

Clinical reactivity to ingestion challenge with mixed mold extract may be enhanced in subjects sensitized to molds

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ABSTRACT

Manifestations of mold allergy are classically associated with inhalation of mold spores leading to symptoms of asthma and other respiratory illnesses. It is largely unknown, however, whether ingestion of aeroallergenic molds, mold spores, or other fungi found in food can also elicit hypersensitivity reactions in mold-sensitive individuals. The aim of this study was to evaluate the association between exposure to molds by oral challenge and elicitation of symptoms in mold- versus nonmold-sensitive individuals. Thirty-four adult atopic subjects were randomized into mold-sensitive groups based on skin test reactivity by skin percutaneous testing (SPT) and/or intradermal (ID) testing to a mixed mold (MM) extract preparation. All subjects underwent a single-blinded, placebo-controlled food challenge to the MM preparation. A modified scoring system was used to grade the clinical severity of symptoms elicited by challenge. All subjects tolerated challenges to the maximal oral mold dose concentration. However, higher symptom scores after challenge were found in mold-sensitive subjects compared with nonmold-sensitive subjects ($p = 0.01$). When mold-sensitive subjects were compared based on SPT and/or ID reactivity, higher symptom scores and lower symptom-eliciting concentrations of mold were associated with the SPT reactive subgroup compared with the subgroup with ID reactivity alone. In summary, based on our challenge results and scoring model, mold-sensitive subjects compared with nonmold-sensitive subjects experienced cumulatively higher symptom scores after oral challenge to an MM extract preparation. Future studies are warranted to confirm whether ingestion of aeroallergenic molds in food may be another contributor to symptoms in mold-sensitive individuals.

(Allergy Asthma Proc 30:433–442, 2009; doi: 10.2500/aap.2009.30.3254)

Key words: Allergy, dose, extract, food, fungi, mold, ingestion challenge, sensitivity, skin test, symptom score

Molds that play important roles in the induction of allergic disease(s) are found in water, soil, and air and are otherwise ubiquitous in the environment.^{1–3} Typically, symptoms of mold allergy manifest as asthma, allergic rhinitis, and other respiratory tract disorders after inhalation of aeroallergenic mold spores by mold-sensitive individuals.¹ Studies have shown that nearly 10% of the population manifest IgE responses to molds.² Furthermore, widespread IgE cross-reactivity has been noted between mold species.^{2,4,5} In a large population study of atopic individuals, 19% were sensitized to at least one mold and 10% were sensitized to two or more molds.⁶ Other epidemiological observations have shown that, in a large population of asthmatic individuals, 16% were sensitized to *Aspergillus fumigatus* and 21% were sensitized

to other molds,⁷ and, in a population of symptomatic office workers exposed to indoor molds, frequencies of sensitization to common aeroallergenic molds such as *Alternaria* spp. and *Cladosporium* spp. approached 20–30%.⁸ High sensitization rates to molds, coupled with ubiquitous exposure to cross-reactive mold species from a variety of environmental sources, raise concerns that allergenic exposure and reactivity to molds may occur by ways other than the respiratory route.

The oral route, e.g., could represent a plausible alternative route for allergenic mold exposure, because many different species of aeroallergenic molds, mold spores, and/or other related proteinaceous materials from molds or other fungi are frequently found in or encountered as natural or airborne contaminants of foods or beverages^{9–13} or even drugs (e.g., penicillins from *Penicillium* spp.). In support of this oral route possibility are accumulating data on hypersensitivity reactions to mushrooms, moldy cheeses, breads, wines, fermented products, and other foods or beverages containing molds.^{14–18} Also, because evidence of IgE cross-reactivity between different allergenic species of the fungal kingdom exists,^{4,5} the potential for oral allergy manifestations, which are routinely observed in pollen-sensitized individuals who ingest certain cross-reactive plant-derived food proteins, could be a plausible phe-

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Supported in part by the Young Faculty Support Award from the Foundation of the American College of Allergy, Asthma, and Immunology, 2002–2003

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nomenon for mold-sensitive individuals ingesting hidden or known sources of mold-containing foods as well.¹⁹ For example, studies have shown that individuals specifically sensitized to fungal species of class *Basidiomycetes* manifest allergic symptoms not only after ingestion of mushrooms and other commonly related fungi,^{20,21} but also after inhalation^{21–23} of *Basidiomycetes* spores. Importantly, there have been reports of allergic reactions after ingestion of food sources such as *Aspergillus*-derived α -amylases^{24,25} in bread and a commercial meat-substitute product (e.g., Quorn) containing mycoprotein derived from *Fusarium* mold^{26,27}; these studies subsequently found that individuals reacting to these foods were sensitized and were clinically reactive to aeroallergenic proteins from these molds.^{24–27} These independent clinical observations of allergic cross-reactivity suggest that a link exists between the oral and respiratory routes of mold allergen sensitization and provocation.

