8 Organic Dust Toxic Syndrome

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CONTENTS

I. Introduction

A. Organic Dust Toxic Syndrome (ODTS)

Organic dust toxic syndrome (ODTS) and hypersensitivity pneumonitis (HP) are associated with inhalation of high concentrations of organic materials, particularly agricultural materials such as dust from grain, hay, or silage contaminated with microorganisms (Pratt and May 1984; Lecours et al. 1986; May et al. 1986; Pratt et al. 1990). Although initial work was associated with farming, one should recognize that workers involved in wood processing, large-scale production of animal feed, treatment and disposal of sewage and garbage by composting, and various bioindustrial processes may be exposed to high concentrations of microorganisms and their products. HP, also known as extrinsic allergic alveolitis, has been recognized for a very long time and occurs in a variety of occupational settings (Parker et al. 1992). Farmer's lung disease (FLD) is the most familiar form of HP in agriculture. The term "pulmonary mycotoxicosis" was applied to ODTS to differentiate it from FLD and to underscore the apparent importance of fungi and/or their metabolic products (Emanuel et al. 1975). Attempts to implicate mycotoxins in the syndrome were limited, in both number and scope, and failed to reveal significant amounts of those few mycotoxins which were sought in dust samples collected from outbreaks of ODTS (May et al. 1986). New mycotoxins continue to be described and the fumonisins, for example, now considered an especially important group because of the frequency of their occurrence and the severity of their toxicity, were not known until 1988. The presence of high levels of fungi and bacteria in the dust has since been found to be a hallmark of the syndrome (Dutkiewicz et al. 1989; Olenchock et al. 1990). ODTS is a non-infectious flu-like illness, and is characterized by fever, malaise, myalgia, and a neutrophilic inflammation of the lower respiratory tract (Lecours et al. 1986; Parker et al. 1992). Hypersensitivity pneumonitis (HP) has many features in common with ODTS, including similar exposure settings and clinical symptoms (Emanuel et al. 1975; Pratt and May 1984; Parker et al. 1992). Although both illnesses appear to involve inflammation of lung parenchyma, they may not be mediated by the same mechanisms. Notably, HP is characterized by a lymphocytic infiltrate into the lower airways, suggesting that it may be due to a cell-mediated hypersensitivity reaction. Prevalence of HP in individuals chronically exposed to potential antigens ranges from 0.03 or 0.42% in farming populations to as much as 15% in office workers exposed to contaminated ventilation systems (Parker et al. 1992). Outbreaks of ODTS are characterized by a much higher attack rate than is observed in farmer's lung disease;

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there is usually no correlation between presence of precipitating antibodies and illness; clinical findings suggest that ODTS results from nonspecific immune mechanisms (Von Essen et al. 1990). An interesting example of such a clustered outbreak was provided by Brinton et al. (1987) in a case report of a college fraternity party in which baled straw had been used on the basement floor of the fraternity house. Of the 67 students who attended the party and answered a questionaire, 82% became ill. Risk of illness was related to length of time spent at the party and duration of illness ranged from 4.5 h to 7 days. Studies among farmers in Sweden (Malmberg et al. 1990a) and in Finland (Husman et al. 1990) have suggested that ODTS occurs 30 to 50 times more commonly than farmer's lung disease. In general, episodes of HP can be triggered by lower levels of exposure than can episodes of ODTS, consistent with the concept that HP is mediated by a hypersensitivity reaction. On the other hand, innate immune mechanisms which do not require activation of antigen-specific immune mechanisms could occur in a much higher proportion of the exposed population but might require exposure to higher levels of the inciting agent(s).

There is no sharp distinction between HP and ODTS and therefore diagnosis and differentiation may be difficult, particularly in a single affected individual (Parker et al. 1992; Weber et al. 1993). Mamolen et al. (1993) described an outbreak of "humidifier fever" which illustrates this difficulty. The outbreak was associated with operation of a humidifier which had not been cleaned for several months. The humidifier was set to operate automatically and it began to run at a time when the ventilation system was shut down because of a plugged intake filter. There were positive serologic reactions among print shop workers to extracts of organisms isolated from the humidifier, but the presence of antibodies could neither distinguish ill from well workers, nor identify causative organisms. Furthermore, although the evidence favored a diagnosis of ODTS in this instance, previous exposure to the humidifier contaminants appeared to be a risk factor in that "those who worked in the shop for at least 3 months showed a fourfold increase in relative risk."

B. Composition of Organic Dust

The majority of outbreaks of ODTS have been associated with agricultural dust, predominantly grain and hay dust. The composition of these dusts is highly complex and may contain the essential components of the individual grains including a wide variety of cereal grains, nongrain plant matter, fungi including mycelium, spores, and mycotoxins, bacteria, bacterial metabolites and cell membrane components (e.g., endotoxin), animal matter including insects, mites, rodents, bird particles and excreta, fertilizers, pesticides and other agricultural chemicals, and inorganic material such as soil and silica dust (Becklake 1980).

