Mold and Mycotoxins: Effects on the Neurological and Immune Systems in Humans

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I. Introduction

The potential harmful effects of exposure to molds in inhabited buildings were recognized in early Biblical times. In the Old Testament (King James Version, Oxford 1888 Edition, Chapter XIV: Verses

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34 thru 47) Leviticus put forth a detailed protocol for the remediation of contaminated structures, including the destruction of dwellings and personal belongings if remediation failed. Currently it is recognized that water intrusion into buildings leads to amplification of molds (Andersson *et al.*, 1997; Gravesen *et al.*, 1999; Hodgson *et al.*, 1998; Jaakkola *et al.*, 2002; Johanning *et al.*, 1996; Nielsen, 2003; Peltola *et al.*, 2001), which often requires remediation.

Fungal fragments occur in indoor air as biocontaminants (Burge, 1990; Gorney *et al.*, 2002). Potentially toxic and immunogenic byproducts of fungi and molds include mycotoxins (Croft *et al.*, 1986; Johanning *et al.*, 2002; Nielsen *et al.*, 1999; Nieminen *et al.*, 2002; Tuomi *et al.*, 1998, 2000); 1,3-alpha-D-glucans (Andersson *et al.*, 1997), extracellular polysacharrides (EPS) (Duowes *et al.*, 1999; Notermans *et al.*, 1988; Wouters *et al.*, 2000); exodigestive enzymes (EDS) (Monod *et al.*, 2002), and solvents (Claeson *et al.*, 2002). In addition, trichothecenes, ochratoxin A, sterigmatocystin, and other mycotoxins have been identified in ventilation duct dust and in the air in buildings where occupants have experienced adverse health effects related to mold exposure (Croft *et al.*, 1986; Engelhart *et al.*, 2003; Jarvis, 2002; Johanning *et al.*, 2002; Nieminen *et al.*, 2002; Skaug *et al.*, 2000; Smoragiewicz *et al.*, 1993; Tuomi *et al.*, 1998). The worst-case scenario appears to be repeated episodes of water damage that promote fungal growth and mycotoxin production, followed by drier conditions leading to release of spores and hyphal fragments (Nielsen, 2003).

Occupants of affected structures develop multiple organ symptoms and have adverse effects of the upper and lower respiratory system, central and peripheral nervous system, skin, gastrointestinal tract, kidneys and urinary tract, connective tissue, and the musculoskeletal system (Anyanwu *et al.*, 2003a; Croft *et al.*, 1986; Gunnbjornsdottir *et al.*, 1998; Gray *et al.*, 2003; Hodgson *et al.*, 1998; Jaakkola *et al.*, 2002; Johanning *et al.*, 1996; Kilburn, 2002; Sailvilahti *et al.*, 2000). Human illness caused by fungi can result via one or all of the following: (1) mycotic infections (mycoses) (Anaissie *et al.*, 2002; Eucker *et al.*, 2001; Fraser, 1993; Grossi *et al.*, 2000), (2) fungal rhino-sinusitis (Braun *et al.*, 2003; Ponikau *et al.*, 1999; Thrasher and Kingdom, 2003), (3) IgE-mediated sensitivity and asthma (Barnes *et al.*, 2002; Lander *et al.*, 2001; Zureik *et al.*, 2002), (4) hypersensitivity pneumonitis and related inflammatory pulmonary diseases (Erkinjuntti-Pekkanen *et al.*, 1999; Ojanen, 1992; Patel *et al.*, 2001; Sumi *et al.*, 1994), (5) cytotoxicity (Desai *et al.*, 2002; Gareis, 1995; Jones *et al.*, 2002; Nagata *et al.*, 2001; Poapolathep *et al.*, 2002), (6) immune suppression/modulation (Berek *et al.*, 2001; Bondy and Petska, 2000; Jakab *et al.*, 1994), (7) mitochondrial

toxicity (Hoehler, *et al.*, 1997; Niranjan *et al.*, 1982; Pace, 1983, 1988; Sajan *et al.*, 1997; Wei *et al.*, 1984), (8) carcinogenicity (Dominguez-Malagon and Gaytan-Graham, 2001; Schwartz, 2002), (9) nephrotoxicity (Anyanwu *et al.*, 2003c; Pfohl-Leszkowicz *et al.*, 2002), (10) the formation of nuclear and mitochondrial DNA adducts (Hsieh and Hsieh, 1993; Petkova-Bochatrova *et al.*, 1998; Pfhlohl-Leszkowicz *et al.*, 1993). Finally, in the infectious state, molds secrete extracellular digestive enzymes (EDE) that cause tissue destruction, angioinvasion, thrombosis, infarction and other manifestations of mycosis (Ebina *et al.*, 1985; Kordula *et al.*, 2002; Kudo *et al.*, 2002; Monod *et al.*, 2002; Ribes *et al.*, 2000; Vesper *et al.*, 2000).

The pathological and inflammatory conditions associated with *Stachybotrys chartarum* in infants with pulmonary hemosiderosis have been characterized. *S. chartarum* isolated from the lungs of an affected infant produced a hemolysin (stachylysin), a siderophore, and a protease (stachyrase) (Kordula *et al.*, 2002; Vesper *et al.*, 2000). Stachylysin has also been demonstrated in the serum of adults ill from a building-related exposure (Von Emon *et al.*, 2003). In rodents, its presence has been demonstrated by an immunocytochemical method following installation of *S. chartarum* spores into lungs. The hemolysin increases in concentration from 24 to 72 hours following instillation of spores, indicating that production/release is a relatively slow process (Gregory *et al.*, 2003). In addition, strains of *S. chartarum* produce different quantities of toxic trichothecenes (Jarvis *et al.*, 1998). In an earthworm model, stachylysin increased the permeability of blood vessels, causing leakage through the vessel endothelium and walls (Vesper and Vesper, 2002). Additionally, pathology may result from the interference of pulmonary surfactant synthesis by *S. chartarum* spores and isosatratoxin-F in juvenile mice. Ultrastructural changes in type II alveolar cells—with condensed mitochondria, increased cytoplasmic rarefaction, and distended lamellar bodies with irregularly shaped lamellae—have been observed following exposure to *S. chartarum* (Mason *et al.*, 1998, 2001; McCrae *et al.*, 2001; Rand *et al.*, 2001). Thus, hemolysins, siderophores, and proteases also appear to have an important role in the pathogenesis of mold infections.

