Mixed Mold Mycotoxicosis: Immunological Changes in Humans Following Exposure in Water-Damaged Buildings

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ABSTRACT. The study described was part of a larger multicenter investigation of patients with multiple health complaints attributable to confirmed exposure to mixed-molds infestation in water-damaged buildings. The authors present data on symptoms; clinical chemistries; abnormalities in pulmonary function; alterations in T, B, and natural killer (NK) cells; the presence of autoantibodies (i.e., antinuclear autoantibodies [ANA], autoantibodies against smooth muscle [ASM], and autoantibodies against central nervous system [CNS] and peripheral nervous system [PNS] myelins). A total of 209 adults, 42.7 ± 16 yr of age (mean ± standard deviation), were examined and tested with (a) self-administered weighted health history and symptom questionnaires; (b) standardized physical examinations; (c) complete blood counts and blood and urine chemistries; (d) urine and fecal cultures; (e) thyroid function tests (T4, free T3); (f) pulmonary function tests (forced vital capacity [FVC], forced expiratory volume in 1 sec [$FEV_{1.0}$], and forced expiratory flow at 25%, 50%, 75%, and 25–75% of FVC [FEF_{25}, FEF_{50}, FEF_{75}, and FEF_{25-75}]); (g) peripheral lymphocyte phenotypes (T, B, and NK cells) and mitogenesis determinations; and (h) a 13-item autoimmune panel. The molds-exposed patients reported a greater frequency and intensity of symptoms, particularly neurological and inflammatory symptoms, when compared with controls. The percentages of exposed individuals with increased lymphocyte phenotypes were: B cells (CD20+), 75.6%; CD5+CD25+, 68.9%; CD3+CD26+, 91.2%; CD8+HLR–DR+, 62%; and CD8+CD38+, 56.6%; whereas other phenotypes were decreased: CD8+CD11b+, 15.6% and CD3–CD16+CD56+, 38.5%. Mitogenesis to phytohemagglutinin was decreased in 26.2% of the exposed patients, but only 5.9 % had decreased response to concanavalin A. Abnormally high levels of ANA, ASM, and CNS myelin (immunoglobulins [Ig]G, IgM, IgA) and PNS myelin (IgG, IgM, IgA) were found; odds ratios for each were significant at 95% confidence intervals, showing an increased risk for autoimmunity. The authors conclude that exposure to mixed molds and their associated mycotoxins in water-damaged buildings leads to multiple health problems involving the CNS and the immune system, in addition to pulmonary effects and allergies. Mold exposure also initiates inflammatory processes. The authors propose the term "mixed mold mycotoxicosis" for the multisystem illness observed in these patients.

<Key words: immune hyperactivation, immunotoxicity, mitogenesis, molds, mycotoxicosis, mycotoxins, proinflammatory immune toxicity, toxic encephalopathy>

THE POTENTIAL HARMFUL EFFECTS of exposure to mixed molds in inhabited buildings were recognized in early Biblical times. In the Old Testament,[1] Leviticus put forth a detailed protocol for the remediation of mold-contaminated structures, including the destruction of dwellings and personal belongings if remediation failed. Today, it is recognized that water intrusion into buildings leads to amplification of molds,[2–7] often requiring remediation.

Potentially toxic and immunogenic byproducts of fungi and molds include mycotoxins; 1,3-alpha-D-glucans; extracellular polysaccharides (EPS); enzymes; and solvents.[8–19] Occupants of affected structures can develop symptoms in multiple organ systems, including the upper and lower respiratory systems, central and peripheral nervous systems, skin, gastrointestinal tract, urinary tract, connective tissue, and the musculoskeletal system.[3,6,20–26] Human illness can result from 1 or all of the following: mycotic infections, or mycoses[27–32]; immunoglobulin (Ig)E-mediated sensitivity and asthma[33–35]; hypersensitivity pneumonitis and related inflammatory pulmonary diseases[36–39]; cytotoxicity[40–44]; immune suppression/modulation[45–47]; mitochondrial toxicity[48–51]; carcinogenicity[52,53]; nephrotoxicity[54]; and the formation of nuclear and mitochondrial deoxyribonucleic acid adducts.[55–58] Finally, in the infectious state, molds secrete exodigestive enzymes (EES) that cause tissue destruction, angioinvasion, thrombosis, infarction, and other manifestations of mycosis.[27,59–63]

We have evaluated and treated more than 209 patients who presented with multiorgan system symptoms resulting from exposure to molds in their homes, schools, or workplaces. Recognizing the complexity of health problems associated with mixed mold exposure, we undertook a multicenter investigation of patients with chronic health complaints attributed to exposure to mixed colonies of indoor fungi and molds. We employed detailed health and environmental history-gathering questionnaires, environmental monitoring data, physical examination, pulmonary function testing protocols, routine clinical chemistries, neurocognitive testing, and 16-channel quantitative electroencephalograms (QEEGs). In addition, we measured lymphocyte phenotypic markers (on T, B, and natural killer [NK] cells), antibodies to molds and mycotoxins, neuronal antigen antibodies, and leukocyte apoptosis. Herein we present data on symptoms, alterations in peripheral lymphocyte phenotypes, and autoantibodies observed in adult patients. Future communications will detail pulmonary abnormalities, antibodies to molds and mycotoxins, and neurobehavioral and QEEG changes observed in these patients, and will report the statistically significant multisystem correlations observed. Currently, we refer to the illness of these individuals as a "mixed mold mycotoxicosis" involving the immune system, the lungs, and the central and peripheral nervous systems, as well as a generalized inflammatory and irritant response to exposure to spores, hyphal fragments, mycotoxins, solvents, and other byproducts (e.g., EPS and EES).

Materials and Method

Patients. Two hundred nine adult patients with a history of exposure to mixed colonies of molds resulting from structural water intrusion in residential, workplace, or school-based settings were included in this study. Adults were considered to be males older than 12 yr of age and females older than 11 yr of age. The patients, 42.7 ± 16 yr of age (mean ± standard deviation), were evaluated from early 1994 through June 2003 and comprised 126 females (43.1 ± 15.2 yr) and 83 males (42.3 ± 17.1 yr). Patients involved in litigation numbered 71 (40.1 ± 16.7 yr) and nonlitigants numbered 1,368 (44.5 ± 15.3 yr). Litigation status was uncertain for 4 adult patients. Females under age 11 and males under age 12 were not considered as adults with respect to immune parameters and symptoms, and were therefore excluded from the data presented in this report, although many of these children were clinically ill.

Questionnaires. We asked the patients to complete 2 self-administered questionnaires developed by 1 of the authors (MRG), seeking information from the following areas of concern: (a) medical history, (b) occupational and general environmental history, (c) lifestyle and habits, and (d) a review of systems. The symptom frequency review entailed questions on 58 specific symptoms. In accordance with methods provided by Ziem,[64] we report on the 38 most frequently experienced symptoms. In brief, the symptoms were scored by the patient as occurring: 1 = daily to almost daily, 2 = several times a week, 3 = weekly, 4 = several times a month, 5 = monthly, 6 = rarely, if ever (considered a negative response), and 7 = unsure. For statistical purposes, the scores were reversed to reflect the ascending frequency of the reported symptoms when tabulated. The mean value and standard deviation for the frequency score for each symptom were determined for the whole group, for males alone, for females alone, and for litigant and nonlitigant patients.

Controls for the historical questionnaires were obtained by auditing responses to the same questionnaires administered to 28 consecutive adult patients presenting to our general medical clinic for initial "database" comprehensive physical examinations. The results from the first 28 consecutive Ziem symptom audits were used as controls for comparison with the mold-exposed patients' responses.

Physical examinations. Each patient underwent a thorough physical examination, performed by MRG. A standardized form for entering relevant physical find-

ings was used to facilitate uniformity of the exam and recording of findings. Clinical laboratory samples were collected by certified phlebotomists at the community hospital located adjacent to the clinic in which the exams were conducted. All samples were either processed at the local hospital's American College of Pathology (ACP)-accredited clinical laboratory facilities, or forwarded to the appropriate ACP-accredited reference laboratories. Guidelines of the U.S. Centers for Disease Control and Prevention (CDC) were followed for the handling of all lymphocyte tissue cultures tested.

