Fungal Spores: Hazardous to Health?

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Fungi have long been known to affect human well being in various ways, including disease of essential crop plants, decay of stored foods with possible concomitant production of mycotoxins, superficial and systemic infection of human tissues, and disease associated with immune stimulation such as hypersensitivity pneumonitis and toxic pneumonitis. The spores of a large number of important fungi are less than 5 μm aerodynamic diameter, and therefore are able to enter the lungs. They also may contain significant amounts of mycotoxins. Diseases associated with inhalation of fungal spores include toxic pneumonitis, hypersensitivity pneumonitis, tremors, chronic fatigue syndrome, kidney failure, and cancer. Key words: mold, fungi, mycotoxin, lung disease, toxic pneumonitis. — Environ Health Perspect 107(suppl 3):469-472 (1999).


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Background

Fungi are heterotrophic, filamentous organisms that by virtue of their dependence on external sources of organic carbon and their rigid cell walls are confined to a saprobiic and/or parasitic lifestyle in which they absorb soluble nutrients through the cell membrane. The fungi, together with the bacteria, are responsible for decay of organic matter and the fungi have been estimated to comprise approximately 25% of the biomass of the earth (1). As such, they are among the principal microorganisms involved in biodeterioration and are found universally in human habitats and occupational settings. The possible role of airborne microorganisms in indoor air health problems as a result of energy-saving measures was accentuated by Tobin et al. (2) and a number of studies have noted a strong relationship between mold growth in homes and respiratory symptoms (3-5). When one considers that urban residents typically spend more than 90% of their time indoors (6), it is readily recognized that very large numbers of people, both adults and children, are potentially exposed to indoor air contaminants. Although spore concentrations are usually much lower in homes than in agricultural workplaces, concentrations as high as 450,000 colony-forming units/m³ have been reported (7). In some of these homes, the toxigenic Stachybotrys chartarum (= Stachybotrys atra) was prominent on the walls and in the air. This article focuses on health effects due to mycotoxins, but fungi in general also cause other diseases such as infections, allergy, and inflammation.

Mycotoxins and Mycotoxigenic Fungi

Excluding mushroom toxins, approximately 350 to 400 fungal metabolites are considered to be toxic. Most of these are relatively small molecules of greater than 200 and the majority are less than 500 mass units (8). Perhaps the most important mycotoxins in agriculture are the aflatoxins, the 12,13-epoxytrichothecenes, the fumonisins, and ochratoxin. Species belonging to the genera Apurellus, Penicillium, and Fusarium are common contaminants of agricultural commodities, and some of the mycotoxins produced by these species are produced by fungi common in house dust (2). In addition, some toxigenic fungi produce many different mycotoxins. For example, the Penicillium verrucosum complex (P. verrucosum, P. aurantiogriseum, P. viridicatum, P. crustosum, and P. solitum) produce nearly 20 different mycotoxins (9) and S. chartarum produces several trichothecenes including the highly potent macrocyclic trichothecenes as well as a variety of other mycotoxins (10). Other toxigenic fungi include species of Alternaria, Paecilomyces, Rhizopus, Trichoderma, and Trichothecium. All of these fungi occur commonly in soil, agricultural products, grain dust, and house dust (2).

