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REVIEW ARTICLE

Studies of Sick Building Syndrome. IV. Mycotoxicosis

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ABSTRACT

There has been increasing public attention to the potential health risks of mold exposure, particularly in wet buildings. A variety of molds has been isolated from both damaged homes and businesses, including agents that secrete toxigenic materials. One area that is attracting particular notice is the relative toxigenic potential of mycotoxins. Although exposure to molds can produce significant mucosal irritation, there are very few data to suggest long-term ill effects. More importantly, there is no evidence in humans that mold exposure leads to nonmucosal pathology. In fact, many of the data on toxigenic molds are derived from animal toxicity studies, and these are based primarily on ingestion. Although every attempt should be made to improve the quality of indoor air, including avoidance of molds, the human illnesses attributed to fungal exposure are, with the exception of invasive infections and mold allergy, relatively rare. In this review we discuss selected aspects of the microbiology of mycotoxin-producing molds and their potential role in human immunopathology with respect to wet building environments.

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INTRODUCTION

One area that is attracting increasing public health attention is the frequent presence of toxigenic molds in buildings that are water damaged. Indoor environments contain numerous potentially harmful substances, such as dust mites, animal danders, formaldehyde, ozone, volatile organic vapors, and toxigenic fungi (1–3). However, there are variable data on how and to what extent such substances can affect human health. *Stachybotrys chartarum* (also known as *S. atra*) is one example of a number of indoor fungi known to produce potent mycotoxins. Mold grows on water-saturated, cellulose-rich products, such as paper, cardboard, and wallboard, and on agricultural materials, including hay, straw, and cereal grains (4–6). The fungus has been implicated, without adequate data, in cases of nonspecific indoor air quality complaints in adults and in cases of pulmonary hemorrhage in infants (7). To understand the potential effects of indoor mold contamination, a discussion of mycotoxins is necessary.

MYCOTOXINS

Numerous fungi, including *S. chartarum*, *Fusarium*, and *Trichoderma*, produce mycotoxins, which are very toxic organic compounds believed to induce mycotoxicosis following ingestion or inhalation. Mycotoxicosis is different from mycosis, a disease caused by fungal growth within a host. It is also different from type I hypersensitivity reactions to foreign proteins inhaled with fungal spores. Human toxic illness from mold exposure is currently felt to be due to exposure to mold spore toxins rather than mold growth within the body.

Dust in buildings may contain mycotoxins including trichothecenes (Table 1), aflatoxins, zearalenone, and secalonic D (8). Investigators have identified two groups of trichothecenes: the "simple" group and the "macrocyclic" group. At first, most trichothecenes were believed to be produced by species of *Fusarium*, but other common indoor fungi such as species of *Acremonium*, *Stachybotrys*, *Penicillium*, and *Aspergillus* have been shown to produce them as well (7,9–13) (Tables 2 and 3). The trichothecenes have been studied in animal models due to concerns about their potential for biological warfare.

Since these macrocyclic trichothecenes produced by *S. chartarum* are related molds and may cause disease in animal models, in a way similar to that observed in stachybotryotoxicosis, they are considered to be responsible for the pathologic effects (14). Moreover, fungal toxicity both in vivo and in vitro correlates closely with the levels of satratoxins in their spores (15). Mycotoxins interfere with

Table 1

Trichothecenes Identified in Dust/Grain

Satratoxins
Trichoverrols
Verrucarol
Verrucarins
Trichoverrins
T-2 toxin
Nivalenol
Deoxynivalenol (DON)
Diacetoxyscirpenol

Source: Ref. 8.

Table 2

Most Common Fungi Identified in Indoor Air

Penicillium
Alternaria
Cladosporium
Acremonium
Aspergillus
Thrichoderma
Dreschslera
Epicoccum
Stachybotrys

Source: Ref. 16 and Ref. 59.

Table 3

Trichothecenes-Producing Fungi

Fusarium
Cephalosporium
Giberella
Aspergillus
Cladosporium
Memnoniella
Myrothecium
Penicillium
Stachybotrys
Trichothecium
Thrichoderma

Source: Ref. 16 and Ref. 59.

protein synthesis by interacting with eukaryotic 60S ribosomes (16). Hence, they have a deleterious effect on leukocytes and rapidly dividing cells.

