

within the network was between AHR and miR-30a-5p, with a Bayes factor⁵ (a Bayesian log-likelihood ratio) of 42.9 (Fig 1, arrow thickness), indicating that a dependence between the 2 is $e^{42.9}$ times (4.3×10^{18}) more likely than independence; mean Bayes factor for edges within Fig 1 is 14.8 ± 12.8 .

Logistic regression analysis did not reveal any miRs significantly associated with 14-year AHR remission (see Fig E3 in this article's Online Repository at www.jacionline.org). This is indicative of the nonlinear combination of miRs in our BN; microRNA acting in networks is consistent with the way many genes are targeted by multiple miRs, each of which may have an additive or even negative effect on expression. These effects are sometimes masked and missed by single-miR analysis.⁶ Similarly, no miRs were significantly associated with sex in a logistic regression test, both before and after adjusting for baseline PC₂₀. At least 6 of the 11 miRs included in our predictive model have previously been associated with asthma or pulmonary conditions: mirR-106a, mirR-126, mirR-19, mirR-30, mirR-146, and mirR-25.

Our BN included the baseline PC₂₀ value in the prediction of AHR remission at 14 years after baseline, achieving an AUC of 86.5%. We also tested the predictive ability of the model based only on microRNAs, removing the baseline PC₂₀ and recomputing parameters of a BN based on the remaining 11 miRs. This model achieved an AUC of 80.0%, suggesting good prediction, but which was significantly different from the full model's 86.5% ($P = .034$). The microRNA-only BN was not strongly correlated with baseline PC₂₀ values ($r^2 = 0.26$), showing that the miRs provide additional, orthogonal information about future AHR remission.

We examined the expression and function of miR-30a-5p in airway smooth muscle (ASM) cells *in vitro*. Analysis of miR-deep sequencing data⁷ showed that miR-30a is among the most abundant miRs expressed in the human primary ASM cells. Because other miR-30 family members (miR-30d, miR-30e) are also highly expressed in ASM cells, we transfected mimics of these miRs in ASM cells and examined their effects on ASM growth and hypertrophy *in vitro*. There was no significant effect by any of the miRs on ASM growth (data not shown). However, miR-30a, miR-30d, and miR-30e all significantly increased the size of ASM cells ($P < .01$; see Fig E5 in this article's Online Repository at www.jacionline.org), indicating potential roles for miR-30 family members including miR-30a in ASM hypertrophy and AHR in those with asthma.

Although the present model should be validated in other longitudinal asthma cohorts, this work demonstrates that microRNAs have the potential to be powerful prognostic biomarkers for future AHR remission and asthma prognosis. Additional longitudinal profiling of microRNAs alongside longitudinal clinical observation will be key in moving these models forward.

Michael J. McGeachie, PhD^{a,b}
Joshua S. Davis, MD^{a,b}
Alvin T. Kho, PhD^{b,c}
Amber Dahlin, PhD^{a,b}
Joanne E. Sordillo, ScD^{a,b}
Maoyun Sun, PhD^d
Quan Lu, PhD^d
Scott T. Weiss, MD^{a,b}
Kelan G. Tantisira, MD^{a,b}

From ^athe Channing Division of Network Medicine, Brigham and Women's Hospital, ^bHarvard Medical School, ^cComputational Health Informatics Program, Boston Children's Hospital, and ^dProgram in Molecular and Integrative Physiological Sciences, Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, Mass. E-mail: michael.mcgeachie@channing.harvard.edu.

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Primary respiratory and food allergy to mealworm



To the Editor:

Mealworm (*Tenebrio molitor* L.) has great potential as a new sustainable protein and is currently introduced in commercially available burgers in a number of European countries. Recently, we showed there is risk of mealworm allergy in the majority of patients with shrimp allergy.¹ Here we address the risk of mealworm sensitization and allergy in subjects without shrimp allergy. We evaluated primary sensitization in mice (C3H/HeOuJ) and allergy in 4 domestic and professional mealworm breeders with allergic symptoms on occupational exposure or ingestion. Mealworm or shrimp extract in PBS (20 mg of protein) with 10 μ g of cholera toxin (CT; List Biological Laboratories, Campbell, Calif) was administered by means of gavage. Control animals received PBS and CT only. Sensitization profiles (specific IgE [sIgE], immunoblot, and the basophil activation test [BAT]), allergens involved (liquid chromatography-mass spectrometry) and the development of clinical allergy to shrimp (open food challenge) and mealworm (double-blind, placebo-controlled food challenge [DBPCFC]) were investigated (for details, see the [Methods](#) section in this

