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Attributes of *Stachybotrys chartarum* and its association with human disease

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Mold contamination and toxicities are not limited to crops and animals; they are also a concern in human health. Molds occur in outdoor and indoor environments, and water-damaged buildings harbor and provide substrate for several mold species. Of these, *Stachybotrys chartarum* poses a particular threat to occupants. Patients with building-related symptoms and infant idiopathic pulmonary hemorrhage often have histories of living in moldy, water-damaged buildings. Although a causal connection is far from being unequivocally proven, *S chartarum* has been associated with such clinical conditions. These illnesses could be attributed in part to mycotoxins released by *S chartarum*. Recently, a hemolysin released by this mold was found to be hemolytic in vitro and in vivo. In addition, allergenic proteins have been characterized from *S chartarum*. The exact mechanism of *S chartarum* pathogenesis has not yet been defined. Moreover, a causality-effect relation is not yet established. This review summarizes available information on the pathogenic attributes of *S chartarum* and calls for well-controlled objective studies. (J Allergy Clin Immunol 2004;113:200-8.)

Key words: *Environment, mold, mycotoxin, Stachybotrys, building-related symptoms, stachylysin*

Fungi are ubiquitous in nature; approximately 100,000 species of fungi have been recognized thus far. They are found in air, water, soil, and plants and on virtually every surface. However, only a few hundred of them are known to cause diseases. The harmful molds or their products enter or come into contact with the body surface from the environment.

It has long been postulated that susceptible individuals might get sick when exposed to molds that grow in water-damaged buildings. In this regard, the most frequently cited fungus is *Stachybotrys chartarum*, which also goes by the older names *Stachybotrys atra* and *Stachybotrys alternans* (the term *S chartarum* is used in this review) and is popularly known as *black mold*. *S chartarum* is dematiaceous and is a member of the division *fungi imperfecti*.

Abbreviations used
IPH: Idiopathic pulmonary hemosiderosis
PH: Pulmonary hemorrhage

Recently, there have been a growing number of studies of moisture- and mold-damaged buildings that aimed at correlating damp indoor building environments and disease.[1,2] Furthermore, in recent years there has been widespread reportage in the news media about black mold and lawsuits filed in which claims have been made for compensation for property damage or health injury as a result of exposure to toxic mold in residential or office buildings. Several studies have suggested some associations between the presence of molds and various disease manifestations; however, strong supporting evidence for a causal relationship is still lacking. These studies were reviewed previously, and readers interested in in-depth coverage of the subject can refer to the articles by Kuhn and Ghannoum[3] and Miller et al.[4]

In this review, we focus on exposure to mold products and on their pathogenicity; included is a discussion of mechanisms underlying mycotoxicosis and allergic conditions. Special emphasis is given to *S chartarum*, which is purported to cause animal and human diseases as a consequence of its toxic metabolic products. Discussion of infections caused by pathogenic molds is beyond the scope of this article.

FUNGAL ORGANISMS IN THE OUTDOOR ENVIRONMENT

Conidia and spores of fungi are always present in the air, vegetation, and soil and can be found on virtually any surface. The numbers and species present in these environments vary with geographic location, climate, season, time of day, and so forth; this is true of spores of myco-

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toxin-producing fungi. A comprehensive list of molds in dust and air has been compiled by Miller et al[5]; among these molds are *Penicillium*, *Rhizopus*, *Cladosporium*, *Alternaria*, and *Aspergillus* species as major outdoor molds. Takahashi[6] reported that species of *Cladosporium*, *Alternaria*, *Aspergillus*, and others were the dominant fungi in outdoor air in Yokohama, Japan.[6]

People engaged in agriculture and factory workers who handle moldy grains or process feed or peanuts are often exposed to molds. Mold is considered an important contaminant of agricultural produce. *Aspergillus* species have an affinity for oilseeds; however, mere *Aspergillus* colonization does not designate any hazardous effect. Inadequate drying or improper storage of agricultural products might facilitate toxin production from aflatoxin-producing *Aspergillus* strains. Likewise, *Aspergillus ochraceus* has been isolated from drying or decaying vegetables, seeds, nuts, and fruits. *Penicillium verrucosum* occurs in grain in temperate zones; this mold has been recovered from barley and wheat and even from meat products in Europe. *Penicillium janthinallum* and *Penicillium simplicissimum* occur in soil and decaying vegetation and are capable of producing the tremorgenic toxins janthitrem and verruculogen. *Penicillium crustosum* is found in food; however, the tremorgenic toxin penitrem A is produced only under special conditions.[7] Exposure of workers to (outdoor) mold in silos might cause illnesses that are due to hypersensitivity pneumonitis rather to than any toxic effects.