Clinical laboratory tests. The following standard diagnostic laboratory tests were performed by Clinical Laboratory Improvement Amendment and Medicare-certified national reference and specialty laboratories: complete blood count (CBC); comprehensive metabolic panel (CMP); urinalysis; urine culture (if indicated); stool (occult blood, fungal, and mold) culture; erythrocyte sedimentation rate (ESR); C-reactive protein (CRP); thyroid profile (thyroid-stimulating hormone [TSH], free T4, and free T3); antinuclear autoantibodies (ANA); and rheumatoid factor (RF). All clinical laboratory tests were compared with the laboratories' published expected reference ranges, according to generally accepted procedures and practices. Serology was performed for the following herpes viruses: herpes I, II, VI, and varicella; Epstein Barr; and cytomegalovirus.

Peripheral lymphocyte phenotype determination. Whole venous blood was sent in laboratory-provided silicon-treated, sodium-heparinized glass e-vac tubes by overnight courier to Antibody Assay Laboratories (AAL), Santa Ana, California, following procedures prescribed by the lab to ensure that the cells were viable in accordance with CDC requirements. AAL performed mitogen tests with phytohemagglutinin (PHA), and with pokeweed and concanavalin A (Con A). Tuberculin purified protein derivative (PPD), tetanus toxoid, and *Candida albicans* mannoprotein were used as control mitogens. Any blood samples that failed to meet CDC requirements were discarded and redrawn. Quality assurance was performed using negative and positive controls. Mononuclear cells were isolated using Ficoll-isopaque density gradient centrifugation.[65] Not all of the 209 adults had all of the tests performed; therefore, *n* varies slightly for some of the parameters, as noted in the relevant tables.

Peripheral white blood cells (i.e., total white cell count and total lymphocyte count) were enumerated by AAL. In addition, the percentages of the following lymphocyte phenotypes were determined: B cells (CD20+); T cells (CD3+); T-helper (CD4+) and T-suppressor (CD8+) cells; interleukin (IL)-2 receptor-bearing T cells (CD5+CD25+); activated T cells (CD3+CD26+ and CD3+HLA–DR+); activated T-suppressor cells (CD8+CD38+ and CD8+HLA–DR+); complement receptor-bearing T-suppressor cells (CD8+CD11b+); and NK cells (CD3–CD16+CD56+). Monoclonal antibodies to CD antigens were purchased from Becton Dickinson (Los Angeles, California), except for CD26 (Beckman Coulter [Miami, Florida]). Flow cytometry was performed using a Coulter Epic XL MCL flow cytometer (Beckman Coulter), in accordance with the manufacturer's instructions.

Mitogenesis. Mitogenesis responses to PHA and Con A were evaluated on peripheral lymphocytes of all 209 patients, using the colorimetric MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay.[66] Viable cells activate the MTT, which is measured colormetrically at 570 nm with an EAR 400 microplate reader (SLT Labinstruments [Salzburg, Austria]). Mononuclear cells were isolated and suspended in 0.1 ml RPMI 1640 medium at 10^6 cells/ml. They were cultured in RPMI 1640 supplemented with 10% fetal calf serum and antibiotics (i.e., penicillin and streptomycin) (Irvine Scientific [Santa Ana, California]). Cells from each individual were tested at 3 different concentrations of the mitogens for optimum stimulation. The tests were performed in triplicate and were reported as the average of 3 concentrations.

NK cell function testing. NK cell function was evaluated by AAL, using K562 cells (immortal cell line) (Coriell Institute for Medical Research [Camden, New Jersey]) as target cells. In brief, the patient's NK cells were incubated with K562 cells and a fluorescein derivative that is hydrolyzed by live K562 cells. The percentage of cells that retained the dye following killing was measured with an Epics Flow cytometer. The result is expressed as a percentage of kill.

Autoantibody determinations. Autoantibodies against smooth muscle (ASM), brush border (ABB), parietal cells (APC), mitochondria (AMIT), and nucleic acids/nucleoproteins (ANA) were determined with standard indirect immunofluoresence.[67,68] The controls for ASM and ANA were reported previously.[69] Immunoglobulin (Ig)A, IgM, and IgG antibodies to myelin sheath were detected with frozen monkey spinal cord (CNS) and sciatic nerve (PNS) as substrates, employing an indirect immunofluorescent technique for which antihuman immunoglobulin conjugated to fluorescein was used.[68,69] The controls for antimyelin antibody testing consisted of 32 chiropractic students (20 males and 12 females), 29 ± 9 yr of age, as reported previously.[69] Antibodies against thyroglobulin and thyroid peroxidase were measured by automated assays performed using chemoluminescence on DPC Immulite 2000 (Diagnostic Products Corp. [Los Angeles, California]), with DPC reagents.

Statistical analysis. All statistical analyses were performed using Statistica 10.0 for Windows (StatSoft, Inc. [Tulsa, Oklahoma]). Statistical tests included basic descriptive statistics, critical *t* tests, critical *z* tests, odds ratios, and analysis of variance (ANOVA).

Results

Physical examinations and routine diagnostic tests. Physical exams revealed (a) nasal mucosal hyperproliferation and inflammatory changes, (b) relative alopecia, (c) cough and wheezing, (d) frequent balance problems (Romberg positive), and (e) increased dermal fluorescence on ultraviolet inspection. The CBC, CMP, ESR, CRP, RF, thyroid panel, urine analyses, urine cultures, and stool analyses and stool cultures did not vary from expected values, except for a low mean total bilirubin of 0.40 (reference range: 0.4–1.0).

Serology tests for all viruses were negative for either active or reactivation infections. Lymphadenopathy indicative of acute or reactivation herpes viral infections was absent in all cases.

Symptoms. Initially, litigants were compared with nonlitigants for each symptom. Critical *t* tests for each symptom revealed no difference between the 2 groups (data not shown). The responses of males vs. females for each symptom were also compared (data not shown). Critical *t* tests showed that females had a greater frequency of the following symptoms: excessive fatigue, headache, memory problems, "spaciness"/disorientation, lightheadedness, slurred speech, weak voice, spasms, coordination problems, vision changes, rash, cold intolerance, heat intolerance, chest discomfort, excessive thirst, swallowing problems, flushing skin, rapid pulse, palpitations, bruising, and swelling ankles.

Results for males and females were grouped together, and the frequency of each symptom expressed by the mold-exposed patients was compared with frequencies reported by the 28 controls (Table 1). As indicated in the table, exposed patients had an increased frequency of expression for the following symptoms: excessive fatigue, headache, nasal symptoms, memory problems, spaciness and disorientation, sinus discomfort, coughing, watery eyes, throat discomfort, slurred speech, lightheadedness, dizziness, weakness, bloating, insomnia, spasms, coordination problems, vision changes, rash, chest tightness, and wheezing.

Peripheral lymphocytes. The percentage of lymphocyte phenotypes measured in the peripheral blood of the patients was compared for litigants vs. nonlitigants. Critical *t* tests revealed no significant difference between the 2 groups for each lymphocyte phenotype (data not shown). Table 2 summarizes our observations following a comparison of males vs. females with respect to each phenotype. Critical *t* tests revealed no difference between males and females. As a result of these observations, all data were grouped into an "all patients" category. The mean percentages of total lymphocytes for all patients exceeded the expected laboratory ranges (95% confidence intervals [CIs]). The percentages of complement-receptor–bearing suppressor cells and NK cells were within expected laboratory ranges, but were on the low side of the 95% CI for each cell type. The percentages of individuals with results outside of the expected laboratory ranges for each phenotype also are presented in Table 2.

Mitogenesis. The results of mitogenic stimulation with PHA and Con A are summarized in Table 2, along with laboratory expected ranges at 95% CI. The average mitogen responses to PHA and Con A were on the low side of the expected ranges. The percentages of all patients with mitogenesis below the expected ranges were 26.2% for PHA and 5.9% for Con A.

NK cell activity. NK cell (CD3–CD16+CD56+) activity was normal for all study subjects.

Autoantibodies. IgG, IgM, and IgA antimyelin antibodies against CNS and PNS myelin were compared for litigants vs. nonlitigants and for males vs. females. Critical *z* tests revealed no difference between males and females (data not shown). Similarly, no difference was observed in myelin autoantibodies when litigants were compared with nonlitigants, except for PNS IgA (< 0.05 and > 0.02, respectively). However, given the number of tests applied (6), this difference was considered insignificant. Thus, antimyelin antibodies were grouped for all patients and compared with controls (Table 3). Observations for antibodies (IgG, IgM, and IgA) against neurofilament antigen in the mold-exposed patients, compared with controls, are presented in Table 4.

