

IgE-mediated inhalant allergy in inhabitants of a building infested by the rice weevil (*Sitophilus oryzae*)

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The rice weevil (Sitophilus oryzae) is known as a stored product pest. Occupants living in a factory used previously as a granary and known to be contaminated by the rice weevil developed rhinitis and asthma. To study the role of the potential insect allergens, extracts were prepared from whole body and a grain dust of the rice and grain weevil (Sitophilus granarius). The extracts were coupled to cyanogen bromide-activated paper disks. Skin prick tests (SPT) were performed using common inhalant allergens as well as the insect allergen preparations. In cases of a positive SPT, specific serum IgE to the insect products was measured. Histamine release studies with peripheral leukocytes (HR) and studies using immunoblot techniques were performed. Fifteen of 39 subjects living in the infested rooms demonstrated positive SPT to the extracts of insect origin. Histamine equivalent wheal size induced by the whole body extract of the rice weevil required 3–160 µg protein/mL. Five of 15 subjects (with positive SPTs) had elevated specific IgE-levels to the insect material. RAST-inhibition studies indicated cross allergenicity between rice and grain weevils, and also between the frass of both beetles. Five of 15 subjects were positive in the HR assay (Ag30, allergen concentration for 30% HR for the whole body extract: range, 10⁻³ to 400 µg protein/mL, maximum release: 26% to 89%). IgE binding was detected after SDS-PAGE by immunoblot techniques at different locations: 35–38, 54, 67, 70, and >94 kD. These results indicate that many subjects continuously exposed to allergens of the rice weevil make insect-specific IgE antibodies and, therefore, are at high risk of developing IgE-mediated respiratory disorders.

INTRODUCTION

Inhalant allergy to arthropods is of major social, economic, and medical importance since it has been recognized that inhalation of insect-derived material can cause allergic reactions.^{1–3} Rhinitis, conjunctivitis, and asthma have been attributed to inhalant sensitivity to the scales, hair, and other emanations of insects for many years. The taxonomic class of insects consists of a large number of orders, which are separated and classified into several families. Thus, there is a complex variety of insect-derived allergens in the environment, which might occur in appropriate size and in sufficient numbers for entry into the respiratory tract where they may in-

duce sensitization in the same manner as pollen or other plant- and animal-derived allergens.¹ It is unclear how many insects cause inhalant allergy, nor is it understood how insect allergens become airborne. It has been suggested that protein debris from insects are part of environmental dust.⁴

The number of "well documented" cases of respiratory allergy caused by inhaled insect materials is relatively small. It has been shown that infestation by Coleoptera (beetles, weevils) can cause immediate and late phase type reactions after inhalation of grain dust.^{5,6} The rice weevil (*Sitophilus oryzae*, Fig 1), a beetle and closely related to the grain weevil (*Sitophilus granarius*), is known as a stored product pest. Their larvae grow up in the intact grain of different cereals. An approximately 120-year-old factory in Berlin, formerly used as a granary and now as housing space for flat share communities, was found to be extensively contaminated by the

rice weevil because cereal products remained under the floor. The finding that many occupants of this building had rhinitis and bronchial asthma led to our investigation of the allergens of the rice weevil.

METHODS AND MATERIALS

Thirty-nine subjects (8–45 years old, mean age 28) who had given informed consent and lived in the factory were evaluated by case history and SPT. The patients were asked about allergic symptoms, the time symptoms occurred during the day and the onset of symptoms when the patient was in contact with other hypersensitivity inducing factors.

Allergen Source

After identification and taxonomic classification of the rice weevil as the insect predominantly infesting the factory, this beetle and another close relative (grain weevil) were chosen for further study. All insect materials were generous gifts of Dr. Rassmann (Biological Institution of

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Figure 1. The rice weevil, *Sitophilus oryzae*.

the Federal Government Authority of Germany, Department of Stored Product Protection, Berlin, Germany). The insects were cultured in glass flasks containing exclusively rye grains. The containers were closed using a stopper with small airholes. The adult species of the female beetles put their eggs into the intact grain in which the larvae grow until they leave the cocoon-like hull of the grain as perfectly developed beetles. After several weeks, the culture contains only adult beetles of one species and a flour-like material, "frass," consisting of fragments from feces and debris of their larvae and powdered rye grains. Both fractions, beetles and frass, were completely separated by a careful sieving procedure and stored separately.