C. Fungi Associated with ODTS

Fungi found on grains and other agricultural commodities are often considered to be either field fungi or storage fungi. Field fungi are those that invade or colonize the developing or mature seed while it is still on the plant (Christensen 1957) and may exist as pathogens or saprobes. Prominent field fungi include species from the genera *Alternaria*, *Cladosporium*, *Fusarium*, *Helminthosporium*, *Diplodia*, *Rhizopus*, and *Mucor*. The rusts (Uredinales) and smuts (Ustilaginales) are pathogens of cereals. Moisture levels in grain during storage are generally unfavorable for both plant pathogens and saprobic field fungi, which tend to die out during storage (Christensen 1957). Mislivec and Tuite (1970) reported that certain species of *Penicillium* (*P. oxalicum*, *P. funiculosum*, and *P. cyclopium*) are consistently isolated from freshly harvested corn. The so-called storage fungi are those species which develop with time under the conditions of reduced water availability generally prevailing during storage. These are predominantly species of *Aspergillus* and *Penicillium*, but the mycobiota of moist or heated grain may be richer and more diverse. Species of *Aspergillus* commonly isolated from stored grains include *A. candidus*, *A. flavus*, members of the *A. glaucus* group, *A. ochraceous*, *A. restrictus*, and *A. versicolor*. *P. aurantiogriseum* (also known as *P. cyclopium*), *P. brevicompactum*, and *P. viridicatum* are among the most frequently isolated *Penicillium* species (Mislivec and Tuite 1970). Although the concept of field and storage fungi is useful, the distinction is not absolute and some species considered storage fungi can be isolated from grain even after harvest (Mislivec and Tuite 1970).

Studies of the mycobiota of grain dust are limited, for both settled and airborne dust. As

expected, the species found in grain dust reflect the species found in the corresponding grains and vary with the type of grain examined and the moisture and heating history of storage (Farant and Moore 1978). Previous studies in our laboratory have shown that *Aspergillus fumigatus*, *A. niger*, *A. terreus*, and *Penicillium spinulosum* can occur as the predominant fungi in samples associated with outbreaks of ODTS (Dutkiewicz et al. 1989; Olenchock et al. 1990; Pratt et al. 1990), and *A. candidus*, *Eurotium amstelodami*, and *Cladosporium cladosporioides* are often the predominant fungi in agricultural dust. Particularly striking in many of these samples are members of the *A. glaucus* group. These are xerophilic fungi common on cereal grains (Raper and Fennell 1965; Ito et al. 1973; Le Bars and Escoula 1973; El-Sharouny et al. 1988). *Eurotium umbrosum*, a member of this group, has been reported to be particularly abundant in hays associated with FLD in Finland and it reacted with most sera from FLD patients in precipitin tests in that country (Terho and Lacey 1979). *E. umbrosum* is regarded by some authors (Pitt 1985; Pitt and Hocking 1985) as a synonym of *Eurotium herbariorum*, which is closely related to *Eurotium rubrum*. *E. amstelodami* and *E. rubrum* were reported to be both toxic and common in industrial feed mixtures (Borkowska-Opacka and Truszczynski 1979). Eduard et al. (1988) reported that *Aspergillus fumigatus*, *Paecilomyces variotti*, and *Rhizopus rhizopodiformis* were the predominant species of fungi collected on filters in the sorting and trimming plants of Norwegian sawmills. Land et al. (1987) reported that isolates of *A. fumigatus* isolated from sawmills in Sweden produced tremorgenic mycotoxins.

D. Current Concepts for Etiology of ODTS

A number of possible mechanisms have been suggested for ODTS, including direct activation of complement (Olenchock and Burrell 1976; Lewis and Olenchock 1989; Olenchock et al. 1990) and the presence of polyclonal cell activators (i.e., agents which stimulate cells of the immune system in a nonspecific way), resulting in production of various mediators of inflammation (Willoughby et al. 1985). Polyclonal cell activators (PCA) may stimulate as much as 10^6 times the number of cells with 60-fold greater mediator release than by antigen stimulation (Willoughby et al. 1985). PCAs may be especially significant with respect to macrophages because whereas antigen can only stimulate these cells indirectly via lymphokines from sensitized lymphocytes, most PCAs can activate macrophages both directly and indirectly (Willoughby et al. 1985). Activation of macrophages can result in enhanced glucose oxidation, secretion of enzymes, synthesis and release of complement components, release of prostaglandins and reactive oxygen intermediates, release of cytokines and enhanced phagocytosis, as well as enhanced bactericidal and tumoricidal activities (Willoughby et al. 1985). Potential inflammatory agents of grain dust and hay include fungi and bacteria and their metabolities, plant lectins, insects, mites, and animal dander. Thus, there is strong evidence that the etiologic mechanism of ODTS involves inhalation of organic materials containing numerous inflammatory substances. Zinc oxide fumes are also the cause of an occupational flulike postinhalation syndrome called metal fume fever (MFF) which is clinically indistinguishable from ODTS (Blanc and Boushey 1993). The clinical similarity between MFF and ODTS is parallelled by the sharp increase in polymorphonuclear cells (PMN) in bronchoalveolar lavage (BAL) fluid without the lymphocytosis observed in HP and these authors have hypothesized that cytokines (including IL-1 and TNF) mediate metal fume fever. In vitro data supporting this hypothesis include the fact that zinc can promote cytokine release and can potentiate cellular response to cytokines (Winchurch et al. 1987; Scuderi 1990; Tanaka et al. 1990).