Recognizing the complexity of health problems associated with multiple mold exposure, we have previously reported a multi-center investigation of patients with chronic health complaints from exposure to multiple colonies of indoor fungi and molds. We utilized detailed health and environmental history–gathering questionnaires, environmental monitoring data, physical examination, pulmonary function testing protocols, routine clinical chemistries, measurements of lymphocyte phenotypic

markers (on T, B, and NK cells), antibodies to molds, mycotoxins, neuronal antigen antibodies, leukocyte apoptosis, neurocognitive testing, 16-channel quantitative EEGs (QEEG), nerve conduction studies (NCS), brainstem auditory evoked potentials (BAER), visual evoked responses (VER), and other neurological testing. The following is a summary of our findings on symptoms, pulmonary function, alterations in peripheral lymphocyte phenotypes, autoantibodies, and neurological abnormalities observed in patients by us and others. Currently we refer to the illness of these individuals as a ''mold mycotoxicosis'' involving the immune system, the lungs, the central and peripheral nervous systems, and generalized inflammatory and irritant responses to exposure to spores, hyphal fragments, mycotoxins, solvents, and other byproducts (e.g., EPS, EDS).

II. Water Damage and Associated Molds

A. Mycobiota

Water intrusion into buildings can lead to an amplification of molds. Molds growing on building materials (e.g., wall board, particle board, plaster board, ceiling tiles, carpeting) are classifiable according to their water activity, a_w (Nielsen, 2003) as follows: (1) primary colonizers have an a_w of <0.8 with an optimal water requirement approaching 1 for growth. The group includes *Penicillium chrysogenum* and *Aspergillus versicolor*, followed by other species of *Aspergillus* (*niger, fumigatus, sydowii, ustus*), several *Eurotium* species, *Penicillium* species (*brevicompactum, commune, corylophilum, pelicans*), *Paecilomyces variotti* and *Wallemia sebi*. (2) Secondary colonizers requiring a minimum of between 0.8 and 0.9 a_w include species of *Alternaria, Cladosporium, Phoma*, and *Ulocladium*. (3) Tertiary colonizers (water-damage molds) that require 0.9 a_w or greater include the most toxic molds: *Chaetomium globlosum, Stachybotrys chartarum, Memnoniella echinata*, and *Trichoderma* species (*viride, citrinoviride, harzianum* and *longibrachiatum*). For a more detailed review, see Nielsen (2003).

B. Mycotoxins Produced by Toxigenic Molds

Fungi produce many metabolites, which are believed to play a crucial role in their natural habitats. In addition, many of the metabolites have been identified. Those that are toxic to animals and humans are called *mycotoxins*. Paradoxically, antibiotics isolated from molds are mycotoxins and are beneficial to humans. Table I lists the molds commonly found in water-damaged buildings and the toxic metabolites

TABLE I

TOXIGENIC MOLDS IN WATER-DAMAGED BUILDINGS

Mold	Metabolites	Health concern
Stachybotrys chartarum	Spirocyclic drimanes; satratoxins G, H and F; hydroxyroridin E, verrucarin J; trichodermin; dolabellanes; atrones B and C; stachyotryamide; stachyotrylactams; stachylysin	Pulmonary hemosiderosis; Induces proinflammatory cytokines
Alternaria tenuissima	Alternariols; tentoxin; tenuazonic acids; altertoxin I	Unknown
Aspergillus flavus	Aflatoxin B1; kojic acid; aspergillic acid; 3-nitropropionic acid; cyclopiazonic acid	Carcinogenesis; aspergillosis
Aspergillus fumigatus	Fumigaclavines; fumitoxins; fumitremorgens; gliotoxins; tryptoquivalines; verruculogen	Tremors and CNS injury; Immune damage by gliotoxin; aspergillosis;
Aspergillus niger	Ochratoxin A	Nephropathy
Aspergillus ochraceous	Ochratoxin A, penicillic acid; xanthomegnin; viomellein, vioxanthin	Nephropathy
Aspergillus ustus	Kotanins	Unknown
Aspergillus versicolor	Sterigmatocystin; 5-methoxy-sterigmatocystin	Carcinogenesis; aspergillosis
Penicillium chrysogenum	Secalonic acid D	Unknown
Chaetomium globosum	Chaetomins; chaetoglobosins A and C	Cytoxicity; inhibition of cell division
Memnoniella echinata	Griseofulvin; dechlorogriseofulvins; trichodermin; trichodermol	Unknown
Penicillium brevicompactum	Mycophenolic acid; botryodiploidin.	Toxic (mutagenic)
Penicillium expansum	Patulin; citrinin; chaetoglobosin; Roquefortine C	Immune toxicity, cytotoxic; tremorgenic
Penicillium polonicum	Verrucosidins; penicillic acid; nephrotoxic glycopeptides	Tremors; cytotoxicity; nephropathy
Trichoderma species	Trichothecenes; trichodermol; trichodermin; gliotoxin; viridin	Toxicity associated with trichothecenes

This table summarizes the toxigenic molds found and/or identified in water-damaged buildings. The mycotoxins isolated from the molds and their general toxic effects are also summarized. The information in this table was obtained from the review Nielsen (2003).

(mycotoxins) that they produce with general statements on their toxicity. Readers are referred to the literature cited in this chapter and in Nielsen (2003) for more detailed information.

C. Human Exposure

Humans can be exposed to mycotoxins and metabolites of molds in the indoor environment via (1) ingestion (contaminated foods, dirt, and dust) (2) the skin (contaminated clothing and surfaces), and (3) inhalation. Inhalation is the primary mode of exposure in the inhalation of spores (3 to 7 μm), hyphal fragments, and particulate matter down to 0.05 μm. It has been shown that particles smaller than spores can be shed from colonies of molds (Gorney *et al.*, 2002; Kildeso *et al.*, 2000). Large quantities of particles $\leq$ 0.03 μm can be released from colonies, creating a 300-fold higher concentration of fungal fragments as compared with the number of spores released (Gorney *et al.*, 2002). There is no apparent correlation between the number of particles and the number of spores. Factors that influence the release of spores and particulates include low humidity (stimulates release), ventilation, external wind speeds, human activity, and pressure shocks (e.g., elevators, doors). Finally, because it is difficult to quantify the particulate matter shed by colonies, very few meaningful correlations have been found between spore concentrations and adverse health effects on humans from indoor exposure to toxigenic molds (Nielsen, 2003). Thus biomarkers for molds and mycotoxins have been and need to be further developed for exposure assessment.

One successful approach has been to use DNA adducts to determine exposure to aflatoxin B1 (Makarananda *et al.*, 1998) and ochratoxin A (Pfhohl-Leszkowicz, 1993a,b). However, another effective approach has been the development of immune assays to detect the presence of antibodies to mold-specific antigens and mycotoxins. Also, an appreciation of the adverse health effects can be obtained by utilizing neurophysiological, neuropsychological, and immunological diagnostic procedures (see below).

