDF

membrane (Immobilon, Merck Millipore, Germany) for immunoblotting analysis as described above. Membranes were incubated with patient sera, diluted 1:30 (v/v) diluted in 5% skim milk-TBST, overnight at 4 under constant agitation and probed with 1:10,000 (v/v) anti-human IgE peroxidase antibody (Abnova, Taiwan) for 1.5 h at room temperature. Fluorescence was visualized by ECL Chemiluminescent Substrate as described previously.

2.8 l Competitive inhibition ELISA

In inhibition experiments, coating of the microtiter plates was performed as described above for ELISA.[17] Two individual sera that showed positive reactions against the hexamerin-2 protein were chosen for inhibition studies. Sera were first incubated for 1 h at room temperature with an equal volume of the inhibitor solution. The final concentrations of the purified proteins used as inhibitors ranged from 0.005 μg/ml to 500.000 μg/ml. Mixtures containing the sera and the inhibitor (or serum without inhibitor as a control) were incubated in the wells for 2 h at room temperature. After this, the ELISA was continued as previously described.

2.9 l Statistical analysis

Statistical significance was analysed by using GraphPad Prism 6.0 (GraphPad Inc., California, USA) and SPSS 24.0 software (SPSS Inc., Chicago, USA), and the values are presented as the mean ± S.D. (standard deviation). A t-test was employed to analyse the nonparametric data. A P value of less than 0.05 was considered statistically significant.

3 l RESULTS

3.1 l Clinical characteristics

According to the retrospective questionnaire survey of 94 persons with locust exposure history from 2000-2019, we found that 13.8% (13 persons) reported locust-induced symptoms. There were 8 males (8/49, 16.3%) and 5 female patients (5/45, 11.1%) with suspected locust allergy (Figure 1A). The clinical symptoms after locust exposure were different between male and female patients. Half of the male patients complained shortness of breath with asthma, while all the female patients had atopic dermatitis-like symptoms, and 80% of them suffered from nasal symptoms (Figure 1B).

A cross-sectional survey investigated 57 (24 males, 33 females) scientists and students in our two separate laboratories in Beijing and Baoding. The latency period from the beginning of exposure to the onset of symptoms varied, ranging from approximately one month to 20 years, with a mean interval of 3.9 years. Twenty-three out of 57 individuals (40.4%) were sensitized to locusts according to the SPT (Figure 2A, Table S1). Although the number of allergic patients was similar among males (11) and females (12), males had a higher sensitization ratio (45.8%, 11/24) than females (36.4%, 12/33) (Figure 2A). Among the 23 locust-sensitized subjects, 18 were diagnosed with occupational allergy, with allergic rhinitis as the most common symptom, followed by atopic dermatitis and asthma, whereas the other 5 subjects were diagnosed as being atopic to locusts (Figure 2B, Table S1). More male patients complained of allergic rhinitis and asthma, while 70% of female patients suffered from atopic dermatitis (Figure 2B). One student (P3) had a severe allergic reaction to locusts, with difficulty breathing and asthma and even anaphylaxis once he went into the insectary. The total IgE level in three subjects was less than 60 kU/L, while in others, the level ranged from 69.3 to 1920 kU/L (Table S1). Moreover, 9 of the 23 locust-sensitized individuals were also sensitized to cockroaches, as determined by the SPT (Table S1). The duration of exposure was a risk factor for occupational allergy (Figure 2D). Therefore, occupational exposure lasting for 2-3 h per day (OR=4.225) and continuing for one and half years (OR=2.842) obviously increased the risk of locust allergy. We also found that previous allergy history showed an effect on allergy to locusts. Among locust-sensitized subjects, 14 patients (14/23, 60.9%) had a previous allergic history, whereas 9 patients (9/23, 39.1%) were not previously allergic (P<0.001). This indicated that past history of allergy might be a risk factor for locust allergy (Figure 2C).

3.2 l Identification of the major allergens in locusts

To further confirm the sensitization of the persons exposed to locusts, we conducted *in vitro* experiments as well. The sIgE for locust proteins in patients' sera was detected by ELISA; the sIgE levels of the allergic group (absorbance at 450 nm was 1.174±0.136) and atopic group (0.603±0.198) were significantly higher than those of the non-atopic group (0.177±0.014) and healthy group (0.174±0.007) (Figure 3A).