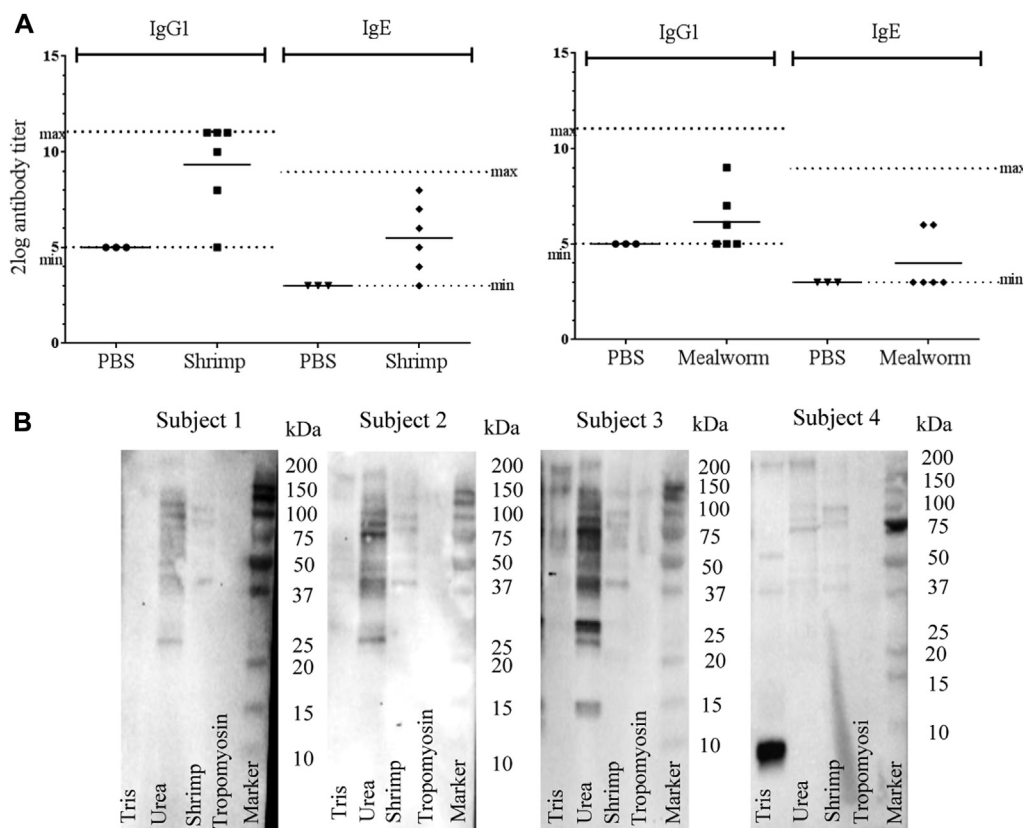


FIG 1. IgE-binding profile in mice and human subjects. **A**, Antibody responses (IgE and IgG₁) to mealworm extract, shrimp extract, and control (PBS) in a mouse model on day 16. Data represent the mean log₂ antibody titer $\pm$ SEM. Horizontal dotted lines represent detection limits of the assays (minimum/maximum). **B**, Subjects show IgE binding to allergens in both the mealworm and shrimp extracts. No binding is seen to Pen a 1 (shrimp tropomyosin) IgE from patients with mealworm food allergy; subjects 3 and 4 additionally bind to proteins with low molecular weight.

article's Online Repository at www.jacionline.org). The study was approved by the local ethics committee (NL43731.041.13).

Mealworm induced extract-specific IgG₁ in 3 of 6 animals and extract-specific IgE in 2 of 6 animals (Fig 1). Shrimp led to the induction of extract-specific IgG₁ and IgE in 5 of 6 animals, illustrating the potency of both extracts to induce primary sensitization. We used a model previously described by Bowman and Selgrade,² which is known to successfully differentiate between known allergenic proteins (eg, Ara h 1 and β -lactoglobulin) and low/nonallergenic proteins (eg, gelatin and beef tropomyosin) in C3H/HeO/J mice.

All 4 human subjects had mealworm allergy during professional or domestic mealworm breeding. The 2 professional breeders had symptoms of inhalant allergy when entering the mealworm rearing room. They sporadically consumed mealworms in small amounts without symptoms (2-5 years of exposure and sporadic ingestion of $\sim$ 1 g of mealworm per serving). One of the domestic breeders (subject 4) had rhinoconjunctivitis after 2 years of exposure, which progressed to dyspnea after starting domestic rearing. Both domestic breeders consumed higher amounts of mealworm than the professional farmers and encountered progressive food-induced allergic symptoms when eating mealworm (7-9 years of exposure and 10-50 g of mealworm per serving on 5-10 occasions); anaphylaxis did not

occur. None reported shrimp or any other food allergy. Only subject 4 had mild rhinoconjunctivitis to house dust mite (HDM) and birch pollen (Fig 2 and see Table E1 in this article's Online Repository at www.jacionline.org).

All 4 were sensitized to mealworm in CAP tests, skin prick tests (SPTs; Fig 2), and BATs. Only subject 2 showed minor sensitization with no clinical allergy to shrimp. Although all subjects were sensitized to some common inhalant allergens (tree and grass pollen and animal dander), titers were very low (see Table E1). Immunoblotting showed that IgE from all subjects bound to similar proteins in mealworm, although with different intensities (Fig 1). When comparing the subjects with a reported respiratory allergy to mealworm (subjects 1 and 2) with those with food allergy (subjects 3 and 4), on the immunoblot of subject 3, additional 15-kDa and 10-kDa proteins were observed in the immunoblot of subject 4. All subjects had a negative oral food challenge result for shrimp, and only subject 3 and 4 had positive DBPCFC results for mealworm.