Deoxynivalenol (DON), a type B trichothecene (belongs to the "simple" group), is the most common trichothecene contaminant of cereal grains in many countries including the United States and Canada (17). In animals, a minimal ingestion of this toxin can lead to a syndrome characterized by reduced food consumption and weight loss, while higher ingested amounts induce vomiting, perhaps by altering brain neurotransmitters including serotonin. Additionally, DON, like other mycotoxins, is an immunomodulator that can enhance or suppress the immune system depending upon the dose and duration of exposure (18,19). Other trichothecenes with immunosuppressive activity include the T2-toxin, which is produced by species of *Fusarium* and diacetoxyscirpenol. When given to mice, T2-toxin inhibits antibody responses and delayed hypersensitivity (skin graft survival). T2-toxin has been shown to increase the *Salmonella* pathogenicity in experimentally infected chickens and in a murine model of Japanese encephalitis virus (20). This toxin has also been shown to modulate apoptosis in human promyelocytic leukemia cells (21,22). *Stachybotrys chartarum* has been shown to produce another immunosuppressant substance, FR901459, a member of the cyclosporine family, which prolongs skin graft survival in rats (23).

Apoptosis, in vivo and in vitro, has been shown to be inhibited or enhanced by mycotoxins (19,21,22, 24,25). Recently, in both an animal model and human leukemic cells, satratoxins were found to modulate apoptosis through activation of three groups of mitogen-activated protein kinases (MAPKs) (26). Satratoxin G was the most potent, both as a cytotoxic agent and as an apoptotic inducer, followed by roridin A, verrucarin A, T2-toxin, satratoxin F, satratoxin H, nivalenol, and vomitoxin.

Effect of Mycotoxins on Lung Tissue and Surfactant Production

Although several outbreaks of illness in humans have been attributed to respiratory exposure to mycotoxins, the causal link between fungal contamination in indoor environment and adverse pulmonary effects has yet to be firmly established (4,6,7,27). However, in animals, acute exposure to *S. chartarum* spores has been shown to result in severe pulmonary injury. Nikulin et al. injected mice intranasally with highly toxic or nontoxic spores of *S. chartarum* (15). These spores contained several mycotoxins, including satratoxins (trichothecene mycotoxins) and stachybotrylactones (immunosuppressant), whereas the nontoxic spores contained no trichothecenes. Severe inflammation and alveolar hemorrhage were detected in the lungs of the mice treated with a high dose (1×10^5) of toxic spores. A dose-response was seen; mice receiving 1×10^3 toxic spores developed inflammatory pulmonary changes that were less severe than those in mice receiving 1×10^5 toxic spores. Mice receiving 1×10^5 nontoxic spores developed significantly milder inflammatory changes, and animals injected with 1×10^3 of nontoxic spores did not show inflammatory changes at all.

Similarly, Rao et al. administered toxin-containing or methanol-extracted *S. chartarum* (reduced mycotoxin content) spores intratracheally to rats (28). Their data showed that methanol-extracted spores had reduced toxicity. These methanol-extracted spores did not affect the animals in a significant way as measured by bronchoalveolar lavage fluid analysis. This same group demonstrated, in rats, that intratracheal instillation of a large quantity of *Stachybotrys* spores resulted in severe injury in a time course assayed by bronchoalveolar lavage. The primary injury in the rats was cytotoxicity and inflammation with hemorrhage. Measurable injury was detected as soon as 6 hr, suggesting early release of mycotoxins. In contrast to direct instillation of mold spores, mice exposed to experimental conditions of significant surface growth of toxigenic *Stachybotrys* and high airflow did not have significant pulmonary effects. This observation may be due to the inability of the *Stachybotrys* growth as a slimy mold to become airborne without appropriate environmental conditions (29). Similarly, the ability of *Stachybotrys* to grow or produce mycotoxins may be affected by its environment and growth substrate.

