BOOK CHAPTER

Mycotoxins and their effects on humans Download PDF

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The term “mycotoxin” describes a chemically diverse group of low molecular weight organic substances produced primarily by moulds, although some yeasts and basidiomycetes (mushrooms) have the capacity to form mycotoxins. Excluding compounds produced by mushrooms, moulds are known to produce about 300–400 compounds that are recognized as mycotoxins. [1]

Mycotoxins form in hyphae where they may remain, be incorporated into conidia during conidiogenesis, or be expelled into the environment. These substances are thought to be produced by fungi as ecologic survival aids designed to reduce competition for nutrients and living space by other fungi and organisms such as bacteria, insects, and arachnids. Depending on the mycotoxin, various effects on protein, DNA, RNA synthesis or disruption of cell membranes are produced, resulting in either impaired cellular function or death. [2]

Given the adverse effect of these compounds upon cellular metabolism, the saprophytic to parasitic nature of fungi, and their frequency in the environment, it is no surprise that both humans and animals are exposed to mycotoxins that may result in harmful effects. This has been the case throughout recorded history, with mycotoxins even being implicated in one of the Ten Plagues of Egypt. [3]

There are three primary manners in which humans and animals may be exposed to mycotoxins; eating contaminated food like cereals and grains, by skin contact and subsequent absorption of the mycotoxins, or by inhalation of mycotoxins or fungal elements containing mycotoxin. Of the three, the first is the most common and may result from pre-harvest growth of mycotoxin-producing fungi on the grain, fungal growth during storage, or by contamination of surfaces under conditions favorable for fungal growth. Mycotoxin contamination of grain can be a significant problem in underdeveloped countries where reliance on a single grain food source with heavy intake of the cereal is common. [4] In these environments, *Aspergillus* spp., which produce aflatoxins and ochratoxins, *Penicillium* spp., which produce ochratoxins, and *Fusarium* spp., which produce trichothecenes and fumosins, are the most relevant species. [4]

A few mycotoxins can be absorbed through the skin or mucous membranes, and may induce necrosis in addition to systemic effects on other rapidly dividing tissues such as the gastrointestinal tract and the hematopoietic system. At

present, some trichothecenes are known to possess this property. These mycotoxins have relevance with respect to the production and use of biologic weapons and are thought to contribute to indoor health problems following water damage in buildings.

Absorption of mycotoxins through the respiratory system, by inhalation of mycotoxin-containing fungal elements, conidia or hyphal fragments, is the route of entry into the human body which has recently become of significant interest. Environments where this can occur include not only industrial processes that involve fungi or commodities contaminated with fungi, such as milling, but also indoor living or working settings with a high amount of mould growth. This latter situation, which has been associated with a somewhat variable and as yet not well-defined complex of signs and symptoms in affected individuals ("toxic mould syndrome"), has not only generated a high level of interest in mycotoxins. It has also illustrated a primary problem related to the association of mycotoxins with medical syndromes or diseases: that is, it has been extremely difficult to definitively link a syndrome or disease with a mycotoxicosis. As has been pointed out by a number of authors, the simple presence of a species of mould does not necessarily imply the presence of mycotoxin. [1,2] The conditions necessary for production of mycotoxin by a given species may be different from those necessary for growth of the mould. [2] In addition, not all strains of a given species are capable of mycotoxin production and, of those that do produce the substances, there can be significant variability in efficiency of production. [1] Further, the effects of mycotoxins are influenced by a large number of factors, including not only the specific mechanism of action of the specific mycotoxin but also the amount and duration of exposure, the general health, age, and sex of the subject, and a host of synergistic effects related to genetics, diet, and interaction with other potential pathogenetic facilitators such as alcohol, infectious agents, and deficits in caloric or vitamin intake. [1]

These difficulties have been particularly vexing with respect to clarifying the association of mycotoxins with chronic conditions that require a long time to develop. Acute intoxications have been less problematic in some aspects, although by no means has the association been clear in all cases. Fortunately, as advancements in both epidemiology and molecular medicine have occurred at a rapid rate over the last several decades, some of these associations have been clarified. Some syndromes and diseases have been clearly established as related to mycotoxins, others that were initially thought to have an association have been demonstrated to either not be linked or have a tenuous or weak association at best, while others require more study before any conclusion can be reached.

Aflatoxins

Aflatoxins are difuranocoumarin derivatives, of which there are over a dozen, with types B_1, B_2, G_1, G_2 being the major types, and B_1 being the main aflatoxin produced. Aflatoxins are produced by *Aspergillus* spp., primarily *A. flavus* and *A. parasiticus*, but a few other species in the genus may also

produce these substances. [1] The commodities affected include corn, figs, cottonseed, peanuts, certain tree nuts, and tobacco.

The primary target organ is the liver, where cytochrome P450 enzymes convert aflatoxins to a reactive form, which can bind to both proteins and DNA. [1] Detoxification involves conjugation of this reactive form by a glutathione S-transferase, with subsequent excretion of the conjugate. There appears to be a significant difference in susceptibility to aflatoxins amongst different animal species, and it is thought that differences in both cytochrome P450 and glutathione S-transferase systems underlie these differences. [1]

Acute aflatoxicosis

Acute toxicosis from aflatoxins has been associated with contaminated grains, particularly maize, and has manifest as an acute hepatitis with centrilobular necrosis and steatosis by histopathologic examination. [5][6][7][8] Mortality was up to 25% in a large series of cases in India. The lethal dose for acute toxicosis for aflatoxin has been approximated to be 10–20mg and in this series, some patients were estimated to have ingested up to 6 mg in a single day. [1][6][7]

