

Occupational asthma induced by garlic dust

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Background: Garlic dust has not been a frequently encountered cause of IgE-mediated disease.

Objective: We report on 12 patients (all of them garlic workers) with the clinical criteria for occupational asthma.

Methods: Skin prick tests and serum-specific IgE determinations were performed with common inhalants, garlic, and other members of the Liliaceae family (onion, leek, and asparagus). Bronchial challenge test with garlic powder was performed in all patients. Garlic and onion extract proteins were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis. Immunoblot and IgE immunoblot inhibition analyses were performed with patients' sera on extracts of garlic, onion, and pollens of *Phleum pratense* and *Chenopodium album*.

Results: Garlic sensitization was demonstrated by bronchial challenge test in seven patients (group 1) and ruled out in the remaining five (group 2). Clinical data were similar in both groups. The patients with garlic allergy had a mean age of 27 years, and all of them had pollen allergy; sensitization to other members of the Liliaceae family was also common. Electrophoresis of garlic extract revealed two major protein bands at approximately 12 and 54 kd. During IgE immunoblotting, the pool of sera reacted with garlic proteins mainly at 54 kd. Preincubation with onion, *Phleum*, and *Chenopodium* partially abolished the IgE binding to several allergens of garlic.

Conclusion: We report on seven patients in whom an occupational garlic allergy was demonstrated. Garlic allergy is relatively rare but seems to affect young subjects with pollen allergy, and sensitization to other members of the Liliaceae family is common. The results of this study confirm the presence of some structurally similar allergens in garlic, onion, and certain pollens. (*J Allergy Clin Immunol* 1997;100:734-8.)

Key words: Occupational asthma, garlic, onion, grass pollen, allergens

Garlic (*Allium sativum*) is a perennial plant of the Liliaceae family. Onion, leek, asparagus, and tulip belong to the same family.

Garlic is known to be a relatively potent contact allergen. Contact dermatitis is frequently diagnosed in professional cooks or persons who handle garlic bulbs, but it has not been a frequently encountered cause of IgE-mediated disease.

Abbreviations used

BSA:	Bovine serum albumin
PBS:	Phosphate-buffered saline
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
SPT:	Skin prick test

The epidemiology of allergy in occupational exposure to garlic is unknown. In 1940, Henson¹ reported the first case of garlic-induced occupational asthma, and to date, only isolated cases of asthma and rhinitis have been reported.²⁻⁵

Twenty percent of the whole harvest of garlic from the European Union (i.e., 68,000 tons) is obtained from the Spanish province of Cuenca. Every year in this area (from November to July), more than 6000 families work on garlic farms. Despite this high level of exposure, only a few patients report garlic-related respiratory symptoms.

In this report we present the results of a clinical study carried out on a group of 12 patients with rhinitis and asthma in whom garlic occupational allergy was suspected.

METHODS

Patients

All patients were first seen with respiratory symptoms associated with occupational exposure to garlic. Occupational respiratory disease was suspected when there were at least three of the following symptoms: cough, wheezing, chest tightness, difficulty breathing, blocked or runny nose, sneezing, and running or itchy eyes after exposure to garlic.

The study was authorized by the hospital's ethical review committee, and informed consent was obtained in all cases.

Twelve patients entered the study (7 women and 5 men). The mean age of the patients was 27.6 years (SD, 9.9 years), and all of them reported rhinoconjunctivitis. Seven patients also reported perennial asthma, and 10 had exacerbations of symptoms during the spring. Nine patients worked as harvesters in the garlic fields, 10 in storage factories, and five in spice-manufacturing factories. Each patient's medical history was obtained by questionnaire during a personal interview. Questionnaire items included job description, length of garlic exposure, date of first reaction to garlic, frequency and nature of reactions, and presence of other concomitant allergies.