The percentages of patients with increased ANA and ASM autoantibodies were compared for males vs. females and for litigants vs. nonlitigants (data not shown). The percentages of males and females with elevated ANA autoantibodies were 25.9% and 31.7%, respectively, and with elevated ASM autoantibodies were 34.6% and 30.3%, respectively. Similar values for litigants vs. nonlitigants were 27.5% and 30.6%, respectively, for ANA, and 27.5% and 25%, respectively, for ASM. Critical *z* tests revealed no differences in the percentages for males vs. females or for litigants vs. nonlitigants. Results for male and female patients were then combined and compared with the controls on the basis of odds ratios. The controls' values for ANA and ASM were 1.8% and 14.6%, respectively ($n = 55$). The ORs at 95% CI for ANA and ASM were 11.00 and 2.58, respectively, both being significant (Table 5).

Discussion

We made the following observations with respect to the 38 most frequently reported symptoms in our study (Table 1): (a) The mixed-mold–exposed patients expressed symptoms at a greater frequency than the controls; (b) Exposure to mixed molds caused significant morbidity, leading affected individuals to seek medical assistance; (c) The difference in symptoms between litigants and nonlitigants was not significant; (d) A statistically significant increase was seen in the frequency of

Table 1.—Frequency of Occurrence of the 38 Most Frequently Reported Symptoms, in Mold-Exposed Patients vs. Controls

Symptom*	Mold-exposed patients (n = 209) $\bar{x}$	*SD*	Controls (n = 28) $\bar{x}$	*SD*	*p*	*p* females
Excessive fatigue	5.8	1.9	4.3	2.1	0.0001	0.001
Headache	5.2	1.9	4.1	2.0	0.005	< 0.006
Nasal symptoms	5.1	2.2	4.1	2.0	0.02	
Memory problems	5.1	2.1	3.3	1.6	0.0002	0.005
"Spaciness"/disorientation	4.8	2.3	3.2	1.8	0.0007	< 0.01
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	0.002	< 0.02
Lightheadedness	4.4	2.2	3.2	1.4	0.006	0.01
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	3.0	1.7	0.008	
Bloating	4.2	2.2	3.2	1.6	0.02	
Insomnia	4.1	2.2	3.8	2.0	NS	
Weak voice	4.1	2.2	2.8	1.4	0.003	0.02
Spasms	4.0	2.2	3.8	2.1	NS	0.04
Coordination problems	4.0	2.2	2.9	1.4	0.01	0.009
Vision changes	3.9	2.3	2.9	1.4	0.02	0.008
Rash	3.9	2.2	2.9	1.7	0.02	0.02
Numbness	3.9	2.2	3.4	1.7	NS	
Cold intolerance	3.9	2.4	3.1	1.8	NS	0.002
Heat intolerance	3.8	2.4	3.6	2.0	NS	0.003
Chest tightness	3.8	2.2	2.6	1.3	0.006	
Chest discomfort	3.7	2.2	3.0	1.3	NS	0.02
Frequent urination	3.7	2.3	3.8	2.1	NS	
Excessive thirst	3.6	2.3	3.4	2.0	NS	0.01
Ringing ears	3.6	2.2	4.4	2.4	NS	
Wheezing	3.6	2.0	2.6	1.3	0.02	
Swallowing problems	3.2	2.0	3.0	1.7	NS	0.008
Skin flushing	3.1	2.1	2.8	1.6	NS	0.04
Bladder control problems	3.1	2.0	2.8	1.4	NS	
Rapid pulse	3.0	2.0	2.6	0.9	NS	0.04
Palpitations	2.8	1.9	2.4	0.8	NS	0.003
Bruising	2.8	1.7	2.4	0.9	NS	0.003
Swelling ankles	2.7	1.8	2.6	1.5	NS	0.02
Hearing changes	2.7	1.8	2.6	1.5	NS	

Notes: $\bar{x}$ = mean, *SD* = standard deviation, and NS = not significant.
*Symptoms were compared for females vs. males.

symptoms among women compared with men; and (e) The most frequently reported symptoms were neurological (i.e., headache, memory difficulty, slurred speech, spaciness, lightheadedness, dizziness, weakness, coordination problems, and changes in vision), state of well-being (excessive fatigue, bloating, rash, discomfort, and muscle spasms), and ophthalmic and upper/lower respiratory (nasal symptoms, sinus discomfort, coughing, watery eyes, throat discomfort, weak voice, chest tightness, and wheezing). Overall, the symptom complex we observed was consistent with observations reported by others.[3,6,12,20-26] The preponderance of symptoms involving the CNS and state of well-being are reflective of injury to the CNS, as reported by Kilburn[25] and Anyanwu et al.[26] The increased frequency of symptoms in females is consistent with their greater representation in several other clinical conditions (e.g., fibromyalgia and related disorders,[70,71] autoimmune diseases,[72-74] and exposure to molds[6]). The greater representation of females with respect to symptoms may suggest that xenobiotics, estrogenic solvents, and/or mycoestrogens in their mold-contaminated environs play a role in their illnesses.[75,76] And, finally, the absence of a difference in symptoms between litigants and nonlitigants supports the assertion that individuals who exercise their legal rights through litigation do not exaggerate their symptoms, nor are they prone to malingering.[77-79]

The lymphocytes measured in our mold-exposed pa-

Table 2.—Lymphocyte Phenotypes Observed in Males vs. Females, and in Combined Sexes (All Patients)

Cell type	Designation	Expected range (95% CI)	% variation from expected	All patients* (n = 206)			Males (n = 83)			Females (n = 123)		
				$\bar{x}$	*SD*	% abnormal	$\bar{x}$	*SD*	% abnormal	$\bar{x}$	*SD*	% abnormal
B	CD20+	5, 15	> 15	17.9	6.1	75.6	18.1	6.9	75.6	17.7	5.5	75.6
IL-2 receptor-bearing T	CD5+CD25+	0, 8	> 8	9.2	4.7	68.9	8.9	4.7	70.7	9.4	4.7	67.5
Activated T	CD3+CD26+	0, 30	> 30	45.9	11.8	91.2	44	12.2	91.5	47	11.3	91.0
	CD3+HLA–DR+	0, 7	> 7	11.8	5.9	95.1	11.9	7.5	92.7	11.7	4.5	96.7
Activated and suppressor	CD8+CD38+	0, 8	> 8	15.4	6	56.6	15.6	6.4	54.9	15.3	5.8	57.8
	CD8+HLA–DR+	0, 3	> 3	4.7	5	62	5.2	6.4	61	4.4	3.7	63.6
Complement-receptor–bearing suppressor	CD8+CD11b+	5, 45	< 5	5.7	3.7†	15.6	6.3	4§	18.3	5.3	3.5**	13.1
Natural killer	CD3–CD16+CD56+	5, 20	< 5	7.6	11	38.5	9.8	16.1	48.8	6.1	3	31.7
Phytohemagglutinin	PHA	96, 195	< 96	104	23.5‡	26.2	104	21.4#	25	104	25**	27
Concanavalin A	Con A	94, 354	< 94	108	27.2‡	5.9	108	23.9#	6.3	108	29.2**	5.7

Notes: CI = confidence interval, $\bar{x}$ = mean, and *SD* = standard deviation. Not all tests were performed on all individuals, as noted.
**t* and *z* tests of males and females revealed no significant differences; therefore, the 2 sexes were combined to form All Patients.
†n = 204.
‡n = 202.
§n = 82.
#n = 80.
**n = 123.

Table 3.—Percentage of Individuals with Antibody Titers > 1:4 against Central Nervous System (CNS) Myelin and Peripheral Nervous System (PNS) Myelin for Each Isotype, in Mold-Exposed Patients vs. Controls

	CNS myelin antibodies						PNS myelin antibodies					
	IgG		IgM		IgA		IgG		IgM		IgA	
Subject	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
All patients	199	67.3	201	43.3	201	55.7	201	61.7	201	45.8	201	60.2
Controls*	32	12.5	32	12.5	32	12.5	32	12.5	32	12.5	32	6.25
Odds ratio	14.40		5.34		10.59		14.40		5.39		19.10	
95% CI	4.85, 42.9		1.8, 15.8		3,58, 31.3		4.86, 42.87		1.82, 15.93		4.44, 82.6	

Notes: Ig = immunoglobulin, and CI = confidence interval.
*The percentage of controls that exceeded 1:4 had antimyelin titers of 1:8.

Table 4.—Percentage of Individuals with Abnormally High Titers to Neurofilament Antigen, in Mold-Exposed Patients vs. Controls

	% abnormal		
Subject	IgG	IgM	IgA
Patients (*n* = 93)	6.45	31.6	36.2
Controls (*n* = 100)	0.0	2.0	2.1
Odds ratio	Infinity	22.615	27.767
95% CI		5.224, 99.900	6.437, 119.784

Notes: Ig = immunoglobulin, and CI = confidence interval.