Allergen Preparation

After freezing the insects at -20°C for four hours, the whole bodies of the arthropods were crushed with a pestle. These materials (individually for the rice and the grain weevils) and the flour-like frass (individually for both species) were used to extract antigenic material, as described elsewhere.⁷ In brief, 5 g of the different raw materials were extracted overnight at 4°C in slowly

rotating 50-mL tubes (Falcon) containing 50 mL of a 0.01 M potassium phosphate buffer (pH 7.0) supplemented with 3 mM sodium azide (NaN_3) as an antimicrobial agent. The homogenate was centrifuged for 30 minutes at $40,000\text{ g}$ at 4°C . To remove low molecular weight, pharmacologically active compounds, the supernatants of the crude extracts were dialyzed against a potassium phosphate buffer using tubing with a molecular weight cut off of approximately 3 kD (Serva, Germany). The total protein content was measured by the Lowry assay.⁸

Skin Prick Tests

Skin prick tests (SPT) were performed with glycerinated allergenic extracts purchased from Allergopharma (Reinbek, Germany) and ALK (Horsholm, Denmark) as well as histamine (1 mg/mL) and physiologic saline as a positive and negative control. The inhalant seasonal and perennial allergens prevalent in northern and central Europe were tested: birch, timothy grass, mugwort pollen, cat, dog, and horse dander, two house dust mites *Dermatophagoides pteronyssinus* and *farinae*, and *Alternaria*. None of the patients had taken medications that might interfere with SPT results. Additionally, skin tests with insect extracts were performed on the volar forearm using the following concentrations of the insect materials: 6.8 mg/mL protein (rice weevil whole body extract), 9.5 mg/mL (grain weevil whole body extract), 4.4 mg/mL (rice weevil frass extract), and 6.9 mg/mL (grain weevil frass extract). Skin tests with the allergen extracts listed above were done in all patients included in the study. The tests were considered to be positive when the wheal size induced by the allergen was at least $>100\%$ of the wheal size induced by the histamine positive control.

In cases of a positive reaction to the arthropod material, titrated SPTs were performed with several dilutions of the insect extracts: 1:1,

1:10, 1:100, 1:1000, 1:10000. After 15 minutes, the wheals were marked and documented with a strip of tape. The diameters were calculated using the formula $(D_{\text{max}} + d_{\text{min}})/2 = d_{\text{mean}}$ and plotted against the extract concentrations using a logarithmic scale.

Specific Serum IgE and Inhibition Experiments

The sera of the patients with a positive SPT to the insects and nonallergic control sera ($n = 4$) were measured by RAST using allergen disks prepared with the dialyzed extracts according to the directions of Ceska et al.⁹ and Maasch et al.¹⁰ For the preparation of the allergen disks, 20 μg of protein were used for the coupling to each CNBr-activated paper disk, since this amount of protein has been shown to provide sufficient binding of antigenic material to the solid phase even in cases of crude, not well characterized allergens [R. Wahl, personal communication]. After the coupling, the amount of protein bound to each disk was measured. For nonspecific binding, the allergen disks were tested with the nonallergic control sera.

The allergenic activity of the insect extracts was determined by RAST-inhibition¹⁰⁻¹² using allergen disks prepared with the dialyzed extracts and the sera of the patients (diluted 1:10 in sample diluent, Pharmacia, Sweden). As the indicator for bound specific IgE, 125-I-anti-human-IgE (Pharmacia) was used.

Histamine Release from Peripheral, Washed Leukocytes

After sedimentation of 20 mL peripheral blood for 90 minutes in a solution containing 5 mL 6% dextran (molecular weight = 60 kD, Knoll, Germany), 2 mL 0.1 M EDTA, 0.6 mL 50% glucose (Braun, Germany), the basophil containing supernatant was centrifuged (400 g , 4°C). The pellet was gently shaken and washed twice by centrifugation (400 g , 4°C) with 50 mL of a buffer

containing 25 mM Tris, 0.1 mL of 20% HSA, either complemented with 0.1 M EDTA or without EDTA. The cells were next resuspended in the same buffer without EDTA, but with 5 mM CaCl_2 and 5 mM MgCl_2 .