Skin tests

Skin prick tests (SPTs) were performed on the volar aspect of the forearm. Saline solution and histamine hydrochloride (10 mg/ml) were used as negative and positive control tests, respectively. Wheals were measured by using the largest diameter and

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its perpendicular. The test results were considered positive when the mean diameter of the wheal was 3 mm or greater than that observed with the negative control. The test panel included standardized domestic and storage mites, molds, animal danders, *Chenopodium* species, mugwort, grass, and tree pollens. Commercial extracts of garlic, onion, leek, and asparagus (Leti, Spain) and garlic powder diluted in saline solution (10 mg/ml) were used for SPTs. In addition, skin tests following the prick-by-prick method with fresh garlic, onion, leek, and asparagus were also performed.

Bronchial provocation tests

Bronchial provocation tests were performed when the clinical status of the patients was stable, when they were not being treated, and when baseline pulmonary function test results were at least 80% of expected normal values. Nonspecific bronchial hyperresponsiveness was assessed by methacholine challenge according to a standardized protocol.⁶

Specific inhalation challenges were performed with garlic powder. The method described by Pepys and Hutchcroft⁷ was used. Subjects were instructed to pour the garlic powder from one tray to another located 20 cm below it. Subjects inhaled the generated dust at tidal volume and normal frequency for 5 minutes or less if symptoms appeared. FEV₁ was recorded at baseline and at 1, 5, 15, 30, 60, and 120 minutes after challenge. Peak expiratory flow rate was also measured before the challenge test was performed and every hour thereafter for a total of 10 hours.

Oral challenge test

Oral challenge test with garlic dust was performed only when patients complained of symptoms after garlic ingestion or when garlic ingestion was stopped at least 1 month before beginning the study. Patients received gelatin capsules of garlic powder. Dosage levels were increased at 1-hour intervals until a dose of 1600 mg was reached or until clinical symptoms appeared.

In vitro tests

Specific IgE tests for positive inhalants, garlic, and onion were performed in all 12 patients with the CAP method (CAP System; Pharmacia, Uppsala, Sweden).

Preparation of extracts

Garlic, onion, and pollens of *Phleum pratense* and *Chenopodium album* were extracted with 50 mmol/L ammonium bicarbonate (pH 8.5) at a 10% (wt/vol) ratio. After stirring for 60 minutes at room temperature, the soluble components were separated from the insoluble ones by centrifugation at 27,000 g at 4° C for 20 minutes. The supernatant was collected, dialyzed against distilled water, centrifuged again as indicated, filtered through a 0.45 µm pore membrane, and lyophilized.

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE analyses of garlic and onion were performed according to the method of Fling and Gregerson.⁸ The gel was stained with Coomassie Brilliant Blue (BioRad) and used for immunoblotting as described below.

Protein transfer and immunoblotting

Protein bands from the SDS-PAGE gels were electrophoretically transferred to a polyvinylene difluoride membrane (Millipore, Bedford, Mass.) in transfer buffer according to the method of Towbin et al.⁹ After transfer, the membrane was

TABLE 1. Clinical features of the patients

	Group 1 (n = 7)	Group 2 (n = 5)
Mean age (yr)	27.4	27.8
Sex (F/M)	3/4	4/1
Allergy to pollens	7	4
Olive	5	2
Grass	4	3
Artemisia	6	4
<i>Chenopodium</i>	6	4
Mean PD ₂₀ -methacholine (breath units)	75	47
Job category		
Harvest	5	4
Storing factories	5	5
Spice factories	3	1
Duration of exposure (yr)	19	16
Mean age at onset (yr)	17.7	16.6
Mean time of evolution (yr)	9.4	10.2
Positive SPT responses		
Garlic	6	1
Onion	4	1
Leek	3	2
Asparagus	4	0
Positive CAP response to garlic (mean kU/L)	5 (4.7)	0 (<0.35)
Positive CAP response to onion (mean kU/L)	6 (8.23)	0 (<0.35)
Positive garlic inhalation challenge	7	0
Rhinitis + asthma	4	0
Rhinitis	2	0
Asthma	1	0

PD₂₀, Provocative dose of methacholine causing a 20% fall in FEV₁.

saturated for 30 minutes with phosphate-buffered saline (PBS) containing 1% bovine serum albumin (PBS-BSA 1%). The membranes were then incubated with pooled sera from group 1 diluted 1:5 in PBS-BSA 1% for 12 hours. After four washes, the membranes were incubated with anti-human IgE antibodies for 12 hours. The bands were visualized by autoradiography.