Both Gram-negative and Gram-positive bacteria are consistent components of ODTS, often in large numbers. These organisms are the source of a number of compounds such as peptidoglycan, muramyl dipeptide (MDP), N-formyl-methionyl-leucyl-phenylalanine (FMLP), and lipopolysaccharide (LPS) which are potent inflammatory agents that can act as mitogens, adjuvants, and chemoattractants and can activate inflammatory cells (Malmberg and Rask-Andersen 1993). Although bacteria and their products are beyond the scope of the present chapter, they are omnipresent and must be considered to play a role in the etiology of ODTS. Several excellent reviews on the properties of endotoxin in relation to its inflammatory and immunomodulating properties have been published (Morrison and Ryan 1979, 1987; Morrison 1983; Wilson 1985; Burrell 1990).

In a study of 143 farms in Sweden, Malmberg et al. (1990a) reported that some ODTS reactions

occurred when dusts contained very low levels of endotoxins, suggesting that factors other than endotoxin could contribute to toxic febrile reactions. Since mold and actinomycete spores are usually present in large numbers in dust associated with ODTS, these authors suggest that excessive activation of alveolar macrophages and the subsequent release of acute phase reaction mediators may cause ODTS reactions. The remainder of this chapter will focus on fungal spores and components of the fungal cell wall.

II. Potential Role of Fungal Spores

A. Mycotoxins

Emanuel et al. (1975) used the term "pulmonary mycotoxicosis" to describe cases of atypical hypersensitivity pneumonitis, i.e., what we now recognize as ODTS. Early but limited attempts to find mycotoxins in agricultural dust samples associated with ODTS were unsuccessful (May et al. 1986) and the term pulmonary mycotoxicosis has fallen into disuse. On the other hand, spores of certain fungi have been shown to contain appreciable quantities of mycotoxins (Wicklow and Shotwell 1983; Sorenson et al. 1987), and when one considers that exceedingly high concentrations of spores ($\geq 10^9$ CFU/m^3) can be found in agricultural environments (Darke et al. 1976), exposure to mycotoxins in such situations is certainly possible. Presently, very little information is available on the airborne levels of any mycotoxin in agricultural dust other than aflatoxin, and even less is known of the airborne levels of mycotoxins in indoor air. A much more extensive data base will be needed in order to assess the role of mycotoxins in ODTS. If such studies reveal that mycotoxins are generally low or absent in dust shown to cause ODTS, alternative explanations will be needed. Even if they are not part of the normal etiology for ODTS, the presence of mycotoxins in such dusts may exacerbate or complicate the disease outcome. Inhalation of freshly generated zinc oxide fumes during welding operations with galvanized metal or melting brass produces a syndrome virtually indistinguishable from ODTS (Blanc and Boushey 1993) and there is little reason to suspect the presence of physiologically relevant concentrations of mycotoxins in these aerosols.

1. Mycotoxigenic Fungi

Bennett (1987) defined mycotoxins as ". . . natural products produced by fungi that evoke a toxic response when introduced in low concentrations to higher vertebrates by a natural route". Although this definition includes mushroom toxins as well as toxins elaborated by saprobic fungi in agricultural products, it is common to exclude mushroom toxins from this concept. At present, approximately 350 to 400 fungal metabolites are considered to be toxic and most of these are relatively small molecules of greater than 200 but less than 500 mass units (Samson 1992), although the fumonisins are greater than 750 mass units. Perhaps the most important mycotoxins in the restricted concept above are the aflatoxins, the 12,13-epoxytrichothecenes, and ochratoxin. The most important mycotoxigenic species belong to the genera *Aspergillus*, *Penicillium*, and *Fusarium* (Samson 1992). For example, the *P. verrucosum* complex (*P. verrucosum*, *P. aurantiogriseum*, *P. viridicatum*, *P. crustosum*, and *P. solitum*) have been shown to produce nearly 20 different mycotoxins (Frisvad and Filtenborg 1989). These fungi are common contaminants of agricultural commodities and some of the mycotoxins produced by these species are known to be produced by fungi common in house dust (Tobin et al. 1987). Other toxigenic fungi include species of *Alternaria*, *Paecilomyces*, *Rhizopus*, *Trichoderma*, and *Trichothecium*. All of these fungi are commonly found in soil, agricultural products, grain dust, and house dust (Tobin et al. 1987).