Table 5.—Odds Ratios (ORs) and 95% Confidence Intervals (CIs) for Antinuclear Autoantibodies (ANA) and Autoantibodies against Smooth Muscle (ASM)

Autoantibody	OR	95% CI
ANA	11.00	2.56, 46.50
ASM	2.58	1.26, 6.26

Note: Autoantibodies for males and females were combined and compared with combined controls A and B.

tients demonstrated increased expression of various activation markers when compared with expected laboratory ranges (Table 2), as follows: CD3+CD26+ (activated T cell), 91.2% of patients; CD3+HLA–DR+ (class II major histocompatibility molecule [MHC] and marker of activation), 95.1%; CD5+CD25+ (IL-2 receptor-bearing T cell), 68.9%; CD20+ (mature antigen-producing B cells), 75.6%; and CD8+HLA–DR+, 62%. In light of these observations, the functional role of each of these activation markers should be considered. Expression of CD26, reflecting cellular activation, is diagnostic of (or prognostic for) a variety of nonallergic clinical conditions (e.g., autoimmune disorders, various tumors, hematological malignancies, and inflammatory conditions)[80] and is also increased in individuals ill from exposure to other xenobiotics.[69,81] HLA–DR, a class II MHC, recognizes either allogenic (self) MHC molecules or foreign protein, which means that it has recognized foreign antigens bound to self class II MHC molecules.[82] HLA–DR is expressed on immune cells in inflammation,[83] asthma,[84] autoimmune diseases,[85] and neurological disorders.[86] CD25 is considered to be a natural regulatory marker of T cells and plays a major role in IL-10 production and in controlling the immune response to self and foreign antigens.[87,88] CD20 (B) cells produce antibodies and are the source of immunoglobulins directed against foreign and self-antigens; they also play a central role in autoimmunity. Currently, these cells are the focus of anti-CD20 compounds directed toward immunotherapy in B-cell malignancies and autoimmune diseases.[89,90] Collectively, the increased presence of activated T cells, and increased B cells, implies a *proinflammatory* state. Moreover, the reduction of CD8+CD11b+ (complement-receptor–bearing suppressor) cells is commensurate with increased expression of activation markers. This level of overexpression of activation markers is related to dramatic antigenic stimulation in patients with a history of mixed mold exposure. In addition, the increase in HLA–DR+ expression reflects the presence of increased autoimmunity. In the aggregate, this situation represents a proinflammatory, immune toxic state.

The effects of mold exposure on the human immune system have been reported previously. Johanning et al.[6] found a significant decrease in CD3 T cells, along with a slight decrease in mitogenesis to both Con A and PHA, following mold exposure in a water-damaged building. However, activation markers were not studied. In children exposed to high levels of residential mold contamination (vs. control children from a low-contamination environment) there was a significant increase in CD3CD45RO (memory T cell) expression, with a concomitant decrease in the helper/suppressor ratio that persisted for 12 mo.[91] Finally, animal feed pro-

duction workers exposed to mixed mycotoxins, with aflatoxin concentrations of 1.55 to 6.25 ng/m^3, had an increase in tumor necrosis factor-alpha (TNF-α).[92] In addition, the animal feed workers had a shift in lactic dehydrogenase (LDH) isoenzymes, with a significant increase in LDH1 (spleen) and LDH3 (lungs). These observations corroborate the immune changes reported herein and support the conclusion that exposure to mixed molds and their byproducts causes the expression of immune markers of activation, as well as at least 1 inflammatory cytokine—TNF-α. Furthermore, the decreases we observed in the percentage of peripheral blood NK cells and response to PHA further support the concept that immune dysregulation is occurring, and represents a "promoter" state for the expression and development of malignancies.

The subjects in our study exhibited a high risk for producing autoantibodies to nuclei, smooth muscle, CNS and PNS myelin, and neurofilament (Tables 3–5). The presence of autoantibodies ANA, ASM, and CNS and PNS myelin has been reported following exposure to other xenobiotics.[69,81,93] High titers of ANA are associated with various types of connective tissue injury and/or connective tissue diseases.[94] ASM antibodies are nonspecific, occurring in a variety diseases, including autoimmune hepatitis,[95,96] vascular events,[97] rheumatoid arthritis,[98] *Mycoplasma pneumoniae*,[99] bronchial suppuration,[100] autoimmunity,[101] and asthmatic bronchitis.[102] The antimyelin autoantibodies—initially identified in Guillian-Barre syndrome[68]—are now recognized to represent several different neuronal antigens, including various gangliosides, tubulin, chondroitin sulfate, and sulfatide, found in neuropathies.[103–108] Thus, we have recently incorporated neuron neurofilament antigen into our protocol and have found increased neurofilament antibodies in these patients (Table 5). In summary, individuals exposed to mixed molds produce several different autoantibodies. Work is in progress to determine the significance of these antibodies in conditions such as lupus erythematosus, autoimmune neuropathy, and a multiple-sclerosis–type syndrome.

A systemic shift in the Th1/Th2 balance to a Th2 immune profile (e.g., TNF-α, IL-4, and IL-10 cytokines) has been reported for Gulf War veterans and chronic fatigue patients, as well as in asthma and lupus erythematosus.[109–112] Shift to Th2 profile leads to an increase in various diseases that are exacerbated by decreased Th1. Therefore, future research into immunological alterations should include testing for Th2 profile and cytokines, particularly because CD5+CD25+ (IL-2) and multiple autoantibodies were present in the patients in our study.

The single limitation of our study needs to be addressed. The immune profiles (Table 3) were compared with expected laboratory ranges, rather than with healthy control subjects. However, the absence of controls should not be considered excessively limiting in the evaluation of immune profiles of these mold-exposed patients. First, the percentage of individuals with increased activation expression on T cells greatly exceeded the maximum expected range as published by the testing laboratory. For example, the maximum percentages of total recorded lymphocytes for controls in regard to CD5+CD25+, CD3+CD26+, CD3+HLA–DR+, CD8+CD38+, and CD8+HLA–DR+ were 8%, 30%, 7%, 8%, and 3%, respectively. The percentages of mold-exposed patients that exceeded these values were 68.9%, 91.2%, 95.1%, 56.6%, and 62.0%, respectively, for each of the phenotypes. In addition, the mean percentages for CD20+, CD5+CD25+, CD3+CD26+, CD3+HLA–DR+, CD8+CD38+, and CD8+HLA–DR+ cell counts exceeded the laboratory's 95% CI, which further supports the preceding observation. This represents a greatly increased frequency of activation markers when compared with maximum expected ranges. Second, expression of the autoantibodies ANA, ASM, CNS and PNS myelin, and neurofilament was significant in the mold-exposed patients vs. controls. Thus, the presence of autoantibodies is commensurate with immune activation, and, finally, the antineuronal antigen (neurofilament)-specific antibodies are strongly associated with a wide array of degenerative neurological disorders of undetermined origin.

In this study, we have shown that individuals exposed to mixed colonies of molds in water-damaged buildings have several abnormalities among their immune parameters. These include (a) immune activation markers, with elevated CD26+, HLR–DR+, CD25+, and CD38+ phenotypes in the peripheral blood; (b) the presence of autoantibodies (ANA, ASM, CNS and PNS myelin, and neurofilament); and (c) decreased complement-receptor–bearing T-suppressor (CD11b+) cells. Future research should be directed toward clarifying the Th1/Th2 profile, and accompanying cytokines, in humans affected adversely by mixed mold exposure. Also, efforts should be made to correlate the abnormal immune parameters with other measured abnormalities found in individuals exposed to mixed colonies of structural fungi and molds, and their associated mycotoxins, extracellular polysaccharides, exodigestive enzymes, hyphae fragments, and spores.