Because *Aspergillus* and *Fusarium* spp. are two of the major mold aeroallergen species in patients with nasorespiratory allergies,^{2,3,28} observations of allergic reactions after consumption of these molds have generated questions of whether individuals sensitized to mold aeroallergens are also at increased risk for allergic reactions from oral exposure to aeroallergenic molds in food or drink products.^{4,27,29} Some authors have also recommended general avoidance of mold- or fungal-containing products in mold-sensitive individuals.^{14,15} Despite these observations and recommendations, however, there are no published prospective studies that have examined the clinical impact of oral exposure to aeroallergenic molds, at quantities or concentrations consistent with everyday exposures from consuming foods and/or beverages. The present study was performed to evaluate the potential provocatory role of this oral mold exposure by assessing symptoms elicited in mold-sensitive patients compared with non-mold-sensitive controls after oral challenge with known quantities of aeroallergenic mold extracts.

METHODS

Patient Selection

Adult subjects >18 years of age referred to Georgetown University Medical Center for evaluation of a variety of skin, respiratory, or gastrointestinal allergies were screened for this study. Entry criteria included a documented history of allergic disease symptoms and a positive reaction by skin percutaneous test (SPT), determined by a wheal diameter of ≥ 3 mm larger than the negative control, to at least one inhalant or mold allergen by a screening skin test battery.

A health questionnaire, physical examination, and a baseline forced expiratory volume in 1 second (FEV₁) determination were performed on all subjects. Subjects

with a positive SPT to the oral challenge vehicle (i.e., apple juice), a baseline FEV₁ of <70% of age predicted values or with an active, comorbid systemic disease were excluded from the study. The study was reviewed and approved by the Georgetown University Institutional Review Board, and all patients provided informed consent before enrollment.

Materials

The initial screening skin test battery of allergenic extracts was administered using Multi-Test II applicators, kindly provided by Lincoln Diagnostics, Inc. (Decatur, IL). Allergenic extracts included common environmental mold species (*Aspergillus*, *Epicoccum*, *Mucor*, *Trichophyton*, *Alternaria*, *Hormodendrum*, *Penicillium*, *Cladosporium*, *Monilia*, *Fusarium*, *Pullularia*, *Rhizopus*, and *Stemphylium*), applied either individually or as part of mixes. The panel also included other inhalant allergens (oak, beech, maple, Timothy grass, bluegrass, short ragweed, plantain, and mugwort pollens; cat hair; dog hair; American cockroach; and house-dust mite *Dermatophagoides farinae*), all kindly provided by Antigen Laboratories, Inc. (Liberty, MO). Histamine and glycerine were used, respectively, as positive and negative skin test controls.

A mixed mold (MM) extract stock solution (10 mg/mL of individual mold in glycerine) was prepared by addition of equal volumes of 10 individual, commercial extract preparations of common mold aeroallergens^{2,3,30} (at initial concentrations of 100 mg/mL of mold in glycerine): *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium oxysporum*, *Alternaria tenuis*, *Epicoccum nigrum*, *Hormodendrum cladosporioides*, *Pullularia* sp., *Penicillium notatum*, *Mucor racemosus*, and *Trichophyton rubrum*. Undiluted and serial 10-fold dilutions of the MM stock solution were prepared for both specific skin testing and oral challenges, yielding MM concentrations ranging from 10 mg/mL (undiluted, 1:1) to 0.001 mg/mL (1:10,000). These concentrations were prepared to simulate a possible exposure dose range (in mg) of ambiently ingested aeroallergenic molds in foods. Mold extracts were used for this study rather than whole crude mold preparations obtained from cultures to minimize the potential for harm from ingestion of mold toxins,¹⁰ which are significantly removed during mold extract preparation.² Also, extracts permitted direct correlation of dermal and oral reactivity to a homogeneously defined mold source. An undiluted commercial source of apple juice, sterilized through a 10- μ m filter (Millipore, Billerica, MA) to remove mold contaminants, was used as a methodological control for SPT and oral challenges.