2. Occurrence of Mycotoxins in Spores

Although few investigators have examined spores of various fungi for mycotoxins, the presence of mycotoxins has been demonstrated in the spores of several species of toxigenic fungi, including *Alternaria alternata* (Häggblom 1987), *Aspergillus fumigatus* (Parker and Jenner 1968; Palmgren and Lee 1986; Land et al. 1995), *Aspergillus flavus* and *Aspergillus parasiticus* (Wicklow and Shotwell 1983), *Fusarium graminearum* (Miller 1992), *Fusarium sporotrichioides* (Miller 1992) and *Stachybotrys atra* (Sorenson et al. 1987). Mycotoxins found in spores include deoxynivalenol (Miller 1992), fumitremorgen and verruculogen (Land et al. 1995), fumigaclavine C (Palmgren and Lee 1986), T-2 toxin (Miller 1992), tryptacidin

(Parker and Jenner 1968), alternariol and alternariol monomethylether (Häggblom 1987), and the macrocyclic trichothecenes satratoxins G and H (Sorenson et al. 1987). Gliotoxin, a known metabolite of *A. fumigatus* which has been demonstrated in tissues infected by *A. fumigatus* (Bauer et al. 1989) and *Candida albicans* (Shah and Larsen 1993), was not detected in spores of *A. fumigatus* (Land et al. 1989). The fact that several mycotoxins have been found in spores – in a high proportion of species in which attempts were made to find them – suggests that their presence in spores of toxigenic species is much more common than is currently appreciated. The vast majority of mycotoxins are nonvolatile and therefore mycotoxin exposure by inhalation is most likely to occur via inhalation of spores. Autrup et al. (1991), in a study of occupational exposure of Danish workers in animal feed production, showed that 7 of 45 workers exposed to feed contaminated with low levels of aflatoxin B_1 (0–26 μg/kg) had detectable levels of aflatoxin B_1 bound to serum albumin, confirming systemic exposure. Two other recent reports of human disease thought to be due to inhalation of mycotoxins are noteworthy. Di Paolo et al. (1993) reported acute renal failure in a female agricultural worker exposed to grain dust in an enclosed granary which they stated was "... undeniably due to inhalation of ochratoxin of *Aspergillus ochraceous*". Although the authors did not detect ochratoxin in airborne dust in the granary, they were able to isolate *A. ochraceous* from a sample of wheat from the granary, extracts of ground moldy grains contained material which was identical to authentic ochratoxin A on thin-layer chromatography, and they were able to demonstrate acute kidney failure in experimental animals (rabbits and guinea pigs) exposed for 8 h to aerosols generated by their natural movement on moldy wheat in their cages. Gordon et al. (1993) 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 the young man's exposure, the similarity of his syndrome to that of an animal model, and the lack of an alternative explanation despite extensive testing, the authors proposed that his illness may have resulted from inhalation of tremorgenic mycotoxin(s).

3. Effects of Mycotoxins on Macrophages

T-2 toxin, patulin, and penicillic acid were shown to be acutely toxic to rat alveolar macrophages 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 (Gerberick and Sorenson 1983; Gerberick et al. 1984; Sorenson et al. 1985, 1986). Ayral et al. (1992) showed that the trichothecenes diacetoxyscirpenol (DAS) and deoxynivalenol (DON) 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 Mavet (1989) demonstrated inhibition of phagocytosis of *Pseudomonas aeruginosa* by murine peritoneal macrophages in the presence of 0.001 μM of T-2 toxin. Trichothecene mycotoxins are also known to have immunosuppressive effects (Tryphonas et al. 1984, 1986) and gliotoxin is a potent immunosuppressive metabolite of *A. fumigatus* (Eichner et al. 1986). Jakab et al. (1994) reported that phagocytosis by alveolar macrophages from rats exposed to AFB_1 in aerosols (estimated dose = 16.8 μg/kg) was suppressed and the effect persisted approximately 14 days. AFB_1 administered by intratracheal instillation (75 μg microcrystalline AFB_1) also suppressed release of tumor necrosis factor and impaired phagocytosis by peritoneal macrophages as well as primary splenic antibody responses. Therefore, it is clear that mycotoxins can be present in agricultural dusts and the mycotoxins can have a profound toxic effect on AM.

B. Cell Wall Composition

Fungal cell walls are typically rigid structures able to withstand a variety of environmental insults and are composed of several different components which include polysaccharides, proteins, lipids, pigments, and inorganic salts. Of these, polysaccharides commonly make up some 80% of cell wall dry weight, proteins range from 3 to 20%, and lipids and the other components are present in smaller amounts (Ruiz-Herrera 1992). Although nucleic acids have been detected in cell wall preparations of various fungi, they are not true components of the cell wall. Cell wall components can be considered to belong to either of two main

functional components: structural components, consisting of chitin, cellulose, and β-glucans, and cementing agents consisting of β-glucans, α-glucans, chitosans, polyuronides, lipids, inorganic salts, and pigments (Ruiz-Herrera 1992). Hydrolysis of cell wall polysaccharides indicates that glucose is the most abundant sugar in cell walls of organisms representative of all fungal taxa, followed by glucosamine, galactose, and mannose. Galactosamine, glucuronic acid, rhamnose, and xylose are present in lower amounts and are not universally present in the various taxonomic groups (Ruiz-Herrera 1992).