III. Symptoms, Upper and Lower Respiratory Tract

A. Symptoms

Occupants of water-damaged buildings express multiple organ symptoms. Table II summarizes observations made on 209 adults exposed at home and/or at the workplace. Complaints significantly different from controls occurred as follows: (1) central nervous system (headache,

TABLE II

FREQUENCY OF SYMPTOMS

Symptom[a]	Mold Patients (N =) 209	Controls N = 28	P value
Excessive Fatigue	5.8 ± 1.9	4.3 ± 2.1	0.0001
Headache	5.2 ± 1.9	4.1 ± 2	0.005
Nasal Symptoms	5.1 ± 2.2	4.1 ± 2	0.02
Memory Problems	5.1 ± 2.1	3.3 ± 1.6	0.0002
Spaciness	4.8 ± 2.3	3.2 ± 1.8	0.0007
Sinus Discomfort	4.7 ± 2.2	3.6 ± 1.8	0.01
Coughing	4.6 ± 2.2	3.2 ± 1.6	0.001
Watery Eyes	4.6 ± 2.1	3.4 ± 1.7	0.004
Throat Discomfort	4.5 ± 2.1	3.4 ± 1.7	0.008
Slurred Speech	4.5 ± 2.3	3.1 ± 2	0.002
Lightheadedness	4.4 ± 2.2	3.2 ± 1.4	0.006
Joint Discomfort	4.4 ± 2.3	3.7 ± 2.1	NS
Dizziness	4.3 ± 2.1	3.1 ± 1.4	0.005
Weakness	4.2 ± 2.3	3 ± 1.7	0.008
Bloating	4.2 ± 2.2	3.2 ± 1.6	0.02
Insomnia	4.1 ± 2.2	3.8 ± 2	NS
Weak Voice	4.1 ± 2.2	2.8 ± 1.4	0.003
Spasms	4 ± 2.2	3.8 ± 2.1	NS
Coordination Problems	4 ± 2.2	2.9 ± 1.4	0.01
Visual Changes	3.9 ± 2.3	2.9 ± 1.4	0.02
Rash	3.9 ± 2.2	2.9 ± 1.7	0.02
Numbness	3.9 ± 2.2	3.4 ± 1.7	NS
Cold Intolerance	3.9 ± 2.4	3.1 ± 1.8	NS
Heat Intolerance	3.8 ± 2.4	3.6 ± 2	NS
Chest Tightness	3.8 ± 2,2	2.6 ± 1.3	0.006
Chest Discomfort	3.7 ± 2.2	3 ± 1.3	NS
Urine Frequency	3.7 ± 2.3	3.8 ± 2.1	NS
Excessive Thirst	3.6 ± 2.3	3.4 ± 2	NS
Ringing Ears	3.6 ± 2.2	4.4 ± 2.4	NS
Wheezing	3.6 ± 2	2.6 ± 1.3	0.02
Swallowing Problems	3.2 ± 2	3 ± 1.7	NS
Flushing Skin	3.1 ± 2.1	2.8 ± 1.6	NS
Bladder Control	3.1 ± 2	2.8 ± 1.4	NS
Rapid Pulse	3 ± 2	2.6 ± 0.9	NS

(*continued*)

TABLE II (Continued)

Symptom[a]	Mold Patients (N =) 209	Controls N = 28	P value
Palpitations	2.8 ± 1.9	2.4 ± 0.8	NS
Bruising	2.8 ± 1.7	2.4 ± 0.9	NS
Swelling Ankles	2.7 ± 1.8	2.6 ± 1.5	NS
Hearing Changes	2.7 ± 1.8	2.6 ± 1.5	NS

This table summarizes the frequency of symptoms of the 38 most frequently reported symptoms in the patients vs the controls. To obtain these data, a total of 209 patients filled out questionnaires. Critical t-test analysis was performed and p values are given for each symptom of patients vs controls (NS = Not Significant).

[a]The symptoms compared were for females versus males. The females had significantly greater frequency for 21 of the 38 reported symptoms (data not shown; see Results section).

short-term memory loss, lightheadedness, dizziness, blurred vision, tinnitus, and cognitive function loss), (2) the upper respiratory tract (nasal congestion and chronic sinusitis), (3) the lower respiratory tract (cough, wheezing, chest tightness, exertional dyspnea, and irritation of the throat), and (4) general ill feeling (excessive fatigue, weakness, joint aches and pains, and rashes) (Campbell *et al.*, 2003; Gray *et al.*, 2003). In addition, others have shown similar increases in the incidence of neurological and respiratory symptoms in individuals ill from mold exposure in water-damaged buildings (Hodgson *et al.*, 1998; Johanning *et al.*, 1996; Kilburn, 2002; Vojdani *et al.*, 2003). Vojdani *et al.* (2003) reported that patients exposed to molds had significant increases in recurrent flu-like illnesses, anxiety, and symptoms of severe allergies. It has become increasingly obvious that exposure to multiple toxigenic molds in water-damaged buildings leads to an increased incidence of multiple organ symptoms in the affected individuals.

B. Upper Respiratory Fungal Infections

Symptoms of upper respiratory involvement include nasal congestion, sinusitis, sinus pain, and nasal bleeding (chronic rhinosinusitis). Individuals with this condition do not respond to ordinary antibiotic therapy.

Several reports have appeared in the literature demonstrating that a large proportion of individuals with chronic rhinosinusitis (CRS) have infections with molds and yeast. CRS is characterized by the presence of eosinophilic mucin, fungal hyphae, Charcot-Leyden crystals, and the presence or absence of polyposis (Ponikau *et al.*, 1999; Taylor *et al.*,

2002). The incidence of fungal involvement in different case studies was 82% to 100% (Braun *et al.*, 2003; Dosa *et al.*, 2001; Ponikau *et al.*, 1999) and 100% (Taylor *et al.*, 2002). The fungal genera isolated and cultured from nasal secretions include such indoor contaminants as *Aspergillus* sp., *Alternaria, Chaetomium, Cladosporium, Epicoccum, Penicillium, Phoma, Trichoderma*, and others (Dosa *et al.*, 2002; Ponikau *et al.*, 1999; Taylor *et al.*, 2002). The isolation of fungi and the presence of eosinophils and eosinophilic mucin rule out type I (IgE) hypersensitivity (allergy) and strongly point to the role of invasive fungi as the cause of CRS (Braun *et al.*, 2003; Ponikau *et al.*, 1999).