Health Effects Linked with Inhalation of Mycotoxins

Although extensive literature has been developed since the discovery of the aflatoxins, few studies have been conducted to document the occurrence of these substances in airborne grain or other organic dust or to estimate the inhalation hazard to workers and others exposed to contaminated airborne dust. Several studies have provided evidence for the association of cancer in humans with inhalation of aflatoxin-contaminated dust, e.g., lung cancer (11-13) or colon cancer (14). Olsen et al. (13) noted elevated risks for liver cancer and cancers of biliary tract among animal feed workers that increased by 2- to 3-fold significance after a 10-year latency. Their daily pulmonary exposure was estimated to be approximately 170 ng. Atrup et al. (15) used measurements of aflatoxin bound to serum albumin as an index of exposure and showed that 7 of 45 workers exposed to feed contaminated with low levels of aflatoxin B₁ (AFB₁; 0-26 μg/kg) had detectable levels of AFB₁, bound to serum albumin, confirming systemic exposure. Zarba et al. (16) demonstrated that aerosol inhalation is an effective route of exposure to AFB₁ in rats. In their experiments, approximately 2% of the administered dose became bound to liver DNA and the amounts of DNA adducts were statistically different among the treated groups. These workers (17) also demonstrated that nose-only aerosol in exposure of rats at an estimated dose of 16.8 μg/kg body weight suppressed alveolar macrophage (AM) phagocytosis, with the effect persisting for approximately 2 weeks. These findings indicate that inhalation exposure to AFB₁ is an occupational hazard where exposure to AFB₁-laden dust is common.

Inhalation exposure to spores of S. chartarum has also been associated with episodes of human illness. Andrassy et al. (18) reported an outbreak of illness in which workers exposed to heavily contaminated with S. chartarum unanimously complained of dyspnea, airway obstruction, sore throat, bloody nose or nasal secretions, conjunctivitis, and inflammation of the

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Two other recent reports of human disease thought to be due to inhalation of mycotoxins are noteworthy. Di Paolo et al. (30) reported acute renal failure in a female agricultural worker exposed to grain dust in an enclosed granary, believed to be due to inhalation of ochratoxin in the spores of Aspergillus ochraceus. Ochratoxin was not demonstrated in airborne dust in the granary, but the authors were able to isolate A. ochraceus from a sample of wheat from the granary. Ochratoxin A was observed in extracts of ground moldy grains, and Di Paolo and colleagues were able to demonstrate acute kidney failure in experimental animals (rabbits and guinea pigs) exposed for 8 hr to aerosols generated by their natural movement on moldy wheat in their cages.

Gordon et al. (31) reported tremorgenic encephalopathy in a young man exposed to high concentrations of grain dust contaminated with several species of fungi known to be capable of producing tremorgenic mycotoxins. Because of the circumstances of exposure, the similarity of his syndrome to that of an animal model, and the lack of an alternative explanation, the authors proposed that his illness may have resulted from inhalation of tremorgenic mycotoxin(s).

**Mycotoxins in Spores**

Species of fungi in which mycotoxins have been reported in the spores include Alternaria alternata (32), Aspergillus fumigatus (33-35), Aspergillus flavus and Aspergillus parasiticus (36), Fusarium graminearum (1), Fusarium sporotrichioides (1), and S. chartarum (37). Several different mycotoxins were demonstrated in these investigations including deoxynivalenol (1), fumitremorgen and verruculogen (35), fumigaclavine C (34), T-2 toxin (1), trypOCIcacin (33), alternariol and alternariol monomethyl ether (32), and the macrocyclic trichothecenes satratoxins G and H (37). Gliotoxin has been demonstrated in tissues infected by A. fumigatus (38) and Candida albicans (39) but was not detected in spores of A. fumigatus (40). It is likely that mycotoxins occur in spores of toxigenic species much more commonly than is currently appreciated, as they have been found in spores in a high proportion of species in which attempts were made to find them. In a study in Scotland, extracts from spores of 47% of a group of 83 isolates collected from damp public sector housing in Scotland were cytotoxic to the human embryonic hybrid fibroblast lung cell line MRC-5 (41).

These findings seem to support earlier findings of health hazards in epidemiologic studies of the inhabitants of damp, moldy houses (42). Spores of *P. aurantiogriseus* containing the benzodiazipine metabolite aurantihine cause nephrotoxicity and pathology typical of Balkan endemic nephropathy when mixed with feed and fed to rats (43). These findings confirm the presence of aurantihine in the spores and suggest that workers and others who handle infected grain may be at risk of exposure by inhalation. The vast majority of mycotoxins are nonvolatile and therefore mycotoxin exposure by inhalation is most likely to occur via inhalation of spores.