Bartnicki-Garcia (1968) has suggested that the fungi can be grouped into eight different chemotypes according to polysaccharide composition and that this grouping suggests an evolutionary pathway consistent with other phylogenetic markers. Of these chemotypes, the predominant fungi seen in agricultural materials are to be found in the chitin-glucan type (mycelial ascomycetous and anamorphic (imperfect) forms). Other frequently encountered chemotypes represented in agriculture and wood products are mannan-glucan chemotype (ascomycetous yeast) and the mannan-chitin chemotype (hemibasidiomycetous forms). Both of the latter chemotypes contain yeasts common in environmental samples.

Germination of fungal spores generally involves formation of an independent structure or extension of an inner spore wall, usually the innermost layer of multilayered spore walls rather than all layers the spore wall, and thus significant differences in composition might be expected. Two common differences are in pigments and sporopollenin, both of which are thought to enhance survival. Spore walls may contain lesser amounts of chitin than grown mycelium, and this is well illustrated by *Trichoderma viride*, in which the mycelium contains 12 to 22%, whereas the spore has no chitin at all (Benitez et al. 1976). Melanin content can be as high as 21% of the dry weight of cell wall.

1. Mannans and Mannoproteins

Galactonommans (GM) have been found to be important immunogens of several species of *Aspergillus* and antibodies specific for purified galactomannans have been used successfully for the detection of fungal contamination of foodstuffs (Kamphuis et al. 1989), serum (Rogers et al. 1990), and urine (Rogers et al. 1990). GM is a major cell wall component in *Aspergillus* species and is also secreted into the growth medium as an exoantigen. Secreted GM from *A. fumigatus* was shown to be composed of a branched core containing α(1→2)- and β(1→6)-linked mannose, with β(1→5) galactofuranose and/or β(1→4) galactopyranose moieties linked linearly in side chains terminated by galactofuranose nonreducing end units (Latgé et al. 1991). Stynen et al. (1992) developed monoclonal antibodies (MAbs) against *A. fumigatus* and *A. flavus* in rat lymph node cells. MAbs from seven different clones were shown to have the IgM isotype with kappa light chains which all reacted with 5 *Aspergillus* species (*A. fumigatus*, *A. flavus*, *A. niger*, *A. versicolor*, and *A. terreus*) but not with *Sporothrix schenkii*, 7 species of *Candida*, or with 15 different species of bacteria tested.

Treatment of purified GM with 0.01 N HCl at 100 °C overnight, a treatment which removes β(1→5) galactofuranose residues of the galactose-containing side chains (Latgé et al. 1991), resulted in loss of antibody binding activity (Stynen et al. 1992). Further evidence that MAb binding occurs with the galactose-containing side chains was provided by ELISA inhibition studies with synthetic β(1→5) galactofuranose oligomers (Stynen et al. 1992).

Stynen et al. (1992) investigated crossreactivity of two of the Mabs by determining avidity constants of these MAbs for exoantigens and purified cell wall polysaccharides from different fungal species. Although avidity constants for several of the fungi (*Fusarium solani*, *Trichoderma viride*, *Candida albicans*, *Saccharomyces cerevisiae*, and *Cryptococcus neoformans*) were too low to be calculated, others (*Penicillium digitatum*, *Trichophyton rubrum*, *Trichophyton interdigitalis*, *Botrytis tulipae*, *Wallemia sebi*, and *Cladosporium cladosporioides*) were comparable to those obtained for *A. fumigatus*, indicating that these galactomannans occur in a variety of fungi.

The MAbs bound intensely to germ tubes and young conidia, but weakly to mature conidia, suggesting that the immunoreactive material was less accessible because of the presence of hydrophobic outer layers on the surface of mature conidia (Stynen et al. 1992).

Mannoproteins of the cell walls of *Candida albicans* have been shown to be powerful immunomodulators in the peritoneal cavity of mice, inducing natural killer and macrophage-mediated

cytotoxicity (Scaringi et al. 1988). An extract containing soluble mannoprotein stimulated the generation of peritoneal cells capable of lysing YAC-1 and P-815 tumor cell lines in vitro. YAC-1 cells and the P-815 cells were destroyed by natural killer cells and activated macrophages respectively. Two major constituents of this mannoprotein were separated by ion-exchange chromatography. One, designated F2, was as active as the crude extracts in inducing lymphoproliferation, production of interleukin-2 and interferon-γ (IFNγ), and generation of cytotoxicity against a natural killer-sensitive target cell line (Torosantucci et al. 1990).