Stachybotrys chartarum spores and a trichothecene (isosatratoxin F) also interfere with surfactant production. Mason et al. (30) treated fetal rabbit alveolar type II cells with varied concentrations of *S. chartarum* spores or isosatratoxin F. They found a diminished uptake of choline into desaturated phosphatydylcholine (DSPC), a main component of surfactant. The authors also noted that exposure of the cells to *S. chartarum* spores or isosatratoxin F resulted in altered alveolar surfactant phospholipid concentrations.

S. chartarum and Pulmonary Hemorrhage/ Hemosiderosis in Infants

The most serious human disease attributed to *S. chartarum* was described in 1994. The Centers for Disease Control (CDC) reported 10 infants (ranging in age from 1 to 8 months) from Cleveland, Ohio, with acute idiopathic pulmonary (IPH) hemorrhage (31,32). In addition, 5 of the 10 infants had recurrent pulmonary hemorrhage after returning to their homes, and 1 of the 10 infants died of respiratory failure. At first, the CDC conducted a case-control study and found an association between pulmonary hemorrhage and mold growth, including *S. chartarum* (33,34). Study of strains of *S. chartarum* isolated from the homes of the children revealed highly toxigenic strains that could produce an arsenal of mycotoxins, including macrocyclic trichothecenes (13). Similar cases have since been reported elsewhere. Elidemir et al. analyzed the bronchoalveolar lavage fluid of a child from Texas with pulmonary hemorrhage and isolated a strain of *Stachybotrys* (Houston strain) from the fluid (35); *Stachybotrys* was also isolated from an infants' water-damaged home (36).

Nonetheless, isolates of *S. chartarum* from case homes of sick infants were not necessarily more toxic than isolates from control homes (13). In addition, *S. chartarum* was not isolated from all the case homes, and in some cases, other species of fungi, such as those of *Penicillium* and *Trichoderma*, were found. These facts led some investigators to hypothesize that exposure to *Stachybotrys* mycotoxins alone may not be sufficient to cause pulmonary hemorrhage and that additional exposure to other factors like tobacco smoke is probably needed (37).

We should note, however, that the ability of a pathogen to proliferate within a mammalian host is frequently dependent on its ability to obtain iron from the host (38). Through the production of iron-chelating agents, fungi utilize siderophores or hemolysins (39–41). Recently, Vesper et al. demonstrated that some strains of *S. chartarum* can produce both hemolysins and siderophores (42,43). They analyzed 16 strains of *S. chartarum* isolated from homes of infants in Cleveland, Ohio, with pulmonary hemorrhage, or from control homes, and an additional 12 non-Cleveland strains. Out of the eight case strains, five produced hemolysin compared to three out of 12 non-Cleveland strains. Moreover, more strains in the case strains group produced highly toxic spores. The hemolytic activity of the case strains and the fact that four of the sick infants suffered from hemoglobinuria along with the pulmonary hemorrhage suggest that hemolysins might play a role in the pathogenesis of pulmonary hemorrhage in these infants. Furthermore, Vesper et al. recently demonstrated that Houston case strains of *S. chartarum* can produce a hydroxamate-type siderophore, hence revealing another mechanism that enables them to obtain iron.

In 1997, the CDC convened a group of advisers to examine the controversial correlation of *Stachybotrys* and the Cleveland area infant cases. Following a thorough examination of the data, this group concluded that it was impossible to prove a definite causal association between infant pulmonary hemorrhage and exposure to potent mycotoxins. They noticed many obstacles in the studies reviewed, including the inconsistent definition of water damage, differences in collecting data, and differences in the methods used to define fungal contamination or exposure. Moreover, in some of the cases, isolation of molds from the case homes was done months after the acute episode of pulmonary hemorrhage and did not necessarily reflect the fungal burden at the time of the illness. They also mentioned the rarity of pulmonary hemorrhage in infants compared to the abundance of water-damaged environments, and the lack of pulmonary hemorrhage as a prominent symptom in previously documented outbreaks of *S. chartarum*-related illness in animals and humans (27). Nonetheless, because of the initial Cleveland-area CDC investigation, the American Academy of Pediatrics' Committee on Environmental Health did establish guidelines regarding the toxic effects of indoor molds. They recommended that infants under the age of 1 year should avoid chronically moldy, water-damaged environments. They also recommended measures to control and prevent indoor mold growth (44). Further investigation into the possible toxic effects of indoor fungi as a possible cause of infant pulmonary hemorrhage is underway.

