

Immediate-type hypersensitivity reaction to ingestion of mycoprotein (Quorn) in a patient allergic to molds caused by acidic ribosomal protein P2

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Background: Quorn is the brand name for a line of foods made with so-called "mycoprotein," which springs from the mold *Fusarium venenatum*. Since the introduction on the food market, there have been complaints from consumers reporting adverse gastrointestinal reactions after ingestion of mycoprotein. To date, it is not clear whether the reported symptoms are IgE-mediated. **Objective:** The aim of the study was to describe for the first time a case history of an asthmatic patient with severe hypersensitivity reactions to ingested mycoprotein and to identify and characterize the potential allergen that might be responsible for this.

Methods: The sensitization pattern of the asthmatic subject was characterized, and food allergy to mycoprotein was assessed by double-blinded placebo-controlled food challenge. Afterward, specific IgE antibodies of the serum of this patient were used to screen a *Fusarium culmorum* cDNA expression library. The coding sequence of one enriched cDNA-clone was expressed in *Escherichia coli* to produce a recombinant protein that was further purified and immunologically characterized. **Results:** The patient showed high sensitization to many known aeroallergens but apart from Quorn not to any other tested food samples. The deduced amino acid sequence of the enriched cDNA-clone (*Fus c 1*) showed large identity to the 60S acidic ribosomal protein P2 which is highly conserved among several species and also described as minor allergen in other mold species. The frequency of IgE reactivity of sera from *F. culmorum*-sensitized subjects to *rFus c 1* was approximately 35%. By enzyme allergosorbent test inhibition, we found 65% inhibition of mycoprotein IgE reactivity by *rFus c 1*. On the opposite we found reduced IgE reactivity of *rFus c 1* of 68% by using mycoprotein as inhibitor.

Conclusions: Sensitization to mold allergens by the respiratory tract and subsequent oral ingestion of cross-reactive proteins may lead to severe food-allergic reactions. Thus, the 60S acidic ribosomal protein P2 of *F. venenatum* probably is the reason

for the described severe hypersensitivity reactions of the patient to Quorn-mycoprotein because of its potential cross-reactivity to the *F. culmorum* allergen *Fus c 1*. (J Allergy Clin Immunol 2003;111:1106-10.)

Key words: Case report, mold allergy, food allergy, cross-reactivity, DBPCFC, recombinant allergen

Mycoprotein (Quorn) is a food produced for human consumption by Marlow Foods Ltd (Stokesley, United Kingdom) by continuous fermentation of *Fusarium venenatum* on a glucose substrate. It has been on sale in the United Kingdom since 1985 and is currently widely available in many leading retailers in and outside the United Kingdom (in Belgium since 1992, the Netherlands since 1993, Ireland since 1994, Switzerland since 1995, and Sweden since 1998). Since the introduction of Quorn on the food market, there have been complaints from consumers reporting symptoms of gastric cramping and vomiting after ingestion of Quorn mycoprotein, with a delay time of approximately 1 to 4 hours between consuming Quorn and development of symptoms. The frequency of reported complaints in the United Kingdom has been estimated to be 1 per 370,000 and in Switzerland 1 per 80,000. An investigation of the allergenicity of mycoprotein was performed on 33 mycoprotein production workers and on 10 consumers who had adverse gastrointestinal reactions to the ingestion of Quorn.¹ Although the mycoprotein workers were screened for specific IgE antibodies to mycoprotein by RAST, consumers were assayed for specific IgE antibodies to mycoprotein and assessed by skin prick testing (SPT) with mycoprotein extract. The results of the study showed that two of the production workers had specific RAST binding $\geq 2\%$, but none reported symptoms. On the other hand, 2 of 10 patients referred to the hospital after ingestion of mycoprotein and vomiting had a mycoprotein SPT ≥ 2 mm, but none had a significantly raised RAST. The authors concluded that the gastrointestinal symptoms were not IgE-mediated. However, extensive cross-reactivity between mycoprotein and common molds (*Cladosporium herbarum*, *Aspergillus fumigatus*, and *Alternaria alternata*) found by means of RAST and RAST inhibition studies on patient sera suggested a potential for patients allergic to molds to react adversely to ingested mycoprotein.