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Psychological, Neuropsychological, and Electrocortical Effects of Mixed Mold Exposure

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ABSTRACT. The authors assessed the psychological, neuropsychological, and electrocortical effects of human exposure to mixed colonies of toxigenic molds. Patients (N = 182) with confirmed mold-exposure history completed clinical interviews, a symptom checklist (SCL-90-R), limited neuropsychological testing, quantitative electroencephalogram (QEEG) with neurometric analysis, and measures of mold exposure. Patients reported high levels of physical, cognitive, and emotional symptoms. Ratings on the SCL-90-R were "moderate" to "severe," with a factor reflecting situational depression accounting for most of the variance. Most of the patients were found to suffer from acute stress, adjustment disorder, or posttraumatic stress. Differential diagnosis confirmed an etiology of a combination of external stressors, along with organic metabolically based dysregulation of emotions and decreased cognitive functioning as a result of toxic or metabolic encephalopathy. Measures of toxic mold exposure predicted QEEG measures and neuropsychological test performance. QEEG results included narrowed frequency bands and increased power in the alpha and theta bands in the frontal areas of the cortex. These findings indicated a hypoactivation of the frontal cortex, possibly due to brainstem involvement and insufficient excitatory input from the reticular activating system. Neuropsychological testing revealed impairments similar to mild traumatic brain injury. In comparison with premorbid estimates of intelligence, findings of impaired functioning on multiple cognitive tasks predominated. A dose-response relationship between measures of mold exposure and abnormal neuropsychological test results and QEEG measures suggested that toxic mold causes significant problems in exposed individuals. Study limitations included lack of a comparison group, patient selection bias, and incomplete data sets that did not allow for comparisons among variables.
<Key words: dose-response relationship, neuropsychological testing, Symptom Checklist-90-R, quantitative electroencephalograph, toxic encephalopathy, toxic mold>

NEUROTOXICITY can cause irreversible nervous system damage related to cell death or permanent alterations of cell structure and receptor sensitivity. Clinical signs are classified as organi/c mental impairments, seizures, movement disorders, involvement of cranial nerves or spinal peripheral nerves, and neuromuscular dysfunction.[1] Neurotoxic exposure and injury are assessed by careful neurological and neuropsychological evaluation, complemented with functional imaging of the brain.

Occupants of mold-infested structures develop multiorgan symptoms that involve the upper and lower respiratory systems, central and peripheral nervous systems, skin, gastrointestinal tract, connective tissue, immune system, and musculoskeletal system.[2-18] Complaints of neurocognitive dysfunction are prevalent among the symptoms reported.[2,3,18-22] A large body of literature exists on the effects of various neurotoxins on neuropsychological functioning, including cognitive

impairment.[23] However, only 4 studies have reported measurements of neurobehavioral changes related to mold and mycotoxin exposure.[18,20–22] The changes described include impairments in balance, reaction time, cognition, verbal learning, recall, visual spatial learning, memory, attention/concentration, and set shifting.

Only a few complementary neuroimaging studies have been published in regard to assessment of the effects of mixed mold exposure on the central nervous system. Adolescents with suspected acoustic mycotic neuroma resulting from environmental exposure to toxic molds had abnormal brainstem evoked potentials.[2] In another study, abnormal electroencephalogram (EEG) examinations in 7 of 10 patients exposed to toxic mold were reported. All 10 patients had frontal-temporal theta wave activity, which indicated diffuse changes characteristic of metabolic encephalopathy. Abnormal brainstem auditory evoked potentials were demonstrated in 9 of these patients, and 4 of the 10 patients showed clear abnormalities.[3]

All of the patients in the current study reported wide-ranging symptoms, including headache, dizziness, visual changes, cognitive impairment, and emotional dysregulation. Their illness has been defined as "mixed mold mycotoxicosis."[19]

It is likely that studies of mold-induced neurotoxicity will yield findings similar to other studies of neurotoxicity from other causes. Single photon emission computed tomography (SPECT) has been used to complement and define the effects of toxic exposure on the central nervous system. A review of the literature on the use of SPECT scans following neurotoxic exposure confirmed that abnormalities can exist from months to years after exposure has ceased, and can involve asymmetrical abnormalities with hypoperfusion in the frontal, parietal, and temporal lobes.[24] Moreover, in 33 workers with encephalopathy following toxic exposure, 94% had abnormal SPECT scans. The most frequent areas of abnormality were the temporal lobes (67.7%), frontal lobes (61.3%), basal ganglia (45.2%), thalamus (29.0%), parietal lobes (12.9%), and motor strip (9.7%).[25]

In recognition of the complexity of health problems associated with mixed mold exposure, a multicenter investigation of patients with chronic health complaints from mold exposure was undertaken. We used generally accepted, standardized, detailed health and environmental history questionnaires, environmental monitoring data, physical examination, accepted pulmonary function testing protocols, routine clinical chemistry, standardized measures of specific immune markers (T, B, and natural killer [NK] cells), measures of antibodies to molds, neuropsychological testing, and 19-channel quantitative EEG (QEEG). The results of this project are being reported in a series of papers. The study presented herein was conducted to assess the psychological, neuropsychological, and electrocortical effects of mixed toxic mold exposure.

Materials and Method

Patients. Adult patients (N = 182) with a history of exposure to mixed colonies of molds and their associated mycotoxins (confirmed with environmental and serologic testing) as a result of structural water intrusion in residential, workplace, or school-based settings,[19] were included in this multicenter study of data gathered from chart review. All patients had been referred for evaluation of health problems related to toxic mold exposure. Because of the prominence of neurological symptoms and complaints of cognitive dissonance, patient assessments included the following neuropsychological and neurophysiological evaluations: a structured psychometric symptom checklist, neuropsychological testing, and QEEGs. The patients (age 42.7 ± 16 yr [mean ± standard deviation]) were evaluated from September 1999 through June 2003. The group comprised 126 females (age 39.3 ± 18.1 yr) and 83 males (age 36.6 ± 21.1 yr). All of the patients were evaluated for this study. Test results were compared against a national normative database in all cases. Inasmuch as our study was based on data from chart reviews, the numbers of subjects for each measure varied slightly.

Measures of psychological distress. Each patient was evaluated with a standard clinical interview and a psychometric self-report symptom inventory—the Symptom Checklist 90-R (SCL-90-R).[26] Patients rated each item on a 5-point scale of distress, ranging from *not at all distressed* (0) to *extremely distressed* (5). Their ratings were computer-scored and produced normalized t scores for 9 symptom dimensions (i.e., Somatization, Obsessive-Compulsive, Interpersonal Sensitivity, Depression, Anxiety, Hostility, Phobic Anxiety, Paranoid Ideation, and Psychoticism) and 3 global indices (i.e., Global Severity Index, Positive Symptom Distress Index, and Positive Symptom Total). For comparison, we used an adult nonpsychiatric patient normative database because it best represented the individuals examined in this study.

Neuropsychological testing. We selected neuropsychological tests on the basis of specific patient complaints, clinical efficiency, and time constraints. All tests chosen had appropriate and comparable norms for adults and children. To obtain estimates of premorbid and general intellectual functioning for all patients, we administered the Vocabulary subtest from the Wechsler Adult Intelligence Scale, 3rd ed. (WAIS-III) and/or from the Wechsler Abbreviated Scale of Intelligence (WASI).[27]

Three subtests from the WAIS-III were administered. The Digit Span subtest measures attention and working

memory capacity. The Digit Symbol Coding subtest measures visual motor learning and psychomotor speed. The Symbol Search subtest is an indicator of visual scanning and concentration.

Two subtests from the Delis-Kaplan Executive Function System (D-KEFS) were selected to measure executive or higher-level cognitive functions.[28] The D-KEFS Color-Word Test produces baseline measures of color naming and word reading to compare with executive measures of inhibition and inhibition switching. The D-KEFS Trail Making Test provides baseline measures of visual scanning, number sequencing, letter sequencing, and motor speed, as well as an executive measure of number-letter switching.

The Integrated Visual and Auditory Continuous Performance Test (IVA-CPT) is a computerized test designed to evaluate auditory and visual attention over time.[29] The IVA-CPT produces global composite scores consisting of a Response Control Quotient (a positive way to describe the problem of response inhibition) and an Attention Quotient (a positive way to describe problems of inattention, loss of focus, and slow processing speed). Resulting measures are normed with a mean of 100 and a standard deviation of 15.

QEEG. Brain electrical activity was recorded from 19 cortical positions in accordance with the International 10/20 electrode-placement system, using a Lexicor Neurosearch Digital EEG acquisition system (Lexicor Research Center [Boulder, Colorado]). Electrodes were positioned on the scalp using appropriately sized electrocaps. We used impedance measurements for each cortical site to ensure accurate data collection. The raw data were edited for artifacts and then subjected to quantitative and neurometric analyses of amplitude, power, and mean frequency, using the NxLink database.[30–32] (The NxLink database has received a 510(k) clearance from the FDA [July 1998, #K974748], indicating that construction of the database was scrutinized for good manufacturing practices, and signifying the legitimacy of marketing claims made concerning the database.)

