

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

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 polysaccharides (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

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infants (Burge, 1990; genic byproducts of 1986; Johanning *et al.*, Tuomi *et al.*, 1998, extracellular poly- *et al.*, 1988; Wouters *et al.*, 2002), and mycotoxins, ochratoxin A identified in vent- occupants have experi- re (Croft *et al.*, 1986; *et al.*, 2002; Nieminen 1993; Tuomi *et al.*, repeated episodes of mycotoxin production, spores and hyphal

the organ symptoms respiratory system, gastrointestinal tract, the musculoskeletal system (Gunnbjornsdottir 1998; Jaakkola *et al.*, 2000). All of the following: 2002; Eucker *et al.*, rhinosinusitis (Braun 2003), (3) IgE- 2002; Lander *et al.*, bronchitis and related asthma (Kanen *et al.*, 1999; 2004), (5) cytotoxicity (Nagata *et al.*, 2001; immunomodulation (Berek *et al.*, 2001), (7) mitochondrial

toxicity (Hoehler, *et al.*, 1997; Niranjana *et al.*, 1982; Pace, 1983, 1988; Sajana *et al.*, 1997; Wei *et al.*, 1984), (8) carcinogenicity (Dominguez-Malagon and Gaytan-Graham, 2001; Schwartz, 2002), (9) nephrotoxicity (Anyanwu *et al.*, 2003c; Pfohl-Leschkowicz *et al.*, 2002), (10) the formation of nuclear and mitochondrial DNA adducts (Hsieh and Hsieh, 1993; Petkova-Bochatrova *et al.*, 1998; Pfohl-Leschkowicz *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 (*brevi-compactum*, *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 globosum*, *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

Mold
<i>Stachybotrys chartarum</i>
<i>Alternaria tenuissim</i>
<i>Aspergillus flavus</i>
<i>Aspergillus fumigatus</i>
<i>Aspergillus</i>
<i>Aspergillus ochraceus</i>
<i>Aspergillus</i>
<i>Aspergillus versicolor</i>
<i>Penicillium chrysogenum</i>
<i>Chaetomium globosum</i>
<i>Memnoniella echinata</i>
<i>Penicillium</i>
<i>Penicillium</i>
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<i>Penicillium</i>
<i>Trichoderma</i>
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MOLDS

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TABLE I
TOXIGENIC MOLDS IN WATER-DAMAGED BUILDINGS

Mold	Metabolites	Health concern
<i>Stachybotrys chartarum</i>	Spirocyclic drimanes; satratoxins G, H and F; hydroxyrordin E, verrucarins J; trichodermin; dolabellanes; atrones B and C; stachyotryamide; stachyotrylactams; stachylisin	Pulmonary hemosiderosis; Induces proinflammatory cytokines
<i>Alternaria tenuissima</i>	Alternariols; tentoxin; tenuazonic acids; altertoxin I	Unknown
<i>Aspergillus flavus</i>	Aflatoxin B ₁ ; kojic acid; aspergillilic acid; 3-nitropropionic acid; cyclopiazonic acid	Carcinogenesis; aspergillosis
<i>Aspergillus fumigatus</i>	Fumigaclavines; fumitoxins; fumitremorgens; gliotoxins; tryptotoxins; verruculogen	Tremors and CNS injury; Immune damage by gliotoxin; aspergillosis
<i>Aspergillus niger</i>	Ochratoxin A	Nephropathy
<i>Aspergillus ochraceus</i>	Ochratoxin A, penicillic acid; xanthomegnin; viomellein, vioxanthin	Nephropathy
<i>Aspergillus ustus</i>	Kotanins	Unknown
<i>Aspergillus versicolor</i>	Sterigmatocystin; 5-methoxy-sterigmatocystin	Carcinogenesis; aspergillosis
<i>Penicillium chrysogenum</i>	Secalonic acid D	Unknown
<i>Chaetomium globosum</i>	Chaetomins; chaetoglobosins A and C	Cytotoxicity; inhibition of cell division
<i>Memnoniella echinata</i>	Griseofulvin; dechlorogriseofulvins; trichodermin; trichodermol	Unknown
<i>Penicillium brevicompactum</i>	Mycophenolic acid; botryodiplodin	Toxic (mutagenic)
<i>Penicillium expansum</i>	Patulin; citrinin; chaetoglobosin; Roquefortine C	Immune toxicity, cytotoxic; tremorgenic
<i>Penicillium polonicum</i>	Verrucosidins; penicillic acid; nephrotoxic glycopeptides	Tremors; cytotoxicity; nephropathy
<i>Trichoderma species</i>	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 $\leq 0.03 \mu\text{m}$ 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 (Pfihl-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,

Symptom
Excessive Fatigue
Headache
Nasal Symptom
Memory Problem
Spaciness
Sinus Discomfort
Coughing
Watery Eyes
Throat Discomfort
Slurred Speech
Lightheadedness
Joint Discomfort
Dizziness
Weakness
Bloating
Insomnia
Weak Voice
Spasms
Coordination Problems
Visual Changes
Rash
Numbness
Cold Intolerance
Heat Intolerance
Chest Tightness
Chest Discomfort
Urine Frequency
Excessive Thirst
ringing Ears
Wheezing
Swallowing Problems
Flushing Skin
Bladder Control
Rapid Pulse

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

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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).

^aThe 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.*,

Controls N = 28	P value
4 = 0.8	NS
4 = 0.9	NS
6 = 1.5	NS
6 = 1.5	NS

frequently reported symptoms in patients filled out questionnaires.
 = symptom of patients vs controls

females had significantly greater
 = Results section).

ziness, blurred vision, respiratory tract (nasal respiratory tract (cough, and irritation of the fatigue, weakness, joint 2003; Gray *et al.*, 2003). cases in the incidence of individuals ill from mold *et al.*, 1998; Johanning 3). Vojdani *et al.* (2003) significant increases in toms of severe allergies. are to multiple toxigenic increased incidence of iduals.

