

Eosinophilia and gastrointestinal symptoms after ingestion of shiitake mushrooms

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Background: Shiitake mushrooms are a dietary staple in Asia and are increasingly popular worldwide. A cholesterol-lowering study with shiitake showed that 17 of 49 participants withdrew because of rash or abdominal discomfort, and two had marked eosinophilia. One of these latter participants was subsequently challenged for 14 days with shiitake powder and again had eosinophilia.

Objective: We investigated whether ingestion of shiitake mushroom powder induces eosinophilia or symptoms.

Methods: We studied 10 normal persons. Each participant ingested 4 gm shiitake powder (open label) daily for 10 weeks (trial 1), and the protocol was repeated in these same subjects after 3 to 6 months (trial 2). Blood counts and serum samples were obtained biweekly (trial 1) or weekly along with stool specimens (trial 2). Eosinophil major basic protein and IL-5, IgE, and IgG antishiitake antibodies were measured in sera. Eosinophil-derived neurotoxin was measured in stool extracts. We defined responders as subjects having peak eosinophil counts four or more times their average baseline counts.

Results: Each trial had four responders, and trial 2 had one new and three repeat responders. Eosinophilia ranged from 400 to 3900/mm³. Responders had increased blood eosinophils, serum major basic protein, stool eosinophil-derived neurotoxin, and factors that enhanced eosinophil viability. Antishiitake IgE was not detected, and antishiitake IgG increased in two responders. Gastrointestinal symptoms coincided with eosinophilia in two subjects. Symptoms and eosinophilia resolved after discontinuing shiitake ingestion.

Conclusions: Daily ingestion of shiitake mushroom powder in five of 10 healthy persons provoked blood eosinophilia, increased eosinophil granule proteins in serum and stool, and increased gastrointestinal symptoms. Shiitake ingestion suggests a model to study the eosinophil's role in the blood and gastrointestinal tract. Finally, our report raises concerns of possible adverse systemic reactions to this increasingly popular food. (*J Allergy Clin Immunol* 1998;101:613-20.)

Key words: Shiitake mushroom, eosinophilia, eosinophil major basic protein, eosinophil-derived neurotoxin, gastrointestinal symptoms, IL-5

The shiitake mushroom (*Lentinus edodes*) is the most commonly eaten mushroom in Asia and is second in the

Abbreviations used

EDN:	Eosinophil-derived neurotoxin
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
MBP:	Major basic protein

global market.¹ Its popularity in the West stems presumably from its pleasing flavor and texture, resistance to spoilage, and ease of cultivation. The shiitake has a long and rich history in Asian culture, with medicinal use dating to the Ming dynasty.² In Japan, shiitake mushrooms are widely believed to have both preventive and therapeutic values, and an extract, lentinan, is produced for both purposes by food and pharmaceutical companies.³ To lend experimental support for the long list of traditionally claimed benefits of the shiitake mushroom, many scientists have studied its biochemical properties and biologic effects. Among the demonstrated effects of shiitake are lowering of plasma lipids,⁴ inhibition of platelet aggregation,⁵ antiviral and antimicrobial activity,⁴ inhibition of tumor growth,⁴ and augmentation of natural killer cell activity.^{6,7} On the basis of its immunopotentiating effects, lentinan has been used in clinical trials in Japan for the treatment of cancer.^{8,9}

During a 10-week study of the cholesterol-lowering effect of 4 gm/day shiitake mushroom powder ingestion, 17 of 49 subjects in the treatment arm withdrew because of either rash (seven subjects) or abdominal discomfort (10 subjects). Two subjects had marked peripheral blood eosinophilia (9700/mm³ and 2760/mm³) at the time they stopped ingesting mushrooms, although their eosinophilia resolved after discontinuation of mushrooms (D. Jacobson, J. O. Hill, University of Colorado, Personal Communication, 1994). The cause for the findings was unknown; however, food allergy seemed unlikely because the reaction developed after prolonged exposure.