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Abbreviations used

DBPCFC: Double-blinded placebo-controlled food challenge
EAST: Enzyme allergosorbent test
SPT: Skin prick test

To the best of our knowledge, this is the first report of a patient allergic to mold reacting with allergic skin and respiratory symptoms after ingestion of a Quorn product. These symptoms probably are the result of cross-reactivity between the mycoprotein derived from *F venenatum* and the 60S acidic ribosomal protein P2, which we identified as allergen Fus c 1 from *Fusarium culmorum* and what was also described as allergen for the molds *C herbarum*, *A fumigatus*, and *A alternata*.

METHODS

Case report

A 41-year-old man was referred for allergologic evaluation of an acute attack of asthma with urticaria/angioedema by his general practitioner. The patient had a 30-year history of perennial bronchial asthma, for which he currently inhaled salbutamol as required and fluticasone on an irregular basis. At the time of referral he reported asthma attacks with a frequency >2 per week and nocturnal attacks >2 per month and requirement of inhalation of salbutamol on a daily basis. Unexpectedly he had rhinoconjunctivitis, urticaria/angioedema, laryngeal edema, and an asthma attack upon resuming work drilling a sheet of plaster and wastepaper (Fermacell, Fels-Werke GmbH, Goslar, Germany) approximately 1 hour after ingestion of a Quorn product with potatoes for lunch. After intravenous administration of 4 mg dimetinden and 100 mg prednisolone by the general practitioner and inhalation of 0.2 mg salbutamol, the symptoms promptly resolved. The referring general practitioner questioned the possibility of a reaction to the dust generated by drilling of the sheets of Fermacell, inasmuch as he had been informed by the Swiss Toxicologic Information Center that Quorn was known to cause gastrointestinal reactions, whereas the patient did not have these.

Skin testing

Skin prick tests were performed on the flexor side of the forearm with a standardized prick needle (Stallerpoint, Stallergènes, Antony, F) with routine inhalants, cow's milk, egg white, egg yolk, potato (Soluprick SQ, ALK, Horsholm, DK), and different molds (Alyostal, Stallergènes Lab, Antony Cedex, F) according to Table I. Histamine dihydrochloride (10 mg/mL) was used as a positive control and the glycerol-containing diluent on the prick solution as a negative control. Reactions were recorded after 15 minutes. A wheal with a diameter of at least 3 mm was considered positive. Scratch tests were performed with Fermacell dust, native mycoprotein (Quorn), and the patient's collected house dust.

Serologic testing

Specific IgE to different molds (Table I) were measured through use of the CAP/Fluorescent-enzyme immunoassay System (Pharmacia Diagnostics, Uppsala, Sweden). A value of at least 0.7 kU/L (class 2) was considered positive.

Lung function tests

Spirometric measurement of FEV₁, FVC, and the FEV₁:FVC ratio were measured by a Vitalograph and performed at admission.

TABLE I. Results of skin and serologic testing for specific IgE antibodies of subjects allergic to Quorn against common molds and mycoprotein

Allergen	SPT	Serologic testing (CAP)	
		kU/L	CAP class
<i>Alternaria</i> ssp	Negative	38.6	4
<i>Aspergillus</i> ssp	+	15.7	3
<i>Aureobasidium</i> ssp	n.t.	18.7	4
<i>Botrytis</i> ssp	++	19.3	4
<i>Chaetomium</i> ssp	+++	n.t.	n.t.
<i>Cladosporium</i> ssp	++	10.3	3
<i>Curvularia</i> ssp	n.t.	10.1	3
<i>Epicoccum</i> ssp	++	25.5	4
<i>Fusarium</i> ssp	++	24.4	4
<i>Helminthosporium</i> ssp	++	26.7	4
<i>Merulius</i> ssp	+	n.t.	n.t.
<i>Mucor</i> ssp	+	2.78	2
<i>Penicillium</i> ssp	+	16.2	3
<i>Phoma</i> ssp	n.t.	23.7	4
<i>Pullularia</i> ssp	+++	n.t.	n.t.
<i>Rhizopus</i> ssp	+	9.7	3
<i>Stemphylium</i> ssp	+++	11.2	3
<i>Trichoderma</i> ssp	n.t.	5.48	3
<i>Trichotecium</i> ssp	++	n.t.	n.t.
Mold mix	n.t.	26.5	4
Mycoprotein (Quorn)	+++	n.t.	n.t.

+, Wheal 3 to 5 mm; ++, wheal 5 to 7 mm; +++, wheal 7 to 10 mm; n.t., not tested.

Evaluation of case history of adverse reactions to foods

A detailed interview revealed no indication of adverse reactions to foods. The possibility of reactions to mold-containing foods, for example, cheese with penicillium growth on it, fermented soy products, and mushrooms was specifically addressed. The patient had previously eaten and tolerated these mold-associated foods.