To determine the molecular weights of possible locust allergens, the locust extracts separated by SDS-PAGE were transferred to a PVDF membrane for immunoblotting with a serum pool of 10 sera (Table S1: P1-P10). Although the bands from the locust extracts ranged from 30 to 80 kDa, significant major bands at 56 and 70 kDa were observed (Figure 3B). When the locust extract immunoblots were probed with 9 individual sera, only a 70-kDa band was observed in all 9 sera (Figure 3C).

3.3 l The ˜70-kDa protein is the locust hexamerin-2 protein

To identify the protein with the band at ˜70 kDa, we analysed the 70-kDa IgE-binding band of the locust extracts by LC-MS/MS. Peptide spectrum matching gave the highest sequence coverage (43%) with a hexamerin-2-like protein (NCBI: ACU78069.1), annotated as a storage protein, the juvenile hormone (JH)-binding protein in locusts (Figure 4B).

Then, we cloned the cDNA of the locust hexamerin-2 protein ORF, which covers 2031 nucleotides. The nucleotide sequence could be translated to a protein sequence with 677 amino acids, and the first 20 amino acids were predicted as a signal peptide. The calculated molecular mass of the hexamerin-2 protein was 78663 Da, with a pI of 5.76. According to sequence structure analysis, the hexamerin-2 protein has 3 domains, and there is evidence to indicate that these domains are Hemocyanin-N domain, Hemocyanin-M domain and Hemocyanin-C domain (Figure 4A, C).

3.4 l Immunological characterization of the hexamerin-2 protein

To determine the sensitization of the hexamerin-2 protein, we used IgE against the purified hexamerin-2 protein from locusts. The levels in the sera of 18 patients with occupational locust allergy (absorbance at 450 nm was 1.546±0.198) were markedly higher than those in 5 atopic patients (1.239±0.099), as determined by ELISA. Hexamerin-2 could not be detected by non-atopic sera (0.520±0.083) and controls (0.524±0.044) (Figure 5A). Immunoblotting was performed using individual sera from 9 locust-allergic patients, and the individuals reacted positively to the hexamerin-2 protein, while the control reacted negatively (Figure 5B).

To determine whether the hexamerin-2 protein is the major allergen in locusts, we chose two patients' sera (P2 and P3) with positive reactions to the hexamerin-2 protein for ELISA inhibition assays. The maximal inhibition of the binding of patients' IgE antibodies to the coated hexamerin-2 protein was approximately 60%. Therefore, the hexamerin-2 protein is a major sensitive protein source in locusts (Figure 5C, D).

3.5 l Conservation of thehexamerin-2 protein in insects

We constructed a phylogenetic tree of the hexamerin-2 protein of different grasshoppers, and the peptide sequences obtained were aligned with those of four grasshopper species (*Chondracris rosea, Heteropternis respondens, Oedaleus asiaticus, Orinhippus tibetanus*) (Figure S1). The similarity of the sequences of the hexamerin-2 protein of different grasshopper species was more than 93% (Figure S2). The hexamerin-2 protein of locusts shares 36.1% similarity with the allergen Bla g 3 (*Blattella germanica*); it also shares 53.3% similarity with the allergen Cr-PI and 49.6% allergen similarity with Per a 3, both of which were identified from *Periplaneta americana*(Figure S3). By sequence alignment between the locust hexamerin-2 protein and shrimp hemocyanins, we found that their sequence similarity was only approximately 29% (Figure S4). Therefore, the hexamerin proteins of locusts are species specific, despite being conserved in insects and arthropods.

4 l DISCUSSION

Locusts are an important insect group inducing occupational allergies, causing sensitization and the development of allergic symptoms in a high proportion of exposed persons. However, little information is available on the prevalence of the allergic diseases. To date, this is the largest study on occupational locust allergy. In

this study, a retrospective survey of 94 individuals and cross-sectional survey of 57 individuals confirmed that locusts can cause allergic reactions during laboratory exposure. In 57 students and staff of our laboratory, 18 subjects with occupational allergy and 5 atopic subjects were identified by SPT. This indicates the risk of potential allergy in the exposed subjects, and the occupational allergy should be integrated into practitioner training priorities.