C. Lower Respiratory Tract

Molds can cause lung disease by different mechanisms: allergic asthma (Jaakkola *et al.*, 2002; Zureik *et al.*, 2002), infections (e.g., aspergillosis) (Fraser, 1993; Sumi *et al.*, 1994), and inflammation (e.g., hypersensitivity pneumonitis, and farmer's lung disease) (Fan, 2002; Ojanene, 1990, 1992; Patel *et al.*, 2001). Chest x-rays can be used to detect pathological changes associated with infections (e.g., aspergillosis and granulomatous lesions). Pulmonary function testing (PFT) is used to diagnose airway restriction caused by allergies to molds as well as inflammatory conditions (hypersensitivity pneumonitis and farmer's lung disease). PFT measures flow rates in the airways of the lungs. The forced vital capacity (FVC) is the maximum amount of air expelled during forced expiration. The fraction of the vital capacity expired in one second is the FEV_1. The importance of these measurements arises from the fact that during disease states, (e.g., asthma), the FVC may be normal while the FEV_1 is reduced because of increased airway resistance. However, these two measurements do not discriminate between the airways of different caliber and therefore are not able to distinguish between the status of the large, medium, and small airways. Airborne particulate matter and spores (bioaerosols) from fungi range from 0.03 to 10 microns. ''Respirable particles'' range from 5 microns to 0.005 microns and are capable of reaching the small airways and alveoli of lungs. Therefore, PFT measurements used must also detect inflammatory or obstructive changes within the small airways. The PFT measurements most suited for small airway obstruction are FEF 75% and FEF 25–75%. These measure the flow rates at 75% and 25–75% of the exhalation and are indicative of air flow through the small airways. A reduction in these PFT values is evidence of small airway obstruction. The results presented in Fig. 1 show the mean and standard deviation of PFT values in

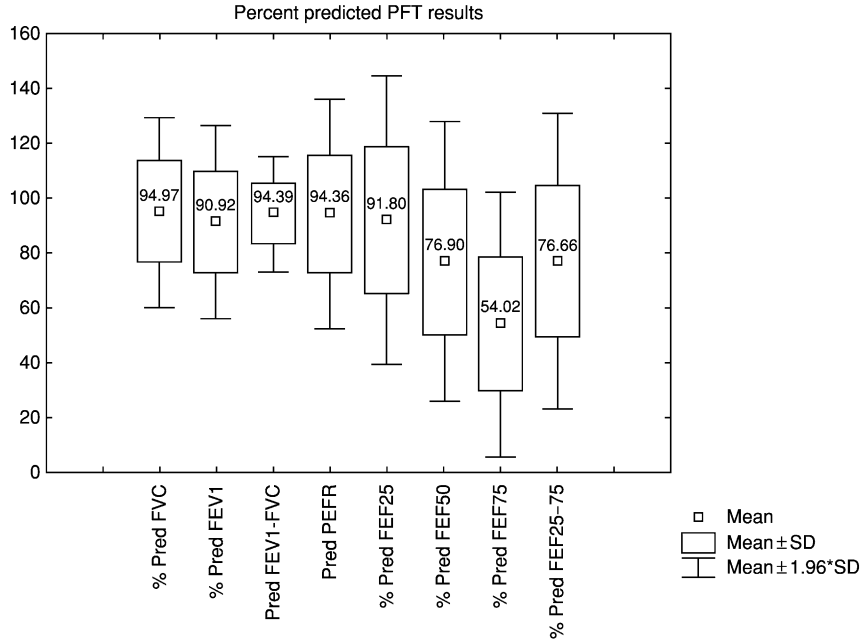

Figure 1. The results of PFT testing on individuals exposed to molds in water-damage structures.

individuals with symptoms of airway obstruction following exposure to multiple colonies of molds in water-damaged buildings. The FEF 75% is the most significantly affected parameter, demonstrating that the airway symptoms are probably the result of obstruction of the small airways in these individuals.

Small airway obstruction separates these patients from the typical occurrence in asthmatic patients, which is generally more global, involving all levels of the bronchial tree. The observed small airway obstruction indicates that particulates from <0.3 to 5 microns are being delivered to the alveoli in the deepest regions of the lung. This model is supported by the lack of a rise in mycotoxin-specific IgA (see Table VI) and the findings of Rand *et al.* (2002, 2003) and, thus, represents the most likely exposure route of relevance in patients exposed to indoor bioaerosols when multiple mold colonies are present. Therefore, the FEF 75% appears to be a biomarker that can be used to identify injury to the small airways as result of particulates containing mycotoxins, EPS, and EDEs (Rand *et al.*, 2002, 2003).

D. Proinflammatory Cytokines and Biomarkers

Proinflammatory cytokines and other biomarkers have been demonstrated to be elevated in the nasal lavage fluid of individuals with upper respiratory symptoms in moldy buildings versus control subjects. Thirty-two full-time employees in a school building contaminated with *A. fumigatus* and *A. versicolor, Eurotium, Exophiala, Phialophora, Rhodotorula, Stachybotrys, Trichoderma, Ulocladium, Willenia*, and actinomycetes had increased concentrations of alpha-tumor necrosis factor (TNF), interleukin 6 (IL-6), and nitric oxide (Hirvonen *et al.*, 1999). Furthermore, Walinder *et al.* (2001) demonstrated increased concentrations of eosinophilic cationic protein, myeloperoxidase, and albumin in the nasal lavages of occupants in buildings with mold infestation of the gypsum board, insulation, wallpaper, and wood. Multiple genera, including *Stachybotrys*, were identified. Finally, Nielsen *et al.* (2001) have shown that an extract of metabolites from *Stachybotrys* independent of macrocyclic trichothecenes and atranones is capable of inducing in vitro macrophage production of alpha-TNF and IL-4. This suggests that in addition to mycotoxins, other metabolites (e.g., spirocyclic drimanes) have a role in the nasal inflammatory process seen in mold exposure individuals (Nielsen, 2003; Nielsen *et al.*, 2001). Further support comes from Leino *et al.* (2003), who have shown that exposure of mice to spores from *S. chartarum* increases monocytes, neutrophils, and lymphocytes in bronchial alveolar lavage fluid (BAL). The infiltration of inflammatory cells was associated with the induction of proinflammatory cytokines (IL-1, IL-6, TNF-alpha), chemokines (CCL3/MIP-1, CCL4/MIP-1, and CCL2/MCP-1), and mRNA levels in the lungs. This effect was independent of the mycotoxin satratoxin produced by this mold. Furthermore, the effects were observed with no significant increase in IgE, IgG2a, and IgG1 antibody levels after exposure to *S. chartarum*.

IV. IgA, IgG, and IgE Antibodies to Molds and Mycotoxins

Molds release antigenic determinants (e.g., EPS, EDS, and proteins) that elicit an antigen-antibody response. In addition, mycotoxins can act as haptens, binding to proteins, forming a new antigenic determinant (NAD). The immune system then recognizes the NAD as foreign and makes antibodies directed against the NAD.