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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 FEV₁. The importance of these measurements arises from the fact that during disease states, (e.g., asthma), the FVC may be normal while the FEV₁ 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

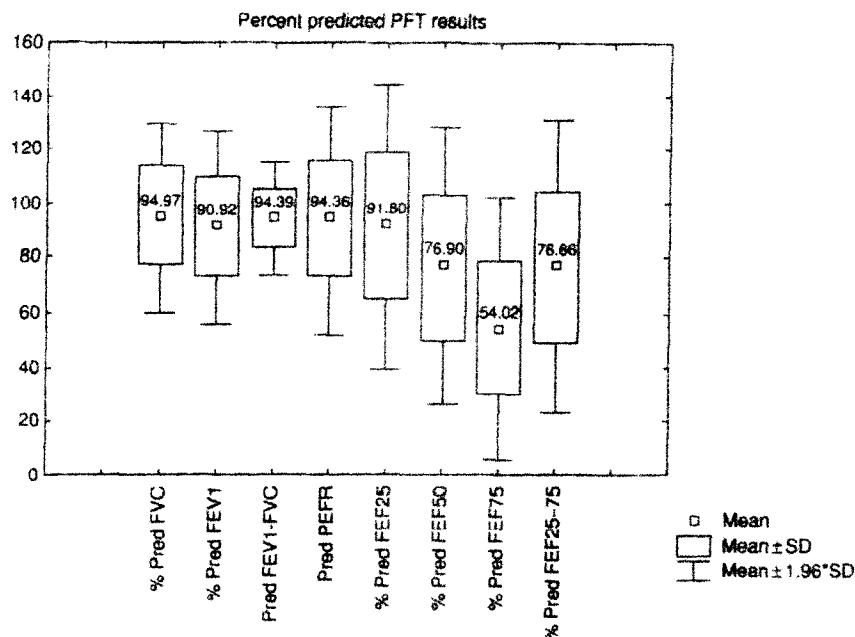


Figure 1. The results of PFT testing on individuals exposed to molds in water-damage structures.

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).

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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 microorganisms and toxins to epithelial

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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.

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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.

Antibody	N = 500	N = 500	Score	Values	N = 500	N = 500	Score	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 oligosporus*, 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 Toxicogenic 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: phytohemagglutinin (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 occurred 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 dysrhythmia 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 (ABN) 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."

REFERENCES

- Abbas, A. K., Lichtman, A. H., and Pober, J. S. (1994). In *Cellular and Molecular Immunology*, 2nd ed., p. 393. WB Saunders and Company, Philadelphia.
- Anaissie, E. J., Stratton, S. L., Dignani, M. C., Summerbell, R. C., Rex, J. H., Monson, T. P., and Walsh, T. J. (2002). Pathogenic *Aspergillus* species recovered from a hospital water system: 3-year prospective study. *Clin. Infect. Dis.* **34**, 780–789.
- Andersson, M. A., Nikulin, M., Kooljalg, U., Anderson, M. C., Rainey, K., Reuula, K., Hintikka, E. L., and Salkinoja-Salonen, M. (1997). Bacteria, molds, and toxins in water-damaged building materials. *Appl. Environ. Microbiol.* **63**, 387–393.
- Apter, A. J., Greenberger, P. A., Liotta, J. L., and Roberts, M. (1989). Fluctuations of serum IgA and its subclasses in allergic bronchopulmonary aspergillosis. *J. Allergy Clin. Immunol.* **84**, 367–372.
- Anyanwu, E. C., Campbell, A. W., and Vojdani, A. (2003a). Neurophysiological effects of chronic indoor environmental exposure on children. *Scientific World Journal* **3**, 281–290.
- Anyanwu, E. C., Campbell, A. W., Jones, J., and Ehiri, J. (2003b). The neurological significance of abnormal natural killer cell activity in chronic toxigenic mold exposures. *Scientific World Journal* **3**, 1128–1137.
- Anyanwu, E. C., Campbell, A. W., Vojdani, A., and Ehiri, J. (2003c). Biochemical changes in the serum of patients with chronic toxigenic mold exposures: a risk factor for multiple renal dysfunctions. *Scientific World Journal* **3**, 1058–1064.
- Baldo, J., Ahmad, L., and Ruff, R. (2002). Neuropsychological performance of patients following mold exposure. *App. Neuropsych.* **9**, 193–202.
- Barnes, C., Buckley, S., Pacheco, F., and Portnoy, J. (2002). IgE-reactive proteins from *Stachybotrys chartarum*. *Ann. Allergy Asthma Immunol.* **90**, 29–33.
- Berger, T., Rubner, P., Schautzer, F., Egg, R., Ulmer, H., Mayringer, I., Dilitz, E., Deisenhammer, F., and Reindl, M. (2003). Antimyelin antibodies as a predictor of clinically definite multiple sclerosis after a first demyelinating event. *N. Engl. J. Med.* **349**, 139–45.
- Bhat, R., Beedu, S., Ramakrishna, Y., and Munshi, K. L. (1989). Outbreak of trichothecene mycotoxicosis associated with the consumption of mold damaged wheat products in Kashmir Valley, India. *Lancet* **1**, 35–37.
- Bigazzi, P. E., Burek, C. L., and Rosen, R. (1986). Antibodies to tissue-specific endocrine, gastrointestinal and neurological antigens. In *Manual of Clinical Laboratory Immunology* (N. R. Rose, H. Friedman, and J. L. Fahey, eds.), p. 762. American Society of Microbiology, Washington D.C.
- Beal, M. F., O'Carroll, C. P., Kleinman, G. M., and Grossman, R. I. (1982). Aspergillosis of the nervous system. *Neurol.* **32**, 473–479.
- Berde, B., and Schild, H. (eds.) (1978). *Ergot alkaloids and related compounds*. Springer, New York.
- Berek, L., Petri, I. B., Mesterhazy, A., Teren, H., and Molnar, J. (2001). Effects of mycotoxins on human immune functions in vitro. *Toxicol In Vitro.* **15**, 25–30.
- Bondy, G. S., and Petska, J. J. (2000). Immunomodulation by fungal toxins. *J. Toxicol Environ. Health B Crit. Rev.* **3**, 109–143.
- Braun, H., Buzina, W., Freudenschuss, K., Beham, A., and Stammberger, H. (2003). "Eosinophilic fungal rhinosinusitis": A common disorder in Europe? *Laryngoscope* **114**, 264–269.
- Burge, H. A. (1990). Bioaerosols: prevalence and health effects in the indoor environment. *J. Allergy Clin. Immunol.* **85**, 687–704.