Kwashiorkor is a childhood disease that has manifestations of severe protein deficiency, hepatic steatosis, and ascites. The disease has been associated geographically with the seasonal occurrence of aflatoxins in food. [9] Animals given dietary aflatoxin demonstrate some of the conditions associated with kwashiorkor, including hypoalbuminemia, hepatic steatosis, and immunosuppression. Many of the manifestations of kwashiorkor in children, including the hepatic steatosis due to decreased apoproteins, the decrease in albumin and other proteins with ascites, and the decrease in antioxidants including glutathione, are consistent with the hepatotoxic effects of aflatoxins. Additionally, aflatoxins have been detected in the livers of children who died with the disease. [10] Despite a fair amount of circumstantial evidence, the case firmly establishing aflatoxins as the perpetrators of kwashiorkor is yet to be made and awaits further studies.

Another disorder associated with hepatic steatosis is Reye's syndrome, an often fatal acute encephalopathy that sometimes occurs after a viral infection, primarily in children or adolescents. The disorder was initially proposed to have a possible association with aflatoxins because of the finding of aflatoxins in some Reye's syndrome patients; however, subsequent studies have not supported this proposal. [11][12][13]

Chronic aflatoxicosis

A good example of how careful epidemiologic studies and improvements in molecular techniques have come together in recent years to clarify the relationship of a mycotoxin to a specific disease is the relationship between aflatoxin B_1 and hepatocellular carcinoma (HCC). While initial studies presented somewhat conflicting results, it has now been accepted by most that aflatoxin

B_1 is involved in the pathogenesis of some cases of HCC, and it has been classified as a Group 1 carcinogen by the International Agency for Research on Cancer. [1]

Worldwide, the major risk factor for development of this malignancy is the hepatitis viruses B (HBV) and C (HCV), with an estimated 50% of cases of HCC associated with HBV and 25% of cases associated with HCV. [14] In several major epidemiologic cohort studies, using the presence of an aflatoxin B_1-DNA adduct whose excision product (aflatoxin B_1-guanine) can be detected in urine, [15] it was determined that aflatoxin B_1 exposure is associated with an increased risk of developing HCC. However, it was only when investigators began to examine the possible relationship between HBV infection and aflatoxin exposure that the true potential impact of this mycotoxin as a co-carcinogen became clear. In a large cohort study from China, aflatoxin exposure revealed a relative risk of 3.4 for development of HCC, the presence of HBV revealed a relative risk of 7.3, and, most importantly, the relative risk for those patients who were positive for both aflatoxin exposure and HBV infection was 59. [16] Additionally, it has been demonstrated that a very specific mutation in the *p53* tumor suppressor gene (G→T transversion of the third base of codon 249), a gene found mutated in many human cancers, has been found frequently in association with HCC in areas of high aflatoxin exposure and very rarely in tumors from patients who reside in areas with little aflatoxin exposure. [16] Both epidemiologic and molecular evidence points to the mycotoxin aflatoxin B_1 as an important factor in the development of liver cancer in patients with HBV.

Ergot alkaloids

These substances are indole alkaloids and there are different classes that have slightly different structures and different relative actions, with their effects including smooth muscle contraction, central sympatholytic activity, and peripheral α-adrenergic blockade. Ergotamine tartrate (an amino acid alkaloid) is the most potent vasoconstrictor and also causes contraction of uterine smooth muscle, although not when given orally. [17] Ergonovine and methylergonovine (amine alkaloids) are very effective at causing uterine contraction (oxytocic effect) while having minimal vasoconstrictive effect without any sympatholytic or α-adrenergic blocking effect. [17] The group with the most sympatholytic and α-adrenergic blocking effect is the dehydrogenated amino acid alkaloids, dihydroergotamine and dihydroergotoxine. [17]

Ergot is produced in sclerotia of the genus *Claviceps*, which are pathogens of grasses and grains, and consists of a mixture of alkaloids, in addition to some other compounds. Ingestion of grains or grain products contaminated with ergot results in ergotism, a mycotoxin poisoning which has been postulated to have been responsible for large outbreaks from antiquity through the 19th century, and is thought by historians to be responsible for hundreds of thousands of deaths. [18][19] Although large epidemic outbreaks of ergotism are no longer seen, prevented by modern agricultural techniques, because of the medicinal use of

ergotamine in the treatment of migraine headaches, iatrogenic ergotism still occurs.

Ergotism

The vasospasm induced by ergot can affect any organ, resulting in ischemia to the tissue and manifestations related to anoxia and infarction of the affected organ. There are two major clinical syndromes associated with ergot poisoning, the signs and symptoms of which have been gleaned from descriptions of affected individuals during the large outbreaks. The two types are the gangrenous and convulsive syndromes. While some symptoms and signs, such as lassitude, nausea and vomiting, diarrhea, back pain, cutaneous ischemic changes in hands and feet, and mental impairment, could be common to both syndromes, gangrene did not usually occur in the patient with the convulsive syndrome and convulsions did not usually occur with the gangrenous syndrome. [17]

The gangrenous syndrome often began with several weeks of intense burning pain (St Anthony's fire), usually in the extremities, the sites most often affected. [17] As the patient continued to consume ergot, ischemia of the affected extremities lead to gangrene, most often dry gangrene, and subsequent auto-amputation of the limb or limbs, as the effect was usually symmetric. As the vasculature of any organ could be involved, intestinal infarction, renal failure, angina pectoris or acute myocardial infarction, or blindness from ischemic infarction of the respective organ could also occur.