S chartarum spores have been reported in the outdoor environment. Unlike the spores of *Aspergillus* species, however, the spores of *S chartarum* do not readily disseminate in the air, because S chartarum spores usually occur in a cluster and are covered with dried slime; they become airborne only when dry and disturbed or when they become attached to dust and the like. The humidity required for growth of *Stachybotrys*—approximately 93% at 25°C—is much higher than what is required for the growth of other molds,[8] though significant quantities of mycotoxins are not produced unless the water activity reaches 0.95.[9] However, increasing temperature and enhanced nutritional status of the substrate can lead to lower moisture requirements. The fungus can survive over the winter; spores stay viable for years to decades.[10] *Stachybotrys*, like many other molds, grows in the presence of other fungi (rather than in isolation) throughout the habitable world.[11,12] Contrary to a general belief, *Stachybotrys* is not a common mold in outdoor or indoor air; instead, the organism is generally found in soil and strata that are rich in cellulose (hay, straw, grain, hemp, plant debris, dead roots, wood pulp, cotton, fabrics, paper, book bindery glue, plant fiber processing facility materials, and so forth).[10]

MOLDS IN THE INDOOR ENVIRONMENT

Molds are transported into the indoor environment through air circulation or are carried indoors by organisms, including human beings, or in the moving of inanimate objects that have molds attached to their surfaces. When the food source, moisture, temperature, and so forth in the indoor environment are favorable, molds can grow. The presence of mold in damp buildings is a major concern and has become a hot topic of discussion during the last decade or so. The most frequently isolated molds in the indoor environment in one study were *Penicillium* (96%), *Cladosporium* (89%), *Ulocladium* (62%), *Geomyces pannorum* (57%), and *Sistronema brinkmannii* (51%).[8,13] In a study conducted in Japan,[6] *Cladosporium*, *Aspergillus*, *Penicillium*, and *Alternaria* species were the predominant indoor molds.

Stachybotrys has a fondness for cellulose,[10] which might promote growth of *Cladosporium*, *Penicillium*, and *Aspergillus* species as well.[14,15] The nutritional and growth requirements of the black mold organism suggest a lack of recovery from laboratory cultures and perhaps underreporting of the incidence of *Stachybotrys*. In the indoor environment, the frequency of isolation of *Stachybotrys* is approximately 13% of dwellings and 5% of samples, as assessed by several studies.[14,16,17] The fungus proliferates rather slowly, facilitating overgrowth by other molds unless appropriate culture substrates (eg, cellulose-based media) are used. Studies using cellulose-based agar techniques have reported a relatively high prevalence of *Stachybotrys*, with positive cultures in up to 30% of water-damaged homes.[18] *Stachybotrys* can sometimes be isolated from other substrates, including pipe insulation, gypsum, glass fiber wallpaper, and aluminum foil.[14] Surfaces that are soiled or that have susceptible paint or paper facilitate mold growth without being damp.

INDOOR MOLD- AND DAMPNESS-RELATED ILLNESS

Although molds are naturally a part of the indoor and outdoor environment, their presence, types, numbers, and biological properties might affect animal and human health. Various illnesses are purportedly caused by indoor molds; these include pulmonary, immunologic, neurologic, and oncologic disorders. In a study by Platt et al,[19] it was found that the occupants of wet, moldy buildings had an increase in subjective complaints.[19] In another study, a similar pattern was observed in more than 6000 American children in 6 states. These studies suggested home dampness to be a strong predictor of various illnesses, including respiratory complaints. The children living in damp homes complained of headache, eye irritation, epistaxis, nasal and sinus congestion, cough, and "cold and flu" symptoms, as well as generalized gastrointestinal disorders.[20] An increased prevalence of asthma was found in moisture-affected schools in other studies.[21,22]