In our study, a long-term retrospective survey indicated that the frequency of suspected occupational allergy was relatively low (13.8%). In particular, males were more sensitive than females to occupational allergy. Although the allergic disorders included atopic dermatitis, allergic rhinitis and asthma, most male patients with allergies exhibited difficulty breathing, as in asthma, while female patients showed a prevalence of atopic dermatitis in both the retrospective survey and cross-sectional survey. However, our cross-sectional survey showed a similar frequency of occupational sensitization (˜50%) to locusts as that observed in previous studies.[7,9,14]For the retrospective survey, we investigated persons who had worked in the locust laboratory for 20 years (long term); some subjects had perhaps forgotten their atopic symptoms, or at that time, the allergy symptoms were not obvious and were not emphasized. However, no association could be demonstrated between atopic status and sensitivity to locusts, probably because of the small sample size of workers investigated. On the other hand, methods of immunological tests are more sensitive and can obtain the higher relevance ratio. Our results demonstrated that occupational exposure for 2-3 h per day and continuing one and half years obviously increased the risk of locust allergy, especially exposure exceeding 2-3 h per day.

Identification of the major allergen is necessary to diagnose locust allergies. Although some studies have shown that locusts can induce allergic reactions, no native allergen has been purified and characterized from migratory locust. In our study, we identified the hexamerin-2 protein as a major allergen of occupational allergy to locusts. We proved for the first time the presence of the hexamerin-2 protein with IgE binding ability in locusts. ELISA inhibition assays indicated that maximal inhibition by the hexamerin-2 protein was approximately 60%, suggesting that the locust hexamerin-2 protein was a major allergen accounting for IgE binding in the two patients whose sera were used for the inhibition experiment. Based on the results, we cannot exclude the idea that the other patients were sensitized against other locust allergens. Therefore, further research to identify other allergens from locusts is necessary.

The hexamerin-2 protein, which is a storage protein in the hexamerin family in locusts, appears to be closely related to locust hemocyanin and associated with JH secretion.[18] On the other hand, the hexamerin-2 protein widely exists in various species of orthopterans.[19] The phylogenetic tree of the hexamerin-2 protein of different grasshoppers and peptide sequence alignment of four grasshopper species (*C. rosea, H. respondens, O. asiaticus,O. tibetanus*) showed that the similarity of the sequence of the hexamerin-2 protein in different grasshopper species was more than 93%. These results indicated that humans are allergic to other locust and grasshopper species as well. The current study assumed that people who are allergic to shrimp might have allergic reactions to locusts, because sera from subjects allergic to shrimp could be used to identify allergens from Bombay locust.[20] In our study, only one subject was allergic to aquatic products among the 23 subjects with locust allergy, so we considered there to be low cross-reactivity between shrimp and locusts. Because the sequence similarity between the hexamerin-2 protein in locusts and hemocyanin in 3 shrimp species was only approximately 29%, cross-reactivity between them could not exist.

Because locusts and cockroaches belong to Orthopteroida within Insecta, allergies to locusts and cockroaches in workers are associated with cross-reactivity. In our study, 39.1% of the locust-allergic subjects tested positive against cockroach (*B. germanica*) in the SPT, and the sequence similarity of the hexamerin-2 protein in locusts and the allergen Blg 3 of *B. germanica* was 36.4%. Moreover, the sequence similarity between the hexamerin-2 protein in locusts and the allergen of *P. americana*was approximately 50%. Therefore, the hexamerin-2 protein of locusts is species specific, despite cross-reactivity between locusts and cockroaches.

Locusts are consumed as food by humans worldwide, in Africa, South America, and Asia, in both rural and urban areas.[21] Commercial farming of migratory locust for food and feed is growing in Southeast Asia, China, Japan, and Korea. They are also served on skewers in some Chinese food markets.[22] Approximately 27 cases of anaphylactic shock caused by consumption of grasshoppers and 27 cases caused by consumption

of locusts were reported from 1980 to 2007.[23] In addition, BBC news reported that eleven people died in the locust swarm in Sudan in 2003, and approximately sixteen thousand people went to a hospital because of asthma induced by locust swarms.[24] Therefore, the development of approaches for the identification of allergens and antibody therapy is urgently needed at the clinical level. In addition, because genomic and transcriptomic information available,[10,25] the hexamerin-2 protein as storage proteins how to be synthesized and function in locusts needs to be further investigated.

It is significant that the hexamerin-2 protein can be utilized to prepare diagnostic reagents in the future. The commercial production of the hexamerin-2 protein as a diagnostic reagent and further applications for detection are expected to improve the diagnosis rate and reduce the rate of misdiagnosis. On the other hand, desensitizers prepared with the hexamerin-2 protein will be beneficial to patients allergic to locusts, effectively relieving symptoms and reducing the health hazards of occupational exposure or other causes of allergies to locusts.