Because 3 of our subjects had higher sIgE levels to mealworm than to any other food or inhalant allergen, mealworm might be the primary sensitizer. Although subject 4 showed slightly higher HDM sensitization compared with mealworm sensitization, this subject had IgE to Der p 1 and 2 (fecal components), but

A														C
Subject	Sex	Age (years)	AA/ AD/ AR	Professional/ Domestic	Years of exposure	Direct inhalant exposure (h/w)	Oral exposure (times consumed)	Average intake (in grams)	Symptoms inhalation	Symptoms ingestion	HDM symptoms	Shrimp symptoms	DBPCFC mealworm	
1	M	46	n/y/n	Prof	2	0.5	< 10	~ 1	C,R	n	n	n	neg	
2	F	22	n/y/n	Prof	5	6	< 5	~ 1	C,N,R	n	n	n	neg	
3	M	28	n/n/n	Dom	7	2-4	< 10	~ 50	n	OA,U	n	n	pos	
4	M	32	n/n/y	Dom	10	2-4	5-10	~ 50	C,N,S,D,W	OA,U	C,N, S	n	pos	
B		Mealworm		HDM					Shrimp					
Subject	CAP (kU/L)	SPT ALK	CAP (kU/L)	SPT Greer	SPT ALK	Der p 1 (ISU)	Der p 2 (ISU)	Der p 10 (ISU)	CAP (kU/L)	SPT Greer	SPT ALK	SPT Stallergen	Pen m 1 (ISU)	OFC shrimp
1	0.75	3+	0	2+	0	0	1.1	0	0	0	0	0	0	neg
2	2.32	3+	0.4	2+	0	0	0	0.6	0.7	2+	2+	2+	0.6	neg
3	2.26	3+	0	2+	0	0	0	0	0	0	0	1+	0	neg
4	14.6	2+	28	3+	2+	9.7	13	0	0	0	0	0	0	neg

FIG 2. Patients' characteristics. **A**, Mealworm exposure and clinical history to direct inhalation (while working with the animal) and ingestion of mealworm, HDM, and shrimp. AA, allergic asthma; AD, atopic dermatitis; AR, Allergic rhinitis; C, conjunctivitis; D, dyspnea; Dom, domestic farmer; F, female; GI, gastrointestinal; M, male; N, nasal congestion; OA, oral allergy; OFC, oral food challenge; Prof, professional farmer; R, rhinitis; S, sneezing; U, general urticaria; W, wheezing. **B**, Sensitization in SPTs, CAP tests, and ISAC tests (with at least 1 subject <0.3 ISU) for mealworm, HDM, and shrimp are included in the table. Der p 1, HDM peptidase C1; Der p 2, HDM NPC2 family; Der p 10, HDM tropomyosin; Pen m 1, shrimp tropomyosin. **C**, Challenge results. neg, Negative; pos, positive.

not Der p 10 (tropomyosin) and arginine kinase, which makes cross-reactivity to HDM less likely. Furthermore, immunoblots from patients with shrimp allergy¹ showed different binding patterns compared with those from the 4 primary patients with mealworm allergy, and their basophil activation to mealworm was stronger than for shrimp (see Fig E1 in this article's Online Repository at www.jacionline.org). Because levels of sIgE to shrimp and HDM were low, inhibition studies were not feasible. However, a role for shrimp as a sensitizer was unlikely because results of food challenges with shrimp were negative.

The 2 subjects with food allergy had higher sIgE levels to mealworm, consumed larger amounts of mealworm (up to ~50 g per meal), and were exposed for a longer period of time (7-9 years) than the 2 subjects with respiratory allergy (~1 g of mealworm and 2-5 years of exposure). This might suggest that occupational exposure for longer periods or oral exposure with higher doses are required for development of mealworm allergy. In line with our study, long occupational exposure resulted in respiratory allergy to mealworm, green bottle fly, and waxmoth.^{3,4}

For both mice and human subjects, IgE binding was shown to known allergens, such as tropomyosin, arginine kinase, and the myosin light and heavy chains (see Tables E2 and E3 in this article's Online Repository at www.jacionline.org). Additionally, both mouse and human IgE recognized 3 new allergens with convincingly high scores and high sequence coverage (~90%). They were identified as highly the homologous (>88% sequence identity) mealworm larval cuticle proteins (LCPs) A1A, A2B, and A3A. IgE binding to LCP A1A was confirmed in 3 of the 4 subjects by using immunoblotting (see Fig E2 in this article's Online Repository at www.jacionline.org).

LCPs might be the dominant allergens in primary mealworm allergy, which is in contrast to cross-reactive mealworm allergy,

where the panallergens tropomyosin, arginine kinase, and myosin heavy chain were dominant.¹ LCPs have not been previously identified as allergens in insects or crustaceans.

LCPs are proteins from the mealworm with a conserved domain in arthropod cuticles known as R&R consensus, and they bind chitin.⁵ The chitin-binding complex links the soft internal tissue to the exoskeleton of the larvae. LCPs from mealworm are most likely both inhalant and food allergens since they were identified (in the Methods section in this article's Online Repository at www.jacionline.org) in subjects with respiratory as well as food allergy to mealworm.

In conclusion, mealworm exposure can lead to primary sensitization in both a mouse model and human subjects and can result in both food and inhalant allergy. LCPs might be major allergens in patients with mealworm allergy.