For IgE-mediated histamine release, 250 μL of the cell suspensions were incubated in duplicate with 250 μL of the arthropod extracts (final dilutions: 1:10, 1:10², 1:10³, 1:10⁴, 1:10⁵, 1:10⁶, 1:10⁷, 1:10⁸) or anti-IgE (final concentrations: 1, 10, 100, 1000 IU/mL) in a water bath (37 °C, 45 minutes). For the determination of total histamine, four 250 μL portions of cells were lysed by 250 μL 0.4 N HClO_4 . Spontaneous HR was determined by incubation of 250 μL cells with 250 μL buffer. After removal of the cells by centrifugation (800 g, 4 °C, 15 minutes), the supernatants were assayed for histamine using an automated fluorometric method.¹³

Histamine release (HR) was expressed as a percentage of the total histamine content after subtracting the spontaneous release. All experiments were performed in duplicate. Histamine release of $\geq 30\%$ was defined as a positive result. The dose response curves, obtained with the insect allergen extracts, were used to calculate the maximum antigen-induced HR (HR_{MAX} = cell reactivity) and the allergen concentration for 30% HR (Ag_{30} = cell sensitivity).¹³

SDS-PAGE/Westernblot

About 200 μL of the insect allergen extracts were separated on a 7.5% to 20% sodium dodecylsulfate-polyacrylamide gel (Sigma, Germany) by electrophoresis (SDS-PAGE) modified after Neville¹⁴ and transferred electrophoretically to a nitrocellulose membrane (Schleicher, Germany). The nitrocellulose was blocked with 0.1% Tween 20 (Sigma, Germany) in 0.1 M Tris-HCl, pH 7.4, 0.1 M NaCl, 2.5 mM MgCl_2 , cut into strips 2 mm wide and incubated with patient sera as described previously.¹⁵ For detection of IgE, an alkaline phosphatase

linked second goat-anti-human IgE antibody (Jackson Inc. Dianova, Germany) was used.

RESULTS

Skin Prick Tests

Fifteen of 39 exposed subjects had positive SPTs to the rice weevil allergen extract. None of the subjects studied showed a positive skin reaction to the saline negative control. The dose-dependent skin test response to the rice weevil obtained in four sensitized individuals is shown in Figure 2. Skin prick tests

with an allergen extract from the frass of the beetle were also found to be positive. Figure 3 shows one example of a titrated prick test result in a patient suffering from asthma after being exposed to the beetles. Nine of the 15 rice weevil SPT-positive subjects also had positive skin test reactions to one or several inhalant allergens. Table 1 summarizes the diagnostic results in all individuals living in the factory who were willing to be studied. Patient 9, with a clear cut case history of rice weevil induced bronchial

	[mg/ml]	A.W.	S.G.	K.L.	I.F.
histamine	1	○	○	○	○
rice weevil (protein)	6.8	○	○	○	○
	6.8×10^{-1}	○	○	○	○
	6.8×10^{-2}	○	○	○	○
	6.8×10^{-3}	○	○	○	○
	6.8×10^{-4}	○	○	○	○

Figure 2. Dose-dependent wheal responses after SPT with an allergen extract from the rice weevil (whole body) in four subjects exposed to the beetle. The total protein content of the extract was measured, and several 10-fold dilutions were applied to titrate the SPT-response. Histamine (1 mg/mL) served as a positive control. No reactions occurred after challenge with physiologic saline used as a negative control.

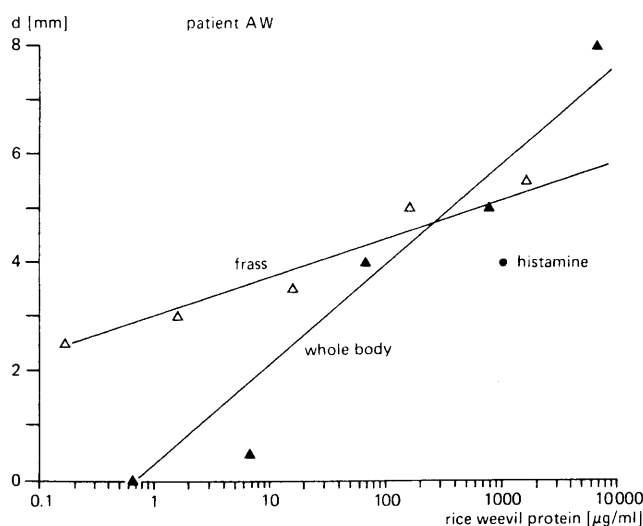


Figure 3. Dose-dependent wheal diameters (d) after SPT using two different extracts from the rice weevil (whole body = black triangles and frass = opened triangles) in one individual with allergic rhinoconjunctivitis and asthma after 6 weeks of daily exposure to the insect. The histamine (1 mg/mL) induced wheal is represented by the black dot.

asthma, did not react to common inhalant allergens by SPT.