2. β1,3-Glucans

β1,3-Glucans are glucose polymers consisting predominantly of chains of glucopyranosyl rings joined by β1,3 linkages with varying degrees of β1,6 side chains. They are thought to exist as stable triple helices under physiological conditions with three glucose polymers held in close proximity in a rigid helical rod-like structure by intramolecular hydrogen bonds (Williams et al. 1991a). Among the few chemically pure β-glucan preparations available commercially are schizophyllan (Yanaki et al. 1983), lentinan (Saito et al. 1977), scleroglucan (Pretus et al. 1991), and glucan phosphate (Williams et al. 1991a). Most are water-insoluble microparticulates, but some have been rendered water-soluble by chemical extraction and chemical derivatization (Williams et al. 1991a,b). There are significant differences between toxicity and immunobiological activity of soluble and insoluble glucan (Bowers et al. 1986). Development of soluble glucans for clinical use was important because of toxic side effects of parental administration of particulate glucan such as hepatosplenomegaly, granuloma formation, and microembolization. Soluble glucan appears to be an equally effective biological response modifier as particulate glucan with none of the associated toxicity (Sherwood et al. 1987).

Numerous investigators have demonstrated the immunomodulating activities of β1,3-glucan (Wooles and Di Luzio 1963; Haba et al. 1976; Morikawa et al. 1985; Patchen et al. 1987; Sherwood et al. 1987; Williams et al. 1987). It is believed that macrophage activation is the central event in the β-glucan immune stimulatory effect (Williams et al. 1983). Glucans enhance both the number and function of macrophages (Patchen et al. 1984; Williams et al. 1987), inducing release of hemopoietic growth factors and stimulating proliferation of hemopoietic progenitors (Patchen et al. 1984). Soluble glucans also enhance production of IL-1 and IL-2 by splenic macrophages (Sherwood et al. 1987) and stimulate production of monocyte chemoattractant protein 1 (MCP-1), which was preceded by an early, transient rise in TNF (Jones and Warren 1992). The rise in BAL fluid monocyte chemotactic activity could be nearly completely neutrolized with antibody directed against MCP-1. Abel et al. (1989) demonstrated that lentinan treatment of resident and thioglycollate-elicited mouse peritoneal macrophages resulted in a dose-dependent stimulation of phagocytosis.

Soluble and particulate glucan have been utilized to treat experimental neoplastic disease (Di Luzio et al. 1979) and glucan therapy was shown to decrease primary tumor weight, reduce hepatic metastases, and prolong long-term survival in mice with experimental sarcoma (Williams et al. 1985).

Fogelmark et al. (1992) compared the effects of inhaled endotoxins and β1,3-glucan, individually and in combination, on the number of inflammatory cells in lung walls and airways. Endotoxin caused an increase in lung lavage leukocytes (neutrophils, macrophages, and eosinophils) at 4 and 24 h after exposure, whereas no significant increase in lung lavage neutrophils was found after exposure to an equal concentration of glucan. The numbers of lymphocytes and macrophages in lung lavage were significantly increased after exposure to endotoxin but decreased 1 day after exposure to glucan. Similarly, the number of lung wall leukocytes was increased after exposure to endotoxin and decreased after exposure to glucan, although there was a small nonsignificant increase in lung wall neutrophils after a 4-h exposure followed by a decline to or below control levels 24 h after exposure. The number of all cell types in lung lavage was less after exposure to a combination of endotoxin and glucan than after exposure to endotoxin alone. The authors also reported a difference in response to different glucan preparations (i.e., naturally soluble glucan or glucan made soluble by NaOH treatment) that produce reactions similar to those produced by endotoxin. These results confirm earlier observations that the structure of the glucan molecule is important for several of its biological activities but also suggest that natural

exposure to glucans in spores or intact cells may not be likely to produce responses similar to those described with purified glucan – either because of its insolubility or because it is masked by the presence of other cell wall components in the spores. Hoffman et al. (1993) demonstrated that fungal β-glucans stimulated rat macrophage release of tumor necrosis factor-α (TNFα) if the cells were treated with less than 500 μg/ml of β-glucan. Treatment with concentrations of β-glucan greater than 500 μg/ml resulted in TNF release comparable to untreated cells with no effect on cell viability. When cells were treated with LPS or INFγ in the presence of high concentrations of β-glucans, TNFα release was also inhibited. These results suggest an immunomodulating role for fungal β-glucans.

De Lucca et al. (1992) demonstrated that β1,3-glucan can form agglutination precipitates with lung surfactant in vitro. The data indicate that agglutination results from the formation of van der Waals or hydrogen bonding and suggest the possibility that abnormalities in surface tension could lead to the symptoms seen in occupational lung disease associated with organic dust.

3. Chitin and Chitosans

Although glucans are more abundant than chitin, the latter is the most characteristics polysaccharide of fungal cell walls and is found in the cell walls of all major groups of fungi (Ruiz-Herrera 1992). Chitin is an unbranched polysaccharide composed of N-acetylglucosamine joined by β-1,4 bonds (Ruiz-Herrera 1992). Chitosan is a deacylated analogue of chitin that has been shown to be a characteristic component of zygomycetous cell walls (Ruiz-Herrera 1992). The relative amounts of chitin and glucans may vary between the walls of somatic hyphae and those of the spore. For example, in *Conidiobolus obscurans*, the mycelial walls contained 15% chitin and 56% β-1,3 glucan, whereas spore walls contained 52% chitin and only 5% glucan (Latgé et al. 1984). Multiporous microspheres prepared from chitin and 80% deacylated chitin were shown to enhance the cytolytic activity of peritoneal macrophages and induce the production of colony stimulating factor in vitro by macrophages, spleen cells, and bone marrow cells (Nishimura et al. 1987).