Determination of Mold-Sensitive Populations

All subjects meeting the initial entry criteria underwent SPT and intradermal (ID) testing with the MM

solutions. SPTs were performed by modified prick test method³¹ on the volar surface of the forearm using small drops of the following preparations: undiluted (1:1) and 1:10 dilutions of the MM stock solution, glycerine control diluent, and undiluted apple juice. ID testing was performed using 0.1 mL of a 1:1000 dilution of the MM stock solution or the glycerine control diluent. Positive dermal reactions in both testing methods were identified as those eliciting wheal diameters of ≥ 3 mm larger than the respective glycerine control. By this method, mold-sensitive subjects (*i.e.*, those with any dermal reactivity to MM) could also be subdivided into two dermally reactive subgroups based on SPT and/or ID testing: 1) SPT⁺/ID⁺ (+/+) and (2) SPT⁻/ID⁺ (-/+).

Oral Challenge

The oral mold challenge doses were prepared by adding 0.1-mL aliquots from serial 10-fold dilutions of the MM stock solution to 9.9 mL of sterilized apple juice. This resulted in total ingested mold doses of 0.0001, 0.001, 0.01, 0.1, or 1 mg for each individual challenge. From these dose concentrations, the lowest concentration of mold to elicit a response was determined and was termed the symptom-eliciting concentration of mold (SECM). Ten milliliters of sterilized apple juice alone was used as the placebo control.

The mold challenge procedure used in the present study was adapted from previously described food challenge studies^{32,33} and involved a single (patient)-blinded, placebo-controlled design. Because the study goal was to record and compare symptoms after challenge to a mixture of mold extracts, none of which were known to be allergenic by the oral route, the single-blinded protocol was chosen because this method allows for more effective screening of ingredients and more focused observation of symptoms specifically elicited by the mold extract challenges.³³ In addition to controls for challenge vehicle-associated effects, a nonmold-sensitive group served as an additional methodological control to validate and establish more physiological baseline symptoms for scoring. Also, to minimize potential scoring bias caused by the single-blinded design, clinical investigators were blinded to the results of each subject's MM skin testing and were thus unaware of individual subject's mold sensitization or control status during administration of the challenge material.

Subjects were instructed to swish and swallow each placebo or MM dose juice challenge at 15-minute intervals until the highest concentration of MM was achieved or the severity of elicited symptoms necessitated termination of the challenge. Symptoms and signs were recorded during each challenge interval period and by telephone interview at 24 hours post-

challenge. Given the expectation that low-dose challenges with mold would result in low-grade symptom elicitation, a modified scoring scale (MSS; Table 1), adapted from food challenge symptom score sheets,³⁴ was developed to capture, record, and grade any and all subjective or objective symptoms or signs appearing during the challenge period. All subjects received a placebo challenge as the initial challenge dose to control for vehicle- or challenge-related effects on baseline symptoms before mold challenge(s). Regardless of whether any baseline symptom(s) or organ system complaint(s) was present after this initial placebo challenge, all subjects began with a baseline symptom score of 0. For purposes of scoring, 0 points denoted absence of new symptoms after MM challenge. Symptoms elicited by MM challenge, which caused an enhancement of baseline symptoms or involved a new organ system category, were each assigned 5, 10, or 15 points, depending on the type and severity of symptoms (Table 1). To quantitatively assess the severity of subjective symptoms, each subject was asked to grade the severity of any baseline or elicited complaint or symptom on an arbitrary scale from 1 to 10, with 1 being the least severe and 10 being the most severe. For scoring purposes, elicited symptoms of $\leq 5/10$ from baseline were determined to be mild and were assigned 5 points while those $>5/10$ from baseline were assigned 10 points. Each graded subjective symptom(s) or objective finding(s) involving any one or more of the six organ systems in Table 1 generated a specific organ category score; the summation of these category scores resulted in the final MSS score. Additional placebo doses were intermittently administered during challenge to further exclude the possibility of placebo-related responses on MSS scores.

Statistics

The data were analyzed and graphically presented using the statistical package SPSS for Windows (Version 11.0; SPSS, Inc., Chicago, IL). Both Mann-Whitney *U* tests and Student's *t*-test methods were used to evaluate and compare median and mean differences, respectively, in symptom scores between study (*i.e.*, mold sensitive) group(s) and control groups. Differences associated with values of $p < 0.05$ were considered significant.