ASSOCIATION OF ALLERGENIC AND TOXIGENIC FUNGI WITH BUILDING-RELATED HEALTH SYMPTOMS

The first report of human illness due to *Stachybotrys* mycotoxins in the United States was by Croft et al. (10). This group reported an outbreak of

unexplained illness that appeared to be related to contamination of the indoor air of a water-damaged home by trichothecenes-producing *S. chartarum*. Reported symptoms included headache, sore throat, diarrhea, fatigue, dermatitis, and depression. Medical evaluations were not obtained. These symptoms improved after removal from the home and repair of the water damage. The authors concluded that trichothecenes caused the symptoms. This report has been often cited in consequent studies and public health statements discussing the potential dangers to human health from exposure to *Stachybotrys*. Since then, mycotoxin-producing fungi have been implicated as potential etiologic agents in a number of unexplained outbreaks of building-related illnesses (4–6) (Tables 4 and 5). There are limitations, and thus controversy in these studies, such as lack of assessment of water damage, lack of assessment of mold in the environment of control subjects in case-control studies, lack of bioassays of *Stachybotrys* and mycotoxin exposure, and lack of physical examination and laboratory data with reported symptoms.

One of the largest cross-sectional studies on the health effects of the indoor environment was conducted in 30 Canadian communities, involving a total of 13,495 children 5–8 years old (45) (Table 6). This survey reported that molds were prevalent in 32.4% of houses, dampness in 14.1%, and water damage in 24.1%. The parents reported that children in damp homes experienced a 25%–50% higher prevalence of respiratory and nonrespiratory symptoms. Exposure to two mold sites resulted in more symptoms, implying a dose–response relation. The observations persisted after elimination of possible confounding variables such as age and sex of the child, the number of household smokers, and the presence of a gas stove (45). In addition, there were no differences between children with known dust and mold allergy and children without these known allergies (45). Similarly, a questionnaire-based study on the health status of employees in a water-damaged office was performed (5). Thirty-nine female and 14 male employees from this office, along with 21 controls, were evaluated. This building was found to have widespread fungal contamination, especially with toxigenic *S. chartarum*. Employees at the problem office site reported more frequent symptoms of lower respiratory system, as well as dermal, ocular, and constitutional symptoms, than controls. In addition, these symptoms correlated with the degree of fungal growth burden. The two groups did not differ in the titers of anti-*S. chartarum*-specific antibodies.

There have been other examples of exposure. Hodgson et al. investigated the outbreak of a disease characterized by mucous membrane irritation, fatigue, headache, and chest tightness that seemed to

Table 4

Outbreaks of Diseases Possibly Associated with Mycotoxins

Reference	Location	Number of Patients	Symptoms	Fungi Isolated
(10)	A house	5	Flu symptoms, sore throat, diarrhea, headache, fatigue, dermatitis, intermittent focal alopecia, and general malaise	*S. chartarum*
(5)	Office building	53	Lower respiratory, dermatological, eye, constitutional, and chronic fatigue symptoms	*S. chartarum*, *Penicillium*, *Aspergillus*
(46)	Courthouse and two associated buildings	14	Mucous membrane irritation, fatigue, headache, chest tightness, and feverishness	*S. chartarum*, *Aspergillus*
(6)	Office building	33	Recurrent upper and lower respiratory infections, fatigue, headache, depression, and difficulty in concentration	*S. chartarum*, *Penicillium*, *Aspergillus*
(60)	Military hospital building	14	Respiratory symptoms, cough	*Sporbolomyces salmonicolor*