Although most fungi are metabolically active throughout a broad temperature range, high moisture and relative humidity are required for their optimal growth.[23] The aforementioned studies and many others suggest an association between subjective complaints and damp environments; however, the links between the illnesses and environmental factors—eg, water damage—are not

TABLE I. Major mycotoxins and related diseases

Mycotoxin class	Related mold(s)	Diseases/toxicity
Aflatoxin	*Aspergillus flavus*, *Aspergillus parasiticus*	Hepatitis, liver cancer, childhood cirrhosis, immunosuppression (?)
Ochratoxin	*Aspergillus ochraceous*, other *Aspergillus* species, *Penicillium verrucosum*	Nephrotoxicity, tumors, hepatotoxicity, embryotoxicity
Fumonisin	*Fusarium moniliforme*, other *Fusarium* species	Gastrointestinal disorders (?)
Trichothecene	*Fusarium* species, *S chartarum*	Skin irritation, gastrointestinal disorders, immunosuppression, hemorrhage, convulsion
Zearalenone	*Fusarium graminearum*, other *Fusarium* species	Gynecomastia in boys (?), precocious puberty, tumor

clear-cut. When such possible links were investigated, etiologies varied considerably.[11,12,24-27] Whereas moisture facilitates mold growth, it also affects mite and ozone levels, as well as off-gassing and salt and acid formation.[28] The fact that dust mites, which are known to be allergic agents and a cause of upper respiratory symptoms, coexist with molds in home environment makes a causal association of mold exposure and building-related symptoms more difficult to prove.[29] Another complicating factor is the fact that gram-negative bacteria and their products (endotoxin, for example) and mycobacteria are often found in water-damaged buildings in association with molds.[30,31] At times, the bacterial load outnumbers the fungal load significantly.[26,32]

MAJOR MYCOTOXINS AND THEIR TOXICITY SPECTRUM

Mycotoxins—secondary metabolic products of fungi that represent a chemically diverse group of organic, nonvolatile, low-molecular-weight compounds—have been associated with food poisoning since ancient times. Mycotoxins have no known direct role in the fungal growth process and are usually produced when conditions favor fungal growth as well as toxin production. Mycotoxin production depends on moisture, pH, growth medium, and temperature; moreover, their presence in the environment has not been quantified. Attempts to detect mycotoxin in indoor environments are often unsuccessful or inconclusive of a specific agent. However, a protein translation (luciferase) assay showed promise for trichothecenes in airborne particulates.[34]

In animals and human beings, mycotoxins cause toxicity and an illness known as *mycotoxicosis*. Toxic mold contamination is a concern in animal and human food supplies. However, the ill effects in human beings are not nearly as well characterized as those in animals.[34] Nikulin et al[35] were successful in establishing a mouse model of pulmonary stachybotryotoxicosis. The use of biomarkers such as β-D-glucan and monoclonal antibody to assess exposure or dose-dependent effects has been suggested, but it is not yet standardized. Mycotoxicosis differs from mycosis in that an infectious fungus need not be involved in the former.

The earliest recognized mycotoxicosis in human beings is gangrenous ergotism, or *St Anthony's fire*, which results from eating rye infected with *Claviceps purpurea* and some other molds. Major epidemics of alimentary toxic aleukia, stachybotryotoxicosis, aflatoxicosis, and so forth have killed thousands of people, pets, and farm animals (including poultry) around the world. Because of its impacts on health and the economy, mycotoxicosis became known to agriculturists, food scientists, environmental scientists, and health professionals during the last several decades.

The diversity in the chemical structures of mycotoxins results in a wide range of clinical signs and symptoms that vary from trivial to very serious in nature. However, it is possible to classify toxicities caused by mold products into 4 categories: acute, chronic, mutagenic, and teratogenic. Of these, acute toxicity, resulting in impairment of functions of the liver and kidney, is of the highest medical importance. Some mold products act primarily by interfering with protein synthesis, their effects ranging from skin sensitivity to necrosis to extreme immunodeficiency; other mold products are neurotoxins that cause alteration in motor function or brain damage.[36] Liver cancer has been associated with consumption of some mycotoxins. Among the wide array of mycotoxins aflatoxins, ochratoxin A, fumonisins, trichothecenes, and zearalenone are considered the most important.[37] Comprehensive details on this subject can be found elsewhere.[1,37] For the purposes of this review, the major mycotoxins, their sources, and their toxicities are summarized in Table I, and we focus on *Stachybotrys*-related toxins.