To determine whether eosinophilia was causally re-

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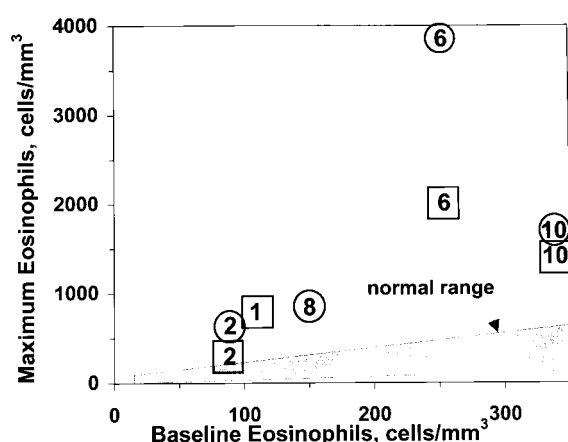


FIG. 1. Peak peripheral blood eosinophil counts of responders (trial 1, numbers in circles and trial 2, numbers in squares) during daily shiitake mushroom powder ingestion (y-axis) in relation to average baseline eosinophil count (x-axis). Range in normal subjects (shaded gray) adapted from Rud.¹⁷ Peak eosinophil counts during two open-label trials are shown with subject's numerical designations. Note that all baseline eosinophil counts fell within normal range.

lated to chronic shiitake ingestion, one of us (VAD) performed a challenge study in one of the subjects (the index subject) who had developed eosinophilia during the cholesterol-lowering trial. Then we fed shiitake mushrooms daily to 10 healthy volunteers for 10 weeks (trial 1) and again after 3 to 6 months (trial 2). Our specific aims in these trials were to determine whether shiitake mushroom ingestion causes eosinophilia in some healthy persons and to assess the changes in eosinophil-active cytokines and eosinophil proteins in blood and stool.

METHODS

Challenge of index subject

During the cholesterol-lowering study, one subject, a 56-year-old man, had severe abdominal cramping prompting an emergency room evaluation that was remarkable for an eosinophilia of 9700/mm³. The challenge study in this index subject was conducted after his symptoms had resolved and his eosinophil counts had normalized. He ingested the same daily dose as that used in the cholesterol-lowering study, but with shiitake mushrooms from a different source. Imported dried shiitake mushrooms were purchased at the local commissary, ground, weighed, and placed into gelatin capsules. An open challenge was conducted consisting of 4 gm of dried shiitake mushroom (imported) powder ingested daily for 14 days. The first dose of shiitake mushroom powder was given under direct supervision without incident. The index subject was monitored for symptoms, and serial blood samples were obtained for complete and differential blood counts. Serum specimens were collected and frozen at -70° C and stored at -20° C until assayed.

Open-label studies 1 and 2

To test if daily ingestion of shiitake mushrooms causes eosinophilia, we conducted a 10-week open-label trial (trial

TABLE I. Blood eosinophils, serum eosinophil MBP, eosinophil viability enhancement, and symptoms: Challenge of index subject

Day of challenge	Eosinophil count/mm ³	MBP (ng/ml)	EVE* (%)	Symptoms
1 (baseline)	100	346	6.6	None
2	300	514	3.6	None
4	300	593	2.1	Mild abdominal discomfort
8	400	935	11.8	Mild abdominal discomfort
12	500	1446	39.7	Mild abdominal discomfort
16 (2 days after challenge)	1600	2150	42.9†	Mild abdominal bloating
33 (17 days after challenge)	200	764	1.7	None

*Eosinophil viability enhancement (EVE) determined by percentage of eosinophils viable after 4 days of culture without added cytokine but with 0.050 ml of subject's serum added. Viability without added subject serum or cytokine in this assay was 5%.

†When eosinophil viability was inhibited with monoclonal antibodies to IL-5, IL-3, and GM-CSF, antibodies to IL-5 reduced viability by 86%, antibodies to GM-CSF reduced viability by 69%, and antibodies to IL-3 did not reduce viability.

1) in 10 healthy subjects using shiitake mushroom powder (vide infra). The same 10 subjects underwent a second 10-week trial (trial 2) after a 3- to 6-month interval without shiitake. Nine men and one woman participated, with an average age of 40.6 years (range, 31 to 63 years). Exclusion criteria were a history of allergy to mushrooms, disease associated with significant eosinophilia, and gastrointestinal disease. Exclusion criteria also included baseline blood eosinophil counts greater than 500/mm³, abnormal serum IgE levels, use of prescription medication (except oral contraceptives), and pregnancy. The woman was of childbearing age, and she was requested to use contraception to prevent pregnancy during the study. To maximize compliance, participants were recruited from among the physicians in the Allergy/Immunology Division (Mayo and Fitzsimons) and the Gastroenterology Division (Mayo). Informed consent was obtained, and all 10 subjects underwent a screening evaluation, including history and physical exam, complete blood count with differential, and serum IgE level. All participants at both sites were asked and reminded not to ingest additional shiitake during the study. The study was approved by the Institutional Review Boards of the Mayo Clinic and Mayo Foundation and Fitzsimons Army Medical Center.