The feeling of insects crawling on the skin (fomication) was a very common symptom associated with the convulsive form early in the course, and with increasing toxicity the poisoned individual began to suffer pain, colonic contractions of the digits and extremities, and weakness. [17] Disorientation, dementia, and sensory disturbances could also occur. Ischemia of the central nervous system sometimes lead to hemiparesis, paraplegia or pseudotabes dorsalis. With very severe poisoning, repetitive seizures culminated in death.

It is thought that the different forms of ergotism may have resulted from differing combinations and quantities of ergot alkaloids that are produced by the different species of *Claviceps* , although this has never been proven, nor will it likely ever be since outbreaks of naturally occurring ergotism are extremely rare in modern times. Possibly lending support to this theory, however, is the fact that the type of ergotism seen today, medical ergotism due to the overdose of the drug ergotamine used to treat migraine headaches, has demonstrated only a single clinical syndrome, that of arterial vasospasm with resultant ischemia. The convulsive syndrome has not been reported in this setting. Cases of medical ergotism are sometimes seen even when the patient is on a low dose of ergotamine when the patient is also being treated with drugs that can inhibit the cytochrome P450 enzymes, as has been reported in HIV-infected patients on the antiretroviral ritonavir or in patients taking the antibiotic macrolides. [20] [21]

Ochratoxin

Ochratoxin A is a dihydroisocoumarin produced by *Aspergillus ochraceus* and at least seven other *Aspergillus* species, including *A. niger* (used in enzyme production for human consumption) and *A. carbonarius* (found on wine grapes). [22] *Penicillium verrucosum* , found on some grains and corn, also produces this mycotoxin. Ochratoxin A has been detected in a host of substances including a variety of grains such as barley, oats, rye, and wheat, and other commodities such as coffee beans, cocoa, various nuts, spices, and wines and beer. [1 22] It is fat soluble and poorly excreted, and as such, finds it way into food animals (particularly pork) through feeds, from which it can be consumed by humans. [19] This substance has a number of biochemical effects, including inhibition of phenylalanine metabolism, inhibition of mitochondrial ATP production, and the stimulation of lipid peroxidation. [1]

Ochratoxicosis

The literature does not contain case reports of acute toxicity caused by ochratoxin in humans. However, it has been shown to be a potent nephrotoxin in all animal species tested, and is felt to be responsible for porcine nephropathy, a significant problem for pork-producing countries. [1] Additionally, animal studies have shown that ochratoxin is hepatotoxic, an immunosuppressant, a teratogen, and a carcinogen. [1]

Ochratoxin is, however, also a good example of how difficult it is to establish mycotoxins as definite etiologic agents in human medical conditions that require a long period to develop. It has been postulated that ochratoxin exposure results in a progressive chronic nephritis in some areas of eastern Europe (Balkan nephropathy) and in tumors of the urinary tract in humans. Some studies have demonstrated increased levels of ochratoxin in foods and in serum in the homes and blood, respectively, of patients with these disorders relative to patients in the same area without these disorders. [1 22]

Studies examining links between mycotoxins and chronic disorders face a variety of difficulties. For example, there is great variation in mycotoxin amounts from crop to crop or commodity to commodity and it is difficult to estimate long-term consumption. There is minimal information on how food preparation methods such as cooking affect mycotoxins. It is known that there is genetic variation amongst individuals in their detoxification abilities (genetic variation in cytochrome P450 enzymes, for example) and because often the methods of assessing exposure are internal methods that are more applicable to estimating individual exposure rather than a population, very large studies are necessary to permit any degree of generalization, and these can be very expensive. As summarized well by Clark and Snedeker, assessing the link between ochratoxin and Balkan nephropathy and urogenital tract tumors has faced these problems and more, and at this time an unequivocal link is not possible. [22] Despite this, there is very suggestive evidence, based on the studies that have been done, that the nephrotoxic and carcinogenic properties of ochratoxin are applicable to

humans. Ochratoxin may act as a co-carcinogen similar to aflatoxin. The International Agency for Research on Cancer has labeled ochratoxin A as a possible carcinogen (category 2B), and many nations have established maximum allowable levels in foods and commodities. [1,22]

Fumonisins

This group of mycotoxins is produced by members of the genus *Fusarium*, with *F. verticillioides* (formerly *F. moniliforme*) probably the most important, but other species such as *F. proliferatum* may also produce fumonisins, as may *Alternaria alternata*. [1] F. *verticillioides* is present on nearly all corn and may cause "seedling blight," "ear rot" or "stalk rot" but also may be present without noticeable adverse effect on the corn. Fumonisin production is strain dependent in all species that potentially produce them.

Biochemically, these mycotoxins consist of a 20 carbon aliphatic backbone with two ester-linked hydrophilic side-chains, and the compounds bear a resemblance to sphingosine. [19] This resemblance appears to play a role in inhibiting sphingolipid metabolism in animals. Fumonisin B1 inhibits N-acyltransferase (ceramide synthase), which is involved in the conversion of sphinganine and sphingosine to ceramide in sphingolipid biosynthesis. [23] Since sphingolipids are major components of cell membranes, their effects are wide-ranging and they are known to cause liver cancer in rats, pulmonary edema in pigs and are the cause of the naturally occurring and severe, fatal leukoencephalomalacia in horses. [24,25,26]

In humans, fumonisins have been noted to have a possible link with esophageal cancer, based on the co-occurrence of a high incidence of *F. verticillioides* contamination of grain and a high incidence of esophageal cancer in areas of South Africa, China, and Italy. [1,19] *F. verticillioides*-contaminated cornmeal has been demonstrated to produce premalignant changes in the esophagus and stomachs of rats and mice, in addition to liver carcinoma. [27] Further case–control epidemiologic studies are necessary to establish the link between fumonisins and esophageal carcinoma in humans.