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Henrike C. H. P. Broekman, MD^{a,d}

André C. Knulst, MD, PhD^{a,d}

Constance F. den Hartog Jager, BSc^{a,d}

Jolanda H. M. van Bilsen, PhD^{b,d}

Florine M. L. Raymakers, RN^a

Astrid G. Kruijzinga, PhD^{b,d}

Marco Gaspari, PhD^c

Caterine Gabriele, PhD^c

Carla A. F. M. Bruijnzeel-Koomen, MD, PhD^{a,b,d}

Geert F. Houben, PhD^{a,b,d}

Kitty C. M. Verhoeckx, PhD^{a,b,d}

From ^athe Department of Dermatology and Allergology, University Medical Center Utrecht, Utrecht, The Netherlands; ^bTNO, Zeist, The Netherlands; ^cthe Department of Experimental and Clinical Medicine, Magna Graecia University of Catanzaro, Catanzaro, Italy; and ^dthe Utrecht Centre for Food Allergy, Utrecht, The Netherlands. E-mail: h.c.h.broekman-4@umcutrecht.nl.

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The benzene metabolite 1,4-benzoquinone reduces regulatory T-cell function: A potential mechanism for tobacco smoke-associated atopic dermatitis



To the Editor:

Exposure to environmental chemicals can contribute to the development or exacerbation of atopic dermatitis (AD). Benzene is one of the chemicals previously been implicated in the etiology of AD.¹ Albeit a ubiquitous environmental chemical, nonoccupational exposure to benzene mainly results from active or passive tobacco smoke exposure in homes or public places. Exposure to benzene has been shown to exert diverse effects on blood cells, in particular on lymphocytes. Although chronic exposure to high levels of benzene may cause leukemia and probably other lymphohematopoietic cancers, already low levels of benzene have been described to decrease the number of peripheral lymphocytes² in humans including circulating CD4⁺ T cells, CD4⁺/CD8⁺ ratios, B cells, neutrophils, and platelets.³

Although benzene itself is not regarded as toxic, its metabolization products are believed to be the main source of benzene toxicity. Benzene degradation in humans preferentially occurs via the formation of phenol from the initial oxidation product benzene oxide.⁴ Phenol is then further degraded to the highly reactive electrophile 1,4-benzoquinone (1,4-BQ; see Fig E1 in this article's Online Repository at www.jacionline.org),

which can readily react with cellular nucleophiles including proteins or DNA. There is a nonlinear relationship between benzene exposure and metabolite formation, causing a favored production of reactive quinones in case of low-dose benzene exposure (<1 ppm).⁵

We have previously reported that prenatal benzene exposure is associated with decreased cord blood regulatory T (Treg) cell numbers and a subsequent increased risk for the development of AD in early childhood.¹ This reduction in immunocompetent cells is likely a contributing factor to the increased risk of AD described in relation to benzene/environmental tobacco smoke exposure. To further elucidate the processes underlying the benzene-associated Treg-cell deficiency, we used an *in vitro* model based on expanded human Treg cells (CD4⁺CD25^{hi}CD45RA⁺; for details see Fig E2 in this article's Online Repository at www.jacionline.org) derived from healthy blood donors. As publicly available RNASeq data revealed that neither resting nor activated Treg cells express the necessary enzymes for the degradation of benzene via phenol (see Table E1 in this article's Online Repository at www.jacionline.org), we directly exposed *in vitro* expanded Treg cells to the benzene metabolite 1,4-BQ. Although Treg-cell exposure (independent donors, n = 5) to 50 nM 1,4-BQ for 24 hours, corresponding to concentrations previously observed in smokers,⁶ had no effect on FOXP3 expression as monitored by fluorescence-activated cell sorting (see Fig E4 in this article's Online Repository at www.jacionline.org), it led to a significant decrease in IL-10 production (see Table E2 in this article's Online Repository at www.jacionline.org; signed rank sum test, *P* = .005) determined by cytometric bead array assays in the cell culture supernatants (cytometric bead array assays performed at least in triplicates; see this article's Online Repository at www.jacionline.org). We hypothesize that this decrease in IL-10 production, a key anti-inflammatory cytokine critical for efficient Treg-cell function, contributed to the impairment in Treg-cell function subsequently observed in *in vitro* suppression assays (Fig 1, A). Exposed Treg cells were cocultured at different ratios with 5(6)-carboxyfluorescein N-succinimidyl ester (CFSE)-labeled responder T cells (CD4⁺CD25[−] as Tconv) of the same donor for 5 days (for details, see this article's Online Repository at www.jacionline.org). 1,4-BQ-exposed Treg cells showed a significant decrease in suppressive function compared with vehicle control-treated Treg cells of the same donor (Fig 1, A; 2-way ANOVA, *P* = .002). Evaluation of cytokine secretion during the duration of the suppression assay revealed a significant increase in IL-13 and granzyme B (GrzB; Fig 1, B; see Table E3 in this article's Online Repository at www.jacionline.org). Elevated GrzB concentrations were most likely related to enhanced production by responder T cells (Tconv) because they seem to be the main source of GrzB, secreting about 25-fold higher amounts of GrzB compared with Treg cells (see Table E4 in this article's Online Repository at www.jacionline.org). This increase in GrzB as an important mediator of cell death and Treg-cell activity might point to an enhanced apoptosis rate in Treg cells, which—in addition to their diminished IL-10 secretion—could account for the relative decrease in Treg-cell suppression by 1,4-BQ.⁷

To evaluate whether these *in vitro* observations can be related to the *in vivo* situation, we additionally studied the effect of benzene exposure in our prospective mother-child cohort (LINA: Lifestyle and Environmental Factors and Their Influence on Newborns

METHODS

Study design

This study was designed to assess whether mealworm could cause primary sensitization and food allergy.