Shiitake mushroom powder

For the open-label studies, domestic, organically raised, sliced and freeze-dried shiitake mushrooms were purchased from the Forest Resource Center (Lanesboro, Minn.). The mushrooms were powdered and mechanically encapsulated by the Mayo pharmacy so that each capsule contained 250 mg of powder. The gelatin capsules were purchased from Shionogi Qualicaps (Greensboro, N.C.). Subjects ingested 4 gm (16 capsules or 4 medium sized mushrooms) of shiitake powder daily for up to 10 weeks in each trial.

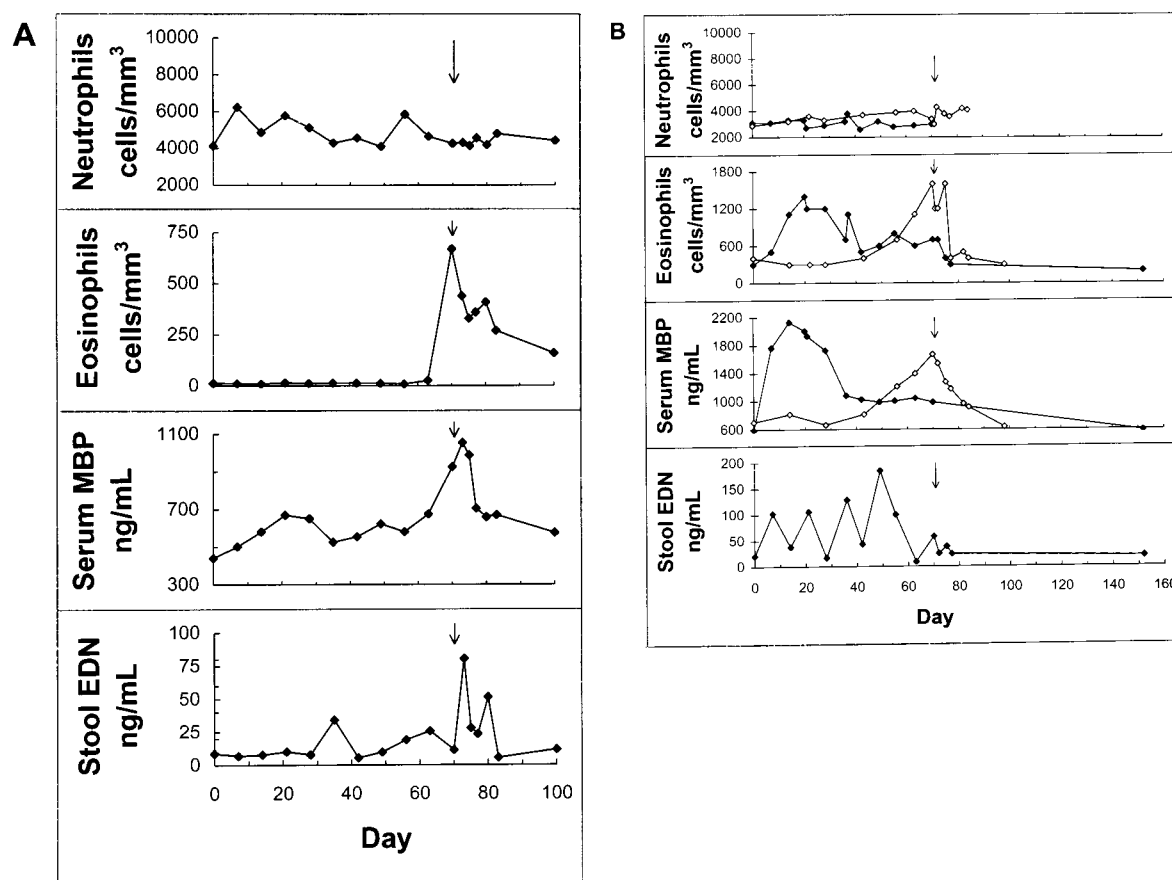


FIG. 2. Patterns of responses to daily shiitake mushroom ingestion in first-time responder (subject 1) in trial 2 (filled diamonds) (A) and repeat responder (subject 10) in trial 1 (open diamonds) and trial 2 (filled diamonds) (B). Shiitake ingestion was from day 1 to day 70; arrows show last day of shiitake ingestion. No data available in B for day 154 neutrophils.