4. Sporopollenins

Sporopollenins are highly insoluble oxygenated polymers of carotenoids (Brooks and Shaw 1968). They have been demonstrated in zygospore walls of *Phycomyces blakesleeanus* (Furch and Gooday 1978) and *Mucor mucedo*, and in the ascospores of several species (Gooday 1981). They are extremely resistant molecules thought to be protective and responsible for the resistance of the walls to physical factors and enzymatic and chemical attack (Ruiz-Herrera 1992). Sporopollenins were not found in somatic hyphae, sporangiophores, or sporangiospores of *M. mucedo* (Gooday 1981). Although no systematic search has been done to find sporopollenins in fungi, they were thought, until recently, to be confined to exine of pollen grains and to the spores of pteridophytes (Gooday 1981). The fungi and spore stages from which sporopollenins have been described (e.g., ascospores of *Neurospora crassa* and zygospores of *M. mucedo*) are not prominent in agricultural dust associated with episodes of ODTS, and therefore, are not likely to be of major importance in the disease.

C. Spore Diffusates and Whole Spores

Robertson and coworkers prepared "diffusates" from conidia of *A. fumigatus* by incubating live conidia in tissue culture medium for up to 3 h and then removing the conidia by centrifugation and filtration. These diffusates inhibited chemotaxis of human PMN and spreading of mouse peritoneal macrophages (Robertson et al. 1987c), inhibited phagocytosis of antibody-coated sheep erythrocytes (Robertson et al. 1987b), and decreased superoxide anion and hydrogen perioxide production (Robertson et al. 1987a). These authors have shown that the active material in the diffusates is dialyzable and thus has molecular weight less than 14 kDa (Robertson et al. 1987a). *A. fumigatus* is an important opportunistic fungus capable of producing life-threatening lung infections in immunosuppressed persons (Warren and Warnock 1982). Release of substances from the spores which inhibit phagocytic cells during early contact in the lung may have great importance in ensuring survival of the spores in the lung and enhancing their pathogenic potential (Robertson et al. 1987c). Gliotoxin has anti-phagocytic and immunomodulating activity, it is produced by *A.*

fumigatus (Müllbacher et al. 1985), and it has been demonstrated in tissues infected by *A. fumigatus* (Bauer et al. 1989). Land et al. (1989) reported that gliotoxin was not present in conidia of *A. fumigatus*, although they were able to demonstrate several other mycotoxins in conidia of this organism. Müllbacher et al. (1985) reported that gliotoxin did not appear in culture filtrates until day 3. Sorenson et al. (1994) reported that diffusates of *A. niger* had a direct chemotactic effect for human PMN and rat AM, but that diffusates prepared from *A. fumigatus* and *A. niger* conidia after 16 h incubation in medium caused a dose-dependent decrease in superoxide anion production stimulated by PMA. That the diffusates did not act as superoxide scavengers is indicated by the fact that diffusates of these species had no effect on superoxide anion production by the xanthine/xanthine oxidase superoxide generation system. Because of the diffusate effect, studies of superoxide anion production by conidia of these species were done with freshly washed conidia.

Sorenson et al. (1994, 1995) demonstrated that conidia of several fungi have the ability to activate complement and stimulate production of LTB_4 and superoxide anion. Complement activation has several important consequences, including production of complement factor C3b, which coats bacteria and fungi and facilitates phagocytosis because of the presence of C3b receptors on the surface of PMN and AM. Activation of complement also results in increased concentrations of C3a and C5a which are potent chemotactic agents and lead to recruitment of large numbers of PMN to the site of activation. C3a and C5a have anaphylatoxic activity, i.e., they can cause contraction of smooth muscles and degranulation of mast cells and basophils with the release of vasoactive amines such as histamine. Olenchock and Burrell (1976) have shown that unimmunized rabbits exposed to small and massive aerosols of *Aspergillus* spores demonstrated postchallenge arterial hypoxia as well as decreased hemolytic complement activity. Unimmunized animals treated with cobra venom factor in a manner known to achieve complement depletion did not respond in this manner. LTB_4 is a potent chemotactic factor for PMN (Smith et al. 1980) and would contribute to recuritment of PMN to the lung following exposure to agents eliciting LTB_4 release. The observation that several species of fungi common in agricultural dust stimulate release of LTB_4 from alveolar macrophages (Sorenson et al. 1994, 1995) suggests that exposure to spores of these species would result in recruitment of PMN to the lung. LTB_4 possesses potent calcium ionophore activity and, in addition to recruiting inflammatory cells to the lung, it can upregulate their function and modulate the immune response (Bjornsdottir and Bush 1993).