For the clinical trial, through insect farms and Internet blogs, 4 subjects were located with histories of symptoms after handling, eating, or other exposure to mealworm. During a screening visit, their medical histories were obtained and, by using SPTs, subjects were examined for sensitization to food allergens (various shellfish and wheat, with the latter being an ingredient of mealworm feed), inhalant allergens (HDM, cat, dog, and birch and grass pollens), and insect allergens (mealworm, cockroach, and silkworm). In addition, BATs and Western blots were performed with mealworm, shrimp, and shrimp tropomyosin extracts. Levels of sIgE for shrimp, HDM, and mealworm were determined by using ImmunoCAP, and sIgE levels to allergen components were measured with the ImmunoCAP ISAC. Immunoprecipitation in combination with liquid chromatography–mass spectrometry was used to identify proteins that bound to IgE in sera from mice and human subjects. All subjects participated in a DBPCFC with mealworm and in an open food challenge with Dutch shrimp (*Crangon crangon*) to exclude shrimp allergy as a cross-reactive source of the mealworm allergy.^{E1}

Shrimp and mealworm extract preparation

Fresh and freeze-dried yellow mealworms (*T. molitor* L.) in the final larval stage were kindly provided by Dutch insect farm Krecia (Ermelo, The Netherlands). Extracts for human trial (SPTs; ALK-Abelló, Hørsholm, Denmark) and *in vitro* testing (Tris and urea extract for immunoblotting and combined Tris/urea extract for BAT) were prepared, as described previously.^{E1} For the animal study, to compare sensitization with a known and possibly similar allergen, shrimp (*Pandalus borealis*) were obtained from a local supermarket. Peeled shrimp (10 g) were freeze-dried, and the protein content was measured by using the Kjeldahl method. A shrimp extract (20% protein) was prepared by homogenizing the shrimp with water using an Ultra-Turrax. Mealworms were washed several times with demineralized water, and protein content was measured by using the Kjeldahl method. Dry mealworms were heated at 95°C for 5 minutes to inactivate endogenous digestion enzymes. After careful removal of the skin, the inner part of the mealworm was mixed with water by using the Ultra-Turrax to obtain a mealworm extract (20% protein). The shrimp and mealworm suspensions were frozen until further use.

Sensitization of mice with mealworm and shrimp extract

The mouse study was conducted with female C3H/HeOJ mice obtained from a colony maintained under specific pathogen-free conditions at Charles River (Sulzfeld, Germany). At commencement of sensitization (day 0), the mice were 7 weeks old. Mice were allowed access to food (cereal-based VRF1 diet; SDS Special Diets Services, Witham, United Kingdom; certified free of mealworms and shrimp) and water *ad libitum*. Mice underwent gavage 2 times at weekly intervals with shrimp/mealworm extract in PBS (20 mg of protein) with 10 µg of CT (List Biological Laboratories). Control animals received PBS and CT only. At day 16, antigen-specific IgG₁ (immunogenicity) and IgE (allergenicity) levels were determined, as described by Smit et al (submitted manuscript). The welfare of the animals was maintained in accordance with the general principles governing the use of animals in experiments of the European Communities (Directive 2010/63/EU) and Dutch legislation (Experiments on Animals Act, 1997). This included approval of the study by The Netherlands Organisation for Applied Scientific Research's Animal Experimental Committee (DEC no. 3640).

Primary sensitization to mealworm in human subjects

Four adult domestic or professional mealworm farmers, each of whom displayed symptoms on occupational mealworm exposure through inhalation or mealworm ingestion, were included in this study. The subjects were selected for suspected mealworm allergy based on suggestive history and sensitization and provided informed consent before participation.

The study was approved by the local ethics committee (NL43731.041.13).

Immunoblotting, BATs, SPTs with extracts from mealworm (ALK-Abelló), shrimp (ALK-Abelló; Stallergenes, Antony, France; and Greer Laboratories, Lenoir, NC), HDM (ALK-Abelló and Greer Laboratories), and ImmunoCAP mealworm (kindly prepared for us by Thermo Fisher, Waltham, Mass), shrimp and HDM (Thermo Fisher), and ImmunoCAP ISAC (Thermo Fisher) tests were performed, as described previously.^{E2}

A DBPCFC was performed in all 4 human mealworm-sensitized subjects, as described previously.^{E2} An open food challenge with boiled Dutch shrimp (*C. crangon*) was performed according to the challenge protocol for shrimp used at our clinic with 7 servings, dosing from 1 mg to 100 g of shrimp.

Both the DBPCFC and the open shrimp challenge were discontinued, and results were considered positive in case of objective symptoms or if a suggestive moderate-to-severe subjective symptom lasted for more than 45 minutes. The days of the DBPCFCs were debinded after a panel review by 3 clinical experts on the outcome.