Monitoring

The subjects were monitored for the development of symptoms, and complete blood counts and serum samples were obtained biweekly (trial 1) or weekly (trial 2). Stool specimens were collected weekly in trial 2. Shiitake capsules were discontinued if the subject had eosinophilia greater than 1500/mm³ or if judged to be clinically warranted. If the preceding findings occurred, monitoring was continued with twice weekly blood counts (trials 1 and 2) and stool collections (trial 2) until symptoms, eosinophilia, or both resolved. Compliance was encouraged and tracked by frequent personal contact with the subjects, by hand delivering the mushroom capsules every 2 weeks, and by collecting the previous weeks' capsule containers for pill counts.

Specimens

Serum specimens were obtained by collecting 10 ml of blood by standard venipuncture technique. The serum was aspirated and stored in freezing vials at -20° C until analyzed.

Stool specimens were collected by using a standard toilet insert and placed in a Styrofoam container on ice until processing. Samples were then diluted with water in a 1:4 ratio by weight and homogenized. One hundred milliliters of homogenate was retained and centrifuged at 1500 g for 20 minutes, and

the supernatant was collected and stored at -20° C until analyzed.

Measurement of eosinophil granule proteins

Serum major basic protein (MBP) levels were determined by an immunoradiometric assay as described previously.¹⁰ A previous study showed that among all of the eosinophil granule proteins, only eosinophil-derived protein (EDN) resisted proteolysis by gastrointestinal fluids.¹¹ Therefore EDN in stool was determined by using a previously described double-antibody radioimmunoassay.¹²

Eosinophil-viability assay and blocking studies

To detect the presence of cytokines active on eosinophils in the subjects' sera, the eosinophil viability and blocking bioassays were performed as described previously.¹³ Briefly, eosinophils purified from healthy donors¹⁴ (0.10 ml suspensions) were dispensed in 96-well, half-area, tissue-culture plates at 2.5×10^4 cells per well. Serum from a trial subject (0.020 ml) was added to each well. Hybri-Care medium (American Type Tissue Collection, Rockville, Md.) containing 50 µg/ml gentamicin, 10% defined calf serum (DCS) and 2 mmol/L L-glutamine was added to each well to provide a final volume of 0.2 ml. For neutralization of eosinophil active