Specific Serum IgE and Inhibition Experiments

Allergen-specific serum IgE to different insect materials was determined in parallel using the RAST. Nonspecific binding did not exceed 400 counts per minute (cpm); thus, positive results were considered to be above 800 cpm to ensure specificity of the IgE binding. The results of eight individuals demonstrating a positive insect SPT are shown in Figure 4. The amount of specific serum IgE is represented by the individual counts per minute but can also be expressed by corresponding classes.

Specificity of the IgE binding was shown by RAST inhibition experiments. Measurement of rice weevil-specific IgE obtained with the whole body extract was inhibited by preincubation of a patient's serum with the whole body extract as well as with the frass extract of the rice weevil. The inhibition curves (Fig 5) were parallel and reached a maximum inhibition of more than 80%. The inhibition curve for the frass extract was shifted to tenfold higher protein concentrations. Similar inhibition curves were also obtained after inhibition of rice weevil specific IgE measurements by preincubation with extracts from the grain weevil (whole body and frass). Conversely, allergen specific IgE determinations to grain weevil allergens were inhibited by preincubation with the rice weevil extract (whole body and frass, data not shown). The protein concentrations for 50% inhibition were found to be in the microgram per milliliter range.

Histamine Release

Dose-dependent histamine release was obtained in five subjects with positive SPT results to the rice weevil (Fig 6). Maximum mediator release of more than 50% of the total histamine content was detectable in all cell donors. One patient (number 3) released nearly 90% of cellular histamine after challenge with $\mu\text{g}/$

Table 1. Diagnostic Results* Obtained in 42 Individuals Using a Whole Body Extract of the Rice Weevil

Subjects	Skin Prick Tests		Specific Serum IgE Rice Weevil	Histamine Release Rice Weevil
	Inhalant Allergens	Rice Weevil		
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	-	(+)
6	+	+	-	-
7	+	+	-	-
8	+	+	-	-
9	-	+	+	+
10	-	+	nd	nd
11	-	+	nd	nd
12	-	+	nd	nd
13	-	+	-	-
14	-	+	-	(+)
14 nonatopic	-	-	nd	nd
10 atopic Subjects	+	-	nd	nd
4 nonexposed Subjects	-	-	-	-

* Results of skin prick tests (SPT), specific IgE and histamine release in 14 sensitized subjects, SPT was considered to be positive when the wheal size induced by the allergen was at least >100% of the wheal size induced by the histamine positive control. Specific serum IgE levels above 800 CPM (twice the nonspecific binding) were considered to be positive. Secretion of $\geq 30\%$ of the total histamine was defined as a positive result in the histamine release assay (nd: not detected). Fourteen nonatopic subjects without and 10 atopic subjects with positive SPTs to common inhalant allergens did not show a positive skin reaction to the rice weevil although they were constantly exposed to the insect. Four nonexposed subjects served as additional controls.

mL concentrations of the rice weevil proteins. The % maximal release for the individual dose-response curves varied over a wide range; thus, the rice weevil protein concentration required for 30% histamine release was found to be between 10^{-3} and $400 \mu\text{g}/\text{mL}$.

Histamine release experiments with different extracts of insect origin demonstrated quite parallel cellular responses to the whole body extract of the rice weevil and the grain weevil as well as an almost identical response to the frass extract of both beetles. The mediator release response to frass extracts was shifted to approximately 20 to 80-fold higher protein concentrations (Fig 7). No histamine release to any of the arthropod extracts was found in four subjects who had not been exposed to the beetles and had not developed indices of specific sensitization (Table 1).