5 l CONCLUSION

We conducted a retrospective survey and cross-sectional survey of occupational allergy to locusts and demonstrated that occupational exposure is an important risk factor for allergies to locusts. We identified the hexamerin-2 protein as a major allergen associated with occupational locust allergy. Natural molecules can be used for component-resolved diagnosis and to investigate locust allergies in the laboratory, to locusts used as food, and to locusts breeding in farms and fields.

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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

LK and LZ conceived and designed the study. YW, HFL and MN conducted the experiments. YW, HFL and CSW analyzed the data. DY collected the information of the subjects. LK and YW wrote the manuscript. All authors read and approved the manuscript.

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Figure and table legends

TABLE 1 Three groups investigated in this study

Group	Number	Number	Male	Female	Investigation Time
Retrospective survey	Retrospective survey	94	49	45	2000-2019
Cross-sectional survey	Cross-sectional survey	57	24	33	2020
Control	10	10	4	6	2020

FIGURE 1. Retrospective survey of locust allergy in a cohort of 94 subjects in a locust laboratory from 2000 to 2019.

A, Number and percentage of subjects allergic to locust (male, female and total). B, Symptoms of locust allergy in subjects (male, female and total). Patients with locust allergy simultaneously had several symptoms.

FIGURE 2. Cross-sectional survey of locust allergy in a cohort of 57 subjects in a locust laboratory in 2020.

A, Number of subjects allergic to locust (males, females and total). B, Suspected allergic disorders to locust in subjects (males, females and total). C, Effect of other allergic histories on occupational allergies to locusts. D, ROC curve of interval exposure and continuous exposure.

FIGURE 3. Identification of the sensitization of individuals exposed to locusts.

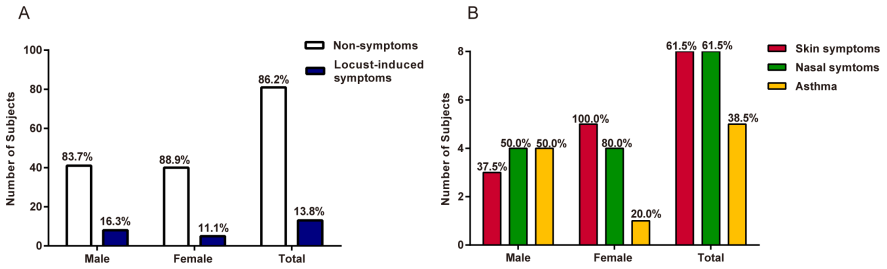
A, Measurement of sIgE for locust protein by ELISA. P values < 0.05 were considered significant. B and C, sIgE immunoblotting. B, Lane 1, protein marker. Lane 2, SDS-PAGE of locust protein. Lane 3, immunoblotting with serum pool from 10 locust-allergic subjects. Lane 4, pooled sera from 10 control subjects. C, Lane 1, protein marker. Lane 2, SDS-PAGE of locust protein. Lanes 3-11, immunoblotting with individual sera from locust-allergic subjects. Lane 12, pooled sera from 10 control subjects.

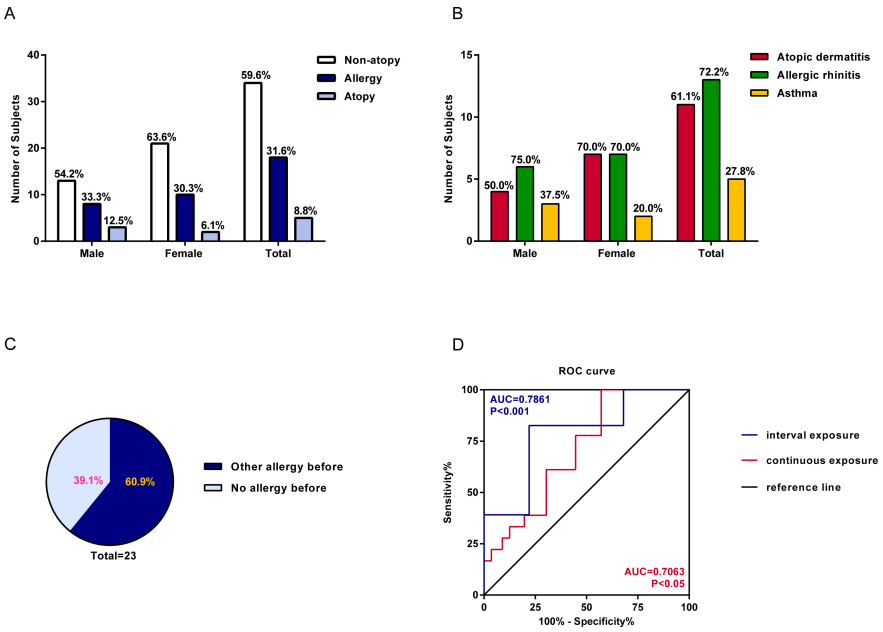
FIGURE 4. Hexamerin-2 protein mass analysis, purification, peptide matches and conserved domains.