Production of reactive oxygen intermediates has been postulated to contribute to the tissue damage seen in pulmonary inflammation. Fungal spores alone were found to stimulate a modest increase in O_2^- production. However, if macrophages were primed by pretreatment with LPS and then exposed to the fungal spores, significant enhancement of O_2^- production was observed (Shahan et al. 1995). Both live and killed spores were active. These results support the concept that the combined exposure to both bacterial and fungal agents may be important in the pathogenesis of ODTS.

Development of fever is an important clinical sign of ODTS and is usually thought to result from IL-1 production. In early experiments to assess the ability of either live or heat-killed spores to stimulate IL-1 production by AM in vitro, Sorenson et al. (1995) were consistently unable to show an increase in IL-1 activity. Endotoxins and their major component, LPS, are well known stimulators of IL-1 production. Natural exposure to organic dust in concentrations sufficient to cause ODTS commonly (invariably?) includes exposure to Gram-negative bacteria and endotoxin. The natural occurrence of fungal spores and endotoxin together in grain dust raises the question of whether the presence of spores could modulate LPS-induced IL-1 production by AM. When AM were treated with spores in combination with suboptimal concentrations of LPS, there was little enhancement of IL-1 production, and IL-1 production was inhibited by *Aspergillus fumigatus*, *A. niger*, and *Eurotium amstelodami* (Sorenson et al. 1995). These results suggest that fungal spores may lack the innate ability to activate IL-1 production/release, at least in vitro, without the presence of T- and/or B lymphocytes, and that fever associated with ODTS may be due to other mediators or to indirect mechanisms, e.g., C5a can stimulate IL-1 release (Lambris 1988). Inhibition of IL-1 production would be expected to diminish, rather than contribute to, symptoms of ODTS. The inhibition of IL-1 production observed in the

present study by *A. fumigatus*, *A. niger*, and *E. amstelodami* suggests the possibility of mycotoxins in the spores. Heat-killed conidia were used in the experiments described and therefore, if mycotoxins were responsible for the inhibition observed, they must have been present in the conidia before autoclaving. We observed striking inhibition of LPS-stimulated IL-1 production at ratios of 5 or 50 spores per AM. When one considers that spore concentrations in heavily contaminated agricultural dusts often reach $10^8/m^3$ and that during moderate activity a worker's ventilation could be $\leq 3\,m^3/h$, exposures of up to 3×10^8 spores/h or 5×10^6/min could be reached. Thus, it is easy to imagine that ratios of 50:1 spores per macrophage may not be uncommon among workers exposed to heavily contaminated agricultural dusts.

Phagocytosis of heat-killed *Saccharomyces cerevisiae* was shown to be mediated predominantly by β-glucan receptors in murine peritoneal macrophages with little involvement of mannose receptors or complement receptors (Goldman 1988). Similar results were found for zymosan-induced arachidonic acid release from rabbit alveolar macrophages. This release was blocked by soluble β-glucan while soluble mannan had no effect (Daum and Rohrbach 1992), indicating that the release was at least in part via a β-glucan receptor. The fact that β-glucan is a major component of the cell wall of a wide variety of fungi suggests that stimulation of alveolar macrophage β-glucan receptor with subsequent release of arachidonic acid may play a role in the pathogenesis of ODTS.

Norn et al. (1993) showed that spores of *Chaetomium globosum, Mucor racemosum*, and *Aspergillus terreus*, while unable to cause histamine release from human basophils in concentrations of 0.1 to 1 mg/ml in the absence of other stimuli, caused a significant enhancement of mediator release when histamine release was triggered by either IgE-dependent or by nonimmunological stimuli. While a number of factors contribute to development of inflammation during exposure to organic dust, the presence of both stimulators and potentiators of histamine release in the same dust may be of major importance.

Siegel et al. (1991) demonstrated concentrations of histamine as high as 0.5 ng/mg in bulk hay and respirable hay dust. Samples from animal housing facilities, such as chicken coops, may contain histamine derived from animal excrement or insect infestation. These sources, however, may not explain the presence of histamine in dust that has no indication of animal contamination. On the other hand, levels of microorganisms are usually high in these samples, and it is possible that they could contribute to total histamine levels in the dust. Several species of bacteria and fungi, some of which are common in agricultural dust, were shown to produce histamine in laboratory culture (Siegel et al. 1992).

III. Conclusions

Organic dust toxic syndrome (ODTS) is associated with agriuclтural and other dust contaminated with microorganisms. Although originally called "pulmonary mycotoxicosis", there is little evidence to support mycotoxin etiology and considerable evidence that other components of microorganisms, such as endotoxin and components of the fungal cell wall, are abundant in organic dust and are able to activate components of the innate immune system, including phagocytic cells and complement. Inflammation is an important part of this response and involves an increased blood supply to the area; increased vascular permeability, permitting entry of large molecules such as soluble mediators of immunity; and recruitment of circulating neutrophils into the site of inflammation by chemotaxis. Therefore, current evidence strongly suggests that ODTS is the result of stimulation of immune cells leading to inflammation. Fungal spores are commonly present in abundance in agricultural dust associated with ODTS and these spores and several of their components (e.g., β1,3-glucan and mannoproteins) have been shown to possess potent inflammatory activity.

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