Immunoblot Studies

After separation of the whole body allergen extract of the rice weevil by SDS-PAGE, IgE binding to single allergens could be shown at five different locations. Their molecular weights were estimated using defined marker proteins. Following IgE-binding, several bands of the allergen extract of the rice weevil could be identified using nine sera of patients with immediate type allergic reactions to the beetle (Fig 8): 70 kD/38 kD ($n = 3$), 70 kD/54 kD/35 kD ($n = 1$), 70 kD ($n = 1$), 38 kD ($n = 1$), >94 kD/67 kD/54 kD ($n = 1$). A negative control serum and two of nine patients' sera showed no IgE binding. Attempts to correlate the individual IgE binding patterns with the degree of sensitization (ie, results for SPT, specific serum IgE, histamine release test) failed; no relationship between the frequency or strength of IgE binding

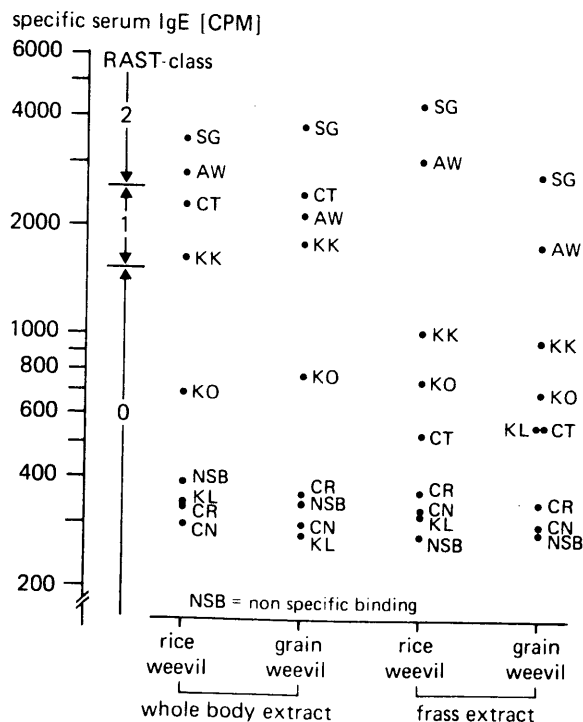


Figure 4. Specific serum IgE levels in eight exposed subjects sensitized to the rice weevil as shown by SPT. The extracts from four different sources (see x-axis) were dialyzed before they were coupled to CnBr-activated paper disks. The counts per minute (cpm, left y-axis) and the RAST classes are given (right y-axis). A specific IgE level was considered positive when binding of the serum IgE exceeded at least twice the nonspecific binding.

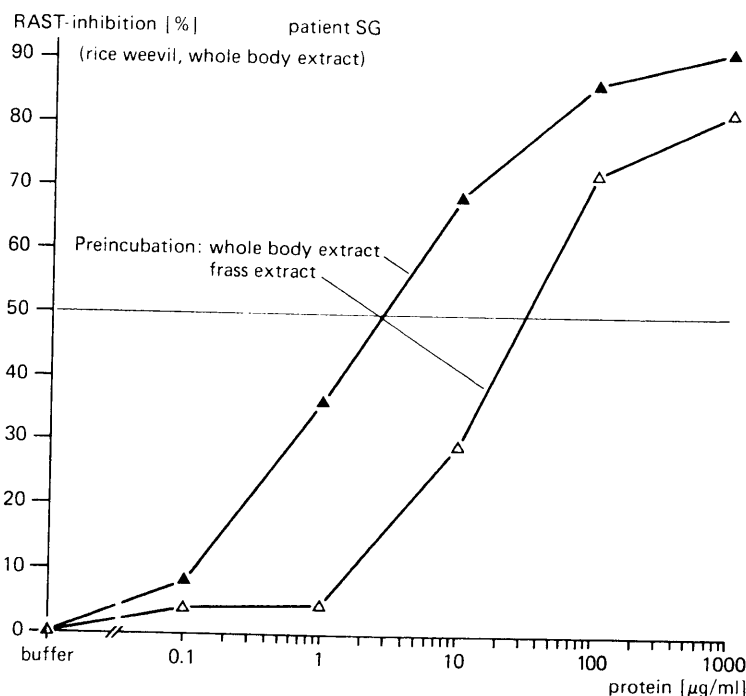


Figure 5. RAST inhibition curves of one subject's allergen-specific serum IgE are shown. Preincubation of the serum with different concentrations (given as total protein) of the whole body extract and also with the frass extract of the beetle abolishes the binding to the solid phase.

and the other diagnostic results could be determined.