The NX-Link database uses the following grouped band frequencies: delta (1.5–3.5 Hz), theta (3.5–7.5 Hz), alpha (7.5–12.5 Hz), and beta (12.5–25.0 Hz). Absolute power magnitude is the amount of electrical activity at each frequency band. Relative power is a proportion of the total absolute power across the different frequency bands. Measures of power reflect different levels of cortical electrical activity and offer insight into cortical regional differences in activation, functional differentiation and integration, and corticothalamic regulation. Decreases in mean frequency are commonly referred to as "slowing," indicating that the average speed of oscillation is decreased relative to the population normal values for that frequency band. The reverse is true of higher-than-expected average frequency, which would be considered "accelerated."

For each QEEG variable, the 5 cortical sites or positions with the most deviant scores were chosen to create a distinct QEEG summary variable. The effects of toxic mold exposure on cortical functioning were related primarily to prefrontal and frontal dysfunctions, with some involvement of other cortical regions. We used these summary variables as predictor variables in forward stepwise regression analyses to determine the amount of variance accounted for by these symptom sets for each neuropsychological testing finding, SCL-90-R factor, and exposure measure.

Data preparation included examination of medication effects. Anxiolytic and narcotic medications were found to have significant effects on some QEEG variables; therefore, our analysis and interpretation excluded such cases.

Measures of exposure and dose-response relationship. Our analyses also took into account the degree of exposure to toxic molds for each individual in the sample. For each patient, we used the following predictor variables: average hours per day present in the building, days of exposure, maximum exposure (calculated as: hr/day × days of exposure), average number of colony forming units (CFUs) per m^3 from all available air samples in a given building, average CFUs × maximum exposure, and whether *Stachybotrys* was present (yes = 1, no = 0). Scores for each of these measures were then used as predictor variables in a forward stepwise regression analysis to determine the amount of variance accounted for by each of these measures of exposure on the SCL-90-R factors, the neuropsychological test measures, and the QEEG results.

Statistical analysis. Repeated measures analyses of variance (ANOVAs) were used to examine the relationships between the QEEG measures, neuropsychological testing results, SCL-90-R factors, and measures of exposure.[33]

Results

Psychological disturbance (SCL-90-R). The descriptive statistics summarizing the group means for 106 of the toxic-mold–exposed population revealed that most of the patients reported a wide range of somatic, affective, and cognitive symptoms, as well as a very high level of general distress. Figure 1 shows that all SCL-90-R scores were significantly elevated. The Global Severity Index had a mean average *t* score of 67—almost 2 standard deviations above the norm. The 4 highest scores on subtests occurred on the Obsessive-Compulsive, Somatization, Depression, and Anxiety subscales.

Examination of the items that made up each of the SCL-90-R scaled scores revealed considerable overlap in symptoms across the categories of cognitive, affective, and somatic symptoms. A factor analysis of all items defined 6 factors. After examination of the indi-

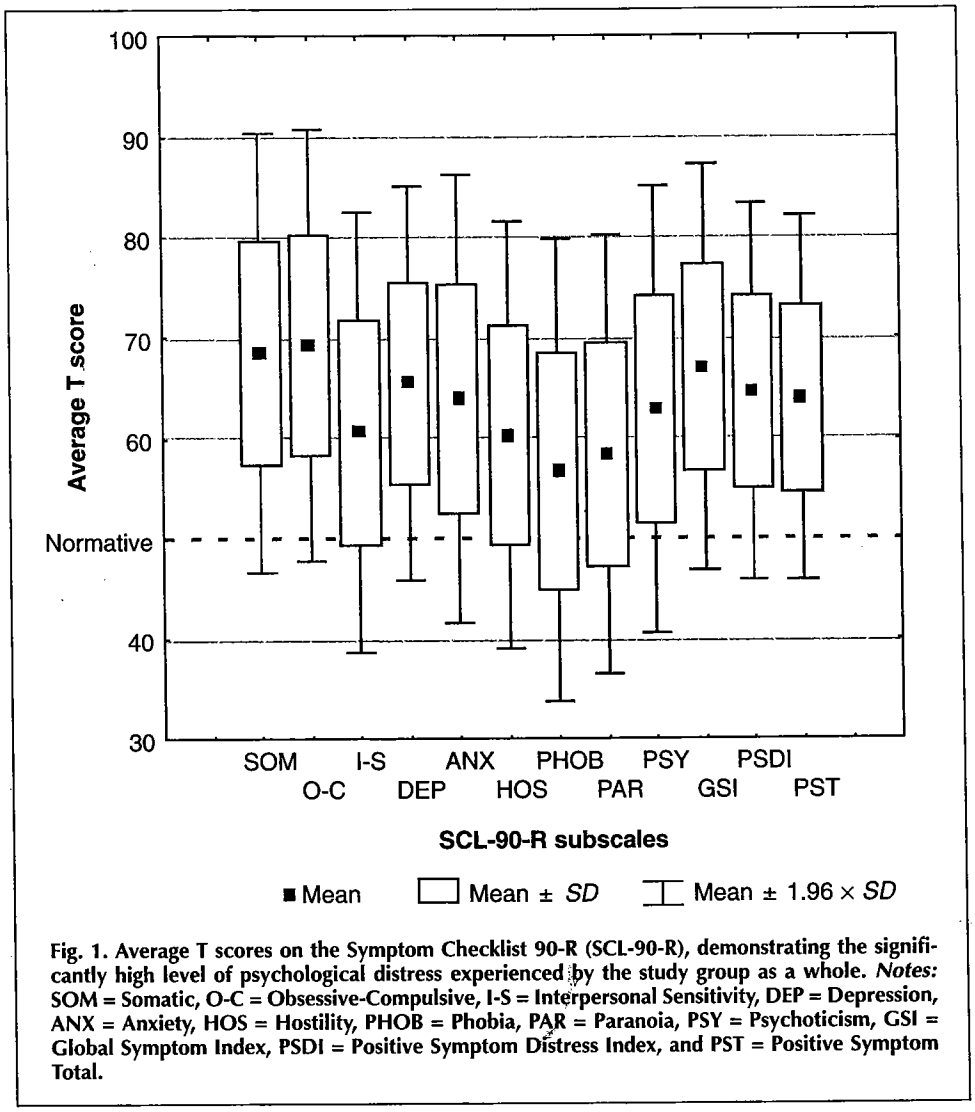

Fig. 1. Average T scores on the Symptom Checklist 90-R (SCL-90-R), demonstrating the significantly high level of psychological distress experienced by the study group as a whole. *Notes:* SOM = Somatic, O-C = Obsessive-Compulsive, I-S = Interpersonal Sensitivity, DEP = Depression, ANX = Anxiety, HOS = Hostility, PHOB = Phobia, PAR = Paranoia, PSY = Psychoticism, GSI = Global Symptom Index, PSDI = Positive Symptom Distress Index, and PST = Positive Symptom Total.

vidual items composing these factors, we numbered and labeled them as follows. *Factor 1: Melancholic Depression* accounted for 32.7% of the total variance in responses. It contained symptom items commonly associated with or reported in depressed states, such as feelings of guilt, worthlessness, hopelessness, and interpersonal distress. *Factor 2: Somatic Complaints* accounted for 6.2% of the total variance in responses. It is straightforward in name and content. *Factor 3: Cognitive Distortion* accounted for 5.5% of the total variance in responses. Described symptoms are usually associated with more severe mental problems, such as paranoia, hypersensitivity, and nervous irritability. *Factor 4: Affective Loss of Control* accounted for 4.9% of the total variance in responses. It contained items reflecting more severe symptoms of agitated depression. *Factor 5: Anxious Depression* accounted for 3.7% of the total variance in responses and contained symptoms that suggested increased arousal, agitated depression, and somatic complaints, such as sleep disturbance, fatigue, tension, nausea, "feeling blue," and worrying too much. *Factor 6: Cognitive Complaints,* which accounted for 3.4% of the total variance in responses, contained 2 items describing concerns about cognitive processes. It is noteworthy that only Factor 1 accounted for any significant amount of variance.

At the time of our analysis, item-by-item data were available for only 52 of the 106 patients who had taken the SCL-90-R. We used scores for each of the 6 factors as predictor variables in forward stepwise regression analyses to determine the amount of variance that these symptom sets accounted for in each neuropsychological testing finding and QEEG finding.