Immunoblotting inhibition

Immunoblotting inhibition studies were performed to determine whether protein from garlic, onion, and pollens (*Phleum* and *Chenopodium* species) were related. Serum pool samples diluted 1:5 in PBS-BSA 1% were preincubated with garlic, onion, *Phleum*, and *Chenopodium* extracts, respectively. Thereafter, polyvinyl difluoride membrane-blotted garlic and onion proteins were incubated with these dilutions, and bound IgE was detected as described above. Controls consisted of a strip of garlic and onion extract incubated with the diluted pool in the presence of garlic and onion, respectively.

Statistical analysis

Patients were classified in two groups according to the results of inhalation challenge tests with garlic (positive or negative). Differences between groups were analyzed on a personal computer with a statistical software package (R-SIGMA; PKWARE, Inc., Glendale, Wis.). Nonparametric statistical tests were used for comparison of quantitative variables (Mann-Whitney U test), and Fisher's exact test was used for comparison of proportions. Probability (*p*) values less than 0.05 were considered significant.

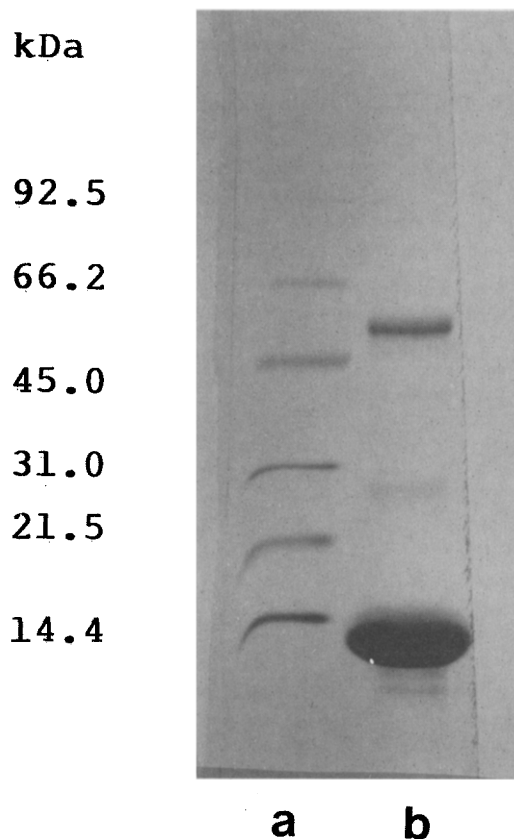


FIG. 1. SDS-PAGE analyses for molecular weight markers (a) and garlic extract (b).