- Busby, M., and Donaghy, M. (2003). Chronic dysimmune neuropathy. A subclassification based upon the clinical feature of 102 patients. *J. Neurol.* **250**, 714–724.
- California Department of Public Services Environmental Health Investigations Branch (2000). Misinterpretation of *Stachybotrys* serology. Available at: <http://www.dhs.ca.gov/ehib/ehib2/topics/Serologyf2.htm>.
- Campbell, A. W., Thrasher, J. D., Madison, R. A., Vojdani, A., and Gray, M. R. (2003). Neural antigen autoantibodies and neurophysiology abnormalities in patients exposed to moulds. *Arch. Environ. Health* **58** (In press).
- Campbell, A. W., Anyanwu, E. C., and Vojdani, A. (2003). Combination of high-dose intravenous immunoglobulins and itraconazole in treating chronic mycotic demyelinating optic neuritis. *Scientific World Journal* **3**, 640–646.
- Ciegler, A., and Bennett, J. W. (1980). Mycotoxins and mycotoxicosis. *Bioscience* **30**, 512–515.
- Challancombe, S. J. (1987). The induction of secretory IgA responses. In "Food allergy and Intolerance" (J. Brostoff and S. J. Challancombe, eds.). WB Saunders, Easturgn, East Sussex, England.
- Chapman, J. (2003). *Stachybotrys (chartarum=atro=alternans)* and other problems caused by allergenic fungi. *Aller. and Asthm. Proc.* **24**, 1–7.
- Claeson, A. S., Levin, H., Blomquist, G., and Sunesson, A. L. (2002). Volatile metabolites from microorganisms grown on humid building materials and synthetic media. *J. Environ. Monit.* **4**, 667–672.
- Cooper, G. S., Miller, F. W., and Germolec, D. R. (2002). Occupational exposures and autoimmune diseases. *Inter. Immunopharmacology* **2**, 303–313.
- Crago, B. R., Gray, M. R., Nelson, L. A., Davis, M., Arnold, L., and Thrasher, J. D. (2003). Neuropsychological and electrocortical effects of mixed mold exposure. *Archives Environ. Hlth.* **549**–552.
- Dales, R., Miller, D., White, J., Dulberg, C., and Lazarovits, A. I. (1998). Influence of residential fungal contamination on peripheral blood lymphocyte populations on children. *Arch. Environ. Health* **53**, 190–195.
- Desai, K., Sullards, M. C., Allegood, J., Wang, E., Schemlitz, E. M., Harl, M., Humpf, H. U., Liotta, D. C., Peng, Q., and Merrill, A. H. (2002). Fumonisin and fumonisin analogs as inhibitors of ceramide synthase and inducers of apoptosis. *Biochim. Biophys. Acta* **1585**, 188–192.
- Dominguez-Malagon, H., and Gaytan-Graham, S. (2001). Hepatocellular carcinoma: an update. *Ultrastruct. Pathol.* **25**, 497–516.
- Dosa, E., Coczi, I., Mojzes, L., Molnar, E. G., Varga, H. M., and Nagy, E. (2002). Identification and incidence of fungal strains in chronic rhinosinusitis patients. *Acta Microbiol. Immunol. Hung.* **49**, 3337–3346.
- Duowes, J., van der Sluis, B., Dockes, G., van Leusden, F., Wijnands, L., van Strien, R., Verhoff, A., and Brunerkeef, B. (1999). Fungal extracellular polysaccharides in house dust as a marker for exposure to fungi: Relations with culturable fungi, reported home dampness, and respiratory symptoms. *J. Allergy Clin. Immunol.* **103**, 494–500.
- Ebina, K., Ichinowatari, S., and Yokota, K. (1985). Studies on toxin *Aspergillus fumigatus*. XXII. Fashion of binding Asp-hemolysin to human erythrocytes and Asp-Hemolysin-binding proteins of erythrocyte membranes. *Microbiol. Immunol.* **29**, 91–101.
- Engelhart, S., Looock, A., Skutlarck, D., Sagunski, H., Lommel, A., Farver, H., and Exner, M. (2003). Occurrence of toxigenic *Aspergillus versicolor* isolates and sterigmatocystin in carpet dust from damp indoor environments. *Appl. Environ. Microbiol.* **68**, 3886–3890.