Identification of IgE-binding proteins

For immunoprecipitation, Dynabeads M-280 Tosylactivated (10 mg; Invitrogen, Carlsbad, Calif) were used according to the manufacturer's instructions. After coating with 0.2 mg of Goat anti-Hu IgE (AP175 Upstate; Millipore, Temecula, Calif), the separate beads were incubated for 1 hour at 37°C with 1 mL of human or mouse serum. Conjugated beads were cross-linked with 5 mmol/L BS³ (Pierce, Rockford, Ill), according to the manufacturer's instructions, to ensure reusability of the beads. The beads were washed 3 times with 0.1% Tween 20 in PBS (pH 7.4), followed by overnight incubation at 37°C with 100 µL of Tris/urea mealworm mixture diluted with 900 µL of PBS. After washing 3 times, proteins were eluted with 2 × 100 µL of 0.1 mol/L glycine, and the pH of the solution was neutralized with 30 µL of 1 mol/L Tris-HCL (pH 8.5). Incubation with mealworm extract was repeated 3 times, and all eluates (800 µL) were pooled. Before analysis, samples were freeze-dried, reconstituted in 250 µL of 0.05% SDS, reduced with 10 mmol/L dithiothreitol (for 1 hour at 37°C), alkylated with 24 mmol/L iodoacetamide (for 1 hour at 37°C), and digested with 600 ng of proteomics-grade trypsin after quenching with 2 mmol/L dithiothreitol (for 20 minutes at 37°C). Peptides were purified by using strong cation exchange stage tips and subsequently injected for mass spectrometric analysis. Of this peptide mixture, 4 µL was analyzed according to Verhoeckx et al,^{E3} with the following minor modifications: the gradient elution was achieved at 350 nL/min flow rate and ramped from 8% B to 35% B in 60 minutes and from 30% B to 100% B in an additional 8 minutes; after 5 minutes at 100% B, the column was re-equilibrated at 0% B for 2 minutes before the subsequent injection. MS detection was performed on a Quadrupole-Orbitrap Mass Spectrometer Q Exactive (Thermo Fisher Scientific, Bremen, Germany) by using a top-12 method with resolution (FWHM; full width at half maximum). Data were processed with Proteome Discoverer 1.3 (Thermo Fisher Scientific), using SEQUEST as search engine, and the Swiss-Prot database was accessed on February 2013 as sequence database (3,123,840 sequences for Metazoa taxonomy). The following search parameters were used: MS tolerance, 15 ppm; MS/MS tolerance, 0.02 Da; fixed modifications, carbamidomethyl cysteine; enzyme, trypsin; maximum missed cleavages, 1; and taxonomy, Metazoa. High-confidence peptides (confidence >99%) were filtered out with Percolator, which was integrated in Proteome Discoverer. Protein hits based on 2 successful peptide identifications were considered valid.

Statistical analysis

Data were collected with IBM SPSS Statistics 21 software (IBM, Armonk, NY). Mice data are presented as means and analyzed with GraphPad Prism software (GraphPad Software, La Jolla, Calif). Antibody levels were analyzed by using the Mann-Whitney *U* test or the Kruskal-Wallis test, followed by the Dunn multiple comparison test.

Although based on a very limited number of cases, the results of this study suggest the occurrence of 3 different phenotypes of primary allergy caused by exposure to mealworm allergens. The 2 occupational workers who had limited oral exposure had occupational respiratory allergy, whereas the domestic breeders who reportedly had more frequent and higher oral intakes had food allergy. One of the 2 never experienced respiratory symptoms during the work with mealworm.

The data suggest that the differences in exposure patterns might result in a different phenotype. Most food-related occupational allergens have not been proved to induce symptoms after ingestion. Whether this lack of evidence is due to underreporting or actual absence of food-induced allergic symptoms in patients with occupational allergy is unclear; data on intake of the work-related food are missing. A few isolated cases have been reported on occupational allergy and the subsequent development of food allergy to the same source of protein. These subjects were reported to work in the garlic, buckwheat, shrimp, and snow crab industries.^{E4-E7}

The risk for mealworm allergy in patients with shrimp allergy through cross-reactivity was shown in our previous study. The present study shows that primary respiratory and food allergy to mealworm can develop as a result of dermal, respiratory, and/or oral exposure. The relative contribution of the oral exposure route cannot be established, although it is remarkable that the 2 subjects who had food allergy to mealworm had a more frequent and higher preceding consumption of mealworm. All 4 subjects with primary allergy to mealworm were atopic but without any food allergy. Thus far, there are no indications that also nonatopic subjects could be at relevant risk for development of primary allergy caused by exposure to or consumption of mealworms.