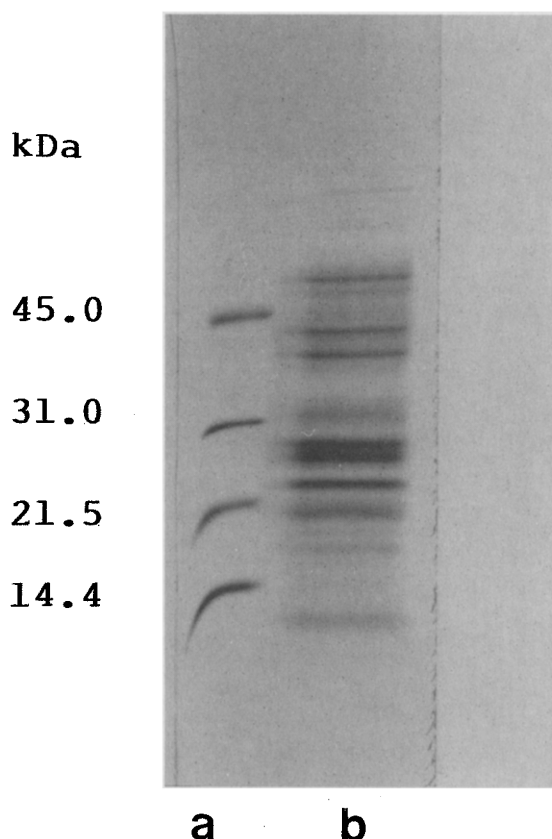


FIG. 2. SDS-PAGE analyses for molecular weight markers (a) and onion extract (b).

RESULTS

According to the results of the specific inhalation challenge test, patients were divided into two groups. Group 1 consisted of seven patients in whom occupational sensitization to garlic was demonstrated by a positive inhalation challenge test response. The remaining five patients (i.e., those having a negative inhalation challenge test response) were placed in group 2.

Table I shows the epidemiologic characteristics of these patients. Clinical history was similar in both groups. It was not possible to differentiate patients allergic to garlic solely by medical history. Data on the different factors of exposure are shown in Table I. Patients with garlic allergy experienced a long exposure, which lasted almost the entire year. They reported garlic exposure since childhood. Mean duration of working activities was 19 years. Symptoms appeared in early life, with a mean age of appearance of 17 years. However, interestingly, the period from onset of symptoms to diagnosis of garlic allergy was rather prolonged, often longer than 10 years. No statistical differences between the groups were found when all of the findings were compared.

Results of the immunoallergic study are summarized in Table I. SPT responses to commercial extracts of garlic, onion, leek, and asparagus were negative in all

patients allergic to garlic except one who was highly sensitive to garlic. All patients from group 2 had negative skin test responses to commercial extracts. However, the skin test responses with garlic dust and fresh bulbs were positive in six of seven patients from group 1 and in one patient from group 2. Skin test responses to other fresh members of the Liliaceae family were positive in four patients with onion and asparagus and in three patients with leek. SPT responses to commercial extracts of onion, leek, and asparagus were all negative.

Results of garlic- and onion-specific IgE determinations are summarized in Table I. Patients from group 2 showed neither garlic- nor onion-specific IgE levels. However, five of seven patients from group 1 had levels of garlic-specific IgE greater than 0.7 kU/L. These five patients also had increased onion-specific IgE levels even quantitatively superior to those observed for garlic.

All patients from group 2 tolerated garlic (both crude and well cooked), onion, leek, and asparagus. When asked, three patients from group 1 reported some type of reaction after garlic ingestion. One patient reported urticaria, another reported asthma and angioedema, and the third reported anaphylaxis. All patients except those with anaphylactic reactions tolerated cooked garlic, onion, leek, and asparagus. An oral challenge test was carried out in two of the three patients who reported