RESULTS

The subject characteristics and results of the oral mold challenge are shown in Table 2. Thirty-four subjects met entry criteria and underwent oral challenge. Twenty were women and 14 were men, and ages ranged from 18 to 58 years (mean, 35.1 years). Of the study subjects, 31/34 (91%) were documented to have allergic rhinoconjunctivitis, 15/34 (44%) had asthma,

Table 1 Modified scoring scale system for assessment of subjective and objective symptoms during oral challenge studies

Grade	Score/ points	Organ System Category					
		General	Skin	Eyes/Nose/Throat	Chest/Lung	Abdomen	Cardiovascular
Severe	15	Sense of impending doom	Marked ERY (>50% of body); new excoriations lesions; generalized URT or angioedema	Continuous rubbing; nose runs freely; throat or nostrils occluded	Dyspnea and accessory muscle use; audible wheezing	>3 Episodes of emesis and/or diarrhea; tense abdomen–writhing in pain	Unconsciousness; Δ mentation; Δ in SBP or DBP > 25 mmHg
Moderate	10	Pacing, poor concentration; discomfort, PR, fatigue, or HA >5/10	Macular or raised rash or fixed ERY (<50%); continuous scratching for >2 min at a time; >3 or <10 hives	Frequent rubbing or sniffle; CONG, PR >5/10	Dyspnea >5/10; frequent cough; wheezing on auscultation ΔFEV ₁ >10%	Nausea, abdominal pain >5/10; 2–3 episodes of emesis or diarrhea ×1 episode	LH, DZ >5/10; Δ in SBP or DBP >10 and <25 mmHg; orthostatic hypotension
Mild	5	Discomfort, PR, fatigue, or HA ≤5/10	ERY-migratory or few areas; occasional scratching; <3 hives	Occasional rubbing or sniffle; CONG, PR ≤5/10	Dyspnea ≤5/10; occasional cough; ΔFEV ₁ 5–10%	Nausea, abdominal pain ≤5/10; emesis ×1 episode	LH, DZ ≤5/10
Absent	0	None	None	None	None	None	None

CONG = nasal/eye congestion; DZ = dizziness; ERY = erythema; HA = headache; LH = lightheadedness; PR = pruritus; Δ = “change in”; FEV₁ = forced

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Table 2 Subject characteristics and results from oral provocation/SBPFCFC to molds

No.	Age/Sex	Medical/Allergic History	Allergens (SPT ⁺)	MM-SPT/ID	SECM (μ g) (*obj)	Symptoms Elicited (*obj)	MSS Score
1	22/M	ARC	DM, C	-/+	>1000	None	0
2	23/M	Asthma, ARC	—	+/+	1000	Δ FEV1 8.5%; asymptomatic	5
3	24/M	Asthma	CO, DM	-/-	>1000	None	0
4	25/M	Asthma, ARC	DM, G, C, D, M01, M02	+/+	1	PR (eyes/ throat) 5/10, LH 5/10, HA 5/10, sleepiness, dry throat	15
5	26/M	Asthma	CO, D, G, M7, 8	+/+	>1000	None	0
6	26/M	ARC	DM	-/-	>1000	None	0
7	27/M	ARC	DM	-/+	10	LH 7/10, HA 6/10, PR 4/10	15
8	27/M	ARC	T	-/-	>1000	None	0
9	28/M	Asthma, FA (shellfish, egg) ANA(hazelnut)	DM, C, M1, 4, 8, 9, 10	+/+	*10	*ERY (conjunctiva)	5
10	33/M	ARC, asthma	DM, T, G, W	-/+	10, *1000	PR 4/10, *Cough (Δ FEV ₁ 7%)	10
11	35/M	ARC	DM	-/+	10	LH 3/10	5
12	40/M	Asthma, ARC, FA (shellfish)	DM, G, T, RW, C, D	-/-	>1000	None	0
13	45/M	ARC, FA (eggs, orange)	DM, T, G, M1, 4	-/+	1	HA 1/10, LH 4/10	5
14	54/M	Asthma/COPD, ARC	C, M01, M02	-/-	100	Watery eyes 2/10	5
15	18/F	ARC, asthma	DM, G, T, RW, C, CO	-/-	100	PR (face) 2/10	5
16	19/F	Systemic lupus, ARC	DM, T, G, W, C	-/-	10	HA 4/10	5
17	22/F	ARC	DM, C	-/-	1000	Erythema, generalized PR 3/10	5
18	24/F	ARC, mold allergy	DM, G, T, C, D, M1-4	-/-	1, *10	PR (nose) 5/10, *turbinate edema	10
19	26/F	FA (nuts), asthma, ARC	DM, T, G, C, D, M01, M02	+/+	>1000	None	0
20	27/F	ARC, FA/OAS (most fruits), nasal polyps	DM, G, T, RW, C, CO, M4, 5, 8	+/+	1	PR (eyes) 4/10	5
21	28/F	SIN, ARC	DM, W, M01	-/+	1000	PR(nose) 7/10	10
22	30/F	EZ/URT, ARC, asthma, ASA	DM, C, T, G, CO, M1-6, 9, 10	-/+	0.1, *10	HA 5/10, *ERY (earlobes)	10
23	35/F	ARC, EZ, FA (shellfish, peanuts)	DM, W, C	-/-	*1	PR (skin/eyes) 3/10, Δ CONG 3/10, *patchy ERY	10