Double-blinded, placebo-controlled food challenge

Double-blinded, placebo-controlled food challenge (DBPCFC) was performed by 2-step spit (local mucosal challenge) and swallow procedure as previously described for celery.² Two different drinks, identical in color, texture, and taste, were prepared. The active drink contained 15 g of dried mycoprotein, 50 g cooked broccoli, 12.5 g broccoli stock, 5 g cream, 50 g yogurt without flavor, 25 g water, and 1 g salt, mixed in a blender. One milliliter of the active drink contained 0.07 g of mycoprotein. The placebo drink contained the same ingredients but no mycoprotein. Apart from mycoprotein, all ingredients were known to be tolerated by the patient.

Screening of *F culmorum* cDNA expression library for IgE-binding clones

A cDNA expression library was constructed and screened for IgE-binding clones by using sera of different atopic subjects to identify and isolate allergens from the mold *F culmorum*, as described in detail.³ In initial experiments, the presence of specific IgE antibodies of these human sera including the serum of the mycoprotein-sensitized patient was verified by immunoblotting and enzyme allergosorbent test, with an in-house Quorn and *F culmorum* extract, respectively (data not shown).

Cloning and expression of an IgE-reactive protein

The complete coding sequence of an IgE-binding clone enriched by four rounds of screening the *F culmorum* library by using the Quorn-atopic patient's serum was obtained by direct colony PCR followed by rapid amplification of 5' cDNA ends (5'RACE). Gene-specific primers were designed that provide *Nde*I and *Bam*HI restriction sites, and the amplified cDNA was subcloned into expression vector pET11a (Novagen, Madison, Wis) to produce a nonfusion protein. The recombinant allergen, further designated as *rFus c 1*, was expressed by using *Escherichia coli* BL21(DE3) cells and purified by electroelution as described.³

Immunoblot analysis, enzyme allergo sorbent test, and inhibition experiments

The IgE reactivity of the purified recombinant allergen *rFus c 1* was assayed by immunoblot analysis and enzyme allergo sorbent test (EAST), with the Quorn-sensitized patient's serum used as described.³ For inhibition experiments, an in-house Quorn extract was either coupled to cyanogen bromide-activated paper disks (10 μ g/disk) as described⁴ or separated by SDS-PAGE and transferred onto nitrocellulose as described.³ For EAST inhibition, 50 μ L of the undiluted serum of the Quorn-sensitized patient was incubated with 50 μ L of various dilutions of inhibitor (Quorn extract: 500, 50, 5, 0.5, 0.05, 0.005 μ g protein/mL; *rFus c 1*: 100, 10, 1, 0.1, 0.01, and 0.001 μ g/mL diluted in incubation buffer). The inhibition data were generated as follows: %inhibition = (extinction_{405nm} [E] serum – [E] serum + inhibitor) / ([E] serum – [E] unspecific binding) \times 100.

For blot inhibitions, 80 μ L serum was preincubated for 15 minutes with either 100 μ g protein of several in house extracts or 10 μ g of *rFus c 1* in 520 μ L incubation buffer. Subsequently, incubations of the blot strips and detection were performed as described.³

RESULTS

Lung function tests

FEV₁ was 2390 mL (normal: 3890 mL), FVC 4430 mL (normal: 4730 mL), and the FEV₁:FVC ratio 0.54 (< 0.7). The FEV₁ did not increase after the use of an inhaled bronchodilator (salbutamol), indicating a fixed obstruction.

Serologic testing, SPT, and DBPCFC of the mycoprotein-allergic patient revealed sensitization exclusively to aeroallergens but not to food allergens

From the routine inhalants, the house dust mites *D pteronyssinus*, *D farinae*, dog danders, cat danders, grass, birch, alder, ash tree, hazel, and rye pollen elicited strong immediate-type reactions (data not shown) in SPT. The results of SPT with molds summarized in Table I showed high sensitization to nearly all of the tested species except of *Alternaria* ssp but indeed to *Fusarium* ssp. The scratch test with native mycoprotein (Quorn) and the patient's collected house dust elicited strong weal-and-flare reactions, whereas Fermacell dust was negative. According to Table I, significant levels of circulating specific IgE antibodies were found for nearly all of the mold species tested including *Alternaria* ssp as well as to *D pteronyssinus* (49.7 kU/L) and *D farinae* (56.4 kU/L).