A. Salivary IgA Antibodies to Molds

IgA antibodies are the first line of defense against foreign invasion by preventing the attachment of microorganisms and toxins to epithelial

cells by complexing antigens (Challancombe, 1987). Recently Vojdani *et al.* (2003) tested for the presence of saliva secretory IgA antibodies against molds and mycotoxins in occupants with upper respiratory symptoms of a water-damaged building. The patients had significantly increased salivary IgA antibodies to *Alternaria, Aspergillus, Chaetomium, Cladosporium, Epicoccum, Penicillium, Stachybotrys*, satratoxin H, and other trichothecenes. It is probable that these IgA antibodies play a role in late-phase type-1 and type-2 hypersensitivity as well as type-3 delayed sensitivities to molds and their byproducts. For example, in farmer's lung disease, serum IgA antibodies against *A. fumigatus* and other molds are elevated and are correlated with the state of the disease (Knutsen *et al.*, 1994; Ojanen, 1992; Ojanen *et al.*, 1990). In addition, serum IgA antibodies to this organism are associated with exacerbations of bronchopulmonary aspergillosis along with elevated IgE, peripheral eosinophilia, and roentgenographic infiltrations (Apter *et al.*, 1989).

B. Serum IgA, IgM, IgG, and IgE Antibodies to Molds

IgA, IgM, IgG, and IgE antibodies to 7 different molds (*Alternaria, Aspergillus, Stachybotrys, Chaetomium, Cladosporium, Epicoccum*, and *Penicillium*), satratoxin H, and other trichothecenes in 40 patients with multiple organ symptoms were compared with 40 age- and sex-matched controls (Vojdani *et al.*, 2003). The exposed individuals occupied a water-damaged building and were tested within days following evacuation of the premises. Quantitative enzyme-linked immunosorbent assay (ELISA) produced the following results: (1) IgG antibodies to the molds and the two mycotoxins were significantly elevated in the patients versus the controls. (2) Levels of serum IgA antibodies for each mold and the mycotoxins were significantly elevated in the patients, with the exception of *Epicoccum*. The highest titers in descending order were found for *Stachybotrys, Penicillium*, and *Chaetomium*. (3) IgM titers were significantly elevated in these patients versus the controls for *Stachybotrys, Cladosporium, Alternaria, Aspergillus*, satratoxin H, and other trichothecenes. No difference in IgM titers were observed between patients and controls for *Chaetomium, Epicoccum*, and *Penicillium*. (4) With respect to IgE antibodies, a significant increase in titers in these patients was found only for *Aspergillus* and satratoxin H. It appears from these observations that randomly selected controls without symptoms and apparent mold exposure have low titers of antibodies to a variety of mold and mycotoxins. However, mold-exposed symptomatic individuals have titers that are significantly elevated over the control values.

In another study, Vojdani *et al.* (2003), utilizing ELISA assay procedures, tested for IgA, IgM, and IgG antibodies against *S. chartarum, A. niger, P. notatum*, satratoxin H, and other trichothecenes in the following three groups: healthy donors (N = 500); 500 patients referred to the laboratory for various diagnostic tests for illnesses without apparent exposure to molds (N = 500); and randomly selected patients referred for illness associated with exposure to molds (N = 500). The results of this study are summarized in Tables III through VI. Briefly, the concentration of IgA, IgM, and IgG antibody titers were lowest in the blood donors, intermediate in the randomly selected patients, and highest in the mold-exposed patients for each of the molds. With respect to satratoxin H and trichothecene antibodies, the antibody titers had a different distribution. When the mold-exposed patients were compared with the healthy controls, IgG and IgM titers were significantly elevated, while IgA titers were not. When the mold-exposed patients were compared with the random patients, only the IgG titers were significantly different. Moreover, on inspection of the data on the random patients, it was noted that the standard deviation (SD) was large and overlapped with the mean value and SD of the mold patients. It appears from these observations that the randomly selected patients may have been exposed to molds without recognition by the attending physician that such exposure might have occurred. Barnes *et al.* (2002) reached similar conclusions. They demonstrated IgE and IgG antibodies to *Stachybotrys chartarum* in 9.4% and 42.2% of the sera of 139 blood donors. They concluded that sensitivity to *S. chartarum* is potentially much more widespread than previously appreciated. This fungus may affect the asthmatic and allergic population through both immunologic and toxic mechanisms. The significance of the fungus in the milieu of allergenic fungi may need to be re-evaluated.

C. Cross-Reactivity of Antibodies to Molds

The use of antibodies to molds as a biomarker of exposure has been criticized (Musmand, 2003). The critique is based on two publications. One is an abstract the full results of which have never been published (Halsey *et al.*, 2001); therefore, it is impossible to determine anything about the methods used in this paper. The second is a position paper published on the Internet by the California Department of Public Services in which not a single experiment was conducted. Recently the question of cross-reactivity between mold antigens (*S. chartarum, A. niger, and P. notatum*) was investigated by using affinity-purified rabbit sera (Vojdani *et al.*, 2004). The results of this study showed that non-immunized rabbits

TABLE III

ANTIBODY LEVELS TO *PENICILLIUM NOTATUM*

Antibody	Healthy Controls N = 500	Mold Patients N = 500	Z Score	P Values	Random Patients N = 500	Mold Patients N = 500	Z Score	P Value
IgG	620 ± 535	2159 ± 2458	13.7	<0.001	1383 ± 1839	2159 ± 2458	5.6	<0.001
IgM	692 ± 551	1692 ± 2442	8.9	<0.001	1241 ± 1530	1692 ± 2442	3.5	<0.001
IgA	640 ± 572	1256 ± 2163	6.1	<0.001	853 ± 1070	1256 ± 2163	3.7	<0.001

Mean ± S.D. IgG, IgM, and IgA antibody levels in ELISA units to *Penicillium notatum* in controls, randomly selected patients and mold-exposed patients with Z test and P values.

TABLE IV

ANTIBODY LEVELS TO *ASPERGILLUS NIGER*

Antibody	Healthy Controls N = 500	Mold Patients N = 500	Z Score	P Values	Random Patients N = 500	Mold Patients N = 500	Z Score	P Values
IgG	618 ± 426	1795 ± 2316	11.1	<0.001	1349 ± 1417	1795 ± 2316	3.7	<0.001
IgM	782 ± 420	1725 ± 2449	8.5	<0.001	1177 ± 1302	1725 ± 2449	4.4	<0.001
IgA	732 ± 595	1346 ± 2456	5.4	<0.001	849 ± 938	1346 ± 2456	4.2	<0.001

Mean ± S.D. IgG, IgM, and IgA antibody levels in ELISA units to *Aspergillus niger* in controls, randomly selected patients and mold-exposed patients with Z test and P values.