Table 5

Indoor Fungi Exposure-Related Symptoms

Symptoms	Objective Way to Evaluate Symptoms
Respiratory tract	
Cough	Peak expiratory flow rate
Shortness of breath	Methacholine
Asthma/wheezing	provocation test
Bronchitis	Skin prick test
Chest tightness	
Ear, nose, and throat	
Runny nose	Eosinophils in nasal lavage
Sneezing	
Sinus congestion	
Sore throat	
Eye	
Irritation	Schirmer test
Watery itchy eyes	Tear film stability
Skin	
Rash	
Flushing	
Gastrointestinal	
Nausea	
Vomiting	
Diarrhea	
CNS/constitutional symptoms	
Anxiety	Physical examination
Depression	
Difficulty in concentration	
Fever, chills, sweat	
Lymphadenopathy	
Flu-like symptoms	
Chronic fatigue symptoms	
Headache	
Myalgia	
Lethargy	

be related to a specific work site (a courthouse and two associated office buildings) (46). The research group identified a number of individuals with respiratory symptoms, and excessive humidity and widespread fungal growth of mainly *S. chartarum*, *Penicillium*, and *Aspergillus* species (mainly *A. versicolor*) was documented in a number of sites throughout the buildings. However, they did not find an association between IgE or IgG antibodies and the presence of symptoms. Sudakin et al. found evidence of fungal contamination and isolated *S. chartarum* in an investigation of a water-damaged office building that generated discomfort in several of the employees (6). The investigators reported a high prevalence of respiratory symptoms and behavioral changes among the workers. Based on former studies, they have suggested that the behavioral changes reported might be the result of exposure to *Fusarium* metabolites, such as fusaric acid, that may act synergistically with trichothecenes to produce these effects (47). Nonetheless, these studies are limited in attempting to establish a causal relationship between mycotoxin exposure and building-related illnesses. In many of the studies, both the degree of exposure to molds and symptoms are based entirely on self- or second-hand reports; therefore, the evidence is weak. Most investigators did not consider other factors that might be present in water-damaged, improperly ventilated environments that have the potential to harm human health. Finally, the prevalence of sick building-related symptoms is strongly influenced by subjective factors such as mental stress (48). In fact, when evaluating 2160 subjects in 67 offices concerning psychological stress and sick building-related symptoms, Ooi and Goh reported a higher prevalence of these symptoms among employees experiencing more physical and mental stress compared to controls (48).

ASTHMA AND OTHER ALLERGIC REACTIONS

The relation between respiratory symptoms, especially in children, and the presence of molds and dampness has been investigated on the basis of mold allergenic potential (49–51). When exposed to airborne fungal spores, atopic individuals may suffer from allergic rhinitis, asthma, and hypersensitivity pneumonitis. It is estimated that fungal allergens are responsible for 20%–30% of the allergic reactions in atopic subjects and for 6% of the allergic reactions in the general population (52). When Brunekreef et al. (53) examined 4625 8–12-year-old children for the association between water-damaged environment and respiratory symptoms, a strong relation between these two factors was noted. However, pulmonary function tests were only slightly, if at all, reduced in the affected children.

Humidity in the environment was associated with asthma and bronchial hyperresponsiveness when studied in a random group of 98 individuals with asthma and 357 controls (54). Not only was the

Table 6

Summary of Studies on Human Exposure to Fungi or Dampness

Reference	Subjects	Study Design	Exposure Assessment	Results	Problems
(45)	13,495 children aged 5–8 years from 30 Canadian communities	Cross-sectional	Parents reported of mold sites, moisture, and flooding	Dose response gradient between number of mold sites and health outcomes (mainly upper and lower respiratory symptoms)	Exposure and outcome variables are based entirely on questionnaire reporting. No measurements of molds concentrations/ humidity. No objective assessment of complaints
(61)	268 female day-care workers from 30 Finnish day-care centers	Cross-sectional	Workers report of water damage and mold odor	Mainly eye, respiratory, and general symptoms	
(5)	53 workers from problem office	Cross-sectional	Sampling of viable and nonviable fungi from indoor and outdoor air analysis of mycotoxins	Increased health problems among office employees Lower levels of mature T lymphocyte cells (CD 3%) in employees than in controls	Controls were 21 subjects without contact with problem office site, but their environment was not tested for the presence of fungi and spores
(57)	1102 employees from six mechanically ventilated buildings in Montreal	Questionnaire-based study	Sampling of fungi and spores in outdoor air and HVAC system. Measures of temperature and humidity	Association of work-related respiratory symptoms with low level allergen exposure	Failure to identify any environmental cause for more than 80% of workers. Not all fungi were measured
(56)	163 office workers: 87 from modern air-conditioned building, 76 from three traditional-style office buildings	Cross-sectional	Environmental evaluation was not done	Atopy does not seem to influence the prevalence of sick building symptoms (SB). Upper respiratory tract seems unaffected by exposure to SB. Alternation in tear film stability seems related to exposure to SB	No exposure assessment. Complaints were evaluated objectively, e.g., spirometry, methacholine test, prick test, and tears sampling
(55)	108 children	Cross-sectional	Pollen counts and fungus spore count	Increased *Cladosporium* and *Epicoccum* spores concentration was associated with decreased peak expiratory flow rate and increased morning cough, respectively	The children were the sole source for symptom information. Indoor fungi that could have important health effects were not measured