Table 2 Continued

No.	Age/Sex	Medical/Allergic History	Allergens (SPT ⁺)	MM-SPT/ID	SECM (μg) (*obj)	Symptoms Elicited (*obj)	MSS Score
24	35/F	ARC, FA (peanut), migraine HA	DM, T	-/+	10	Throat dryness 2/10	5
25	35/F	ARC, FA (crab), migraine HA, asthma	DM, T, W, M01, M02	+/+	1, * ¹ 10, * ² 1000	PR 7/10, HA 5/10, * ¹ migratory ERY, * ² cough (ΔFEV ₁ 17%)	25
26	40/F	ARC, SIN	T	-/-	*10	*Cough (No Δ FEV ₁)	5
27	45/F	ARC, SIN, rosacea	DM, G	-/-	>1000	None	0
28	47/F	FA-ANA (shellfish), ARC, SIN	DM, T	-/+	1	PR(neck)<5/10, CONG <5/10	10
29	51/F	Asthma, ARC, SIN, idio- ANA, HT	W, C, D, M02	+/+	1	PR 7/10, CONG 3/10, HA 3/10	20
30	52/F	Asthma, FA (pepper), ARC, SIN	DM, G, C, D, M01, M02	+/+	1	PR(ear) 2/10	5
31	54/F	Asthma, SIN, ARC, FA/OAS (watermelon, cantaloupe, wheat)	G, M4, M01, M02	+/+	10	HA 6/10, LH 5/10	10
32	55/F	ARC, PCN	DM, T, G, W, C, D, CO	-/-	>1000	None	0
33	57/F	ARC, SIN, HA	DM, RW	-/-	>1000	None	0
34	58/F	ARC, SIN, food interances, CFS	DM, W, M1, 6, 8, 10	+/+	100	DZ 5/10, LH 2/10	5

*⁽ⁿ⁾ = Denotes relative SECM and symptoms elicited due to objective symptoms.

MM = mold mix; ANA = anaphylaxis; ARC = allergic rhinoconjunctivitis; ASA = aspirin sensitivity; EZ = eczema; FA = food allergy; HT = hypothyroidism; Idio = idiopathic; OAS = oral allergy syndrome; PCN = penicillin allergy; SIN = sinusitis; URT = urticaria; Bl = baseline; M1 = Aspergillus fumigatus; M2 = Aspergillus niger; M3 = Fusarium oxysporum; M4 = Alternaria tenuis; M5 = Epicoccum nigrum; M6 = Hormodendrum cladosporioides; M7 = Pullularia; M8 = Penicillium notatum; M9 = Mucor racemosus; M10 = Trichophyton rubrum; M01 = Alternaria, Hormodendrum, Penicillium, Cladosporium, and Aspergillus; M02 = Monilia, Fusarium, Pullularia, Rhizopus, Mucor, and Stenphylium; C = cat; CO = cockroach; D = dog; DM = dust mite; G = grass pollen; T = tree pollen; W = weed pollen; MPD = minimum provoking dose per mold; obj = objective; CONG = nasal/eye congestion; DZ = dizziness; ERY = erythema; HA = headache; LH = lightheadness; PR = pruritus; MSS = modified symptom score; FEV₁ = forced expiratory volume in 1 s.