TABLE V

ANTIBODY LEVELS TO *STACHYBOTRYS CHARTARUM*

Antibody	Healthy Controls N = 500	Mold Patients N = 500	Z Score	P Values	Random Patients N = 500	Mold Patients N = 500	Z Score	P Values
IgG	803 ± 530	2304 ± 2432	13.5	<0.001	973 ± 1234	2304 ± 2432	10.9	<0.001
IgM	629 ± 602	1940 ± 2478	11.5	<0.001	1115 ± 1212	1940 ± 2478	6.7	<0.001
IgA	665 ± 665	1511 ± 2660	6.9	<0.001	760 ± 1086	1511 ± 2660	5.8	<0.001

Mean ± S.D. IgG, IgM, and IgA antibody levels in ELISA units to *Stachybotrys chartarum* in controls, randomly selected patients and mold-exposed patients with Z test and P values.

TABLE VI

ANTIBODY LEVELS TO SATRATOXIN H

Antibody	Healthy Controls N = 500	Mold Patients N = 500	Z Score	P Values	Random Patients N = 500	Mold Patients N = 500	Z Score	P Values
IgG	767 ± 641	1523 ± 1352	11.3	<0.001	1054 ± 1147	1523 ± 1352	5.9	<0.001
IgM	611 ± 648	1320 ± 1590	9.2	<0.001	1160 ± 1170	1320 ± 1590	1.8	<0.060
IgA	715 ± 588	705 ± 868	2.1	<0.440	747 ± 819	705 ± 868	0.78	<0.430

Mean ± S.D. IgG, IgM, and IgA antibody levels in ELISA units to satratoxin H in controls, randomly selected patients and mold-exposed patients with Z test and P values.

develop IgG antibody titers to these molds that increase in concentration with age. The sera from these rabbits gave an impression of up to 52% cross-reaction with *Aspergillus, Penicillium*, and *Stachybotrys*. When using affinity-purified antibodies in cross-inhibition studies, the antigenic cross-reaction between *Stachybotrys* and *Aspergillus* was between 8.6% and 12.3%, and between *Stachybotrys* and *Penicillium* extracts it showed 9.3–9.6% antigenic similarities. Thus, for cross-reaction studies between different molds, affinity-purified antibodies and a sensitive and quantitative assay with natural antigens should be used. When using such an assay, it was concluded that cross-reactions between *Stachybotrys, Aspergillus*, and *Penicillium* exist but are much less widespread than previously believed. Based on these observations, antibodies to molds and mycotoxins as developed by this laboratory methodology are reliable biomarkers of mold and mycotoxin exposure.

D. Antibodies to Extracellular Polysaccharides (EPS)

EPS can cause type I and type III inflammatory processes. They have been shown to be present in mold-contaminated buildings and can be used as a marker of mold contamination and exposure (Duowes *et al.*, 1999; Wouters *et al.*, 2000). Exposure to 1–3 beta-D-glucan caused airway inflammation with symptoms of dry cough, phlegm, and hoarseness (Rylander, 1997; Rylander *et al.*, 1998). IgG antibodies in immunized rabbits against EPS from several mold genera have been reported (Notermans *et al.*, 1987, 1988). The EPS antigens caused the production of fairly specific antibodies, with some cross-reactivity as determined by an ELISA. The EPS antigens produced by species of *Penicillium, Aspergillus*, and *Geotrichum* lost their immunological activity with heating at 100 °C at pH 1.8. The EPS antigens from *Mucor recemosus, Rhizopus olgosporus*, and *C. cladosporoides* were stable under the same conditions. It appears from these data that an ELISA for antibodies to EPS released by various molds could be developed as an additional biomarker for mold exposure.

V. Alterations in T and B Cells, Natural Killer (NK) Cells, and Other Immune Parameters in Humans Exposed to Toxigenic Molds

A. Alterations in Percentage of T and B cells

Peripheral blood lymphocytes can be identified and quantified by using fluorescent antibodies to cell surface antigens. Typical markers for T cells are designated as CD2, CD3, CD4, and CD8. B cells are identified by CD19 or CD20. In addition, other markers can be used to

identify activation of T and B cells, (e.g., CD25, CD26, HLR-DR+, CD8CD11b+). Patients chronically ill from exposure to toxigenic molds in water-damaged office buildings, schools, and homes have altered percentages of lymphocyte markers in their peripheral blood when compared with expected ranges (Gray *et al.*, 2003). The patients had increased B cells (CD20) (75.6%). T cell activation markers increased for the following cell types: CD5CD25 (68.9%), CD3CD26 (91.2%), CD8HLR-DR+ (62%), and CD8CD38 (56.6%). Decreases were observed for CD8CD11b+ (15.6%) and natural killer (NK) cells (CD3CD16CD56, 38.5%). Moreover, Thrasher *et al.* (2004) found that individuals with an ongoing exposure to molds in a water-damaged building had relative increases over controls of the following: total lymphocyte count, T cells (CD2, CD3, CD4, CD8, and CD3CD16), B (CD19) cells, and NK cells (CD3CD16CD56).

B. Mitogen Activity

T and B cells respond to specific and nonspecific antigens by undergoing cell division (mitogenesis). Mitogenesis responses to nonspecific mitogens were as follows: phytohematogglutinin (PHA) was decreased by 26.2% in mold-exposed subjects, while only 5.9% had decreased response to Concanavalin A (ConA) (Gray *et al.*, 2003). PHA stimulates T cells, while Con A causes T and B cells to divide.

Mitotic responses to ConA, PHA, PWM (pokeweed mitogen), and LPS (lipopolysaccharides) were examined in patients with an ongoing exposure to toxigenic molds. In general, mitogenesis to PHA and Con A was significantly elevated over controls, indicating increased response of T cells to nonspecific antigens. In addition, mitogenic response to B cell stimulators (ConA, PWM, and LPS) was also significantly elevated. Although mitogenesis was increased, the patients could be subdivided into three distinct responses to each mitogen as follows: suppression, elevation, and extremely elevated (Thrasher *et al.*, 2004). Analysis of the NK cell (CD3CD16CD56) activity revealed that 42.4% of these patients had decreased killing of target cells. Furthermore, the CD4/CD8 (helper/suppressor) ratio was significantly elevated.

These two studies (Gray *et al.*, 2003; Thrasher *et al.*, 2004) indicated that alterations in the percentages of T and B cells, mitogenesis, and NK cell activity ccurred in mold-exposed humans. The alterations included an increase in activation markers, which may be a result of antigenic stimulation. Furthermore, the changes in mitogenic response to both nonspecific and specific mitogens indicate immune

suppression occurred in some individuals, while others experienced immune stimulation. The decrease in NK cells and their activity may indicate that there was a decrease in immune surveillance, which may have importance with respect to cancer and/or infectious diseases.