Bands with IgG-binding properties were found at the following locations: 14, 43, 70, 78, >94 kD. One sharp and a few weak IgG-bands in the area above 94 kD were also demonstrated using the control serum.

DISCUSSION

The inflammatory reactions to insect allergens and the disease one develops depend on both the intensity and the anatomic location of exposure and the specific nature of the immune response. IgE-mediated hypersensitivity to insect allergens occurring in the eye, nose, or lung may result in symptoms of conjunctivitis, rhinitis and asthma, respectively.² The symptoms in patients with respiratory allergies caused by arthropods are similar to inhalant allergies caused by other factors (eg, pollens).¹ Arthropod-human interactions may result in an immunization without causing disease, and this may explain the frequent finding of arthropod-specific IgG antibodies in both the normal and atopic population.²

In this study, immediate type sensitization to the rice weevil could be shown by positive SPTs, measurements of allergen-specific serum IgE and histamine release studies. Most of the subjects who developed specific sensitization to the insect were also found to be SPT positive to inhalant allergens. One patient, however, without any signs of atopy (number 9), developed an immediate type hypersensitivity with subsequent asthma attacks after continuous exposure to the allergenic products of the beetle. A much greater degree of reactivity to insect allergens has been demonstrated in allergic than in nonallergic individuals, especially in patients with seasonal as well as perennial allergic rhinitis.¹⁶ Increased numbers of positive skin test reactions to insect allergens have been documented in allergic patients as compared with nonallergic subjects.¹⁷ Skin test surveys with insect allergens suggest a

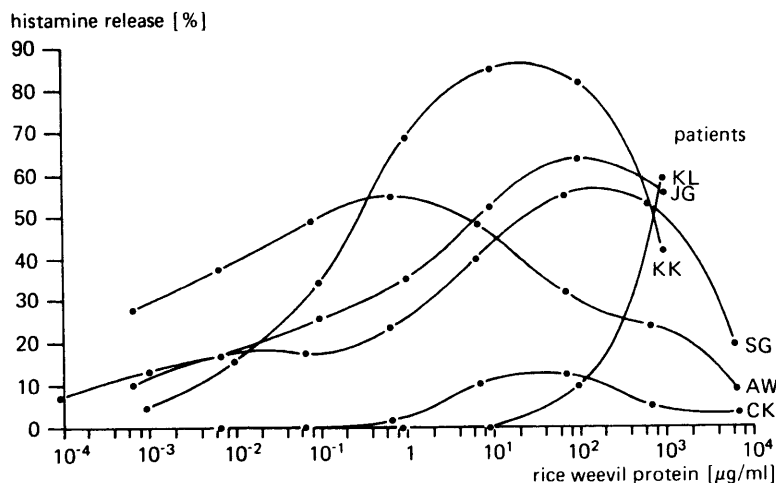


Figure 6. Leukocyte histamine release induced by the whole body extract of the rice weevil. The allergen extract concentration is given as its total protein content. Mediator release is expressed as % of the total cellular histamine content. Control experiments with leukocyte preparations of four nonexposed subjects did not show any significant histamine release (<10% of the total histamine) after incubation with the various insect extracts (data not shown).