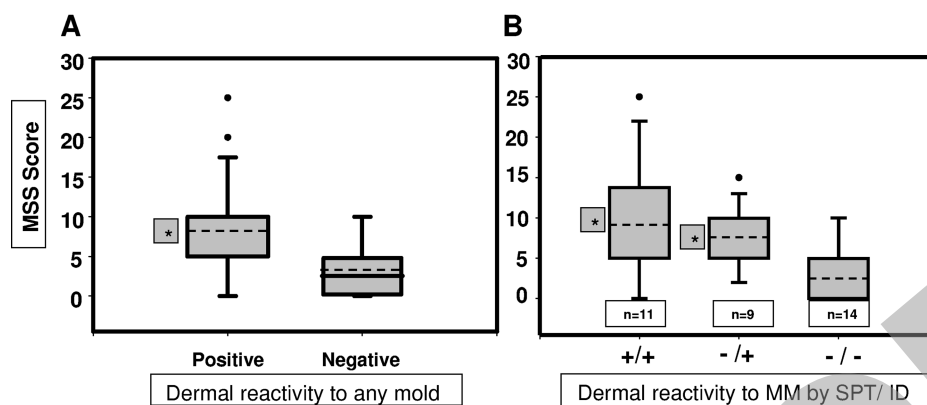


Figure 1. Box plot representations of postchallenge modified scoring scale (MSS) scores between (A) all mold-sensitive (positive; $n = 20$) and nonmold-sensitive (negative; $n = 14$) subjects. The median value is denoted by solid horizontal line (negative group) or the lower line border of the box (positive group). (B) Dermal reactive subject groups based on respective skin percutaneous test (SPT) and intradermal (ID) results (+, positive) and (–, negative) to mixed mold (MM). Each box contains 50% of the data while the upper and lower edges of the box indicate the 75% and 25% percentiles, respectively. Dotted horizontal lines represent the mean MSS. The vertical lines represent the minimum and maximum data values while ● represents outliers; * $p < 0.05$ in both mean and Mann-Whitney U test median scores compared with the control (–/–) group.

11/34 (32%) had food allergies, and 2/34 (6%) had eczema using criteria based on clinical practice parameters. Three of the 34 subjects (9%) reported previous episodes of anaphylaxis to foods without a known mold association.

A total of 20 of 34 subjects had positive dermal reactivity to MM by either SPT or ID (*i.e.*, mold sensitive), and 14 subjects were SPT[–]/ID[–] (–/–) to MM, and thus nonmold sensitive. After oral MM challenge, a significantly higher mean MSS score ($\pm$ SEM) was found in the mold-sensitive group (8.25 ± 1.5) compared with the nonmold-sensitive group (3.2 ± 1.0 ; $p = 0.014$; Fig. 1 A). By Mann-Whitney U test, the median MSS score difference between groups was also statistically significant ($p = 0.0096$). A positive MSS score of ≥ 5 was observed in 17/20 (85%) subjects in the mold-sensitive group compared with 7/14 (50%) in the nonmold-sensitive control group. Three subjects in the mold-sensitive group had symptoms of moderate severity by MSS criteria; all other elicited symptoms in both groups were of mild severity. All subjects completed the oral challenge reaching the final concentration (1 mg/mold). However, one mold-sensitive subject (subject 25), developed a cough and a 17% decrease in FEV₁ at this final dose. Symptoms in this subject resolved promptly within 15 minutes after receiving two inhalations of albuterol. Other than occasional skin flushing or symptoms of pruritus, no significant clinical skin or gastrointestinal symptoms were elicited. Placebo doses administered during the MM symptom elicitation phase had no observed effect on symptom score or symptom reproducibility in any challenge group. Also, no delayed symptoms from the challenge were reported by any subject 24 hours after challenge.

Mold-sensitive subjects comprised two dermal mold reactive subgroups based on skin test reactivity: 11 subjects who were both SPT⁺ and ID⁺ (+/+) and 9 subjects with selective reactivity to ID alone, SPT[–]/ID⁺ (–/+). Of the (+/+) subjects, all individuals reacted by SPT to the undiluted 1:1 MM solution, but none reacted to the diluted 1:10 MM solution. Collectively, compared with the nonmold-sensitive control (–/–) group, either dermal mold reactive subgroup, (+/+) or (–/+), showed a significantly higher mean MSS ($\pm$ SEM) of $8.64 (\pm 2.44)$; $p = 0.035$ and $7.78 (\pm 1.47)$; $p = 0.014$, respectively, after challenge. Median differences by Mann-Whitney U test were also statistically significant between dermal mold reactive subgroups and the nonmold-sensitive group ($p = 0.040$ and $p = 0.015$ respectively) but not between each dermal mold reactive subgroup ($p = 0.409$). However, a tendency toward higher MSS scores was observed for the (+/+) group compared with the (–/+) dermal mold reactive group (Fig. 1 B).

