

Original article

Allergy to millet: another risk for atopic bird keepers

Background: Millet has been reported to induce not very frequent but severe anaphylactic reactions following ingestion. Seven individuals who all kept cage birds experienced allergic reactions after ingestion of millet-containing food.

Methods: We investigated the immunoglobulin E (IgE)-reactivity of these individuals to millet employing immunoblotting, RAST and skin prick tests. As the sensitization possibly occurred via the inhalant route we investigated millet-specific IgE levels of 16 additional sera from bird keepers with proven atopy, in retrospect.

Results: All patients who had experienced reactions after ingestion of millet displayed millet-specific IgE. Sixty-three percent of the atopic bird keepers possessed millet-specific IgE. By means of immunoblotting three major allergens in millet extract were detected.

Conclusions: Our results indicate that millet plays an important role as inhalant allergen for atopic bird keepers. A sensitization to millet may subsequently also elicit food allergy.

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Millet (*Panicum milliaceum*) seed is one of the oldest cultivated crops and has been used in Africa and India as a staple food for thousands of years. In China it was grown as early as 2700 BC where it was the prevalent grain before rice became the dominant staple. Today, millet ranks as the sixth most important grain in the world and is a major crop in Africa and the Indian subcontinent. At the present time the grain is known in Western countries mainly as bird and cattle feed. Because millet contains various high-quality proteins it started to become a more popular crop with the Western population in the recent years.

It is well documented that cereal grains can elicit hypersensitivity reactions sensitizing by both, ingestion and inhalation (1, 2). Some case reports of anaphylaxis after ingestion of food containing millet have been described (3–8). With the increasing popularity of 'natural food diet' millet is more frequently included in various dishes which might give rise to the incidence of millet-related allergic reactions.

In this study we investigated seven patients who experienced allergic reactions after ingestion of food containing millet. The severity of these reactions ranged from the oral allergy syndrome to anaphylaxis. By immunoblotting we characterized immunoglobulin E (IgE) binding components in millet extract. To investigate our hypothesis that the allergic sensitization to millet occurred *via* inhalation of millet particles, we additionally analyzed sera of patients with other allergies, who kept birds and were therefore exposed to millet dust.

Methods

Subjects

In total, 23 allergic subjects were included in the study and were divided into two groups. Group I comprised seven patients who had developed allergic symptoms after ingestion of millet-containing food (Table 1). Interrogation revealed that none of the patients had eaten millet before. As the consumption of millet can be easily avoided none of these patients gave consent to oral provocation tests with millet. Group II comprised 16 patients who attended the Allergy Clinic because of other reasons (Table 2). All patients were exposed to millet dust because they kept a cage bird fed with millet. The IgE reactions were analyzed by radioallergosorbent test (CAP/RAST, Pharmacia, Uppsala, Sweden), immunoblotting and skin prick tests (SPT) with millet extract.

Preparation of millet extract

Millet grains of common millet (*P. milliaceum*) (3 g) were pounded in liquid nitrogen and incubated with 30 ml of phosphate-buffered saline (PBS) overnight at 4°C. The mixture was centrifuged at 15 000 rpm (40 000 g) for 30 min. Protein determination of the extracts was performed with a commercially available protein determinant (BioRad, Inc, Richmond, CA, USA).

Immunoblotting

Immunoblotting was performed according to methods described earlier (9). Briefly, millet extract (25 µg/lane) and *Phleum pratense* extract (25 µg/lane) were separated by 12% SDS-PAGE and blotted onto a nitrocellulose membrane. The membrane was incubated with patients' sera overnight at 4°C. After incubation with ¹²⁵I-labeled

Table 1. Clinical characterization of the patients who experienced allergic reactions after ingestion of millet (group I)

Number	Sex	Age	Total IgE (kU/l)	Symptoms after ingestion	Millet		Budgerigar CAP value	Grass pollen		Other food allergies
					CAP value	SPT		CAP value	SPT	
A	M	13	224	N, H	4	—	0	5	++	Birch-fruit-syndrome
B	F	28	226	OAS	2	n.d.	0	2	+	Birch-fruit-syndrome
C	F	32	17,4	C, D, U	2	++	0	0	—	—
D	F	46	278	R, D, U	5	++	0	2	++	wheat, rye, rice
E	F	62	52.2	N, D, U, H	2	+	0	1	++	Sunflower seeds, birch-fruit-syndrome
F	F	30	377	R	2	—	0	4	+++	—
G	F	40	127	OAS	3	n.d.	0	0	—	Sunflower seeds, rice, corn

C, conjunctivitis; Co, collapse; D, dyspnea; H, hypotension; N, nausea; OAS, oral allergy syndrome; R, rhinitis; U, urticaria; SPT, skin prick test.

anti-human IgE antibody (IBL, Hamburg, Germany), bound IgE was visualized by autoradiography. A serum pool from nonallergic donors served as negative control.