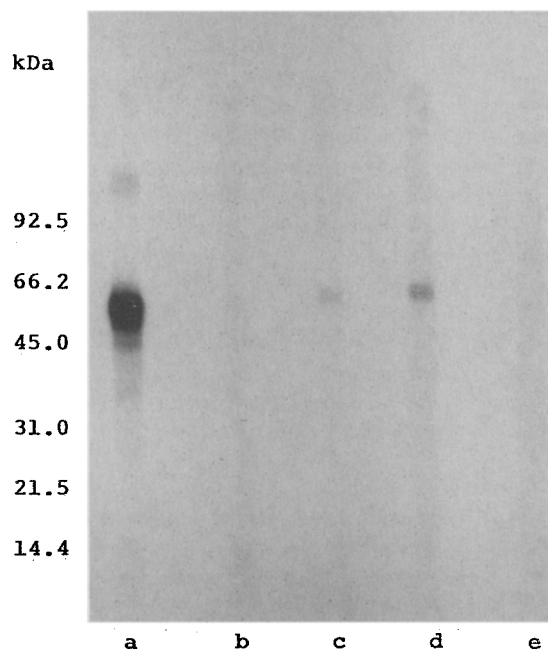


FIG. 3. Inhibition of immunodetection of garlic extract. Inhibition was carried out by preincubating the serum pool with garlic, onion, *Phleum*, and *Chenopodium*. The strips show IgE immunoblot analysis of garlic extract without inhibition (*a*), garlic inhibited with garlic (*b*), garlic inhibited with onion (*c*), garlic inhibited with *Chenopodium* (*d*), and garlic inhibited with *Phleum* (*e*).

symptoms after ingestion of garlic. The patient who reported urticaria tolerated the full dose of garlic dust. The other patient had a 35% decrease in basal FEV₁ and angioedema of the eyelids at a cumulative dose of 500 mg. Oral challenge was not performed in the patient with anaphylaxis.

Table I shows the clinical features of garlic reactions after inhalational challenge with garlic dust. Rhinitis was the most common symptom, often associated with asthma. Immediate response patterns were observed in all seven patients, and two of them had dual bronchial reactions with late bronchoconstriction 7 and 9 hours after challenge. There were no isolated late responses. Reactions were generally of moderate intensity, of short duration, and easily controlled with bronchodilator therapy.

SDS-PAGE

As shown in Fig. 1, garlic extract shows several protein bands in the Coomassie Blue, with the most abundant band having a molecular weight of around 12 kd. A faint protein band was detectable at 54 kd. Onion extract (Fig. 2) revealed a complex protein pattern. Bands ranged in molecular weight from 12 to 92 kd, but there was no major protein band.

Immunoblotting and cross-inhibition of immunoblotting

In IgE immunoblotting, the pool of sera reacted to garlic proteins (Fig. 3), as well as to onion proteins (Fig. 4). In the

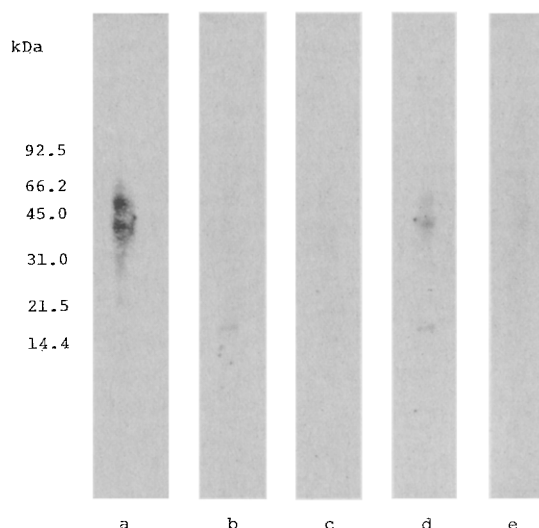


FIG. 4. Inhibition of immunodetection of onion extract. Inhibition was carried out by preincubating the serum pool with onion. IgE-immunoblot analysis of onion with pool of sera (*a*), onion immunoblotting inhibition with garlic extract (*b*), onion inhibition with onion (*c*), onion inhibition with *Chenopodium* (*d*), and onion inhibition with *Phleum* (*e*).

garlic strip (Fig. 3), a very weak allergen band was detectable at 54 kd, and other bands were found at molecular weights of 100, 40, and 33 kd. Bands at less than 14 kd were less clear. The major protein of the garlic extract (around 12 kd) appears not to have allergenic activity. Preincubation of the serum pool with onion, *Phleum*, and *Chenopodium* partially abolished the IgE binding to several allergens of garlic. The garlic extract (positive control) caused complete garlic blot inhibition. In the onion extract (Fig. 4), two main bands of IgE-binding components were detectable at approximately 54 and 43 kd, and two less clear bands were detected at approximately 23 and 17 kd. Preincubation of the serum pool with garlic extract abolished IgE binding to all allergens of onion except the binding at 17 kd. Preincubation with onion and *Phleum* caused complete onion blot inhibition, but *Chenopodium* did not cause inhibition of IgE binding to proteins of onion extract.