- Erkinjuntti-Pekkanen, R., Reiman, M., Kokkarinen, J. L., Tukinnen, H. O., and Terho, E. O. (1999). IgG antibodies, chronic bronchitis, and pulmonary function values in farmer's lung patients and matched controls. *Allergy* **54**, 1181-1187.
- Etzel, R. A., Balk, S. J., and Bearer, C. F. (1998). Toxic effects of indoor molds. *Pediatr.* **101**, 712-714.
- Eucker, J., Sezer, O., Graf, B., and Possinger, K. (2001). Mucormycoses. *Mycoses* **44**, 254-260.
- Ezeamuzie, C. I., Al-Ali, S., Kahn, M., Kahn, Z., Dowaisan, A., Thomson, M. S., and Georgi, J. (2000). IgE-mediated sensitization to mould allergens among patients with allergic respiratory diseases in a desert environment. *Int. Arch. Allergy. Immunol.* **121**, 300-307.
- Fan, L. L. (2002). Hypersensitivity pneumonitis in children. *Curr. Opin. Pediatr.* **14**, 323-326.
- Flannigan, B., McCabe, E. M., and McGarry, F. (1991). Allergenic and toxigenic microorganisms in houses. *J. Appl. Bact. Sym* **70**(suppl), 61-73.
- Forgacs, J. (1962). *Mycotoxins: The neglected diseases, Feedstuffs*, pp. 34, 124.
- Fraser, R. S. (1993). Pulmonary aspergillosis: Pathologic and pathogenetic features. *Pathol. Annu.* **28**, 231-277.
- Gareis, M. (1995). Cytotoxicity testing of samples originating from problem buildings. In "Proceedings of the International Conference: Fungi and Bacteria in Indoor Environments: Health Effects, Detection and Remediation" (Eckart Johanning, S. Chin, and Yang, eds.), pp. 139-144. Saratoga Springs, NY.
- Gordon, K. E., Masotti, R. E., and Waddell, W. R. (1993). Tremorgenic encephalopathy: A role of mycotoxins in the production of CNS disease in humans. *Can. J. Neurol. Sci.* **20**, 237-239.
- Gorney, R. L., Reponen, T., Willeke, K., Schmechel, D., Robine, E., Boissier, M., and Grinshpun, S. A. (2002). Fungal fragments as indoor air contaminants. *Appl. Environ. Microbiol.* **68**, 3522-3551.
- Gravesen, S., Nielsen, P. A., Iverson, R., and Nielsen, K. F. (1999). Microfungal contamination of damp buildings—examples of constructions and risk materials. *Environ. Hlth. Perspect.* **107**(suppl. 3), 505-508.
- Gray, M. R., Thrasher, J. D., Crago, R., Madison, R. A., Campbell, A. W., and Vojdani, A. (2003). Mixed mold exposure: Immunological changes in humans with exposure in water damaged buildings. *Archives Environ. Hlth.* **58**, 410-420.
- Gregory, L., Rand, T. G., Dearborn, D., Yike, A., and Vesper, S. (2003). Immunocytochemical localization of stachylysin in *Stachybotrys chartarum* spores and spore-impacted mouse and rat lung tissue. *Mycopathologia* **156**, 109-117.
- Griem, P., Wulferink, M., Stachs, B., Gonzalez, J. B., and Gleichmann, E. (1998). Allergic and autoimmune reactions to xenobiotics: How do they arise? *Immunol. Today* **134**, 133-141.
- Grossi, P., Farina, C., Fiocchi, R., and Della Gasperina, D. (2000). Prevalence and outcome of invasive fungal infections in 1,963 thoracic organ transplant recipients: a multi-center retrospective study. Italian study group of fungal infections in thoracic organ transplant recipients. *Transplantation* **70**, 112-116.
- Gunnbjornsdottir, M. I., Norback, D., Plaschke, P., Norman, E., Bjornson, E., and Janson, C. (2003). The relationship between indicators of building dampness and respiratory health in young Swedish adults. *Respir. Med.* **97**, 301-308.
- Halsey, J. F., Jensen, J. T., and Miller, J. D. (2001). Immunological responses to *Stachybotrys chartarum* antigen. *J. Allergy Clin. Immunol.* **107A**1034.
- Hayes, W. (1980). Mycotoxins: A review of biological effects and their role in human diseases. *Clin. Toxicology* **17**, 45-83.