By performing DBPCFC, the patient reported swelling of the tongue and a tingling sensation in the mouth after local mucosal exposure to the active meal with doses containing 1.4 g and 2.9 g mycoprotein. After ingestion of 0.9 g, 1.9 g, and 3.7 g mycoprotein, the patient again complained about severe itching of the oral mucosa lasting for 1 hour. Since the patient reported during several provocation steps consistently symptoms of an oral allergy syndrome, and, thus, allergic reaction to mycoprotein was confirmed, the provocation was not continued with higher doses, taking in account the severe reaction after ingestion of Quorn. Provocation with the placebo drink was tolerated without any reaction.

Identification and molecular characterization of the mold allergen *Fus c 1*

Initially, the serum of the patient allergic to Quorn was examined for specific IgE antibodies against *F culmorum* (data not shown) and thereafter used to enrich IgE-reactive clones out of a cDNA-expression library that was originally made for the identification of *F culmorum* allergens. After four rounds of enrichment, all clones were positive. Sequencing, completion of the cDNA, and subcloning of the corresponding open reading frame into a prokaryotic expression vector led to the recombinant allergen called *Fus c 1* (GenBank accession number: AAL79930).³ The protein has an estimated molecular weight of approximately 11 kD and shows a high degree of amino acid sequence identity (86%, 78%, and 76%, respectively) to the 60S acidic ribosomal protein P2, which is highly conserved among several species and was also described as minor allergen in *A alternata* (Alt a 6, P42037; P78982), *C herbarum* (Cla h 4, P42039), and *A fumigatus* (Asp f 8, Q9UUZ6).

Cross-reactivity between *rFus c 1* and mycoprotein was indicated by EAST and immunoblot inhibition experiments

Through the use of the Western blot technique as well as the EAST, the presence of IgE antibodies specific for *rFus c 1* in the serum of the patient allergic to Quorn was confirmed (data not shown). Inhibition of specific IgE binding to Quorn mycoprotein as well as *rFus c 1* was examined first by EAST inhibition with a Quorn extract prepared in our laboratory and *rFus c 1* as inhibitors. The results are presented in Fig 1, A and B. We found a 65% decrease of IgE binding to mycoprotein in the EAST inhibition experiment when 0.01 μ g of *rFus c 1* was applied. Higher amounts of *rFus c 1* did not increase the level of inhibition. By contrast, a dose-related inhibition of up to 99% was revealed by using the mycoprotein itself as inhibitor (Fig 1, A). On the other hand, when we used immobilized *rFus c 1*, dose-related inhibition of up to 98% was observed by using *rFus c 1* as inhibitor, whereas application of mycoprotein resulted in only 68% inhibition at the highest concentration (Fig 1, B). No inhibition was observed with skim milk extract as negative control in both cases (not shown).

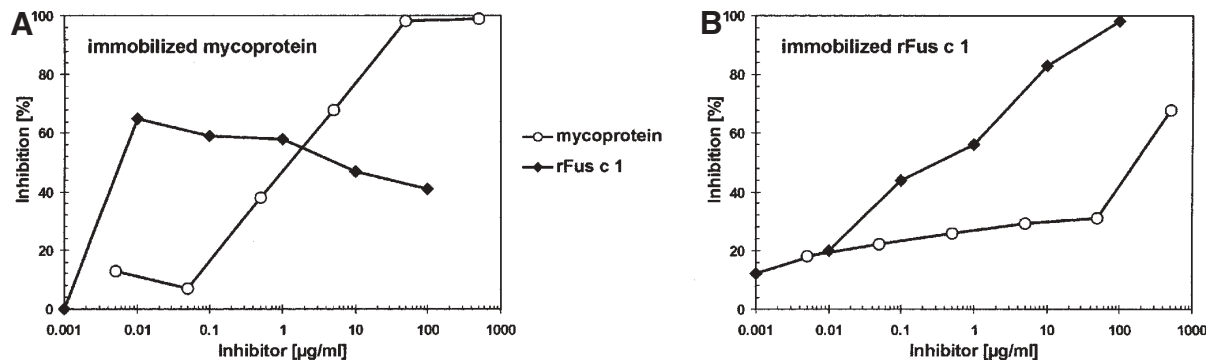


FIG 1. EAST inhibition. **A**, Patient's serum was preincubated for 1 hour with increasing amounts of either Quorn extract or *rFus c 1*. After subsequent incubation with mycoprotein immobilized on paper disks, residual IgE-binding capacity was measured. **B**, Same experiment as before using *rFus c 1* immobilized on paper disks.