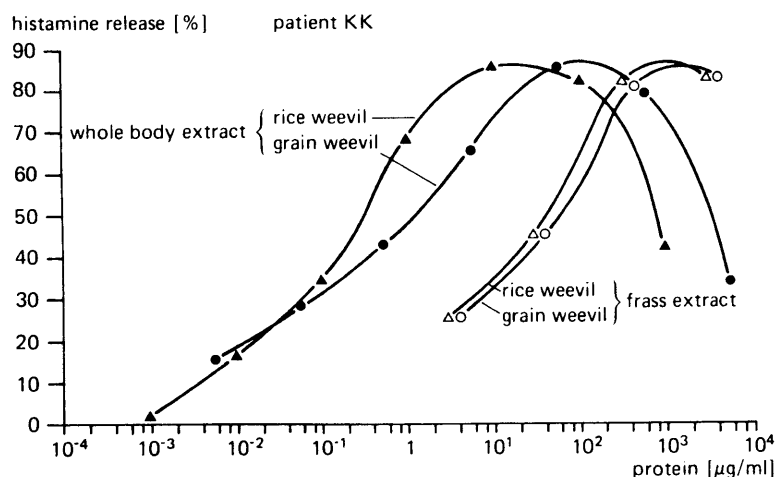


Figure 7. Biologic activity of four insect extracts (whole body and frass of two species: rice weevil and grain weevil) demonstrated in a histamine release assay using leukocytes from a person sensitized to the insect allergens. The extracts are compared on the basis of their total protein content after removal of low molecular weight compounds by dialysis (molecular weight cutoff 3 kD). Histamine release is expressed as % of the total histamine.

high frequency of sensitization in otherwise atopic individuals.

The diagnostic results in our study are quite variable and do not correspond in all cases to sensitization to the insect allergens. Only 5 of 15 subjects, SPT positive to the rice weevil, definitely had IgE-mediated hypersensitivity, shown by the presence of specific serum IgE and concordant positive histamine release to the insect allergens. Skin

test results with arthropod extracts are highly dependent on the quality of the allergen, which should not contain extraneous material and inappropriate compounds, since non-specific skin irritation may occur. Additionally, the lack of detailed data on the major arthropod antigens makes meaningful skin test surveys difficult to conduct and to interpret.¹ Undialyzed whole body extracts of the rice weevil, used for

SPT, produced nonspecific wheals with erythema and local itching that remained for a few days not only in allergic subjects but also in nonexposed, nonatopic controls (data not shown). To remove pharmacologically active irritants, dialysis of the insect allergen extracts prior use was performed, since the crude materials can not only cause nonspecific reactions but might interfere with the assay used (ie, fluorescence by low molecular weight compounds during the histamine assay).⁶

Proteins with allergenic activity were found in the whole body extract as well as in the frass extract of the insects. Some of these individual antigens were capable of binding specific IgE in the serum of the exposed subjects as shown by immunoblot techniques. In two subjects with clearcut histories and concordant diagnostic results, no IgE-binding bands could be demonstrated, which might be explained by the reducing conditions used for the SDS-PAGE. Two molecules of approximately 35–38 kD and 70 kD seem to play a major role in cases of IgE-mediated sensitization to the rice weevil, since the majority of the subjects tested showed specific IgE-binding at these locations. Histamine release studies and RAST inhibition indicated a 10- to 100-fold increased allergenic potency of the whole body extract compared with the frass extract; therefore, it might be speculated that the insects themselves contain the allergens with a major role in the IgE response. It is important to note that all extracts were compared on the basis of their total protein content, which may have no relationship to the allergenic activity.

The material used for our extracts was obtained from the rice weevil, raised only in intact corns of rye grain. The larvae grow up inside the corn and do not leave it before the metamorphosis into the adult beetle is complete. The beetles can be separated by sieving the resulting material, the frass, which appears as a