Evidence that fumonisins may induce neural tube defects in humans arose out of a high incidence of anencephaly and other neural tube defects along the Texas–Mexico border in the early 1990s, associated with an epidemic of equine leukoencephalomalacia that had occurred in Texas. To contain the leukoencephalomalacia outbreak, animals were not allowed to eat corn, as it was contaminated with *F. verticillioides*. Humans, however, continued to consume the corn from this harvest. Careful epidemiologic work by Missmer and colleagues, who conducted a case–control study correlating fumonisin levels in corn tortillas, consumption amounts of tortillas during pregnancy, and a surrogate marker for fumonisin in mother's postpartum serum (sphinganine:sphingosine ratio), suggested that fumonisin exposure in pregnancy played a role in the development of neural tube defects. [28] As these authors noted, other studies had demonstrated that inhibition of sphingolipid biosynthesis adversely affects uptake and binding of folate, and that an animal

model exposed to fumonisins developed neural tube defects, the effect of which was prevented by folate administration, lending further support to the hypothesis related to the teratogenic potential of fumonisins. [28]

Gliotoxin

This mycotoxin is an epipolythiodioxopiperazine, a group of mycotoxins that possess a disulfide bridge that appears to be involved in the toxic effects. [29] Gliotoxin is produced by *A. fumigatus* (and no other members of the genus), *Trichoderma virens* , *Penicillium* spp., and *Candida albicans* . The mechanisms by which gliotoxin induces toxicity are thought to be by conjugating with thiol residues on proteins and by generating reactive oxygen species through oxidation of the reduced dithiol to the disulfide form. [29] Gliotoxin has been demonstrated to have a number of immunosuppressive actions, including the ability to inhibit oxidative killing by neutrophils and macrophages, the phagocytic ability of macrophages, antigen-mediated activation of lymphocytes and cytotoxic T cell activation, the ability of T-helper lymphocytes to secrete γ-interferon, and the activation of transcription factor NF-κB. [30] Additionally, it can induce apoptosis of both macrophages and lymphocytes. [30] The mycotoxin may be a virulence factor in infections caused by *A. fumigatus* and *C. albicans* . The frequency of infections by these organisms in patients with chronic granulomatous disease, who have defects in the oxidative killing of neutrophils, suggests that this mycotoxin is a virulence factor. [31] High levels of gliotoxin have been detected in the serum of animals in models of invasive aspergillosis and in human patients with neoplastic disease and invasive aspergillosis. [30]

The epipolythiodioxopiperazines have been suggested as a potential source for, or a model for the synthesis of, therapeutic agents for several disorders. [29] The disulfide bridge in the toxins imparts an ability to kill cells with increased glutathione levels or with glutathione detoxifying enzymes, as may be seen in some forms of tumor resistance to therapy. [29] Gliotoxin itself has been demonstrated to inhibit an enzyme involved in the function of the *ras* oncogene. [29] This mycotoxin has also been shown to destroy activated hepatic stellate cells, through its ability to induce apoptosis, which are involved in the etiology of the fibrotic process that leads to hepatic cirrhosis. [29]

Zearalenone (F-2 Toxin)

This mycotoxin is mentioned in this brief overview not because of the significant toxicity associated with its exposure to humans, but because it illustrates the diversity with which mycotoxins act. While the use of most mycotoxins as therapeutic agents is theoretic, zearalenone has been administered for specific effects in both animals and humans. Bennett et al have stated that labeling zearalenone as a mycotoxin is actually a misnomer as, in reality, since its structure resembles 17β-estradiol, it is capable of binding to mammalian steroid receptors and has demonstrated estrogenic effects. It would more properly be labeled a “mycoestrogen” or “phytoestrogen.” [1] Whatever label is given to the substance, a synthetic analog has been used as an anabolic agent in livestock, and

the naturally occurring chemical and its synthetic analog have been used to treat postmenopausal symptoms in women. [1]

Zearalenone is produced by *Fusarium graminearum* and several other *Fusarium* species, all of which are found in abundance in association with grains. As such, this has been described as the most common of the mycotoxins associated with *Fusarium* and it has been stated that up to approximately one-quarter of corn consumed by humans contains zearalenone. [32] Pigs are particularly sensitive to its action, and consumption in contaminated feed results in a hyperestrogenic syndrome that may include both infertility and teratogenic effects. [32] The common occurrence of zearalenone and its estrogenic actions have led to a number of hypotheses related to possible adverse effects on humans, including reduced fertility and stimulation of estrogen-sensitive neoplasms. However, to date, epidemiologic studies have not confirmed any demonstrable adverse effects in humans. [1,32]

Trichothecenes

This is a very large group of mycotoxins (over 60) and many are extremely toxic. A few of the most toxic include T-2 toxin, deoxynivalenol or vomitoxin, and diacetoxyscirpenol. While chemically heterogeneous, all are sesquiterpenes and all have a 12,13-epoxytrichothene ring. [1] The genus *Fusarium* is the major producer of these substances but other producers include *Trichoderma* , *Myrothecium* , *Phomopsis* , *Trichothecium* , and, importantly, *Stachybotrys* species. [1] Many of these species are plant pathogens and can be associated with food grain or feed contamination. Like many mycotoxins, trichothecenes act by inhibiting protein synthesis, although different trichothecenes act at different stages in the process. As these substances are so potent, and produced by species that grow readily on plants or plant-based materials, they have not only been associated with significant naturally occurring toxicity, but have also been implicated in toxicity that is man-made (bioterrorism) or man-facilitated (sick building syndrome).