DISCUSSION

To date, only a few studies on allergic occupational asthma caused by inhalation of garlic dust have been published, and consequently, the overall number of cases with a complete clinical and immunologic evaluation is limited.¹⁻⁵

The results of this study, performed in a group of workers exposed to garlic who were thought to have occupational allergy, show that some subjects do indeed have asthma and rhinitis caused by IgE-mediated allergy to garlic. This conclusion is based on the presence of an immediate response (rhinitis, asthma, or both after inhalation challenge) and is supported by skin and serum IgE test results.

Garlic-induced occupational allergy seems to be more frequent in young subjects with pollen sensitization. The development of garlic allergy appears to follow a relatively long period of exposure. Given the high molecular weight of the allergens, it is to be expected that the reactions to garlic exposure are clinically expressed as immediate response, with a few subjects showing dual asthmatic response. In the subjects studied here, garlic-induced occupational asthma was always preceded by rhinitis. Specific food allergy with symptoms such as angioedema, asthma, or anaphylaxis can occur in patients who are highly sensitive to inhalatory garlic. Interestingly, time from onset of symptoms to diagnosis of garlic allergy in all cases was rather long, perhaps because the disease significantly impaired the patients' performance status only at late stage or because there may be a lack of awareness of the problem.

The diagnosis of garlic-induced occupational allergy should be based on a detailed medical history, on in vivo and in vitro demonstration of specific IgE, and on challenge test results. In our experience, commercial garlic extracts are not useful. Positive SPT responses to appropriate garlic extracts (dust or fresh samples) seem much more specific and correlate with the presence of specific IgE as determined by the CAP method. However, the gold standard for specific diagnosis remains the inhalation provocation test.

Susceptibility of patients to garlic dust sensitization may have been enhanced by their previous atopic status. As in other reported cases of garlic-induced occupational asthma or rhinitis,¹⁻³ our patients have atopic constitutions. All of our patients with allergy to garlic had allergy to pollens, mainly those from grasses and weeds of the *Chenopodiaceae* and *Compositae* families. The association between allergy to foods and pollens is well known. Cross-reactions of allergen-specific IgE antibodies with food allergens and pollen proteins have been considered to be the reason for the clustering of hypersensitivities. It is possible that subjects with a history of pollinosis could be predisposed to the development of occupational allergy to garlic. Pollen allergy may be the trigger or risk factor in the development of garlic allergy. In previously published RAST inhibition studies,¹⁰ partial allergenic identity between onion and grass pollen has been demonstrated. Garlic allergens have not been characterized, but our results confirm the presence of several allergens cross-reacting with grass and *Chenopodiaceae* pollens (garlic allergens were almost completely inhibited by these pollen extracts). Additionally, similarities between other pollens and garlic should be sought in further studies.

Skin testing with other members of the *Liliaceae* family also showed immediate positive reactions in the majority of patients, suggesting a cross-reaction with antigens from garlic. In fact, immunologic evidence of crossed allergenicity with other related members of the *Liliaceae* family in patients with contact dermatitis¹¹ and asthma³ induced by garlic has been published. The results of this study suggest that all allergenic epitopes of garlic are present in onion (garlic allergens were completely inhibited by onion extract). In contrast, garlic inhibited all bands from onion, except one at 17 kd, which suggests that onion has at least one allergen that is not present in garlic. All allergenic epitopes from onion are present in grass pollens, but we have not identified cross-reactivity between *Chenopodium* pollen and onion.

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