Trichothecenes are toxic metabolites of some imperfect fungi—eg, species of the genus *Fusarium*, such as *Fusarium trichioides*, *Fusarium solani*, *Fusarium toxicum*, *Fusarium tricinctum*, and *Fusarium graminearum*, and *S chartarum*. Trichothecenes (eg, T-2 toxin and trichodermin) and macrocyclic trichothecenes (eg, roridin A) produce a variety of clinical conditions, including skin irritation, anorexia, vomiting, diarrhea, immunosuppression, hemorrhage, convulsion, and death. Most domestic animals are susceptible to T-2, HT-2, and diacetooxyscirpenol, the major trichothecenes; however, alimentary toxic aleukia, red mold (akakabi) disease, "Taumelgetreide" toxicosis, and other conditions have been reported to occur in human beings as well as in domestic and farm animals.

ALLERGIC REACTION TO MOLDS

Mold-related health injury can result from infection, mycotoxins, or allergic reactions. In general, fungal particles that measure ≥10 μm are deposited in the

nasopharynx, whereas smaller particles can reach the alveoli. Therefore, symptoms of any allergic reaction might be related to the size of the spore or its fragments. However, live fungal cells are often not required for injury by mycotoxins or fungal allergens. Unlike mycotoxins, which are low-molecular-weight proteins, fungal allergens are large proteins or glycoproteins in nature. The exact prevalence of fungal allergy varies significantly on the basis of climatic and environmental factors as well as the testing methods used (readers interested in this subject can refer to a review by Horner et al[38]). In the industrialized countries, including the United States, approximately 2% of the population has been estimated to have fungal allergy. Of special mention is the fact that these allergies are not seasonal.

Most of the fungal organisms in the environment are capable of producing substances with allergenic potential. Of these, allergens have been characterized from only few molds, including *Alternaria alternata*, *Aspergillus fumigatus*, and *Cladosporium herbarum.* The relationship between fungal airborne spores and allergic disease is not clearly understood. Exposure to a mold or its product might not be related to spore formation. Ponikau et al[39] determined the incidence of allergic fungal sinusitis in 210 patients with chronic rhinosinusitis; culture of nasal mucus was positive for fungal species in 96% of the patients, but fungal organisms also grew from culture of nasal mucus from all of the healthy controls. The total and fungus-specific IgE levels in the patients tended to be higher; however, the difference was not significant over IgE levels in the control group.[39] It should be noted that on the average >2 organisms were grown from each subject but that no species of *Stachybotrys* was isolated in this key study.

Exposure to large amounts of organic dusts can result in a condition characterized by fever, chills, myalgias, dry cough, headache, and dyspnea. Symptoms of variable degree usually occur 4 to 8 hours after exposure and are self-limiting. This condition is known as *pulmonary mycotoxicosis* or *organic dust toxic syndrome*. The condition differs from hypersensitivity pneumonitis in that the conidia are transient and not related to a previous exposure and patients are negative for serum-allergic precipitins.[40]