**Effects of Mycotoxins on Alveolar Macrophages and Immune Function**

T-2 toxin, patulin, and penicillic acid were shown to be acutely toxic to rat AM in vitro, causing membrane damage, inhibition of protein and RNA synthesis, inhibition of phagocytosis, and inhibition of the ability of AM to respond to lymphokines (44-47). Ayral et al. (48) showed that the trichothecenes diacetoxyscirpenol and deoxynivalenol reduce phagocytosis, suppress microbicidal activity, and inhibit superoxide anion production and phagosome-lysosome fusion of peritoneal macrophages at concentrations that did not affect cell viability. Similarly, Vidal and Mavey (49) demonstrated inhibition of phagocytosis of Pseudomonas aeruginosa by murine peritoneal macrophages in the presence of 0.001 μM T-2 toxin.

The trichothecene mycotoxins are immunotoxic in rats and mice, causing acute inhibition of antibody and delay of skin graft rejection (50,51). Gliotoxin has antiphagocytic and immunomodulating activity, it is produced by *A. fumigatus* (52), and it has been demonstrated in tissues infected by *A. fumigatus* (38). Gliotoxin also contributes to the pathogenesis of vaginal candidiasis (39). Jakab et al. (17) confirmed earlier reports that dietary exposure to AFB1 impairs innate and acquired host defenses. Subsequent work by these authors also showed that AM phagocytosis was suppressed for approximately 2 weeks following nose-only inhalation exposure to an estimated dose of 16.8 μg/kg. Exposure using infracell instillation (IT) of AFB1 also suppressed AM phagocytosis in a dose-dependent manner, but approximately 10-fold higher doses were required for IT than for inhalation. Animals exposed by IT administration also had impaired release of tumor
necrosis factor-α, primary splenic lymphocyte response, and peritoneal macrophage phagocytosis. These studies show that experimental respiratory tract exposure can suppress pulmonary and systemic host defenses and that inhalation exposure to ABF₁ could lead to increased susceptibility to infection. Richard et al. (53) used killed spores of A. fumigatus as carrier vehicles for ABF₁ in studies of the effect of long-term exposure to aflatoxin. Lung lesions were not observed in unexposed animals, but lesions of varying severity were observed in the lungs of rats exposed to aflatoxin. The authors reported that the general response of the exposed animals appeared to be that of a compromised host.

Although the effects of mycotoxins as a result of dietary exposure is beyond the scope of the present discussion, a number of mycotoxins, especially aflatoxin, the trichothecces, ochratoxin A, patulin, citrinin, and zearalenone, experimentally alter immunity, causing inhibition of natural killer cell activity, impaired resistance to pathogenic microorganisms, suppression of antibody response, lymphoid depletion of the thymus associated with delayed hypersensitivity, and functional alteration of bone marrow cells. This subject has been extensively reviewed by Richard et al. (54), Thurston et al. (55), and Pestka and Bondy (56, 57).

In studies of the acute inhalation toxicity of T-2 toxin in mice, Creasia et al. (58) demonstrated that T-2 toxin, when given by inhalation, was at least 10 times more toxic than systemic administration and at least 20 times more toxic than dermal administration.

Because of technical limitations, it is difficult to accurately assess the role of mycotoxins in human health effects associated with damp environments. For example, demonstration of mycotoxin production in the laboratory does not prove their presence in the environment; we are generally unable to measure mycotoxins in the field at the site of exposure and, with the exception of aflatoxin, there is a general lack of biomarkers of either exposure or effect. In addition, it appears that isolates of many toxigenic species vary in their ability to produce mycotoxins, but it is uncertain to what extent this is due to actual genotypic differences between the isolates or to the effect of the stress of isolation in artificial culture. It is prudent to take the possibility of mycotoxin exposure into consideration whenever known toxigenic species are demonstrated in these environments.

References and Notes


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