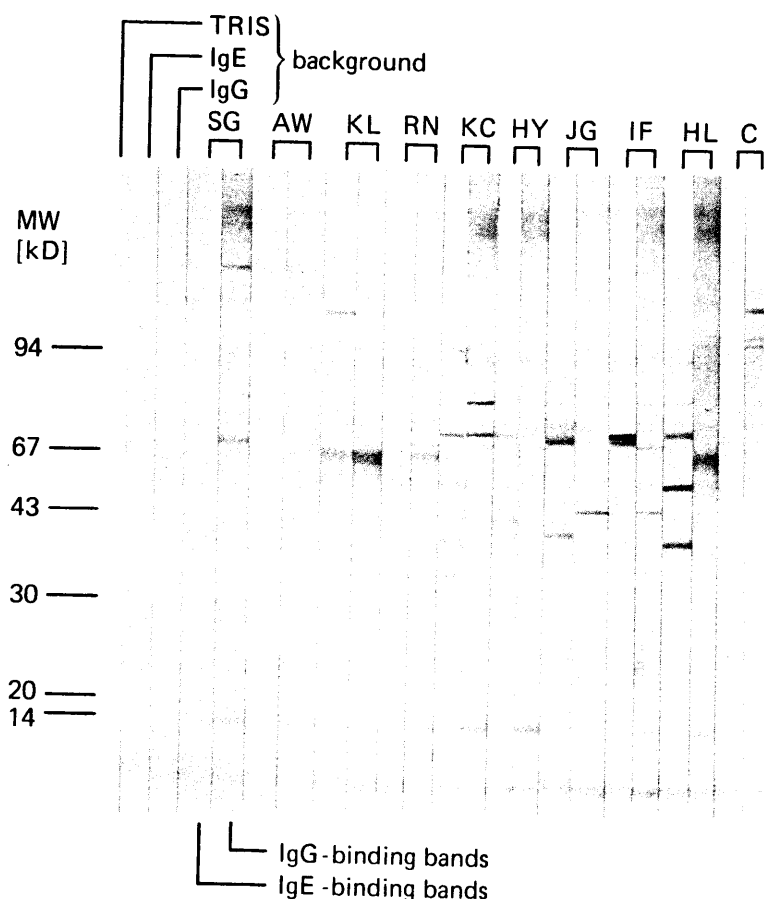


Figure 8. Immunoblot studies after SDS-PAGE separation of a whole body extract of the rice weevil. The three left lanes represent controls, which have not been incubated with patient's sera, showing no nonspecific binding. Sera of nine individuals, exposed and sensitized to the rice weevil, were investigated for IgE and IgG binding to the components of the allergen extract; a control serum (C) from a nonsensitized person has been included.

light-brown-flour-like powder. This material also contains allergenic material, indicated by SPT, specific IgE-, and histamine release results. It is unknown to what extent body parts or feces of the beetle or the larvae are responsible for its allergenicity.

Our results indicate that common allergens exist in the rice and the grain weevil. Both species are closely related biologically which might explain the cross reactivity seen in the IgE response to these allergens. The rice and the grain weevil belong to a family of beetles in which larvae and adults require intact corn for their development. One reason for the dramatic and obvious infestation of the housing space by the rice

weevil, in our study, might be a decrease in adequate food material, which forced the insects to leave the underground area seeking new food sources. In some cases the presence of insects may not be obvious or even suspected.¹⁸ Respiratory disorders caused by infestation of these insects could, therefore, go unrecognized and the incidence of this problem thereby be underestimated.

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NEW X-LINKED RECESSIVE IMMUNODEFICIENCY SYNDROME

The authors describe a family in which several males had had severe infections, including recurrent otitis media, mastoiditis, recurrent pneumonia with bronchiectasis, severe colitis, and fatal sepsis. The patient described in greatest detail remained well until 9 months of age, possibly due to prophylactic antibiotics from early infancy. Progressively protracted diarrhea began at 9 months of age. At 1 and 2 years of age serum IgG concentrations were normal and IgA, IgM, and IgE concentrations were elevated. Small bowel and colon biopsies at 5 years of age disclosed partial villous atrophy and nonspecific colitis, respectively. There was a transient T-cell lymphocytopenia during the first 4 months of life but there were otherwise normal numbers of T and B lymphocytes and natural killer cells and normal distribution of cell subsets. Nitroblue tetrazolium test, complement activity, and adenosine deaminase and purine nucleoside phosphorylase erythrocyte enzyme activities were normal. There was no evidence of maternal T-cell engraftment by HLA typing and karyotyping. The patient's serum was negative for HIV-1 and HIV-2 antigens and antibodies.

T-cell function declined progressively. Antigen-induced T-cell proliferation to tetanus toxoid and *Candida* was normal during the first year of life but later became negative with failure of response to later administration of tetanus toxoid. The MLR activity was very low and cytotoxic T-cell responses were absent but there were mitogen-induced T-cell responses. Repeated immunizations elicited no antibodies to *Bordetella pertussis* and diphtheria toxoid, and antibody titers elicited initially by immunization with poliomyelitis vaccine and tetanus toxoid later became negative.

Studies of DNA from the mother and a sister disclosed nonrandom X chromosome inactivation of T cells and, partially, of B cells but random inactivation of polymorphonuclear cells, a pattern similar to that observed in X-linked severe combined immunodeficiency carriers.

The authors conclude this new X-linked immunodeficiency may be an expression of a new X-linked genetic locus or an attenuated phenotype of X-linked severe combined immunodeficiency.

—RMS

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