When the SECM was compared with dermal mold reactivity, a similar association was observed (Fig. 2). It can be seen that in the (+/+) dermal mold reactive group, 9 of 11 subjects had symptoms elicited by challenge, and, in the majority of responders (5 of 9), SECMs were found at doses of 1 μ g. In the (–/+) dermal mold reactive group, 8 of 9 subjects had symptoms elicited by challenge, and, in the majority of responders (4 of 8), SECMs were found at 10-fold higher doses of 10 μ g. In contrast, in the nonmold-sensitive control (–/–) group, the number of responders and nonresponders were evenly distributed, 7 versus 7, and, of the responders, no clustering of SECMs was observed. There was no consistent association between

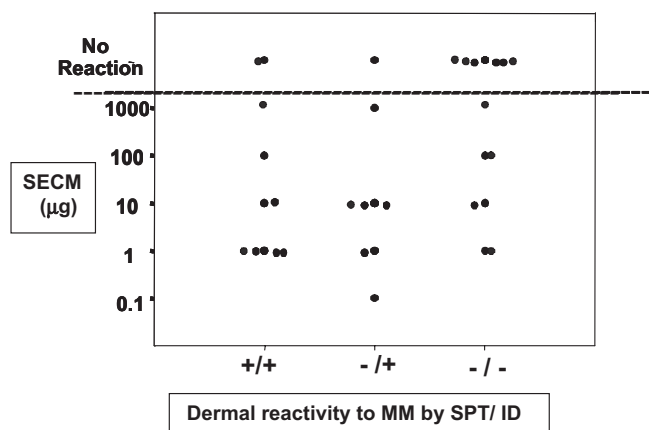


Figure 2. Dot plot representation of symptom eliciting concentrations of mold (SECM; in μg) for subjects in each dermal reactive subgroup. The SECM is represented as the lowest individual mold dose (derived from the mixed mold [MM] concentration), which elicited a subjective and/or objective symptom during challenge. Subjects who had no symptoms elicited by challenge are represented by "no reaction."

SPT or ID wheal size and diameter and MSS score or SECM for any subject group. No significant gender or age-related differences in these parameters were observed.

DISCUSSION

The purpose of the present study was to evaluate the association between the oral route of challenge to low doses of aeroallergenic molds and symptom elicitation in a population of mold-sensitive subjects compared with a nonmold-sensitive atopic control group. All subjects successfully completed challenge to the final concentration of an extract mixture of aeroallergenic molds, and the majority of elicited symptoms in subjects, if any, were mild in severity. However, based on an MSS developed for this study, mean symptom scores were found to be statistically higher in the mold-sensitive group compared with the nonmold-sensitive control group. Although the clinical relevance of this statistical difference in scores still needs to be elucidated and validated by double-blinded challenge studies, this finding suggests that mold-sensitive populations may be more sensitive to the oral provocatory route of aeroallergenic mold exposure, at exposures expected from daily oral ingestion. Exposure to aeroallergenic molds by the oral route may be another source of allergic symptom elicitation in mold-sensitive individuals.

Using a combined approach of dermal mold reactivity, symptom score, and SECM, this study also sought to identify potential diagnostic or clinical characteristics that could predict the most sensitive mold-allergic populations to oral mold challenge provocation. It was found that subgroups of individuals with any dermal

mold reactivity had significantly higher mean symptom scores compared with nonreactive controls. Additionally, although differences in scores were not statistically significant, it was observed that subjects with both SPT and ID reactivity to mold had comparatively higher symptom scores and had consistently lower SECMs than subjects with ID reactivity to mold alone. These results would suggest that a higher degree of dermal mold reactivity, as manifested by SPT reactivity, may correlate with a greater tendency for clinical reactivity to oral mold exposure. However, because no subjects in this study reacted to dilutions lower than the 1:1 MM SPT dilution, an adequate comparison of individuals with varying degrees of dermal reactivity was not achievable to validate this hypothesis.

Clinically significant reactions to mold- or fungi-containing foods have been previously reported in mold-sensitive allergic individuals.^{14–18} Moreover, some studies have suggested a link between the oral and respiratory routes of mold allergen sensitization and provocation.^{4,20–27} Despite these observations, we are aware of only one other controlled challenge study that prospectively examined the clinical impact of oral exposure to mold in a population of patients sensitized to mold aeroallergens.²⁹ In this study, 17 *A. fumigatus*-sensitive individuals were orally fed breads containing *Aspergillus*-derived α -amylase and hemicellulase, and clinical symptoms were graded. No significant increase in clinical symptomatology was observed in individuals after challenge to these mold-containing foods.²⁹ However, comparatively lower doses of allergenic mold protein ($\sim 0.15 \mu\text{g}$ of α -amylase) per challenge were used in this study, and no graded subjective symptoms were reported. Also, the study lacked a control atopic group to study and compare differences in elicited symptom scores between sensitive and non-sensitive individuals.