Results

Table 1 summarizes the clinical data of group I. All patients included in group I were positive in millet-specific RAST and three of five reacted with the millet extract in SPT. None of these patients was sensitized against bird feathers. Group II consisted of atopic bird keepers without (patients 1–4) or with (patients 5–16) allergy to bird feathers (Table 2). Results of group II indicated the existence of patients with specific IgE against millet but not to budgerigar (patients 1–4), patients possessing IgE against millet and budgerigar (patients 5–8) and patients with bird feather allergy but no sensitization to millet (patients 9–16, Table 2). As millet belongs to the *Poaceae* family, RAST and SPT specific for grass pollens were also performed. In group I, five of seven individuals displayed grass pollen-specific IgE (Table 1). Group II included patients possessing specific IgE against millet as well as grasses (patients 1, 3–6, 8), millet-specific IgE but no grass pollen-specific IgE (patient 7), patients positive for grass pollens without IgE against millet (patients 9,10) and patients possessing neither IgE against millet nor grass pollens (patients 11–16; Table 2).

The IgE-binding patterns to millet extract of sera from all patients are shown in Fig. 1. In group I, six of seven patients exhibited millet-specific IgE in the immunoblot. Although patient C had immediately developed anaphylaxis after ingestion of millet and had a positive SPT (Table 1), the serum was negative in immunoblotting. In group II, 10 of 16 patients revealed allergen-specific IgE directed against various proteins in millet-seeds (Fig. 1). Patients 9 and 10 were negative in RAST but showed a weak IgE reactivity in the immunoblot.

In both groups of patients, the IgE-binding pattern to millet extract was very inconsistent and several IgE-binding proteins were detected. The molecular weights of millet allergens ranged from 11 kDa up to 90 kDa. No difference in the recognition of particular allergens

Table 2. Clinical characterization of millet-exposed bird keepers tested in retrospect (group II)

Number	Sex	Age	Total IgE (kU/l)	Millet CAP value	Budgerigar CAP value	Grass pollen	
						CAP value	SPT
1	F	13	881	3	0	3	+
2	F	11	1331	3	0	n.d.	+
3	M	14	247	2	0	4	+
4	M	44	1061	2	0	4	n.d.
5	M	7	439	4	4	2	+
6	M	6	>2000	2	2	3	+
7	F	29	1112	3	3	0	—
8	F	29	>2000	2	4	3	+
9	F	54	545	0	4	2	+
10	F	42	35,7	0	2	3	+
11	F	44	>2000	0	3	0	+
12	F	61	367	0	2	0	n.d.
13	F	40	476	0	5	0	—
14	M	10	1496	0	5	0	—
15	F	61	86.9	0	2	0	—
16	F	53	611	0	4	0	—

between group I and II was observed. Fifteen of 16 patients possessing millet-specific IgE recognized one allergen of approximately 36 kDa. Two proteins of high molecular weight (approximately 70 and 90 kDa) were reactive with the sera from 10 of 16 and 12 of 16 patients, respectively. The control serum pool and the buffer control exhibited no IgE-binding (Fig. 1).

In order to investigate potential cross-reactivities between millet and grass pollen proteins, 10 individuals with typical grass pollen allergy were investigated for IgE reactivity against millet by immunoblotting (Fig. 2). None of these patients ever owned a cage bird. All patients displayed IgE antibodies to the typical grass pollen allergens (Fig. 2B), but no IgE directed against millet proteins was detected (Fig. 2A).

Discussion

We investigated the IgE-binding patterns of seven individuals who experienced allergic reactions after the consumption of millet-containing food. As all of these

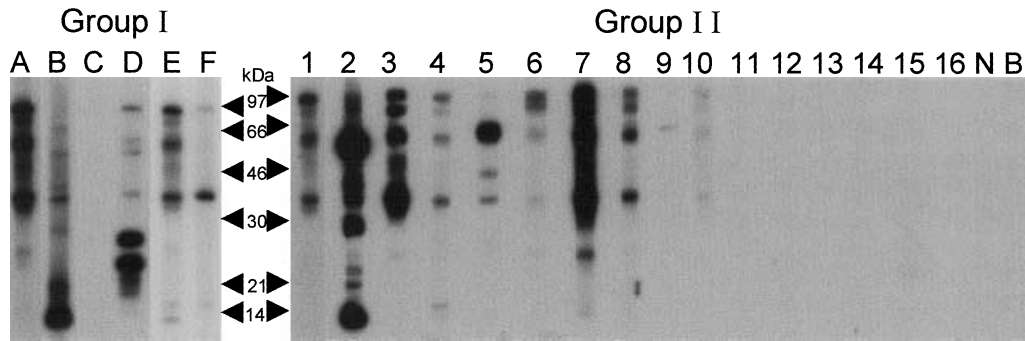


Figure 1. IgE-immunoblots. IgE reactivity of sera from patients tested with blotted millet extract. Group I comprises individuals who experienced anaphylactic reactions after ingestion of millet, Group II comprises millet-exposed atopic bird keepers. N, serum pool of nonatopic individuals; B, buffer control.