C. Autoantibodies

Autoantibodies directed against self-antigens are known to occur in a variety of autoimmune diseases and degenerative neurologic disorders. Antinuclear autoantibodies (ANA) are the ones most commonly recognized and are usually associated with connective tissue disease (e.g., lupus). However, other autoantibodies can be directed against a variety of self-antigens and can also be used as biomarkers of toxic exposure (Thrasher *et al.*, 2002; Vojdani *et al.*, 1992, 1993). Humans exposed to toxigenic molds have abnormally elevated autoantibodies to the following: ANA, anti-smooth muscle, peripheral, and central nervous system myelin and eight different neural antigens including myelin basic protein, ganglioside G1, sulfatide, tubulin, crystallin, filament, MOG, and MAG (Campbell *et al.*, 2003; Gray *et al.*, 2003). Odds ratios for each autoantibody at 95% C.I. was significant, showing an increased risk for autoimmunity. Autoantibodies and autoimmune diseases are recognized as occurring from toxic exposures (Cooper *et al.*, 2002; Griem *et al.*, 1998). For the significance regarding the neural antigen autoantibodies, see Neurological Abnormalities, Section VI.

D. Immune Complexes

Immune complexes occur when antigen and antibodies combine and have been implicated in numerous immunopathologic conditions, including systemic lupus erythematosus, rheumatoid arthritis, glomerulonephritis, and infectious induced inflammation (Abbas *et al.*, 1994). Deposition of immune complexes can occur from cell or tissue specific antibody-antigen reactions resulting in organ injury and/or immune complex diseases (Bigazzi *et al.*, 1986). Thus it would appear from these observations on increased immune complexes that inflammation and autoimmune reactions may exist in mold-exposed patients. Circulating immune complexes containing IgG, IgM, and IgA antibodies can generate a variety of substances associated with muscle damage and the acute phase response that can activate the classic pathway of complement (Sorensen *et al.*, 2003). Autoantibodies are also known to activate the complement system.

E. Concluding Remarks on Immunological Observations

The increase in B cells, activation markers, and helper/suppressor ratio all indicate immune activation has occurred as demonstrated by Gray *et al.* (2003) and Thrasher *et al.* (2004). Increased activation marker (CD45RO) has been reported for symptomatic children with exposure to molds in contaminated homes (Dales *et al.*, 1998). These observations are consistent with production of proinflammatory cytokines as discussed above with antigenic stimulation. In addition, elevated immune complexes are further support for immune activation and antigenic stimulation. The presence of elevated immune complexes is compatible with increased production of antibodies to mold antigens as well as the presence of ANA, anti-smooth muscle, and anti-neural antigen antibodies. The observations on immune alterations discussed above are also consistent with the suggestion that mold exposure causes immune dysregulation (Hirvonen *et al.*, 1999; Wichman, *et al.*, 2002). Recently a review by Anyanwu *et al.* (2003b) showed that natural killer cell activity was adversely affected in patients with chronic exposure to indoor molds and may be implicated in causing neurological abnormalities.

VI. Neurological Abnormalities

Neurological abnormalities caused by mycotoxins from molds have been described in the literature. The neurotoxic mycotoxins include trichothecenes, citreoviridin, patulin, fumonisin, penitrem, verruculogen, rubratoxin, ergot alkaloids, and tremorgens.

Wilson *et al.* were the first to isolate a tremorgenic mycotoxin in 1964. The mycotoxin penitrem has been shown to induce tremors and convulsions in experimental animals (Hayes, 1980). Jorntner *et al.* (1986) and Norris *et al.* (1980) studied the neurological effects of the mycotoxins penitrem A and verruculogen, which are known to cause a neurotoxicity characterized by sustained tremors. Their findings support a primary site of action of both of these mycotoxins as being presynaptic. Mycotoxins, being relatively nonpolar, pass through the blood-brain barrier and thereby have access to synapses. The neurotoxic effects of ergot alkaloids are known to affect the postganglionic parasympathetic synapses (Berde *et al.*, 1978).

Wang *et al.* (1998) in their study suggested that the primary site of trichothecene action is the brain. Chapman (2003) reported how trichothecene mycotoxins from *Stachybotrys* cause neurological disorders by being neurotoxic. The clinical signs of trichothecene mycotoxicosis include eye pain, dyspnea, tachycardia, vomiting, muscle tremors and

weakness, lack of coordination, and confusion. Patients affected develop seizures, central nervous system dysfunction, hypotension, and myelosuppression (Stahl *et al.*, 1985). Studies have shown that exposure to molds can cause optic demyelinating neuritis and multifocal choroiditis (Campbell *et al.*, 2003; Rudich *et al.*, 2003).

The nephrotoxic and hepatotoxic effects of mycotoxins have been well documented in several studies (Anyanwu *et al.*, 2003c; Bhat *et al.*, 1989; Etzel *et al.*, 1998). The mycotoxin rubratoxin was studied by Moss (1971) and was shown to cause liver and kidney damage and lesions of the central nervous system. Ciegler and Bennett (1980) stated that trichothecene mycotoxins cause clinical conditions that include skin irritations, vomiting, anorexia, diarrhea, hemorrhage, and convulsions.

Walsh *et al.* (1985) reviewed a large number of patients with necropsy-proven central nervous system aspergillosis and identified important epidemiological, pathological, and clinical features. In their study, the most common central nervous system lesions were subcortical hemorrhagic infarcts in the cerebral hemispheres or cerebellum, and they found that the most common entry of *Aspergillus* into the central nervous system was the lower respiratory tract. Aspergillosis of the central nervous system, lungs, and at least one other organ was found in almost 66% of the patients. Beal *et al.* (1982), in their neuropathological review, discovered that the pathologic hallmark of neurologic aspergillosis cases was the invasion of fungal hyphae into the blood vessel walls with subsequent necrosis and thrombosis and spread into the surrounding tissues.

A. Neurocognitive Deficits and Central Nervous System Dysfunction

Pena (1970) noted subtle personality changes were observed as an initial sign in cases of disseminated aspergillosis. Young *et al.* (1970) noted in their study of 13 patients with disseminated aspergillosis that all had some degree of lethargy or fatigue. Malkin *et al.* (1998) in their study at National Institute of Occupational Safety and Health reported multiple neurological symptoms in occupants of an office building contaminated by several species of fungi, including *Penicillium, Aspergillus, Alternaria, Candida, Cladosporium, Epicoccum, Fusarium*, and *Pullularia*. Gordon *et al.* (1993) described a patient who after being exposed to *Aspergillus, Penicillium*, and *Rhizopus* developed fatigue, headache, progressive somnolence, slowness of thinking, and severe tremors. The patient had coarse fasciculations of the muscles of the face and tongue and was unable to stand unassisted. His EEG showed a general dysrythmia consistent with a toxic encephalopathy.