The premise for using a low-dose range of aeroallergenic molds for challenge in the present study was to simulate exposures to ambient mold concentrations expected from everyday consumption of foods or beverages. Although the impact of mold-containing products was not directly investigated, allergenic mold species naturally contaminate the food supply through growth on agricultural crops during harvesting and storage, airborne contamination, biotechnical processing, and other methods.³⁰ From a review of the literature and food website resources,³⁵ we observe many different types or groups of foods and beverages that could contain appreciable levels of aeroallergenic mold or other potentially cross-reactive fungal or yeast species (Table 3). These include foods with an inherently higher moisture content, such as fruits^{11,12} and luncheon meats³⁶; products from certain food establishments, such as dairies and bakeries, in which environmental contamination with molds, e.g., *Aspergillus*,

Table 3 Common mold-containing foods and beverages

Food/Food Product Categories	Examples
Cheeses	Soft cottage or cream cheese; shredded or sliced cheeses
Dairy	Yogurt, sour cream, buttermilk
Fresh fruits and vegetables	Cucumbers, tomatoes, grapes, berries, citrus fruits, apples, melons, spinach, fruit salads
Dried and candied fruits	Raisins, apricots, figs, prunes
Jams or jellies	
Fruit juices	
Canned fruits and tomatoes	
Nuts	Peanut butter, unprocessed nuts
Fermented soy products	Tofu, natto, tempeh, soy sauce
Vinegar-containing foods	Mayonnaise, salad dressing, catsup
Pickled foods	Pickles, relish
Breads and baked goods	Pumpernickel, fresh rolls, coffee cakes
Cereal grains, cooked	Oatmeal, flour, corn, pasta
Cooked casserole	
Leftover cooked meat and poultry	
Cured meats	Salamis, hot dogs, sausages, luncheon meats
Meat substitutes	Quorn (mycoprotein)
Alcoholic or malted beverages	Wine, cider, beer (malted barley)

Source: Refs. 9–13, 26, and 35–38.

Candida, *Fusarium*, *Geotrichum*, *Mucor*, *Penicillium*, *Alternaria*, *Botrytis*, *Cladosporium*, and *Saccharomyces*, has been shown to inevitably occur³⁷; and common beverages such as fruit juices, wine, and beer.^{9–11} Molds are also frequently used as cultures or other aids in the processing and fermentation of food products.^{38,39} Given our study findings of the oral reactivity potential to low doses of aeroallergenic mold extracts, many of which are related to common mold species found in foods, it is possible that exposure to cross-reactive mold and fungi from a wide variety of food or beverages could be an important, but hidden or underrecognized, source of allergenic reactivity. This may be especially true in individuals who report allergic reactions or symptoms after ingestion of foods or beverages in which no common food allergen or allergenic food ingredient can be identified. For these individuals, it may be practical to consider or question sensitivities to or presence of molds or fungi in the suspected products. By the same token, if the affected individual is mold sensitive, it may also be sensible to obtain a dietary history to exclude mold-containing food sources as the suspected cause.

Before applying the aforementioned considerations to clinical practice, a few important limitations in the study results should be noted. First, clinical interpretation of the differences in symptom scores and eliciting concentrations of mold between study groups is subject to the fact that the modified scoring system used in this study is not validated as an allergy assessment tool and that some scoring may have been biased

by the single-blinded study design. Although the study used a control group and blinding of the investigators to the skin testing results as safeguards to minimize biased interpretation of symptom score results, a double-blinded study design remains the gold standard method for determining causality between oral dose exposure to a suspected allergen and symptom elicitation. Second, because an MM extract preparation was used as the challenge material, it is therefore difficult to infer which molds specifically, if any, were responsible for eliciting reactions during challenge. Moreover, because extract processing methods may qualitatively or quantitatively alter the active allergenic materials compared with those naturally occurring in the environment,² it can be questioned whether real-life mold contaminant exposures in foods were truly simulated. In conclusion, there is no direct evidence from the study results that molds act as primary food or intestinal allergens. The symptom profile in challenged subjects showed relatively few skin or gastrointestinal symptoms commonly seen with food allergies.²⁶ Given that the oral delivery of mold allergen occurred by a liquid swish and swallow method and not by direct consumption of food, it is entirely possible that eliciting responses to molds occurred *via* more local mucosal routes, such as inhalation of mold in the oral cavity or contact with the sublingual mucosa, rather than by the intestinal route, thus explaining the lack of typical food-allergic symptoms. In this regard, aeroallergenic mold exposure in the oral cavity could represent an-

other possible route or process by which these allergens come into local contact with the nasorespiratory tract to elicit symptoms.

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