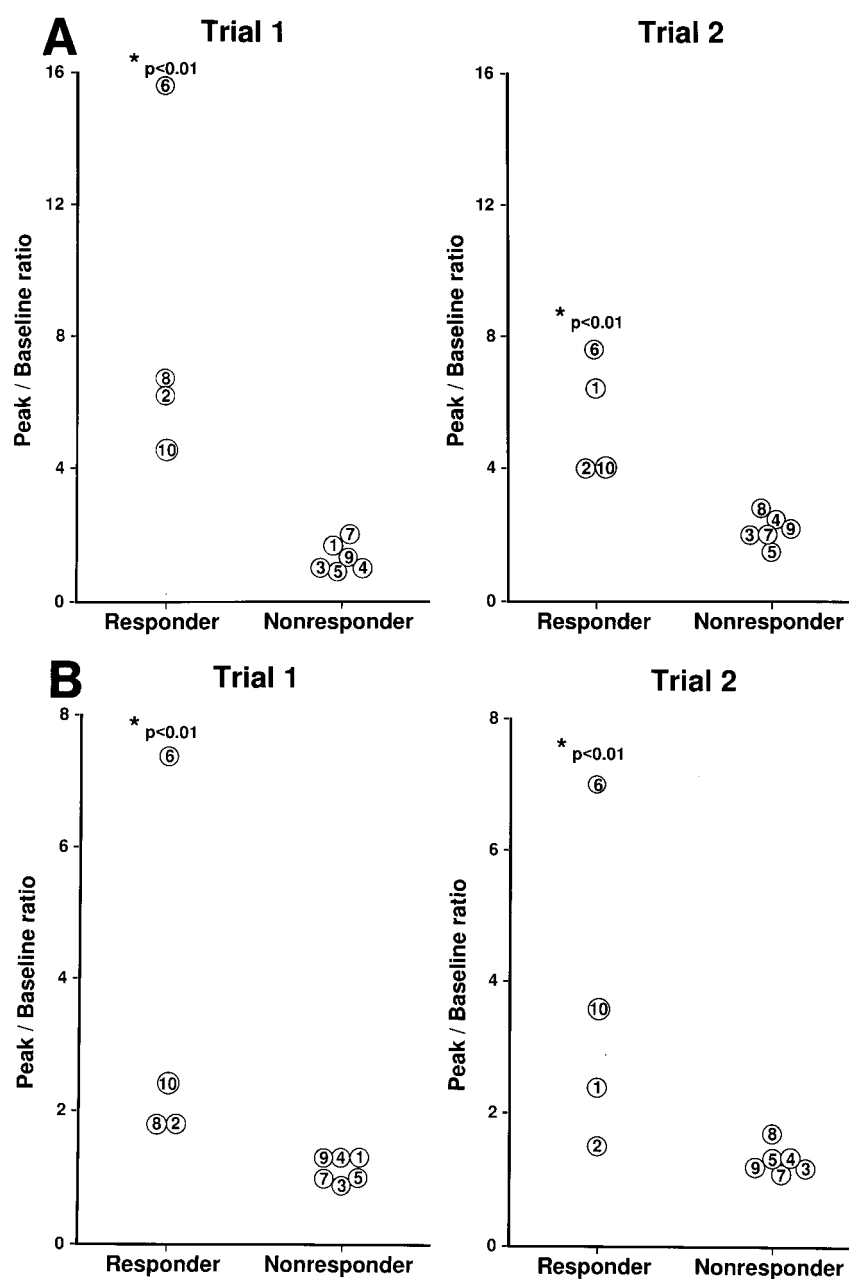


FIG. 3. Comparison of peak-to-baseline ratios of blood eosinophil counts, serum MBP, and stool EDN during daily shiitake mushroom ingestion. **A**, Peak-to-baseline ratios of blood eosinophil counts in responders and nonresponders. **B**, Peak-to-baseline ratios of serum MBP concentration in responders and nonresponders.

cytokines, monoclonal antibodies to IL-5, IL-3, and granulocyte-macrophage colony-stimulating factor (GM-CSF) at 1 mg/ml in phosphate-buffered saline were diluted 1:10, and 0.010 ml was added. After incubation at 37°C and 5% CO₂ for 4 days, the entire cell suspension was transferred to sterile 12 × 75 polystyrene round-bottom tubes. Propidium iodide (Sigma) solution at 1.0 µg/ml was added to the cell suspension to provide a final concentration of 0.50 µg/ml propidium iodide, and cells were enumerated by flow cytometry with a Becton Dickinson FACScan. Viable cells were calculated as the ratio of intact cells not stained with

propidium iodide compared with the total number of stained and unstained cells. In the blocking studies, 5000 cells were counted from each well, and single samples were tested.

IL-5 ELISA

IL-5 levels in serum specimens were assayed with the Quantikine human IL-5 ELISA kit (R&D Systems, Minneapolis, Minn.). The manufacturer's recommended procedure was used except that the sensitivity of the standard curve was extended to 3.9 pg/ml. Single samples were assayed.