prevalence of asthma higher among residents of damp dwellings, but these individuals also had eosinophilia and higher rates of type I hypersensitivity to molds, mainly to *Cladosporium* and *Alternaria*. Airway disease has also been attributed to moisture in day-care centers (55). One hundred and eight children who recorded symptoms, peak expiratory flow rates (PEFR), and hours spent outdoors twice daily were included in the study. The investigators demonstrated that detectable *Cladosporium* and *Epicoccum* spores influenced morning PER and morning cough in a dose–response relation. In another study, 87 office workers from a sick building and 76 controls were evaluated (56). Objective evaluation of reported symptoms was done using spirometry, the methacholine provocation test, skin prick tests, and analysis of ocular symptoms. A higher frequency of ocular and respiratory symptoms was noted among the employees of the sick building than among controls. Prick tests were positive in 20% and 17.4%, and a positive methacholine test was documented in 20.5% and in 16.2%, respectively. No significant differences were measured on spirometry. Interestingly, atopic individuals did not report more sick building symptoms or lower respiratory symptoms. Menzies et al. investigated the same association, by skin prick testing (57). Out of 1102 employees questioned for sick building-related symptoms in six buildings in Montreal, 121 employees were identified. These 121 employees, along with 107 matched asymptomatic controls, participated in the study. With the exception of a small group of employees that had positive skin tests to *Alternaria*, which was detected in their working site, there were no major differences between the symptomatic group and the asymptomatic group. The two groups did not differ in their skin tests results or in the level of contaminants in their working site, which were generally low. They concluded that allergic reaction to molds could explain respiratory symptoms in only a small subgroup of employees.

Nine population-based studies that were undertaken in the previous 10 years have been reviewed (58), and in seven of them at least one positive association was found between mold burden and allergic health outcomes, but these studies are limited by the potential of reporting bias. Furthermore, there are no specific diagnostic symptoms, signs, or laboratory tests for mycotoxicosis, and despite the fact that mycotoxins can harm health, the mechanisms are unclear. Allergies to molds are not uncommon, but immediate hypersensitivity reaction is not involved in the vast majority of the reported studies, nor was atopy a predisposing factor for sick building-related symptoms (5,55,56,58,62,63).

PREVENTION

The most effective treatment of fungus exposure health-related problems is by prevention. Extensive mold problems should be remediated in consultation with professionals trained in industrial hygiene because of the potential of extensive release of mold spores. Due to concerns regarding infants and indoor molds, interim consensus guidelines for mold cleanup are now established (44). However, conclusive evidence regarding bioassays to confirm illness is still needed in order to determine the appropriate degree of avoidance measures to be undertaken.

Although numerous reports and studies deal with the association between toxigenic fungi and health-related problems, the inconsistency and inaccuracy of data regarding environmental factors and sick building-related symptoms and signs in these studies make their interpretation difficult. Furthermore, there are no reliable tests for clinical assessment of toxigenic molds or mycotoxins. As public awareness of the health effects reportedly associated with water-damaged building increases, the issue of "toxic molds" will continue to confront physicians and public health officials. The techniques of collecting and analyzing the presence of fungi and their attendant mycotoxins are evolving, including immunologic assays, PCR detection, and assays for trichothecene toxicity (43). As these techniques improve and become standardized, less controversial and more scientific evaluation of the human risks from exposure to mycotoxins will be possible.

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