- Hirvonen, M.-R., Ruotsalainen, M., Roponen, M., Hyvarinen, A., Husman, T., Kusma, V. M., Komulainen, H., Savolainen, K., and Nevalainen, A. (1999). Nitric oxide and proinflammatory cytokines in nasal lavage fluid associated with symptoms and exposure to mold building microbes. *Resp. Crit. Care Med.* **160**, 1943-1946.
- Hodgson, M. J., Morey, P., Leung, W. Y., Morrow, L., Miller, D., Jarvis, B., Robbins, H., Halsey, J. F., and Storey, E. (1998). Building-associated pulmonary disease from exposure to *Stachybotrys chartarum* and *Aspergillus versicolor*. *J. Occup. Environ. Med.* **40**, 241-249.
- Hsieh, L. L., and Hsieh, T. T. (1993). Detection of aflatoxin B1-DNA adducts in human placenta and cord blood. *Cancer Res.* **53**, 1278-1280.
- Hoehler, D., Marquardt, R. R., McIntosh, A. R., and Hatch, G. M. (1997). Induction of free radicals in hepatocytes, mitochondria and microsomes of rats by ochratoxin A and its analogs. *Biochim. Biophys. Acta* **1357**, 225-233.
- Jaakkola, M., Nordman, H., Pilpari, R., Uitti, J., Laitinen, J., Karajainen, A., Hahtola, P., and Jaakkola, J. J. (2002). Indoor dampness and molds and development of adult-onset asthma: A population-based incident case-control study. *Envir. Hlth. Persp.* **110**, 543-547.
- Jakab, G. J., Hmielecki, R. R., Hemenway, D. R., and Groopman, J. D. (1994). Respiratory aflatoxicosis: suppression of pulmonary and systemic host defenses in rats and mice. *Toxicol. Applied Pharm.* **125**, 198-205.
- Jarvis, B. B. (2002). Chemistry and toxicology of molds isolated from water-damaged buildings. In "Mycotoxins and Food Safety" (J. W. DeVries, M. W. Trucksess, and L. S. Jackson, eds.), pp. 43-52. Kluwer Academic/Plenum Publishers.
- Jarvis, B. B., Sorenson, W. G., Hintikka, E.-L., Nikulin, M., Zhou, Y., Jiang, J., Wang, S., Hinkley, S., Etzel, R. A., and Dearborn, D. (1998). Study of toxin production by isolates of *Stachybotrys chartarum* and *Memniella echinata* isolated during a study of pulmonary hemosiderosis in infants. *App. Environ. Microbiol.* **64**, 3620-3625.
- Jones, C., Ciacci-Zanella, J. R., Zhang, V., Hendeson, G., and Dickman, M. (2002). Analysis of fumonisin B1-induced apoptosis. *Environ. Health Persp.* **109**(suppl 2), 315-320.
- Jornrner, B. S., Erich, M., Katherman, A. E., Huckle, W. R., and Carter, M. E. (1986). Effects of prolonged tremor due to penitrem A in mice. *Drug. Chem. Toxicol.* **9**, 101-116.
- Johanning, E., Biagini, R., Hull, D.-L., Morey, P., Jarvis, B., and Landsbergis, P. (1996). Health and immunology study following exposure to toxigenic fungi (*Stachybotrys chartarum*) in a water-damaged office environment. *Int. Arch. Occup. Environ. Hlth.* **68**, 207-218.
- Johanning, E., Gareis, M., Nielsen, K. F., Dietrich, R., and Martbauer, E. (2002). Airborne mycotoxins sampling and screening analysis. In (H. Levin, G. Bendy, and J. Cordell, eds.), Indoor Air 2002, The 9th International Conference on Indoor Air Quality and Climate, Monterey, CA, June 30-July 5, 2002, Vol 5, pp. 1-6. The International Academy of Indoor Air Sciences, Santa Cruz.
- Karlsson-Borga, A., Jonsson, P., and Rolfsen, W. (1989). Specific IgE antibody to 16 widespread mold genera in patients with suspected mold allergy. *Ann. Allergy* **63**, 521-526.
- Kildeso, J., Wurtz, V. M., Nielsen, K. F., Wilkins, C. K., Gravesen, S., Nielsen, P. A., Thrane, U., and Schneider, T. (2000). The release of fungal spores from water damaged building materials. In (O. Seppanen and J. Sateri, eds.), Proceedings of Healthy Buildings 2000, August 6-10, Espoo Finland. SYI Indoor Air Information Oy, Helsinki, pp. 131-138.
- Kilburn, K. H. (2002). Inhalation of molds and mycotoxins. *Eur. J. Oncol.* **7**, 197-202.

- Knutsen, A. P., Mueller, K. R., Hutcheson, P. S., and Slavin, R. G. (1994). Serum anti-*Aspergillus fumigatus* antibody immunoblot and ELISA in cystic fibrosis with allergic bronchopulmonary aspergillosis. *J. Clin. Immunol.* **93**, 926-931.
- Kudo, Y., Ootani, T., Kumagai, T., Fukuchi, Y., Ebina, K., and Yokota, K. (2002). A novel oxidized low-density lipoprotein-binding protein. *Asp-hemolysin* recognizes lysophosphatidylcholine. *Biol. Pharm. Bull.* **25**, 787-790.
- Kordula, T., Banbula, A., Macomson, J., and Ravis, J. (2002). Isolation and properties of Stachyrase A, a chymotrypsin-like serine proteinase from *Stachybotrys chartarum*. *Infection Immun.* **70**, 419-421.
- Kurup, V., Shen, H.-D., and Banerjee, B. (2000). Respiratory fungal allergy. *Microbes Inf.* **2**, 1101-1110.
- Lander, F., Meyer, H. W., and Norm, S. (2001). Serum IgE specific to moulds, measured by basophil histamine release, is associated with building-related symptoms in damp buildings. *Inflamm. Res.* **50**, 227-231.
- Leino, M., Makela, M., Reijula, K., Haahtela, T., Rauhamaa, H., Tuomi, T., Hintikka, E. L., and Alenius, H. (2004). Intranasal exposure to a damp building mould, *Stachybotrys chartarum*, induces lung inflammation in mice by satratoxin-independent mechanisms. *Clin. Exper. Allergy* **33**.
- Makarananda, K., Pengpan, U., Sriskulthong, M., Yoovathaworn, K., and Sriwatanakul, K. (1999). Monitoring of aflatoxin exposure by biomarkers. *J. Toxicol. Sci.* **23**(suppl 2), 155-159.
- Malkin, R., Martinez, K., Marinkovich, V., Wilcox, T., Wall, D., and Biagini, R. (1998). The relationship between symptoms and IgG and IgE antibodies in an office environment. *Env. Resear.* **76**, 85-93.
- Mason, C. D., Rand, T. G., Oulton, M., MacDonald, J. M., and Scott, J. E. (1998). Effects of *Stachybotrys chartarum* (atra) conidia and isolated toxin on lung surfactant production and homeostasis. *Nat. Toxins* **6**, 27-33.
- Mason, C. D., Rand, T. G., Oulton, M., MacDonald, J. M., and Anthes, M. (2001). Effects of *Stachybotrys chartarum* on surfactant convertase activity in juvenile mice. *Toxicol. Appl. Pharm.* **172**, 21-28.
- McCrae, K. C., Rand, T., Shaw, R. A., Mason, C. D., Oulton, M. R., Hasting, C., Cherlet, T., Thliveris, J. A., Mantsch, H. H., MacDonald, J. M., and Scott, J. E. (2001). Analysis of pulmonary surfactants by Fourier-transform infrared spectroscopy following exposure to *Stachybotrys chartarum* (atra) spores. *Chem. Phys. Lipids* **110**, 1-10.
- Monod, M., Capoccia, S., Lechenne, B., Zaugg, C., Holdom, M., and Jouddon, O. (2002). Secreted proteases from pathogenic fungi. *Med. Microbiol.* **292**, 205-419.
- Musmand, J. (2003). Dose *Stachybotrys* actually cause adverse effects? *Ann. Allergy Asthma Immunol.* **90**, 274-275.
- Nagata, T., Suzuki, H., Ishigami, N., Dhinozuka, J., Uetsuka, K., Nakayama, H., and Doi, K. (2001). Development of apoptosis and changes in lymphocyte subsets in thymus, mesenteric lymph nodes and Peyer's patches of mice orally inoculate with T-2 toxin. *Exp. Toxicol. Path.* **52**, 3309-3315.
- Nielsen, K. F. (2003). Mycotoxin production by indoor molds. *Fungal Genetics Biol.* **39**, 103-117.
- Nielsen, K. F., Gravesen, S., and Neilsen, P. A. (1999). Production of mycotoxins on artificially and naturally infested building materials. *Mycopathologia* **145**, 43-56.
- Nieminen, S. M., Karki, R., Auriola, S., Toivola, M., Laatsch, H., Laatikainen, F., Hyvarinen, A., and Von Wright, A. (2002). Isolation and identification of *Aspergillus fumigatus* mycotoxins on growth medium and some building materials. *Microbiology* **68**, 4871-4875.