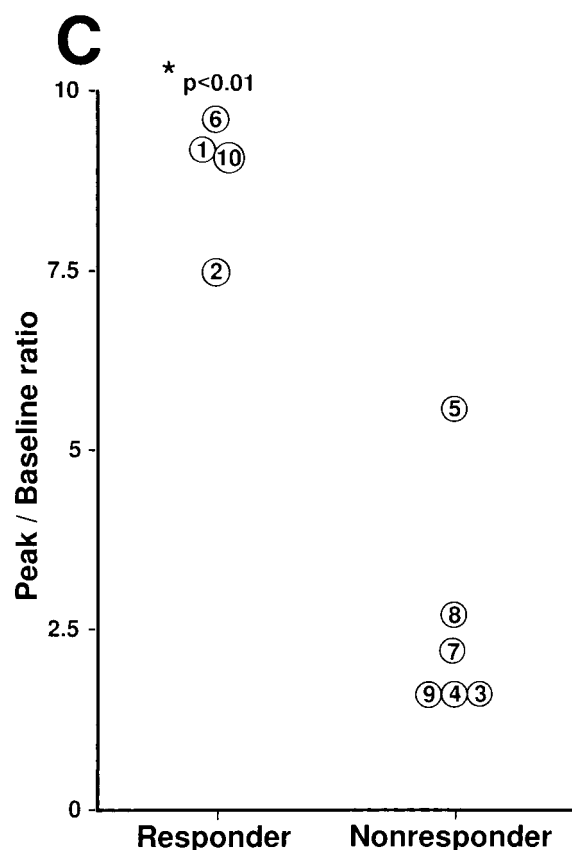


FIG 3. C, Peak-to-baseline ratios of stool EDN concentration in responders and nonresponders. In all figures, median values for groups were compared by using Wilcoxon rank sum test.

Detection of shiitake-specific IgE and IgG antibodies

Shiitake mushroom powder was extracted in phosphate-buffered saline at a ratio of 1:20 mass per volume overnight at 4° C. After centrifugation, the extract was coupled to microcrystalline cellulose beads.¹⁵ The solid-phase shiitake antigen was used to test serum for the presence of IgE antibodies as previously described.¹⁵ To detect IgG antibodies, a method used earlier was modified.¹⁶ Shiitake extract (described above) was diluted 1:500 in 200 mmol/L carbonate buffer at pH 9.2, and 0.10 ml of extract was added to each well of an Immulon-4 microtiter plate (Dynatech Laboratories Inc.). Plates were incubated overnight and washed three times with phosphate-buffered Tween 20 (Sigma). Samples of human sera diluted 1:500 (0.1 ml) were added to each well, and the plate was incubated overnight and washed three times with phosphate-buffered Tween 20. Protein A radiolabeled with iodine 125 was added (0.1 ml), and the plate was incubated overnight. Finally, the plate was washed three times with phosphate-buffered Tween 20, and the bound activity was counted. A pool of normal human sera was used as the negative control.

Data analysis

From previous studies in healthy individuals,^{17,18} a given individual's eosinophil count does vary, but does not exceed twice their average eosinophil count. Therefore we defined responders as subjects having peak eosinophil counts of four or more times their average baseline eosinophil count before

shiitake ingestion. The Wilcoxon rank sum test for nonparametric data was used to compare the median peak-to-baseline ratios between the responder and nonresponder groups for absolute eosinophil counts, stool EDN, and serum MBP. The level of significance was set at a *p* value less than 0.05. Increases of antishiitake IgG were defined as two or more times the mean of the baseline values. Correlation analyses were performed by using Spearman's rank correlation.

RESULTS

Challenge of index subject

While ingesting 4 gm shiitake powder daily for 14 days, the subject experienced mild abdominal discomfort (not severe discomfort as previously experienced in the cholesterol-lowering study), eosinophilia, and an elevated serum MBP level (Table I). By day 12, cytokines that enhance eosinophil viability were detected in the subject's serum (Table I). Four days after the last ingestion of shiitake powder, his symptoms had resolved. Seventeen days after the last shiitake ingestion, eosinophil-related parameters were decreasing to or at normal levels.

Open-label studies 1 and 2

Over the two trials, there were eight positive responses of eosinophilia in five subjects. Four subjects in each 10-week trial were considered to be responders on the basis of peak eosinophil counts four or more times their baseline average, ranging from 400 to 3900/mm³ in trial 1 and 360 to 1900/mm³ in trial 2 (Fig. 1). Three of four responders in trial 1 were also responders in trial 2. One nonresponder from trial 1 (subject 1) became a responder in trial 2. Ingestion of shiitake capsules was stopped for subject 6 in both trials when the eosinophil count exceeded 1500 cells/mm³.

All subjects were questioned several times per week for development of gastrointestinal and dermatologic symptoms. Although two responders developed mild gastrointestinal symptoms, their eosinophilia was less than 1500/mm³; therefore ingestion of shiitake was not stopped. One responder (subject 2) had abdominal cramping with more frequent and looser stools during both trials coincident with eosinophilia, and a second responder during trial 2 (subject 1) had the same symptoms from day 67 to day 72.

Representative patterns of the responses to daily shiitake mushroom ingestion in a first-time responder and a repeat responder are shown in Fig. 2. These graphs show that the increase in eosinophil count is unrelated at any time to the absolute neutrophil count and is accompanied by increases in serum MBP and stool EDN.