STACHYBOTRYS MYCOTOXINS

Effects of *Stachybotrys* during the Russian equine outbreak in the 1940s were attributed to the trichothecenes class of compounds.[10] Experimentally, two-thirds of *Stachybotrys* isolates produce stachybotryotoxins. *S chartarum* is the species most frequently associated with trichothecenes mycotoxicosis; other important trichothecenes-producing molds are *Fusarium*, *Myrothecium verrucaria*, and *Myrothecium roridum*. Although trichothecenes are chemically diverse, some of the best described are satratoxins F, G, and H, roriden E, verrucarin J, and trichoverrols A and B. These toxins have been isolated from a variety of substrates, including dust (satratoxins, trichoverrols, verrucarol, verrucarins, trichoverrins) and grain (T-2 toxin, nivalenol, and derivatives of others).[41] The most potent of these are T-2 toxin, diacetoxyscirpenol (anguidine), deoxynivalenol (vomitoxin), and fusarenon-X. Several mechanisms underlie the action of mycotoxin, such as initiation of protein synthesis (scirpentriol, 15-acetoxyscirpendiol, diacetoxyscirpenol, verucarin A, T-2 toxin) and some of the toxins (eg, trichodermin, trichodermol, crotocol, trichothecolone, trichothecin, verrucarol) have been reported to have activity during elongation or termination phases of protein synthesis.[42] Satratoxins and other trichothecenes have been found to cause apoptosis in addition to inhibition of protein synthesis in vitro.[43] Trichothecenes resist sunlight, ultraviolet light, X-rays, heat (up to 120°C), and acids. They are readily destroyed by alkalis, which allows for detoxification with Na-, K-, Ca-, or NH_3-hydroxide or gaseous ammonia.[44,45] This has important ramifications for building remediation.

THREAT WITH *STACHYBOTRYS*

There is a growing concern that *Stachybotrys*, which was once considered a saprophyte, is a pathogen for equine and nonequine animals, including human beings. Coincident with the epidemics of equine stachybotryosis in the 1940s, a dermatologic and respiratory syndrome developed in human beings, especially fodder-handlers and others who had close contact with musty straw.[46] Therefore, the route of exposure might be direct contact or inhalation rather than ingestion.[47,48] Primary disease manifestations appeared on the skin, with dermatitis on the contact areas. Lesions progressed from hyperemia to crusting exudates to necrosis, with subsequent resolution.[49] When applied to the skin of volunteers, the isolates produced the same local and systemic responses.[50] Respiratory symptoms were described, including catarrhal angina, bloody rhinitis, cough, throat pain, chest tightness, and occasional fever. Some patients experienced transient leukocytopenia. Despite the association of *Stachybotrys* with animal and human disease, scientists have found it difficult to establish *Stachybotrys* as an infectious mold; since the disease process does not fulfill Koch's postulates with the fungus, the illness is attributed to toxin rather than to fungal growth or tissue invasion.

Respiratory symptoms range from benign, such as congestion and cough from rhinitis, to reactive airways disease, to more serious syndromes, including alveolitis, bronchiectasis, and pulmonary fibrosis. A variety of actual syndromes ranging from asthma to allergic bronchopulmonary aspergillosis, hypersensitivity pneumonitis, emphysema, pulmonary fibrosis, and pulmonary hemosiderosis have been reported.[51,52] Whereas some studies indicate adverse pulmonary effects by indoor mold-induced allergic reactions, sound evidence of serious or permanent lung injury is lacking.

The report of a cluster of 10 cases of idiopathic pulmonary hemosiderosis (IPH) in infants from Cleve-

land, Ohio, generated much attention on the part of public health professionals. As reported, the infants presented with severe respiratory disturbances requiring intensive care; symptoms recurred in 50% of them after they returned home. The investigators concluded that the infants were exposed to toxigenic *S chartarum* from their water-damaged building environments.[53-55] The same group examined whether fungal exposure was a risk factor for IPH by air and surface sampling; they concluded that, infants with IPH were more likely than controls to live in homes with toxigenic *S chartarum* and other fungi.[56] In a published overview of the Cleveland investigations and of other, similar cases in the United States,[57] the same researchers stated that rapidly growing infant lungs appear to be vulnerable to toxigenic molds. They considered environmental tobacco smoke a possible trigger for pulmonary hemorrhage (PH).[57] However, because of apparent flaws in these reports, the Centers for Disease Control and Prevention stated that the association between *S chartarum* and IPH was not substantiated adequately, because the methods used in the aforementioned studies did not distinguish between contamination and a clinically meaningful exposure.[58]