Neuropsychological testing vs. normative data. Patients' levels of completed education (if they were born after World War II) and a measure of vocabulary (from the WAIS-III subtest scaled score) are widely accepted methods for estimating premorbid levels of intellectual functioning.[34] The average level of education for the 109 adults in this sample was 14.57 yr, indicating a slightly higher than average level of premorbid functioning. This finding agreed with the average WAIS-III

vocabulary scores for this sample, which was almost 1 standard deviation above normal.

We found statistically significant differences between the high average range scaled score for the group's estimate of premorbid intelligence (the Vocabulary subtest from the WAIS-III) and the group's scaled scores for performance on the D-KEFS Color-Word subtests of Color Naming, Word Reading, Inhibition, Inhibition-Switching ($p < 0.0000$), and the group's scaled scores on the D-KEFS Trail Making Test subtests of Visual Scanning, Number Sequencing, Number-Letter Switching, and Motor Speed ($p < 0.0005$) (Fig. 2). Statistically significant differences ($p < 0.05$) were also found between the scaled score for the Vocabulary subtest from the WAIS-III and the group's scaled scores for performance on other WAIS-III subtest measures of "fluid" intelligence (e.g., attention, working memory, visual motor learning, speed, and visual scanning).

The IVA-CPT, which tests response inhibition and the ability to maintain attention over time, has a normative population mean of 100 and a standard deviation of 15. Figure 3 summarizes the results of the normative scores on the IVA-CPT. All scores were below expectations, given the group's estimated level of intelligence on the WAIS-III Vocabulary subtest and the average level of education. Attention scores were especially depressed. We also found dissociation between response control and attention across both auditory and visual domains of the IVA-CPT. Attentional functioning in this group was significantly impaired ($p < 0.0126$) compared with response inhibition capacities.

QEEG measures vs. normative data. Without regard to any categorical predictors, the group as a whole exhibited a definite pattern of slowing in the faster beta frequency ($F(18, 2,574) = 2.8707$; $p = 0.00005$) and acceleration of the slower delta ($F(18, 2,592) = 2.9554$, $p = 0.00003$) and theta ($F(18, 2,592) = 3.1680$; $p = 0.00001$) frequencies. The QEEG summary variables for these measures identified frontal cortical positions as the most significantly deviant (mean frequency delta: Fp1 Fp2 F3 F4 F8; mean frequency theta: Fp1 Fp2 F3 F4 C4; mean frequency beta: Fp1 Fp2 F7 F8 T3).

There were significant increases in absolute ($F(18, 2,538) = 12.007$; $p = 0.0000$) and relative ($F(18, 2,520) = 9.5775$; $p = 0.0000$) power alpha, and in absolute ($F(18, 2,538) = 6.5807$; $p = 0.00000$) and relative ($F(18, 2,520) = 3.4143$; $p = 0.00000$) power theta. Again, the QEEG summary variables identified the frontal cortical

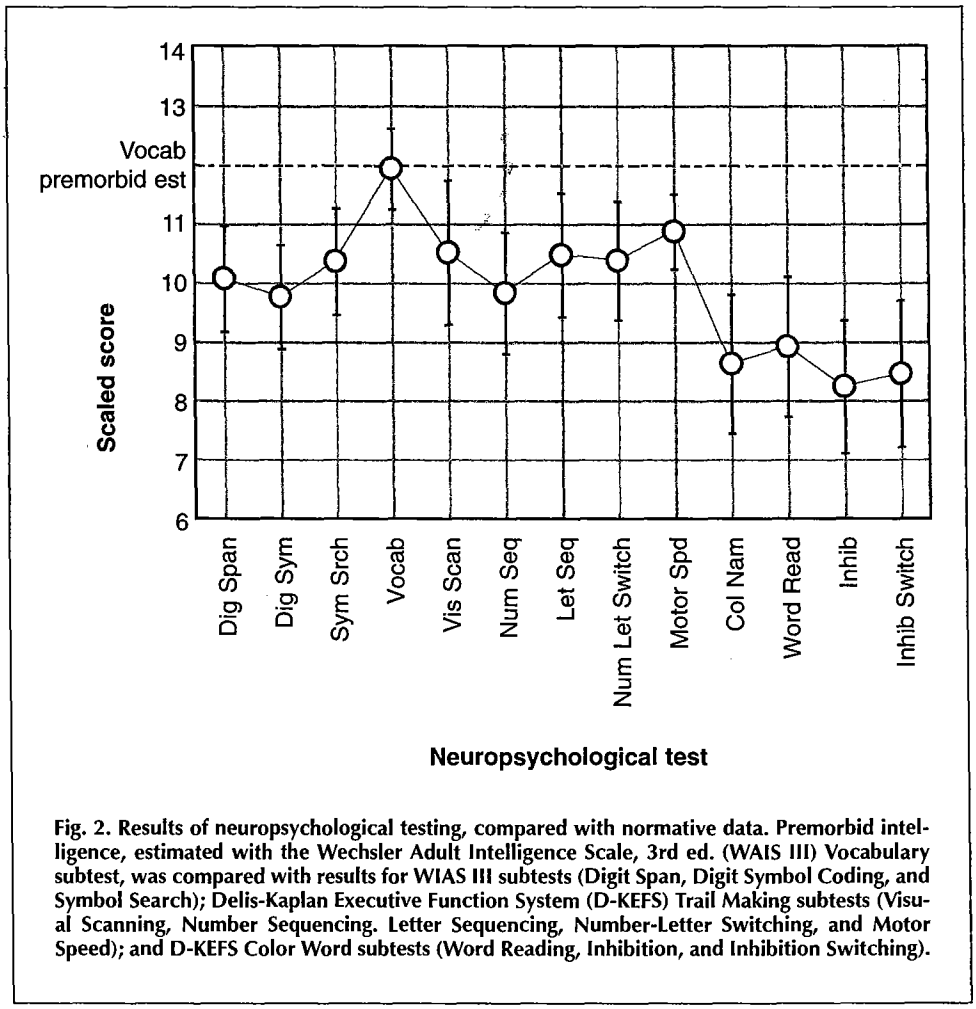

Fig. 2. Results of neuropsychological testing, compared with normative data. Premorbid intelligence, estimated with the Wechsler Adult Intelligence Scale, 3rd ed. (WAIS III) Vocabulary subtest, was compared with results for WIAS III subtests (Digit Span, Digit Symbol Coding, and Symbol Search); Delis-Kaplan Executive Function System (D-KEFS) Trail Making subtests (Visual Scanning, Number Sequencing. Letter Sequencing, Number-Letter Switching, and Motor Speed); and D-KEFS Color Word subtests (Word Reading, Inhibition, and Inhibition Switching).

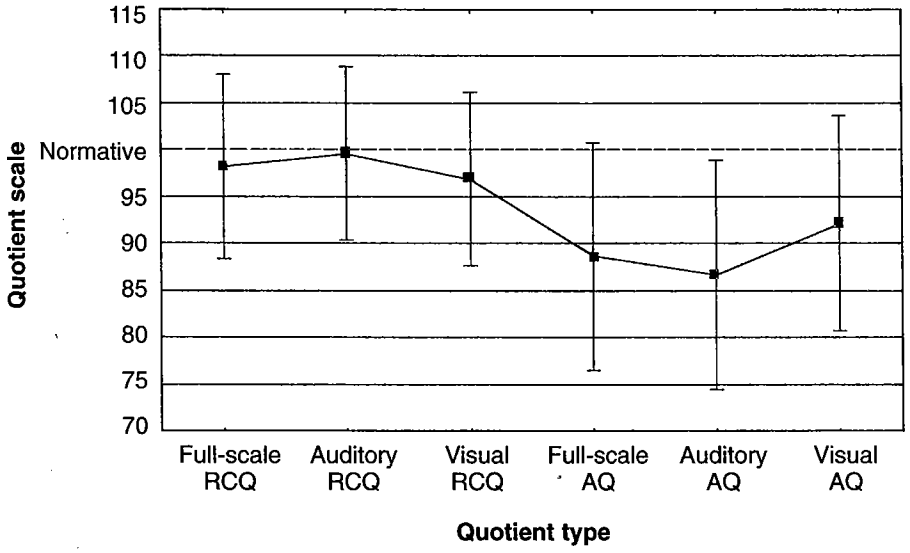

Fig. 3. Normative scores, by analysis of variance, on the Integrated Visual and Auditory Continuous Performance Test of response inhibition and attention. All scores were below expectations given the group's intelligence levels (estimated with the Wechsler Adult Intelligence Scale, 3rd ed. [WAIS III] Vocabulary subtest) and average level of education. Attention Quotient scores were significantly lower than Response Control Quotient scores. *Notes:* $F(5, 410) = 2.9448$, $p = 0.01263$. Vertical bars denote 95% confidence intervals. RCQ = Response Control Quotient, and AQ = Attention Quotient.

areas as being the most deviant (absolute alpha: Fp1 Fp2 F4 F8 Fz; relative alpha: Fp1 Fp2 F4 Fz Cz; absolute theta: F3 F4 F7 F8 Fz; relative theta = Fp1 Fp2 F7 F8 F4).