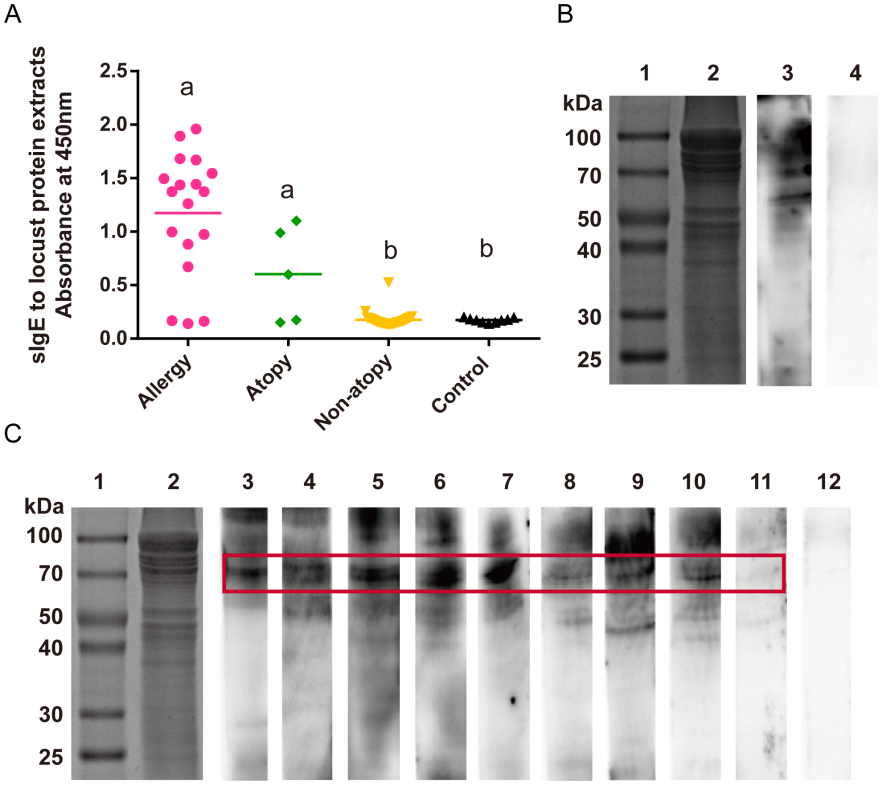
A, SDS-PAGE of the purified hexameirn-2 protein. Lane 1, protein marker. Lane 2, SDS-PAGE of the hexamerin-2 protein. B, Mature protein sequence, peptides verified by the mass spectrum fingerprint are shown in red and highlighted (NCBI accession ACU78069.1). C. Three domains of the hexamerin-2 protein analysed against the NCBI database: hemocyanin-N domain, hemocyanin-M domain, hemocyanin-C domain.

FIGURE 5. Identification of the sensitization of individuals exposed to the hexamerin-2 protein.

A, Measurement of sIgE for the hexamerin-2 protein by ELISA. B, Hexamerin-2 protein sIgE immunoblotting. Lane 1, protein marker. Lane 2, SDS-PAGE of the hexamerin-2 protein. Lanes 3-11, Immunoblotting with pooled sera from 9 locust-allergic subjects. Lane 12, pooled sera from 10 control subjects. C and D, Examination of serum IgE reactivity to the hexamerin-2 protein by competitive ELISA, representing the serum from locust-allergic patients P2 and P3, respectively. P values < 0.05 were considered significant.







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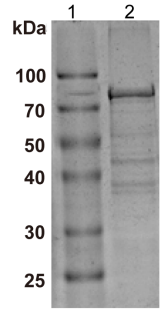

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C