Trichothecene toxicity

Alimentary toxic aleukia is a syndrome that has been attributed to the trichothecenes T-2 and diacetoxyscirpenol. At least 100,000 human deaths during the years 1942–1948 in the former Soviet Union have been blamed on alimentary toxic aleukia, thought to have resulted from eating overwintered grain containing *F. sporotrichoides* and *F. poae* . [1,19] These mycotoxins were never directly identified in the crops but the syndrome has been attributed to the trichothecenes because both species of *Fusarium* have been shown to produce these mycotoxins, the clinical syndrome resembles a similar syndrome in horses which has been shown to be due to trichothecenes produced by *Stachybotrys* , and an experimental syndrome with the same features can be produced in an animal model administered T-2 toxin. [1,32]

In affected patients described in accounts from the Soviet Union, oral mucosal ulcerations and gastroenteritis were followed by pancytopenia due to bone marrow toxicity, with hemorrhage and agranulocytosis. [33] Mortality could be as high as 80% of affected individuals, especially in patients who were also suffering from malnutrition. In many cases, the cause of death was an opportunistic bacterial infection. [32,33]

Although alimentary toxic aleukia has not been reported since the immediate post-World War II period, a syndrome (red mould disease) with some of the components of alimentary toxic aleukia has been reported more recently. This mycotoxin syndrome could be more confidently assigned to trichothecenes produced by *F. graminearum* , using modern analytic techniques. [32] Nausea, vomiting, abdominal pain, diarrhea, chills and headache were the symptoms in a large number of patients with toxicity due to another trichothecene, deoxynivalenol, during 1988 in India. [34] This trichothecene is said to be the most common mycotoxin found in food grains. [1]

Trichothecenes are the major group of mycotoxins thought to be involved in producing a complex set of signs and symptoms, including fatigue, headache, irritation of the eyes, nose and pharynx, a variety of neurologic symptoms such as dizziness and loss of balance, along with cognitive abnormalities such as difficulty in concentrating, and memory loss. This controversial and somewhat poorly defined syndrome has been labeled by a variety of terms, including “toxic mould syndrome,” “sick building syndrome” or “building-related illness,” to name a few. A large array of possibilities in the indoor environment have been postulated to contribute to the etiology of the syndrome, such as organic and non-organic chemicals used in cleaning and construction, poor ventilation, tobacco smoke, noise, even psychosocial factors. [32] Most cogently for this review, mycotoxins have been suggested as a potential cause, as a result of inhaled mycotoxins from mould growing on damp or wet cellulose products, a nutritive source for many moulds and a common component of building materials. This hypothesis was bolstered by the initial findings of extensive *Stachybotrys chartarum* growth in homes of a cluster of children with pulmonary hemorrhage, the demonstration that the isolates produced a variety of potent trichothecenes and the hemolysin stachylysin, and a CDC investigation that suggested a link between the hemorrhage and the mould. [1] A warning to pediatricians issued by the American Academy of Pediatrics about the possible link between mould and the pulmonary hemorrhage contributed to establishing the hypothesis as scientific fact in the perception of many, when in reality, a subsequent CDC report and a number of independent investigations concluded that a definite link between the cases of pulmonary hemorrhage and *Stachybotrys* mycotoxins could not be inferred from the evidence. [1]

The degree of confusion and contradiction surrounding the relationship between the series of infants with pulmonary hemorrhage and mycotoxins is reflective of the entire issue of indoor mould growth, inhalation of mycotoxins by residents of buildings containing mould, and the effects inhaled mycotoxins may have on

exposed individuals. An extensive examination of the various aspects of this controversy is beyond the scope of this review. It is clear that there are a number of species of moulds that grow well in damp indoor environments, including *Penicillium* and *Aspergillus* spp. and *S. chartarum* , to name a few. However, as has been pointed out previously, the presence of mould species capable of producing mycotoxins does not automatically imply that mycotoxins will be produced by mould. [32] However, it has been demonstrated that the potential for airborne mycotoxins in a building with heavy growth of mould does exist. [35] If mycotoxins are present in the indoor environment, will they be present in a sufficient concentration to have an adverse toxic effect? The conclusion of the American Academy of Occupational and Environmental Medicine (ACOEM) is that this is unlikely to be the case. [36] Other authors feel that they have demonstrated adverse neurobehavioral and cognitive effects on individuals in environments contaminated with mould, and that mycotoxins produced by the moulds is the most likely cause. [37][38] Many appear to be taking the view put forth by Kuhn and Ghannoum that, while there is suggestive evidence with respect to indoor mould, mycotoxins, and adverse effects on those exposed in the indoor environment, the evidence is not definitive and further study is required. [32]

Another area where trichothecenes appear to hold a unique position among the mycotoxins is with respect to their use as weapons. As has been mentioned in this review, many mycotoxin effects require long-term and additive exposure to become manifest, and in many cases, the cooperation of the subject in ingesting the mycotoxin, since the majority of these substances require an alimentary route of introduction into the human body. These characteristics make most mycotoxins poor choices for weapons, which is why most experts find the stockpiling of aflatoxin for use as a weapon by the former government in Iraq difficult to understand. [1] However, one of the trichothecenes, T-2, has many characteristics that make it an effective weapon. The substance can be absorbed through a variety of routes, including the gastrointestinal, respiratory and dermal route, it acts immediately rather than requiring a long period of additive exposure, and finally, a very small amount, of the order of milligrams, can be lethal. [1][39] It was alleged that T-2 was utilized in south east Asia by the former Soviet Union, as a component of the bioweapon called “yellow rain” (along with nivalenol and deoxynivalenol), although there is great controversy as to whether these mycotoxins, which were detected on foliage analyzed in the United States, were actually components of a weapon or naturally occurring. [1] Another mycotoxin that is not a trichothecene but has been suggested as possible bio-weapon is α-amanitin from the mushroom *Amanita phalloides* , because of its high toxicity, water solubility, and stability at high temperatures. [39]