During trial 1, peak eosinophilia developed after an average of 49 days (range, 34 to 70 days), but in trial 2 the three repeat responders developed peak eosinophilia after only an average of 18 days (range, 14 to 21 days). In both trials eosinophilia resolved within 1 week after discontinuation of the mushroom capsules. Eosinophil peak-to-baseline ratios were significantly increased (*p* = 0.01) in responders compared with nonresponders

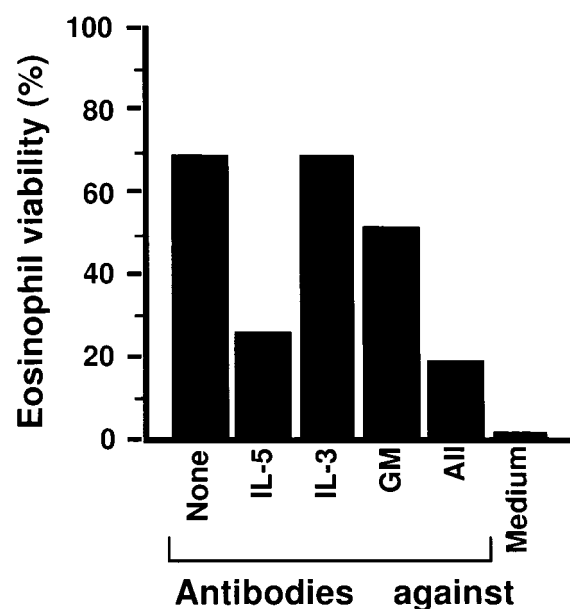


FIG. 4. Blocking of eosinophil viability activity in serum by antibodies to cytokines. Serum from subject 6 in trial 1 was pretreated with anti-IL-5, anti-IL-3, and/or anti-GM-CSF antibody (5 μ g/ml final concentration). Eosinophils from normal donor were incubated with pretreated serum. After 4 days at 37° C, eosinophil viability was determined as described in Methods section. Single samples were counted by using fluorescence-activated cell-sorting technology. Medium shows viability of eosinophils cultured without subject's serum. GM, anti-GM-CSF.

in both trials 1 and 2 (Fig. 3, *A* and Table II). Serum MBP concentrations were significantly increased ($p = 0.01$) in responders compared with nonresponders (Fig. 3, *B* and Table II). Stool EDN concentrations (trial 2 only) were significantly increased ($p = 0.01$) in responders compared with nonresponders (Fig. 3, *C* and Table II). In each responder the peak stool EDN concentration followed the peak eosinophil count by an average of 17 days (range, 3 to 29 days). Peripheral blood eosinophilia in responders correlated significantly with increases in serum MBP (trial 1, $r_s = 0.81$ and trial 2, $r_s = 0.88$; $p = 0.01$ for both trials) and stool EDN (trial 2, $r_s = 0.73$, $p = 0.03$) by Spearman's rank test (peak-to-baseline ratios used for all correlations).

Antishiitake IgE was undetectable in all subjects' sera in both trials. Subject 7 (a nonresponder) had elevated antishiitake IgG levels at baseline (before ingestion of shiitake) in both trials, and the levels did not change during the trials. One of the responders in trial 1 (subject 10) and two of the responders in trial 2 (subjects 6 and 10) had increases in antishiitake IgG levels. The nonresponders' antishiitake IgG levels did not change during either trial.

Eosinophil viability and blocking studies

Sera from all four responders in trial 1 and from two of the three repeat responders in trial 2 (subjects 2 and 6) enhanced eosinophil viability, reflecting the presence

TABLE II. Median peak-to-baseline ratios for eosinophils and granule proteins

	Peak-to-baseline ratio median (range)		
	Eosinophils	Serum MBP	Stool EDN
Trial 1			
R = 4	6.45 (4.6-15.6)*	2.1 (1.8-7.4)*	ND
N = 6	1.2 (0.9-2)	1.2 (0.9-1.3)	ND
Trial 2			
R = 4	7.0 (4.0-7.6)*	3.0 (1.5-7.0)*	9.2 (7.5-9.6)*
N = 6	2.1 (1.5-2.7)	1.3 (1.1-1.7)	1.9 (1.6-5.6)

R, Responder; N, nonresponder; ND, not determined.