- Niranjan, B. F., Bhat, N. K., and Avadhani, N. G. (1982). Preferential attack of mitochondrial DNA by aflatoxin B1 during hepatocarcinogenesis. *Science* **214**(4528), 73-75.
- Nielsen, K. F., Huttunen, K., Hyvarinen, A., Andersen, B., Jarvis, B. B., and Hirvonen, M. R. (2001). Metabolite profiles of *Stachybotrys* isolates from water-damaged buildings and their induction of inflammatory mediators and cytotoxicity in macrophages. *Mycopathologia* **154**, 201-205.
- Norris, P. J., Smith, C. C., De Belleruche, J., Bradford, H. F., Mantle, P. G., Thomas, A. J., and Penny, R. H. (1980). Actions of tremorgenic fungal toxins on neurotransmitter release. *J. Neurochem.* **34**, 33-42.
- Notermans, S., Dufrenne, J., Wijnands, L. M., and Engel, H. W. (1988). Human serum antibodies to extracellular polysaccharides (EPS) of moulds. *J. Med. Vet. Mycol.* **26**, 41-48.
- Ojanen, T. (1992). Class specific antibodies in serodiagnosis of farmer's lung disease. *Br. J. Ind. Med.* **49**, 332-336.
- Ojanen, T., Terho, E. O., Tukaninen, H., and Manttyjarvi, R. A. (1990). Class specific antibodies during follow up of patients with farmer's lung disease. *Eur. Respir. J.* **3**, 257-280.
- Pace, J. G. (1993). Effect of T-2 mycotoxin on rat liver mitochondria electron transport system. *Toxicon*. **21**, 675-680.
- Pace, J. G. (1998). T-2 mycotoxin inhibits mitochondrial protein synthesis. *Toxicon*. **26**, 77-85.
- Patel, A. M., Ryu, J. H., and Reed, C. E. (2001). Hypersensitivity pneumonitis: current concepts and future questions. *J. Allergy Clin. Immunol.* **108**, 661-670.
- Peltola, J., Andersson, M. A., Hahtela, T., Mussalo-Rauhamaa, H., Rainey, F. A., Kroppenstedt, R. M., Samson, R. A., and Salkinoja, M. S. (2001). Toxic-metabolite-producing bacteria and fungus in an indoor environment. *Appl. Envir. Microbiol.* **67**, 3269-3274.
- Pena, C. E. (1970). Aspergillosis. In "The pathology and anatomy of mycoses: human infection with fungi" (R. E. Baker, ed.), pp. 762-831. Springer, Berlin.
- Petkova-Bocharova, T., Stoichev, I. I., Chernozemsky, I. N., Castegnaro, M., and Pfohl-Leszkowicz, A. (1998). Formation of DNA adducts in tissue of mouse progeny through transplacental contamination and/or lactation after administration of a single dose of ochratoxin A to the pregnant mother. *Environ. Mol. Mutagen.* **32**, 155-162.
- Pfohl-Leszkowicz, A., Grosse, Y., Kane, A., Creppy, E. E., and Dirheimer, G. (1993a). Differential DNA adduct formation and disappearance in three mouse tissues after treatment with mycotoxin ochratoxin A. *Mut. Res.* **289**, 265-273.
- Pfohl-Leszkowicz, A., Grosse, Y., Castegnaro, M., Nicolov, I. G., Chernozemsky, I. N., Bartsch, H., Betheder, A. M., Creppy, E. E., and Dirheimer, G. (1993b). Ochratoxin A-related DNA adducts in urinary tract tumors of Bulgarian subjects. *IARC Sci. Publ.* **124**, 141-148.
- Pfohl-Leszkowicz, A., Petkova-Bocharova, T., Chernozemsky, I. N., and Castegnaro, M. (2002). Balkan endemic nephropathy and associated urinary tract tumours: A review on aetiological causes and the potential role of mycotoxins. *Food Addit. Contam.* **19**, 282-302.
- Poapolathep, A., Ohtsuka, R., Kiatipattanasakul, W., Ishigami, N., Nakayam, H., and Doi, K. (2002). Nivalenol-induced apoptosis of thymus, spleen, and Peyer's patches of mice. *Exp. Toxicol. Pathol.* **53**, 441-446.
- Ponikau, J. U., Sherris, D. A., Kern, E. G., Homburger, H. A., Frigas, E., Gaffey, T. A., and Roberts, G. D. (1999). The diagnosis and incidence of allergic fungal sinusitis. *Mayo. Clin. Proc.* **74**, 877-884.