Mycotoxin effects on the human immune system

It has been known for some time that many mycotoxins can influence the immune system of animals, with experiments demonstrating that these substances affect primarily the cellular arm of the immune system. [40] It has been alleged by some who believe that mycotoxins are responsible for the signs and

symptoms associated with the “sick building syndrome,” described earlier, that mycotoxins also affect human immune function. [41] That metabolic products of fungi can influence the human immune system is evident, as the unadecapeptide cyclosporine A, which selectively suppresses the function of T cells to prevent many types of rejection and graft-versus-host disease in human transplants, is a substance that was originally detected as a fermentative product of the mould *Tolypocladium niveum* . The effects of gliotoxin on T cell, macrophage, and neutrophil function, and its possible role as a virulence factor in infection, have been previously described in the section on this mycotoxin.

Recently, more studies have begun to examine the influence of some of the mycotoxins described in this short review in human in vitro systems. Aflatoxin has been a favorite because of its consistent inhibitory effect on cell-mediated immunity in a variety of animal models and the potential for studying the in vivo effects of the mycotoxin in humans, as it is one of the few mycotoxins where long-term exposure to populations can be documented by both environmental detection and in vivo biomarkers. Using aflatoxin-albumin as a biomarker, Turner et al examined whether high levels of aflatoxin could be correlated with a variety of tests to assess T cell, B cell, and mucosal immune function. [42] A correlation of high levels of aflatoxin and reduced levels of secretory immunoglobulin A was noted but the high level of anergy noted in the subjects (50%) could not be correlated with aflatoxin levels. The authors note, however, that such a correlation may require more than the single point in time level that was used in this study.

Using more sophisticated immunologic techniques, other investigators have begun to detect specific defects in immune function in patients chronically exposed to aflatoxin. For example, Jiang et al examined specific characteristics of T cells in the African population tested by Turner et al and were able to correlate a decrease in T cells with an activator inducer molecule (CD69) in subjects with high levels of aflatoxin exposure. [43] The presence of this molecule on a sufficient number of T cells is necessary for a normal immune response to infections. This same study also showed a decrease in effector CD8 T cells that express pore-forming protein and serine proteases, necessary for the killing function of these cells, in those subjects with high aflatoxin exposure. [43] Both these findings suggest the possibility of impaired T cell function in subjects with high levels of aflatoxin exposure.

As biomarkers for other mycotoxins that have been shown to influence immunity in animal models are discovered, the application of modern sophisticated immunologic investigative techniques in future studies should help to clarify the influence of mycotoxins on human immunity.

Mycotoxins associated with mushrooms

Many reviews do not include mycotoxins produced by mushrooms in their body of work although, as pointed out by Bennett et al, the essential difference between the toxins produced by moulds and those produced by mushrooms is

that intoxication with mould-associated toxins results from the unknowing ingestion of contaminated food, while intoxication resulting from mushroom-associated toxins results from the willing ingestion of the poison-containing mushroom, albeit misidentified as an edible non-poisonous species. [1] There are a wide variety of toxins produced by mushrooms, with a variety of clinical effects and a variety of mechanisms of action. An in-depth discussion of even the major ones is beyond the scope of this short review and there are a number of excellent reviews that cover clinical, mycologic, and biochemical aspects. [44,45,46] Several of these substances bear similarity to some of the mould-associated mycotoxins and will be briefly described here.

Perhaps the most famous, and arguably the most potent, mycotoxin associated with mushrooms is amanitin, a bicyclic octapeptide found in *A. phalloides*. As previously mentioned, it has been suggested as a potential bio-weapon. Within 6–24 hours of ingestion, patients begin to suffer abdominal cramping, nausea and vomiting, and diarrhea, followed by a period when liver damage becomes evident with rising liver enzymes and hyperbilirubinemia, which can progress to liver and renal failure and death during the period of 6–16 days after ingesting the toxin. [44] In some cases, the only life-saving therapy available is liver transplant. Mortality has been approximately 20% in adults and this substance is responsible for over 90% of deaths from mushroom poisoning. [44] Similar to many of the mould-associated mycotoxins, it acts at the level of an enzyme involved in protein synthesis, in this case RNA polymerase II, resulting in cessation of transcription and cell death. [44]

Another mushroom associated mycotoxin that can result in a serious clinical syndrome is orellanine. The mechanism of action is unknown but the toxin bears some structural resemblance to the herbicide paraquat. [45] Paraquat is thought to exert toxicity via redox production of superoxide anions, which can induce lipid peroxidation of membranes and deplete cellular nicotinamide adenine dinucleotide phosphate (NADPH). Whether orellanine's structural similarity to the herbicide also extends to mechanism of action is unknown and requires study. Ingestion of orellanine-containing mushrooms (*Cortinarius* sp.) results in nausea and vomiting with diarrhea 36–48 hours after ingestion, followed by the development of acute renal failure in up to half of patients, which may take up to 3 weeks to manifest. [45] Up to half of these patients may develop chronic renal failure, and dialysis or renal transplant may be required. [45]

Gastrointestinal irritation (gyromitrin), hallucinations (psilocybin), seizures and fluctuating central nervous system effects (isoxazoles), and parasympathetic overstimulation (muscarine) are examples of other syndromes associated with mushroom mycotoxins. [45] Like the mould-associated mycotoxins, there are a wide variety of toxins with an equally broad spectrum of effects on humans.