* $p = 0.01$ as determined by Wilcoxon rank sum test.

of cytokines in sera. Sera from nonresponders never enhanced eosinophil viability. Viability was sufficiently enhanced in two responders in trial 1 (subjects 6 and 8) to perform blocking studies to identify the eosinophil active cytokines. Similar to the results from the challenge of the index subject (Table I), these experiments demonstrated that the increased viability was strongly inhibited by the addition of anti-IL-5, partially inhibited by the addition of anti-GM-CSF, and unaffected by the addition of anti-IL-3 (Fig. 4).

IL-5 was undetectable in serum specimens by ELISA (<3.9 pg/ml) at baseline in all subjects but increased in three of the four responders in trial 1 (subjects 6, 8, and 10) (range, 4.2 to 31.6 pg/ml). In trial 2, IL-5 could not be detected.

DISCUSSION

This is the first report of eosinophilia and gastrointestinal changes after shiitake mushroom ingestion. Other immunologic reactions associated with the shiitake mushroom have been described. These reactions have included contact dermatitis, generalized pruritus, and hypersensitivity pneumonitis.¹⁹⁻²¹ However, eosinophilia has not been associated with these findings, and in our study no participant experienced skin or lung symptoms.

Here we have demonstrated that a chance observation of eosinophilia in healthy persons ingesting shiitake mushrooms is, in fact, a real phenomenon that occurs in a substantial percentage (40% to 50%) of healthy persons. The effects of chronic shiitake ingestion are reproducible and due to some component of the shiitake mushroom itself because mushrooms from different sources were tested in the original cholesterol-lowering study, the challenge of the index subject, and the open-label studies reported here. Furthermore, the effects are not due to pesticide or preservative contamination because the mushrooms used in the open-label trials were organically raised and freeze-dried without any additives. The eosinophilic response to shiitake does not appear to be a typical allergic reaction as suggested by the inability to detect antishiitake IgE and by the delayed and gradual time-course of the response. However, the response is likely immune-mediated because it is associated with cytokines that enhance eosinophil

viability and elevations in antishiitake IgG in two of the five responders.

Our findings of increased levels of stool EDN among the responders suggest that the eosinophilic response to shiitake mushrooms is not limited to the peripheral blood, but that the eosinophil population of the gastrointestinal tract increases as well. Thus chronic shiitake ingestion probably stimulates enhanced bone marrow production of eosinophils with trafficking of mature eosinophils through the peripheral blood to the gastrointestinal mucosa. Several lines of evidence support this contention. First, in healthy human beings the gastrointestinal mucosa is the only location where substantial numbers of mature eosinophils are normally found.²² Second, biokinetic studies in healthy animals have shown that eosinophils mature in bone marrow and then migrate through the peripheral blood to the mucosa of the gastrointestinal tract where they may be eliminated by shedding across the mucosa²³ or engulfed by macrophages.²⁴ Alternatively, eosinophils may degranulate and undergo cytotoxicity.²⁵⁻²⁷ Third, in disease the latter mechanisms are important. Degranulation products are increased in bronchoalveolar lavage fluids of patients with asthma²⁸ and are deposited at sites of tissue injury in asthma,^{29,30} eosinophilic-endomyocardial disease,³¹ and eosinophilic gastroenteritis.³² Studies in our laboratory have shown that eosinophil granule proteins are increased in stool, gut lavage fluid, and colonic biopsy specimens from patients with inflammatory bowel disease and eosinophilic gastroenteritis.^{11, 33-35} Furthermore, stool levels of EDN in eosinophilic gastroenteritis mirror the clinical course of the disease.³⁵ Finally, in this study the increase in blood eosinophils preceded the increase in stool EDN. In two responders abdominal cramping and loose stools developed in association with eosinophilia and elevated stool EDN.

These findings are unique in that they show the modulation of a peripheral blood effector cell by a common food substance. In addition, the findings demonstrate the non-IgE-mediated systemic modulation of eosinophils. Our observations raise important issues in several different arenas. First, chronic ingestion of particular foods may cause gut inflammation in the absence of an acute typical allergic reaction, suggesting that eosinophilic gastroenteritis may be caused by an ingested substance even in the absence of classic food allergy. Second, given the rising popularity of shiitake mushrooms in the United States, public health concerns likely need to be considered. Third, from the experimental medical perspective, the results suggest a potential model of eosinophil-mediated gut inflammation. Future studies are needed to identify the active component or components of shiitake and to establish the mechanism by which the reaction occurs.

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