In the immunoblot inhibition experiment (Fig 2), total protein of *A alternata* and *C herbarum* extracts was applied as additional inhibitors to determine potential cross-reactions with Quorn mycoprotein, which was blotted on solid phase. Skim milk extract was again used as negative control. The mold extracts and the Quorn extract completely inhibited IgE binding to all visible mycoprotein bands (the dominant 14-kD band and two very weak bands at about 22 kD and 24 kD). By contrast, *rFus c 1* only inhibited the IgE reactivity to the dominant 14-kD band. No inhibition was observed with skim milk extract used as inhibitor.

DISCUSSION

We described the case of an asthmatic patient who had an acute attack of asthma with urticaria/angioedema after ingestion of a mycoprotein food product. Food allergy to mycoprotein was confirmed by DBPCFC. This is most likely the result of cross-reactivity between the *F venenatum* (source of the mycoprotein Quorn) counterpart to the allergen *Fus c 1*, which was isolated by screening a *F culmorum* cDNA expression library with the serum of the patient allergic to Quorn. The presumption of a potential cross-reactivity between IgE-reactive proteins of the species *F venenatum* and *F culmorum* was confirmed by results of EAST inhibition and blot inhibition experiments. In the EAST, IgE binding to Quorn mycoprotein was inhibited by 65% when the serum was preincubated with 0.01 $\mu\text{g/mL}$ of *rFus c 1* as inhibitor, whereas increased amounts of *rFus c 1* had no additional effect. Since the remaining IgE binding activity of 35% was entirely suppressed by using increasing amounts of mycoprotein as inhibitor, it is likely that this IgE reactivity was caused by the two IgE-reactive proteins of 22 kDa and 24 kDa, which were detected on immunoblots and exclusively inhibited by preincubation of serum with mycoprotein and mold extracts. Significant inhibition of IgE reactivity to *rFus c 1* by mycoprotein in the EAST experiment was observed only at the highest concentration of 500 $\mu\text{g/mL}$. This finding is due to the high amount of immobilized recombinant allergen compared with the relatively low amount of soluble allergen in the extract.

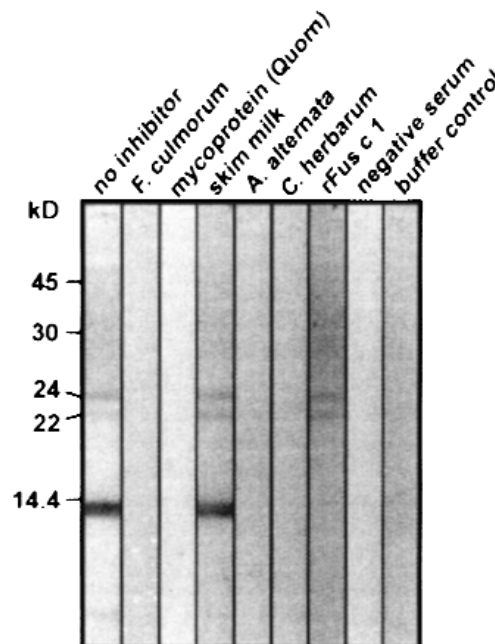


FIG 2. Blot inhibition. For each blot strip, serum from the Quorn-sensitized patient was preincubated with either protein of the in-house extracts as indicated or *rFus c 1*. Detection of the bound IgE antibodies was performed with an alkaline phosphatase-conjugated mouse anti-human IgE antibody and NBT/BCIP as substrate.

The cross-reactivity of *rFus c 1* with IgE against Quorn mycoprotein as well as the cross-reactivity between the mycoprotein and the molds *A alternata* and *C herbarum* revealed by blot inhibition indicates that sensitization to mold allergens absorbed by the respiratory tract and subsequent oral ingestion of cross-reactive proteins may lead to severe food-allergic reactions. A databank search indicated that *Fus c 1* has no significant sequence similarity to any known food allergen. Hence, it is likely that *Fus c 1* is an "incomplete" food allergen, which is able to elicit symptoms in patients with a respiratory sensitization to molds but not to induce an IgE response after ingestion. This is in accordance with our finding that *Fus c 1* is high-

ly susceptible to proteolytic digestion. When added to *F culmorum* extract, which has a very high proteolytic activity,⁵ *rFus c 1* was rapidly degraded (unpublished data). In conclusion, the finding that approximately 65% of the Quorn-specific IgE antibodies in the patient's serum reacted with *rFus c 1* suggests that *Fus c 1* may be an allergen with a high clinical relevance.

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