Conclusion

While most are familiar with the significant morbidity and mortality associated with the wide variety of mycotic infections that can occur in humans, the same

degree of familiarity and concern does not frequently extend to mycotoxins. Due to the difficulty of establishing definitive cause-and-effect relationships between mycotoxins and medical syndromes or disorders, knowledge relating to the pathogenetic potential of the mycotoxins has lagged behind that associated with the mycoses, health officials have not paid sufficient attention to the mycotoxins, and people have suffered and continue to suffer the consequences of exposure. Fortunately, with evolving investigative and epidemiologic techniques, new awareness and interest by the medical and scientific community, and concern on the part of the public, knowledge of the mycotoxins and their medical importance is increasing, which it is hoped will, in turn, lead to better control of exposure and mitigation of their adverse effects. It should be remembered that the fungi that produce these substances are in most cases saprophytic and as such, much more abundant in the environment than the mycoses-causing fungi, with opportunity to affect a larger and more varied population.

References

1. 1. Bennett J.W., and Klich M.: Mycotoxins. Clin Microbiol Rev 2003; 16: pp. 497
   View In Article | Cross Ref

2. 2. Levy M.B., and Fink J.N.: Toxic mold syndrome. Adv Appl Microbiol 2004; 55: pp. 275
   View In Article | Cross Ref

3. 3. Marr J.S., and Malloy C.D.: An epidemiologic analysis of the ten plagues of Egypt. Caduceus 1996; 12: pp. 7
   View In Article

4. 4. Bryden W.L.: Mycotoxins in the food chain: human health implications. Asia Pac J Clin Nutr 2007; 16: pp. 95
   View In Article

5. 5. Shank R.C.: Epidemiology of aflatoxin carcinogenesis. Adv Mod Toxicol 1977; 3: pp. 291
   View In Article | Cross Ref

6. 6. Krishnamachari K.A., Bhat R.V., Nagarajan V., et al: Investigations into an outbreak of hepatitis in parts of western India. Indian J Med Res 1975; 63: pp. 1036
   View In Article

7. 7. Krishnamachari K.A., Bhat R.V., Nagarajan V., et al: Hepatitis due to aflatoxicosis. An outbreak in western India. Lancet 1975; 1: pp. 1061
   View In Article | Cross Ref

8. 8. Ngindu A., Johnson B.K., Kenya P.R., et al: Outbreak of acute hepatitis caused by aflatoxin poisoning in Kenya. Lancet 1982; 1: pp. 1346
   View In Article | Cross Ref

9. 9. Hendrickse R.G., Coulter J.B., Lamplugh S.M., et al: Aflatoxins and kwashiorkor: epidemiology and clinical studies in Sudanese children and findings in autopsy liver samples from Nigeria and South Africa. Bull Soc Pathol Exot Filiales 1983; 76: pp. 559
   View In Article

10. 10. Hendrickse R.G.: The influence of aflatoxins on child health in the tropics with particular reference to kwashiorkor. Trans R Soc Trop Med Hyg 1984; 78: pp. 427
View In Article | Cross Ref

11. 11. Nelson D.B., Kimbrough R., Landrigan P.S., et al: Aflatoxin and Reye's syndrome: a case control study. Pediatrics 1980; 66: pp. 865
View In Article

12. 12. Rogan W.J., Yang G.C., and Kimbrough R.D.: Aflatoxin and Reye's syndrome: a study of livers from deceased cases. Arch Environ Health 1985; 40: pp. 91
View In Article | Cross Ref

13. 13. Hurwitz E.S.: Reye's syndrome. Epidemiol Rev 1989; 11: pp. 249
View In Article | Cross Ref

14. 14. Kirk G.D., Bah E., and Montesano R.: Molecular epidemiology of human liver cancer: insights into etiology, pathogenesis, and prevention from the Gambia, West Africa. Carcinogenesis 2006; 27: pp. 2070
View In Article | Cross Ref

15. 15. Essigmann J.M., Croy R.G., Nadzan A.M., et al: Structural identification of the major DNA adduct formed by aflatoxin B . Proc Natl Acad Sci USA 1977; 74: pp. 1870
View In Article | Cross Ref

16. 16. Groopman J.D., and Kensler T.W.: Role of metabolism and viruses in aflatoxin-induced liver cancer. Toxicol Appl Pharmacol 2005; 206: pp. 131
View In Article | Cross Ref

17. 17. Merhoff G.C., and Porter J.M.: Ergot intoxication: historical review and description of unusual clinical manifestations. Ann Surg 1974; 180: pp. 773
View In Article | Cross Ref

18. 18. Matossian M.K.: Mold poisoning: an unrecognized English health problem, 1550–1800. Med Hist 1981; 25: pp. 73
View In Article | Cross Ref

19. 19. Pitt J.I.: Toxigenic fungi and mycotoxins. Br Med Bull 2000; 56: pp. 184
View In Article | Cross Ref

20. 20. Caballero-Granado F.J., Viciana P., Cordero E., et al: Ergotism related to concurrent administration of ergotamine tartrate and ritonavir in an AIDS patient. Antimicrob Agents Chemother 1997; 41: pp. 1207
View In Article