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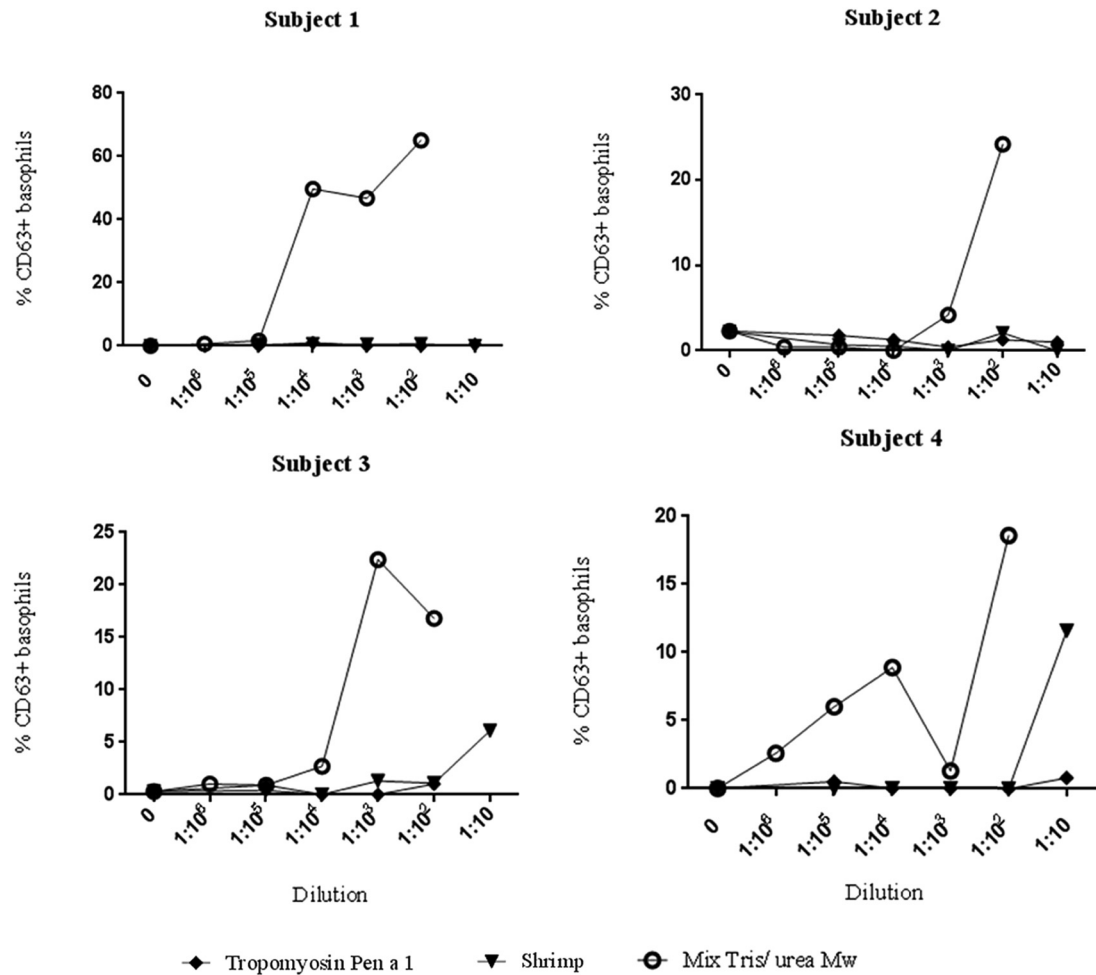


FIG E1. Functional IgE binding. BATs showing basophil activation to mealworm proteins (Tris/urea extract) and shrimp (SPT, ALK-Abelló). No reactivity to Pen a 1 (shrimp tropomyosin) was seen. Clinically, subjects 1 and 2 showed respiratory symptoms to mealworm; subjects 3 and 4 showed food-induced allergic symptoms.

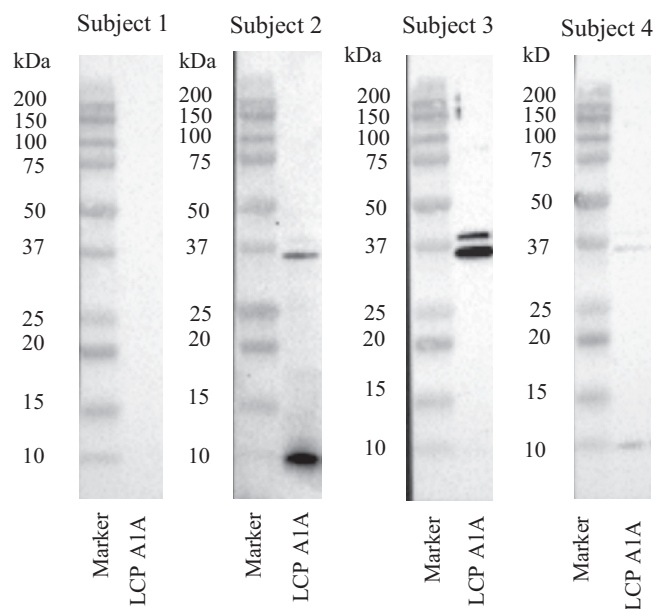


FIG E2. Three patients with mealworm allergy sensitized to the LCP A1A on immunoblotting. Both patients 3 and 4 with food allergy show IgE binding to the mealworm LCP A1A. IgE from 1 subject with inhalant allergy bound to LCP A1A.

TABLE E1. ImmunoCAP ISAC results

Subject no.	Pen m 1	Der p 1	Der p 2	Der p 10	Bla g 7	Ani s 3	Cyn d 1	Phl p 1	Phl p 2	Phl p 4	Phl p 5
1	0	0	1.1	0	0	0	0	0	0	0	0
2	0.6	0	0	0.6	0.6	0.8	2.3	0	0	2.0	0
3	0	0	0	0	0	0	2.0	0	0	2.8	2.1
4	0	9.7	13	0	0	0	14	20	1.2	0	3.6
Subject no.	Cry j 1	Cup a 1	Pla a 2	Can f 5	Fel d 1	Der f 1	Der f 2	Lep d 2	Jug r 2	Jug r 3	MUXF 3
1	0	0	0	0	0	0	2.1	5.4	0	0	0
2	0	0	0.4	0	0	0	0	0	2.4	0	1.7
3	0.5	1.0	1.5	0	0	0	0	0	1.9	0	2.0
4	0	0	0	1.0	6.2	10	24	0	0	0.3	0