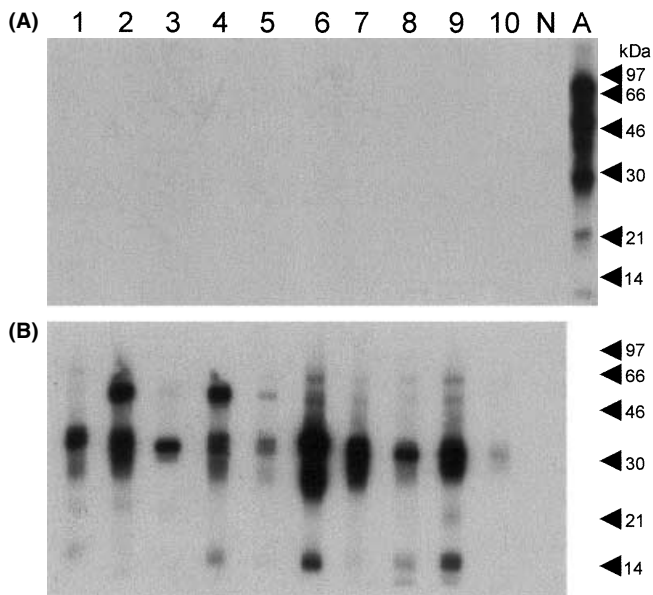


Figure 2. IgE-immunoblots. IgE reactivity of sera from patients with grass pollen allergy tested with blotted millet extract (A) or blotted grass pollen extract (B). N, serum pool of nonatopic individuals; A, positive control.

subjects kept birds at home and used millet as birdseed we assumed that the sensitization to millet occurred *via* the inhalant route (8). To support this hypothesis we investigated the sera of atopic individuals who kept birds and were therefore exposed to millet dust, in retrospect.

The possibility that millet represents a potentially harmful source leading to anaphylactic reactions after ingestion has been described casuistically (3–8). However, to date, no information regarding the allergenic components of millet was available. Six of the seven patients who experienced allergic symptoms after ingestion of millet were positive in IgE-immunoblots (Fig. 1). Inter-

estingly, also 10 of 16 (63%) of millet-exposed atopic bird keepers displayed IgE to allergens in millet extract. One protein of approximately 36 kDa was recognized by 94% of the patients, two allergens of high molecular weights (approximately 70 and 90 kDa) by 62 and 75% of the patients, thus representing dominant IgE-binding components.

A sensitization *via* the inhalant route which subsequently leads to food allergy is frequent in pollinosis (9). Respiratory allergy to indoor allergens can also elicit food hypersensitivity (10). In the case of atopic bird keepers obviously two potential risks for developing food allergy exist: first, the sensitization to alpha-livetin, subsequently causing the ‘bird-egg-syndrome’ (11), and secondly, the sensitization to birdseed leading to allergy to sunflower seeds (12). In this context, our results indicate that millet also represents an important inhalant allergen in atopic bird keepers. Several patients sensitized against millet (patients B, D, E, F, G and 4; Tables 1 and 2) reported respiratory symptoms while cleaning the bird cage. However, none of these patients was allergic against bird feathers, only one reacted to house dust mite allergens. Similar observations have been described previously (5, 8).

According to our results, IgE binding to millet was not based on cross-reactivity with grass pollens, as three of 11 millet-positive patients displayed neither positive RAST nor SPT with grass pollen allergens (patients C, G and 7; Tables 1 and 2). Furthermore, individuals with typical grass pollen allergy who never kept a cage bird displayed no IgE antibodies specific for millet proteins (Fig. 2). From these data we conclude that contact with millet dust is necessary to develop this sensitization and subsequently allergy.

There is evidence that anaphylactic reactions to food are increasing (13). Taken together, our results support that the search for millet-specific IgE should be included in the diagnostic procedure for atopic individuals keeping birds, regardless of whether they react with grass pollens

or the animal itself. The test set-up should include a thorough anamnesis as well as SPT and serology, because the sensitivity of each test might not be satisfactory. In this way, the potential risk of hypersensitivity reactions after consumption of millet might possibly be minimized.

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