21. 21. Liaudet L., Buclin T., Jaccard C., et al: Severe ergotism associated with interaction between ritonavir and ergotamine. BMJ 1999; 318: pp. 771
View In Article | Cross Ref

22. 22. Clark H.A., and Snedeker S.M.: Ochratoxin A: its cancer risk and potential for exposure. J Toxicol Environ Health B 2006; 265:
View In Article

23. 23. Wang E., Norred W.P., Bacon C.W., et al: Inhibition of sphingolipid biosynthesis by fumonisins: implications for diseases associated with . J Biol Chem 1991; 266: pp. 14486
View In Article

24. 24. Gelderblom W.C.A., Marasas W.F.O., Vleggaar R., et al: Fumonisins: isolation, chemical characterization, and biological effects. Mycopathologica 1992; 117: pp. 11
View In Article | Cross Ref

25. 25. Harrison L.R., Colvin B., Green J.T., et al: Pulmonary edema and hydrothorax in swine produced by fumonisin B1, a toxic metabolite of . J Vet Diagn Invest 1990; 2: pp. 217
View In Article | Cross Ref

26. 26. Marasas W.F.O., Kellerman T.S., Gelderblom W.C.A., et al: Leukoencephalomalacia in a horse induced by fumonisin B1 isolated from . Onderstepoort J Vet Res 1988; 55: pp. 197
View In Article

27. 27. Marasas W.F.O., Kreik N.P.J., Fincham J.E., and van Rensburg S.J.: Primary liver cancer and oesophageal basal cell hyperplasia in rats caused by . Int J Cancer 1984; 34: pp. 383
View In Article | Cross Ref

28. 28. Missmer S.A., Suarez L., Felkner M., et al: Exposure to fumonisins and the occurrence of neural tube defects along the Texas–Mexico border. Environ Health Perspect 2006; 114: pp. 237
View In Article | Cross Ref

29. 29. Gardiner D.M., Waring P., and Howlett B.J.: The epipolythiodioxopiperazine (ETP) class of fungal toxins, distribution, mode of action, functions and biosynthesis. Microbiology 2005; 151: pp. 1021
View In Article | Cross Ref

30. 30. Lewis R.E., Weiderhold N.P., Chi J., et al: Detection of gliotoxin in experimental and human aspergillosis. Infect Immun 2005; 73: pp. 635
View In Article | Cross Ref

31. 31. Tsunawaki S., Yoshida L.S., Nishida S., et al: Fungal metabolite gliotoxin inhibits assembly of the human respiratory burst NAPDH oxidase. Infect Immun 2004; 72: pp. 3373
View In Article | Cross Ref

32. 32. Kuhn D.M., and Ghannoum M.A.: Indoor mold, toxigenic fungi, and . Clin Microbiol Rev 2003; 16: pp. 144
View In Article | Cross Ref

33. 33. Joffe A.Z.: [object Object]. In (eds): . New York: Marcel Dekker, 1978. pp. 21
View In Article

34. 34. Bhat R.V., Ramakrishna Y., Beedu S.R., et al: Outbreak of trichothecene mycotoxicosis associated with consumption of mould-damaged wheat products in Kashmir Valley, India. Lancet 1989; 1: pp. 35
View In Article | Cross Ref

35. 35. Neilsen K.F.: Mycotoxin production by indoor moulds. Fungal Genet Biol 2003; 39: pp. 103
View In Article | Cross Ref

36. 36. American College of Occupational and Environmental Medicine. Adverse human health effects associated with molds in the indoor environment.
Online: www.acoem.org/guidelines.aspx?id=850
View In Article

37. 37. Gordon W.A., and Cantor J.B.: The diagnosis of cognitive impairment associated with exposure to mold. Adv Appl Microbiol 2004; 55: pp. 361
View In Article | Cross Ref

38. 38. Kilburn K.: Role of molds and mycotoxins in being sick in buildings: neurobehavioral and pulmonary impairment. Adv Appl Microbiol 2004; 55: pp. 339
View In Article | Cross Ref

39. 39. Russell R., and Paterson M.: Fungi and fungal toxins as weapons. Mycol Res 2006; 110: pp. 1003
View In Article | Cross Ref

40. 40. Richard J.L.: Mycotoxins as immunomodulators in animal systems. In Bray G.A., and Ryan G.H. (eds): Mycotoxins, Cancer, and Health. Pennington Center Nutrition Series. Baton Rouge: Louisiana State University Press,, 1991. pp. 197
View In Article

41. 41. Campbell A.W., Thrasher J.D., Gray M.R., et al: Mold and mycotoxins: effects on the neurological and immune systems in humans. Adv Appl Microbiol 2004; 55: pp. 375
View In Article | Cross Ref

42. 42. Turner P.C., Moore S.E., Hall A.J., et al: Modification of immune function through exposure to dietary aflatoxin in Gambian children. Environ Health Perspect 2003; 111: pp. 217
View In Article | Cross Ref

43. 43. Jiang Y., Jolly P.E., Ellis W.O., et al: Aflatoxin B1 albumin adduct levels and cellular immune status in Ghanaians. Int Immunol 2005; 17: pp. 807
View In Article | Cross Ref

44. 44. Berger K.J., and Guss D.A.: Mycotoxins revisited: part I. J Emerg Med 2005; 28: pp. 53
View In Article | Cross Ref

45. 45. Berger K.J., and Guss D.A.: Mycotoxins revisited: part II. J Emerg Med 2005; 28: pp. 175
View In Article | Cross Ref

46. 46. Karlson-Stiber C., and Persson H.: Cytotoxic fungi – an overview. Toxicon 2003; 42: pp. 339
View In Article | Cross Ref