All patients show some sensitization to both common inhalant and food allergens: values (>0.3) are expressed in ISU. Only scores that were positive in at least 1 subject are shown: Pen m 1, 2, and 4 are from shrimp; Der p 1, 2, and 10 are from HDM; Bla g 7 is from cockroach; Ani s 3 is from *Anisakis* species; Cyn d 1 is from Bermuda grass; Phl p 1, 2, 4, and 5 are from timothy grass; Cry j 1 is from Japanese cedar; Cup a 1 is from Arizona cypress; Pla a 2 is from plane tree; Can f 5 is from dog; Fel d 1 is from cat; Der f 1 and 2 are from HDM; Lep d 2 is from storage mite; Jug r 2 and 3 are from walnut; and MUXF3 is from bromelain.

TABLE E2. IgE-binding proteins identified in mice

Protein (source)	Accession no.	Score	Sequence coverage (%)	Peptides identified	PSM	Mass (kDa)
Beads, mice						
LCP A1A (<i>Tenebrio molitor</i>)	P80681	519	97	15	114	17.7
LCP A2B (<i>Tenebrio molitor</i>)	P80682	300	97	10	70	12.3
LCP A3A (<i>Tenebrio molitor</i>)	P80683	292	97	15	70	14.0
Tropomyosin-like (<i>Tribolium castaneum</i>)	D6X4X3	85	38	12	23	32.3
Actin-87E (<i>Drosophila melanogaster</i>)	P10981	60	22	9	14	41.8
TM-E1A = cuticular protein (<i>Tenebrio molitor</i>)	Q9TXE4	32	27	3	9	23.2
Other known allergens identified						
Chitin-binding protein (<i>Nasonia vitripennis</i>)	K7IX02	29	8	4	7	23.0
Myosin heavy-like (<i>Tribolium castaneum</i>)	D6WVJ3	27	15	9	10	262.1
Troponin T-like (<i>Tribolium castaneum</i>)	D6W953	17	14	5	6	45.7
Myosin light chain 2 (<i>Tribolium castaneum</i>)	D6WZU7	12	9	3	4	31.3
Arginine kinase (fragment [Lasippa tiga])	D1L9H9	11	19	2	2	12.0

Proteins were identified on beads with IgE from mealworm-sensitized mice after incubation with Tris/urea extract by using liquid chromatography–tandem mass spectrometry. The top 6 IgE-binding proteins were identified with liquid chromatography–tandem mass spectrometry based on the highest mean score (pooled sensitized mouse sera [n = 6]) and top 5 known allergens (not in protein top 6). Results were arranged based on highest scores. Identification was based on homology with metazoan proteins in the Swiss-Prot database. Known allergens are noted in boldface and based on arthropod nomenclature. Scores are defined as the sum of individual SEQUEST scores of all the identified peptides assigned to the protein itself. The score is the probability that the observed match is not a random event.

PSM, Peptide-spectrum matches, a value that represents the number of tandem mass spectrometric spectra that matched peptide sequences assigned to that particular protein.

TABLE E3. IgE-binding proteins identified in 4 human subjects

Protein (source)	Accession no.	Score	Sequence coverage (%)	Peptides identified	PSM	Mass (kDa)
Beads, subjects 1-4						
LCP A1A (<i>Tenebrio molitor</i>)	P80681	521	84	15	115	17.7
LCP A2B (<i>Tenebrio molitor</i>)	P80682	436	90	10	97	12.3
LCP A3A (<i>Tenebrio molitor</i>)	P80683	378	97	15	88	14.0
Tropomyosin-like (<i>Tribolium castaneum</i>)	D6X4X3	78	37	12	21	32.3
TM-E1A = cuticular protein (<i>Tenebrio molitor</i>)	Q9TXE4	56	43	6	14	23.2
Myosin heavy chain-like (<i>Tribolium castaneum</i>)	D6WVJ3	55	21	17	20	262
Known allergens identified						
Troponin T-like (<i>Tribolium castaneum</i>)	D6W953	32	19	8	12	45.7
Chitin-binding protein (<i>Nasonia vitripennis</i>)	K7IX02	27	6	3	8	23.0
Arginine kinase (fragment [Stibochiona nicea])	B3TFY5	12	11	3	4	22.6
Myosin light-chain 2 (<i>Tribolium castaneum</i>)	D6WZU7	10	9	3	3	31.3
Allergen Ale o 10 (<i>Aleuroglyphus ovatus</i>)	A1KYZ1	6	5	2	3	33.0

Proteins were identified on beads with IgE from the 4 subjects after incubation with Tris/urea extract by using liquid chromatography–tandem mass spectrometry. The top 6 IgE-binding proteins were identified by using liquid chromatography–tandem mass spectrometry based on the highest mean score (4 subjects) and the top 5 known allergens (not in protein top 6). For sequence coverage, peptides were identified, and PSM values are given as the mean of the 4 sera. Identification was based on homology with metazoan proteins in the Swiss-Prot database. Scores were defined as the sum of individual SEQUEST scores of all the identified peptides, which were assigned to the protein itself. The score is the probability that the observed match is not a random event. Known allergens are noted in boldface and based on arthropod nomenclature.

PSM, Peptide-spectrum matches, value that represents the number of tandem mass spectrometric spectra that matched peptide sequences assigned to that particular protein.