- Potter, P. C., Juritz, J., Little, F., McCaldin, M., and Dowdle, E. B. (1991). Clustering of fungal allergen-specific IgE antibody responses in allergic subjects. *Ann. Allergy* **66**, 149–153.
- Rand, L., Rand, T. G., Dearborn, D., Yike, I., and Vesper, S. (2003). Immunocytochemical localization of stichylisin in *Stachybotrys chartarum* spores and spore-impacted mouse and rat lung tissue. *Mycopathologia* **156**, 109–117.
- Rand, T. G., Mahoney, M., White, K., and Oulton, M. (2002). Microanatomical changes in alveolar type II cells in juvenile mice intratracheally exposed to *Stachybotrys chartarum* spores and toxin. *Toxicol. Sci.* **65**, 239–245.
- Reindl, M., Linington, C., Brehm, U., Egg, R., Dilitz, E., Deisenhammer, F., Poewe, W., and Berger, T. (1999). Antibodies against the myelin oligodendrocyte glycoprotein and the myelin basic protein in multiple sclerosis and other neurological diseases: A comparative study. *Brain* **122**, 2047–2056.
- Ribes, J. A., Vanover-Sames, C. L., and Baker, D. J. (2000). Zygomycetes in human disease. *Clin. Microbiol. Rev.* **13**, 236–301.
- Richard, J. L., Plattner, R. D., May, J., and Liska, S. L. (1999). The occurrence of ochratoxin A in dust collected from a problem household. *Mycopathologia* **146**, 99–103.
- Rudich, R., Santilli, J., and Rockwell, W. (2003). Indoor mold spore exposure: A possible factor in the etiology of multifocal choroiditis. *Am. J. Ophthalmol.* **135**, 402–404.
- Rylander, R. (1997). Airborne 3-beta-D-glucan and airway disease in a day-care center before and after renovation. *Arch. Environ. Health* **52**, 281–285.
- Rylander, R., Norrhall, M., Engdahl, U., Tunsater, A., and Hott, P. G. (1998). Airways inflammation, atopy, and (1→3)-beta-D-glucan exposures in two schools. *Am. J. Respir. Crit. Care Med.* **158**, 1685–1687.
- Sajan, M. P., Satav, J. G., and Battacharya, R. K. (1997). Effect of aflatoxin B1 in vitro on rat liver mitochondrial respiratory functions. *Indian J. Exper. Biol.* **35**, 1187–1190.
- Salvilahti, R., Uitt, J., Laippaia, P., Husman, T., and Roto, P. (2000). Respiratory morbidity among children following renovation of water-damaged school. *Arch. Environ. Health* **55**, 305–410.
- Schwartz, G. G. (2002). Does ochratoxin A cause testicular cancer? *Cancer Causes and Control* **13**, 91–100.
- Shelton, B. F., Kirkland, K. H., Flanders, W. E., and Morris, G. K. (2002). Profiles of airborne fungi in buildings and outdoor environments in the United States. *Appl. Environ. Microbiol.* **68**, 1743–1753.
- Skaug, M. A., Eduard, W., and Stormer, F. D. (2000). Ochratoxin A in airborne dust and fungal conidia. *Mycopathologia* **151**, 93–95.
- Smoragiewicz, W., Cossete, B., Boutrard, A., and Krzystyniak, K. (1993). Trichothecene mycotoxins in the dust of ventilation systems in office buildings. *Int. Arch. Occup. Environ. Health* **65**, 113–117.
- Sorensen, B., Streib, J. E., Strand, M., Make, B., Giclas, P. C., Fleshner, M., and Jones, J. F. (2003). Complement activation in a model of chronic fatigue syndrome. *J. Allergy Clin. Immunol.* **112**, 397–403.
- Stahl, C. J., Green, C. C., and Farnum, J. B. (1985). The incident at Tuol Chrey: Pathological and toxicologic examinations of a casualty after chemical attacks. *J. Forens. Science* **30**, 317–337.
- Steck, A. J., Murray, N., Dellagi, K., Brouet, J. C., and Seligmann, M. (1987). Peripheral neuropathy associated with monoclonal IgM autoantibody. *Ann. Neur.* **22**, 764–767.
- Sumi, Y., Natura, H., Takeuchi, M., and Miyakawa, M. (1994). Granulomatous lesions in the lung induced by inhalation of mold spores. *Virchows Arch.* **424**, 661–668.