Baldo *et al.* (2002) studied the neuropsychological performance of 10 patients exposed to molds (*Stachybotrys atra, Penicillium*, and *Aspergillus*). The patients had a variety of symptoms: fatigue, respiratory problems, recurring bloody noses, nausea, frequent sore throats, and headaches, among others. The mold-exposed patients were impaired on a number of cognitive measures, with the most consistent deficits in visuospatial learning, visuospatial memory, verb, learning, and psychomotor speed. In addition, the mold-exposed patients had evidence of Axis I and Axis II pathology. There was a significant correlation among patient's scores on the Beck Depression Inventory, with a number of neuropsychological tests falling within the impaired range. The authors put forth a model by which to investigate the effects of mold exposure on the central nervous system.

Crago *et al.* (2003) further demonstrated that measures of exposure were highly predictive of neuropsychological test performance using two subtests from the Delis–Kaplan Executive Function System (D–KEFS) to measure executive or higher-level cognitive functions. Significant predictive power was observed for the D–KEFS Trail Making subtests of visual scanning, letter sequencing, number–letter sequencing, and motor speed; the D–KEFS Color–Word Inhibition/Switching subtest; the WAIS-III Digit Symbol Coding and Symbol Search subtests; and the IVA-CPT full-scale attention quotient and the visual and auditory attention quotients. Crago *et al.* (2003) also reported that significant predictive power was found for estimates of degree of exposure and for the QEEG variables of mean frequency delta, relative power theta, relative power alpha, absolute power delta, absolute power theta, and absolute power alpha. In addition, the QEEG findings in confirmed mold-exposed patients indicated a restriction in the range of functioning (narrowed frequency bands) of the frontal lobes, that is, increased (accelerated) mean frequency of the slower frequencies (delta range) and decreased (slowed) higher frequencies (beta range), indicating a collapse toward the middle of the frequency spectrum. These findings, coupled with observed increased levels of absolute and relative power theta and alpha waves in frontal sites, indicated hypoactivation of the frontal cortex consistent with insufficient excitatory input from the reticular activating system anatomically seated in the midbrain.

Finally, Kilburn (2002) reported on both objective neurological tests and neuropsychological evaluation of 20 mold-exposed patients. Objective tests showed impaired balance, reaction time, color discrimination, and visual fields in the mold-exposed patients. Neuropsychological tests showed impaired cognition, verbal recall, and trail making. Pulmonary function testing showed small airway obstruction was observed in 4

patients. Longer durations of exposure and aging appeared to increase the total abnormalities. He concluded ''Moulds appear to cause chemical encephalopathy and these abnormalities.''

Neurophysiological effects of mold exposure have been reported in children as compared with controls (Anyanwu *et al.*, 2003a). Brainstem auditory evoked response (BAER), electroencephalogram (EEG), visual evoked potential (VEP), and somatosensory evoked potential (SSEP) were used to test neurological abnormalities. Three of 10 children had an abnormal EEG following mold exposure. The frontal-temporal theta wave activity in the 10 patients seemed to indicate diffuse changes consistent with metabolic encephalopathies. Also, 1 to 3 hertz delta activity was asymmetric in the right hemisphere of 3 patients. BAER showed abnormalities in 9 patients with both 15′ and 35″ check sizes. A significant delay in waveform V occurred in the majority of patients, representing dysfunctional cognitive process and conductive hearing loss in both ears. VEP showed clear abnormalities in 4 of the children with P100 amplitudes and latencies decreased bilaterally. SSEP showed diffuse polyneuropathy in three patients. The authors concluded that exposure to toxic molds can affect neurological and behavioral status of children.

B. Peripheral Motor and Sensory Neuropathy

Campbell *et al.* (2003) studied 119 patients with symptoms of neurotoxicity with documented measured exposure to molds. These patients complained of fatigue, memory loss, cognitive function loss, headaches, tremors, numbness and tingling, blurred vision, tinnitus, and muscle weakness. Ninety-nine of these patients had abnormal clinical neurological examinations, abnormal findings on neurophysiological testing, and elevated antibodies to neuronal antigens. Nerve conduction studies (NCVs) revealed three groups of abnormal patients (ABM) and one group of normal (NM): mixed sensory motor polyneuropathy (55 ABN); motor neuropathy (17 ABN); sensory neuropathy (27 ABN); and symptoms without neurophysiological abnormalities (20 NM, controls).

C. Neuronal Antibodies

Elevated autoantibodies by ELISA to several neuronal antigens were found in patients with documented measured exposure to molds. The titers of the autoantibodies were significantly elevated over controls. These included IgA, IgG, and IgM antibodies to myelin basic protein, myelin associated glycoprotein, oligodendrocyte glycoprotein, ganglioside GM-1, chondroitin sulfate, crystalline, tubulin, and neurofilament.

D. Demyelination of Peripheral Nerves

Campbell *et al.* (2003) concluded their observations on changes in nerve conduction velocities and the presence of neural antigen autoantibodies as follows: ''The increased latency for motor and sensory nerves observed in the 55 patients with mixed neuropathy is suggestive of a demyelinating process (Busby *et al.*, 2003).'' This was accompanied by a decrease in velocities for the median, ulnar, and peroneal nerves while the tibial nerve had a decrease in the amplitude. All three sensory nerves (median, ulnar, and superficial peroneal) exhibited increased latencies and decreased amplitudes. Thus the polyneuropathy observed in these patients appeared to be a demyelinating process with decreased number and size of fibers (decreased amplitude) and chronic involvement of the nerve (decreased velocities) (Busby *et al.*, 2003; Steck *et al.*, 1987). The motor neuropathies (17 patients) had decreases in latencies (peroneal and tibial nerves), decreased amplitudes (median and peroneal nerves), and decreased velocities (median, ulnar, peroneal, and tibial nerves). This appeared to be a diffuse neuropathy and may involve some demyelination (Berger *et al.*, 2003). Finally, the sensory neuropathies (27 patients) had increased latencies for all three nerves, with that of the superficial peroneal being not significant. The increased latencies and the decreased amplitude of the superficial peroneal suggested demyelination was occurring (Reindl *et al.*, 1999; Willison and Yuki, 2002).

VII. Conclusion

Forgacs noted in 1962 that mold mycotoxicosis was called ''the neglected disease.'' The manifestations and disorders in humans caused by molds and mycotoxins continues to be overlooked or unnoticed by many physicians. Each year studies continue to be published throughout the world medical and scientific literature elucidating and explaining the pathological processes and biomechanisms by which exposure to molds and mycotoxins cause sickness in humans. We have described in this chapter the most recent neuroimmune mechanisms of disease process in humans of molds and mycotoxins. The exact biological and chemical actions through which these mechanisms unfold is not completely understood. However, molds do produce metabolites (mycotoxins, solvents) and shed antigenic materials (spores, hyphae, extracellular polysaccharides, and enzymes), which are toxic (mycotoxins) and or cause immunologic responses (antigens). Science and medicine should continue its work in these areas and bring about the much-needed change from ''the neglected disease'' to ''the accepted disease.''

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