Stachybotrys species can produce a cyclosporin-like immunosuppressive agent capable of increasing skin graft survival in rats.[59] Such findings have become of increasing concern in the light of reports of immune suppression and increased infections in *Stachybotrys*-exposed individuals. Animal experiments show pancytopenia after ingestion of *S chartarum*.[60] Trichothecenes, including T-2 toxin, diacetoxyscirpenol, deoxyivalenol (vomitoxin), and fusarenon-X (produced by *Stachybotrys* and *Fusarium* species), have immunosuppressive effects.[34,61] These compounds are among the most potent small-molecule inhibitors of protein synthesis.[62, 63] In addition, trichothecenes might affect cytokine production.[64,65] Trichothecenes have been associated with decreased resistance to infectious organisms, including *Salmonella*, tuberculosis bacteria, *Listeria*, herpes simplex virus, *Candida*, and *Cryptococcus*.[61] Some mycotoxins, including citrinin, patulin, and even T-2 toxin, can enhance immune function.[66,67] However, in human beings, toxin-related immunologic compromise has not been ascertained. Despite many reported subjective complaints, there is no objective evidence for neurologic compromise by indoor mold exposure to human beings—in particular, exposure to *S chartarum*.[3] Although some trichothecenes are potentially carcinogenic, to date there have been only conflicting reports in animal studies.[68] There is no evidence to support claims that individuals exposed to *Stachybotrys* are at long-term risk of cancer or require cancer surveillance.[69] Although some nonspecific constitutional symptoms have been reported, no reports of hepatobiliary, endocrine, renal, or pregnancy-related effects due to inhalation of *Stachybotrys* spores or toxin have been published.

ASSOCIATION OF *STACHYBOTRYS* SPECIES WITH DISEASES IN WATER-DAMAGED BUILDINGS

Many authors have reported ill effects of water-damaged buildings in relation to *Stachybotrys* species. Hodgson et al[11] reported a building-related illness in Florida; symptoms, which occurred within weeks of patients' moving into the affected building, consisted of mucosal irritation, fatigue, headache, and chest tightness. Although the researchers concluded that the symptom outbreak was likely a result of inhalation of fungal toxins, including satratoxins G and H caused by *S chartarum* and *Aspergillus versicolor* in moldy ceiling tiles, there was in fact no clear evidence (eg, laboratory parameters) to support the claim. Similarly, Johanning et al[2] implied that mycotoxins—namely, satratoxin H and spirocyclic lactones from water-damaged material—were the cause of respiratory and immune problems in occupants of "sick" buildings. Tuomi et al[17] examined Finnish buildings with water damage and identified a host of fungal organisms and mycotoxins (satratoxins G and H, T-2 toxin, and the aflatoxin precursor sterigmonisin) in bulk samples; however, the relationship between the organisms and the toxins was unclear. There are more examples that described the illnesses with few objective measurements and without a clear etiologic link.

In recent years, many schools have been closed and buildings abandoned because of alleged mold problems with water damage. Air samples from 48 US schools were tested for molds because of indoor air quality and health concerns. Fewer than 50% of those schools had indoor fungal counts that were higher than the counts for outdoor air. *Stachybotrys* was not a major component of the isolated mycoflora; however, the mold was present on some visibly wet and mold-contaminated carpet and wall surfaces.[16] This apparent discrepancy signifies that not all spores are detectable, viable, or cultivable. A minimal contribution of *Stachybotrys* was demonstrated by a study by Miller et al.[5] The investigators examined the occupants of 50 Canadian houses that were associated with complaints of unexplainable respiratory or allergic symptoms; the occupants of 6 of these houses had building-related illnesses. However, fungus-related disease could not be established in these individuals.[5,70] On certain occasions, people who became ill from building-related problems recovered after relocation or remediation of the buildings.[16]

PATHOBIOLOGY OF *STACHYBOTRYS* SPECIES

Reaction to the toxins produced by *Stachybotrys* is considered the most important factor in the illnesses under discussion; allergic reactions and infection are considered to be minor mechanisms involved in *Stachybotrys*-related health injury (Fig 1). As noted earlier, skin contact and inhalation are the major means of exposure to *Stachybotrys*. The toxins produced, being nonvolatile