- Taylor, M. J., Ponikau, J. U., Sherris, D. A., Kern, E., Gaffey, T. A., Kephart, G., and Kita, H. (2002). Detection of fungal organisms in eosinophilic mucin using a fluorescein-labeled chitin-specific binding protein. *Otolarygol. Head Neck Surg.* **127**, 377–383.
- Thrasher, J. D., Campbell, A. W., Vojdani, A., Madison, R. A., and Gray, M. R. (2004). Immune alterations in humans chronically exposed to toxigenic molds. (In preparation).
- Thrasher, J. D., Heuser, G., and Broughton, A. (2001). Autoimmunity and other immunological abnormalities in humans chronically exposed to Chlorpyrifos. *Arch. Environ. Health* **57**, 181–187.
- Thrasher, R. D., and Kingdom, T. T. (2003). Fungal infections of the head and neck: An update. *Otolarygol. Clin. North Am.* **36**, 577–594.
- Tuomi, T., Johnson, T., Hemminki, K., Hintikka, E.-L., Lindroos, O., Kalso, S., Koukila-Kähkölä, P., Mussalo-Rauhamaa, H., and Haahtela, T. (2000). Mycotoxins in crude building materials from water-damage buildings. *Applied Environ. Microbiol.* **66**, 1899–1904.
- Tuomi, T., Saarinen, L., and Reijula, K. (1998). Detection of polar and macrocyclic trichothecene mycotoxins from indoor environments. *Analyst* **123**, 1835–1841.
- Vesper, S. T., Dearborn, D. G., Elidemir, O., and Haugland, R. A. (2000). Quantification of siderophore and hemolysin from *Stachybotrys chartarum* strains, including a strain isolated from the lung of a child with pulmonary hemosiderosis. *Appl. Environ. Microbiol.* **66**, 2678–2681.
- Vesper, S. J., and Vesper, M. J. (2002). Stachylysin may be a cause of hemorrhaging in humans exposed to *Stachybotrys chartarum*. *Infect. Immun.* **70**, 2065–2069.
- Vojdani, A., Campbell, A., Anyanwu, E., Kashanian, A., and Vojdani, E. (2002). Antibodies to neuron-specific antigens in children with autism: Possible cross-reaction with encephalitogenic proteins from milk, *Chlamydia pneumoniae* and Streptococcus A group. *J. Neuroimmunology* **129**, 168–177.
- Vojdani, A., Campbell, A., Kashanian, A., and Vojdani, E. (2004). Antibodies against molds and mycotoxins following exposure to toxigenic fungi in a water-damaged building. *Arch. Environ. Health* **58**, 324–336.
- Vojdani, A., Campbell, A., and Brautbar, N. (1993). Immune functional abnormalities in patients with silicone breast implants. *Toxicol. Indust. Health* **8**, 415–429.
- Vojdani, A., Ghoneum, M., and Brautbar, N. (1992). Immune alteration associated with exposure to toxic chemicals. *Toxicol. Indust. Health* **8**, 239–254.
- Vojdani, A., Kashanian, A., Vojdani, E., and Campbell, A. W. (2003). Saliva secretory IgA antibodies against molds and mycotoxins in patients exposed to toxigenic fungi. *Immunopharm Immunotoxicol* **25**, 595–614.
- Vojdani, A., and Thrasher, J. D. (2004). Cross-reactivity between antigens of fungal extracts studied by affinity-purified chromatography antibodies and ELISA inhibition. (Manuscript in preparation).
- Vojdani, A., Thrasher, J. D., Madison, R. A., Gray, M. R., Campbell, A. W., and Heuser, G. (2003). Antibodies to molds and satratoxin in individuals exposed in water-damaged buildings. *Arch. of Environ. Health* **58**, 421–432.
- Von Emon, J. M., Reed, A. W., Yike, I., and Vesper, S. J. (2003). ELISA measurement of stachylysinTM in serum to quantify human exposures to the indoor mold *Stachybotrys chartarum*. *JOEM* **45**, 582–591.
- Walinder, R., Wieslander, G., Norback, D., Wessen, G., and Venge, P. (2001). Nasal lavage biomarkers: Effects of water damage and microbial growth in an office building. *Arch. Environ. Health* **56**, 30–36.
- Walsh, T. J., Heir, D. B., and Kaplan, L. R. (1985). Aspergillosis of the central nervous system: Clinicopathological analysis of 17 patients. *Ann. Neurol.* **18**, 574–582.

- Wang, J., Fitzpatrick, D. W., and Wilson, J. R. (1998). Effects of the trichothecene mycotoxin T-2 toxin on the neurotransmitters and metabolites in discrete areas of the rat brain. *Food Chem. Toxicol.* **36**, 947–953.
- Wei, Y. H., Ding, W. H., and Wei, R. D. (1984). Biochemical effects of PR toxin on rat liver mitochondrial respiration and oxidative phosphorylation. *Arch. Biochem. Biophys.* **230**, 400–411.
- Wichmann, G., Herbarth, O., and Lehmann, I. (2002). The mycotoxins citrinin, gliotoxin, and patulin affect interferon-gamma(greek symbol) rather than interleukin-4 production in human blood cells. *Environ. Toxicol.* **17**, 211–218.
- Willison, H. J., and Yuki, N. (2002). Peripheral neuropathies and anti-glycolipid antibodies. *Brain* **125**, 2591–2625.
- Wilson, B. J., and Wilson, C. H. (1964). Toxin from *Aspergillus flavus*: production on food material of a substance causing tremors in mice. *Science* **144**, 177–178.
- Wouters, I. M., Douwes, J., Dockes, G., Thorne, S., Brunekreef, B., and Heederik, D. J. (2000). Increased levels of markers of microbial exposure in homes with indoor storage of organic household waste. *Appl. Environ. Microbiol.* **66**, 627–631.
- Young, R. C., Bennett, J. E., Vogel, C. L., Carbone, P. P., and De Vita, T. (1970). Aspergillosis: The spectrum of disease in 98 patients. *Medicine* **49**, 147–173 (Baltimore).
- Zureik, M., Neukirch, C., Leynaert, B., Liard, R., Bousquet, J., and Neukirch, F. (2002). Sensitization to airborne moulds and severity of asthma: Cross sectional study from European Community respiratory health survey. *BMJ* **325**(7361), 411–414.