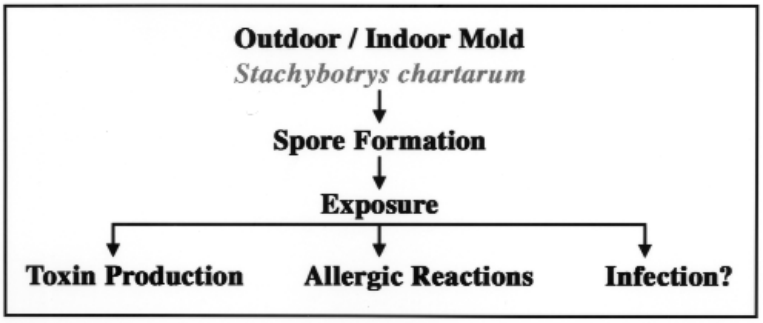

FIG 1. Possible mechanisms causing *S chartarum*–related diseases.

and of high molecular weight, usually do not occur as aerosols. Therefore, exposure is likely through inhalation of airborne particulates containing mycotoxins, such as dust and fungal components.[71]

The exact molecular mechanism of injury in stachybotryosis is not known. Recently, Nagase et al[72] demonstrated that in a cell culture system apoptosis can be induced by satratoxin G mediated by activation of caspase 3 via both caspase 8 and caspase 9 apoptotic pathways; this that was evident by cytosolic release of cytochrome c and fragmentation of nucleosomal DNA.[72] Animal models have been used to understand the pathobiology of stachybotryosis. In mice, intranasal instillation of toxigenic spores resulted in a decrease in platelet count and an increase in leukocyte and erythrocyte counts, hemoglobin concentration, and hematocrit, but no IgG antibodies to *S chartarum* were detected. Inflammatory changes and hemorrhage were detected in the alveoli.[73] Marked ultrastructural changes, including condensation of mitochondria with separated cristae, scattered chromatin, and poorly defined nuclei, and an increase in lamellar body volume were identified in alveolar type II cells of juvenile mice exposed to *S chartarum* conidia or its toxin isosatratoxin F.[74] Rao et al[75] demonstrated that in rats, inflammation and PH result from intranasal *S chartarum* challenge, with biochemical changes in bronchoalveolar lavage fluid. Purified trichothecenes, such as T-2 toxin and *S chartarum* extracts, can cause hemolysis.[76] Recently, stachylysin, a new hemolysin, has been characterized from the Cleveland cluster of *S chartarum*.[77] The hemolytic potential of stachylysin was tested in an earthworm model. The researchers observed that stachylysin was hemolytic, caused dilatations of blood vessels, and was lethal to earthworms. They hypothesized that bloody nose in adults and PH in infants might be due to stachylysin released from *S chartarum* spores.[78] Spores of *S chartarum* strains with different stachylysin-producing abilities were instilled intratracheally in mice and rat pups. Using immunohistochemistry and immunocytochemistry, the investigators detected granuloma after instillation of a control strain with no stachylysin-producing ability, whereas stained areas for stachylysin and phagolysosomes with spore wall fragments were seen after instillation of a stachylysin-producing strain. Therefore, an association of stachylysin with IPH was suggested.[79]

A previous sensitizing exposure is a prerequisite for allergic reaction. Hyperresponsiveness to fungal allergens is typically manifest as rhinitis (hay fever) or asthma. Emerging evidence indicates that a significant, persistent inflammatory component (in addition to IgE-triggered effects) underlies the etiology of asthma.[38] Genetic factors are also known to influence an individual's ability to mount an IgE-mediated reaction.[80] Viana et al[81] found that multiple respiratory exposures to *S chartarum* crude allergen extract cause responses typical of allergic airway disease, as demonstrated by elevated serum and bronchoalveolar lavage fluid proteins and lactate dehydrogenase, neutrophilia, and hyperresponsiveness to increasing concentrations of nebulized methacholine in BALB/c mice. Barnes et al[82] demonstrated that in healthy individuals, IgG and IgE antibodies directed against *S chartarum* could be approximately 50% and 10%, respectively, indicating exposure to *S chartarum* or cross-reacting fungal proteins. They also identified the proteins on immunoblots and found them to be specific for *S chartarum* by inhibition of immunoblot.[82] Van Emon et al[83] developed an ELISA to quantify stachylysin in biological and environmental samples. Despite negligible cross-reactivity with several molds, *S chartarum* was highly reactive to ELISA. The authors also demonstrated that *S chartarum* mycelia produce large amounts of stachylysin in vitro; this suggested that ELISA determination of stachylysin might help screening of exposed individuals.[83] The allergenic potential of (1,3)-β-D-glucan, as indicated by elevated IgE levels, has been demonstrated in a mouse model,[84] and its association with mold exposure in human beings has been suggested.[85] This complex carbohydrate, one of the cell wall components of fungi, has been investigated as a diagnostic marker of systemic mycosis.[86] Moreover, this component is not specific to any particular fungal species. Its exact role in inflammatory response has not yet been determined.[87]

No systemic or localized infection from direct exposure to *Stachybotrys* has been reported in human beings. Recently, Elidemir et al[88] reported identification of *Stachybotrys* from the lungs of a 7-year-old boy. Clinical investigations found hemosiderin-laden macrophages in the child's bronchoalveolar lavage fluid and red blood cell microcytosis; however, no hemolysis was seen on blood smears. Environmental surface sampling resulted

in growth of several fungal species. The authors did not confirm an infection; they did confirm an association of exposure to or recovery of *S chartarum* with IPH.[88] Random amplified polymorphic DNA analysis gave different banding patterns despite common toxicity and hemolytic properties; some characteristic patterns were more common in toxigenic strains.[89] Recently, *S chartarum* was isolated from a patient with PH and hemosiderosis from Houston, Tex. The clinical isolate was found to be capable of producing hemolysin and siderophores and had genetic relatedness with environmental case isolates of the Cleveland cluster.[90]

DISCUSSION

Although the Centers for Diseases Control and Prevention and the American Academy of Pediatrics have suggested some guidelines respecting the challenge posed by toxic molds, whether molds in water-damaged indoor environments are pathogenic has not yet been determined. As of this writing there are only some associations, which are far from establishing any definite relationship between the fungus and the disease. However, in recent years, mold-related illnesses and damage to property have been discussed frequently. One might wonder whether this is due to a general awareness of the health issues or to attractive legal compensations.

This article has reviewed the currently available knowledge regarding the pathogenic attributes of *S chartarum*. Although dampness-related illnesses have been associated with indoor molds, the studies or observations have often failed to substantiate the subjective clinical findings in stachybotryotoxicosis or IPH in infants. The environmental factor is often not clearly understood; not everyone exposed to the molds has gotten sick, nor has a pathogenic mold been isolated from a normally sterile biological sample. The situation becomes worse when mycoflora in the outdoor air is similar to that in the indoor air and susceptible individuals are colonized with similar microorganisms.[39] The presence of potentially toxigenic strains does not imply production of toxin (either in the environment from which the organism was isolated or the laboratory), and the appearance of a toxin in environmental samples does not mean that *Stachybotrys* (or another relevant fungus) is present. Associations with other factors, including genetic predisposition, are sometimes equally important.

Although animal studies have suggested a pathobiologic relationship, it should be mentioned that there are limitations in these animal models. In addition to species variation in response, there is the fact that an animal model often does not represent actual disease in human beings; for example, a bolus dose or an intraperitoneal route does not mimic human stachybotryosis. Therefore, determination of the pathogenicity of potential indoor molds—*Stachybotrys* in particular—is extremely valuable. In addition to the toxicity caused by mycotoxins, the characterization of products from *Stachybotrys*, such as antigenic substances and hemolysin, has advanced our knowledge in recent years. However, further studies are necessary for an understanding of the pathogenicity of this mold in relevant biological systems; this includes studies of sporulation, routes of exposure, adhesion, secretions, and putative virulence factors, as well as host cell protection and damage. These factors must be the subjects of basic biological study in vitro as well as in animals in order for the knowledge to be applied to clinical settings.

CONCLUSIONS

An absence of specific clinical features and a lack of evidence as regards a definitive pathogen make it difficult for a clinician to appreciate the condition of a patient with black mold–associated pathosis. Some environmental factors have been defined. In addition, antigenic and toxic products from the mold have been partially characterized; this helps us to understand the disease associations. Dampness- and mold-associated illnesses, including those caused by *S chartarum*, have a great impact on health and the economy. Therefore, mold-related illnesses need special attention; more in-depth studies should be conducted to advance our understanding of the pertinent biology, ecology, and host-pathogen relationships so that these illnesses can be prevented.

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