The absolute power *Z* scores in the delta frequency band across the scalp were significantly decreased relative to the normative values ($F(18, 2{,}538) = 2.4576$; $p = 0.00058$). Specifically, the decreases were most notable in the prefrontal, parietal, and occipital areas (i.e., Fp1, Fp2, Cz, P3, P4, Pz, O1, and O2)

Exposure measures vs. SCL-90-R, QEEG, and cognitive measures. Measures of exposure were highly predictive of neuropsychological test performance, moderately predictive of QEEG measures, and only slightly predictive of measures of subjective stress on the SCL-90-R.

There were predictive, nonsignificant trends for performance on the D-KEFS Trail Making Test and the Color-Naming subtest (in which maximum exposure, average CFUs, and whether *Stachybotrys* was present in the environment yielded an adjusted multiple $R^2 = .240$, $p < 0.070$); the D-KEFS Word Reading subtest (in which maximum exposure and whether *Stachybotrys* was present in the environment yielded an adjusted multiple $R^2 = .220$, $p < 0.053$); and the D-KEFS Color-Word Inhibition (classic Stroop) subtest (in which maximum exposure, average CFUs, and whether *Stachybotrys* was present in the environment yielded an adjusted multiple $R^2 = .235$, $p < 0.073$).

Table 1 shows the significant predictive power ($p < 0.05$) 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.

It should be kept in mind in reviewing these findings that the sample sizes are smaller than those for other analyses reported herein, which can affect the degrees of freedom and the variability accounted for by these scores. Although some of the values reported in Table 1 are quite high (e.g., WAIS-III Symbol Search subtest adjusted multiple $R^2 = .787$), it is still safe to conclude that measures of exposure account for a significant amount of the variance in neuropsychological test performance on the basis of these findings.

Table 2 shows that 6 of the 8 QEEG summary variables were related significantly to measures of mold exposure. Four of the 6 variables were measures of power in the theta and alpha frequencies. 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.

Exposure measures predicted scores on only 2 of the SCL-90-R factors. The only statistically significant relationship was found between CFU × maximum exposure and a combination of SCL-90-R Factor 3 (Cognitive Distortion) and Factor 6 (Cognitive Complaints), which together yielded an adjusted multiple R^2 of 0.291, $p <$

Table 1.—Relationships between Measures of Exposure and Performance on Neuropsychological Tests

Test	*n*	Variables in model	Significant β weights*	Adjusted multiple R^2	*p*
D-KEFS Trail Making Test: Visual Scanning	19	Hours of exposure, days of exposure, maximum exposure, average CFU, *Stachybotrys* present	Hours of exposure = 0.836 Days of exposure = 3.32	. 350	0.054
D-KEFS Trail Making Test: Letter Sequencing	19	Hours of exposure, days of exposure, maximum exposure, CFU × maximum exposure	Days of exposure = 4.99 Maximum exposure = –4.6	.326	0.047
D-KEFS Trail Making Test: Number-Letter Switching	19	Hours of exposure, days of exposure, maximum exposure, CFU × maximum exposure, *Stachybotrys* present	Hours of exposure =0 .997 Days of exposure = 5.12 Maximum exposure = -4.6	.399	0.035
D-KEFS Trail Making Test: Motor Speed	19	Hours of exposure, days of exposure, maximum exposure, average CFU, CFU × maximum exposure, *Stachybotrys* present	*Stachybotrys* present = 0.963 Days of exposure = 5.08 Maximum exposure = –4.7 CFU × maximum exposure = 0.626 Hours of exposure = 0.642	.705	0.001
D-KEFS Color-Word Inhibition: Switching	18	*Stachybotrys* present, maximum exposure	*Stachybotrys* present = 0.623 Maximum exposure = 0.546	.298	0.028
WAIS-III: Digit Symbol Coding	36	Hours of exposure, days of exposure	Hours of exposure = –0.47 Days of exposure = 0.325	.259	0.002
WAIS-III: Symbol Search	15	Hours of exposure, maximum exposure, average CFU, CFU × maximum exposure	Hours of exposure = –0.64 Maximum exposure = 0.598 Average CFU = 0.804 CFU × maximum exposure = –0.71	.787	0.0004
IVA-CPT: Full-Scale Attention Quotient	30	Maximum exposure, average CFU, CFU × maximum exposure	Maximum exposure = 0.399 Average CFU = –0.40	.300	0.006
IIVA-CPT: Visual Attention Quotient		Maximum exposure, average CFU, CFU × maximum exposure	Average CFU = –0.41	.276	0.009
IIVA-CPT: Auditory Attention Quotient		Maximum exposure, average CFU	Maximum exposure = 0.469	.278	0.004

Notes: The fluctuating sample sizes reflect the fact that the statistics program selected only cases from the chart review for which all data in the model were present. CFU = colony-forming unit.
*$p < 0.05$.

Table 2.—Predictive Power for Estimates of Degree of Exposure and Quantitative Electroencephalogram (QEEG) Variables

QEEG variable	*n*	Variables in model	Significant β weights*	Adjusted multiple R^2	*p*
Mean frequency delta	62	Average CFU	Average CFU = –0.29	.069	0.022
Relative theta	62	CFU × maximum exposure, *Stachybotrys* present, days of exposure, average CFU	None	.096	0.043
Relative alpha	62	CFU × maximum exposure, *Stachybotrys present*, hours/day of exposure, maximum exposure	CFU × maximum exposure = 0.344	.124	0.020
Absolute delta	62	*Stachybotrys* present	*Stachybotrys* present = 0.270	.057	0.034
Absolute theta	62	*Stachybotrys* present	*Stachybotrys* present = 0.255	.049	0.046
Absolute alpha	62	Average CFU, CFU × maximum exposure	None	.086	0.026

Note: CFU = colony-forming unit.
*$p < 0.05$.

0.0124. That is, 29.1% of the cognitive distortion and cognitive complaints were predicted by the variance observed in CFU × maximum exposure. A nonsignificant trend was found between hours of exposure and Factor 3 (Cognitive Distortion), which yielded an adjusted R^2 of 0.064, $p < 0.096$.

QEEG and neuropsychological test performance. We observed several statistically significant relationships between the QEEG summary variables and performance on neuropsychological tests. The data in Table 3 demonstrate that significant QEEG predictors of test performance were found for measures of number and letter sequencing, number-letter switching, motor speed, response inhibition, and visual attention. The specific tests were the D-KEFS Trail Making Number Sequencing, Letter Sequencing, Number-Letter Switching, and Motor Speed subtests; the D-KEFS Color-Word Inhibition subtest; and the IVA-CPT Visual Attention Quotient. QEEG variables showing the greatest predictive power were those that involved the theta or alpha frequency bands and, most often, the mean frequency theta or relative power theta summary variables.

Minimal QEEG predictors of test performance or nonsignificant trends ($p < 0.10$ through $p < 0.051$) were found for measures of visual scanning, color naming, attention, and response inhibition (WAIS-III Symbol Search subtest, D-KEFS Color-Word Color Naming subtest, IVA-CPT Attention Quotient, IVA-CPT Response Control Quotient, and IVA-CPT Auditory Response Control Quotient).

SCL-90-R and cognitive performance. We could address only partially the mitigating influence of psychological factors on cognitive test performance in this study. However, sufficient cases were available to compare the results of the SCL-90-R with Digit Symbol Coding and the results of the IVA-CPT scales.

The SCL-90-R scales and factors did not predict performance on Digit Symbol Coding (adjusted $R^2 = .002$). The SCL-90-R scales of depression and anxiety were found to have the opposite effect on the IVA-CPT Attention Quotient, even though these SCL-90-R scales correlate in this data set at $R = .81$.

We observed a significant relationship for Factor 1 (Melancholic Depression) and Factor 5 (Anxious Depression) with the IVA-CPT full-scale Attention Quotient (adjusted $R^2 = .241$, $p < 0.04$), Auditory Attention Quotient (adjusted $R^2 = .200$, $p < 0.06$), and Visual Attention Quotient (adjusted $R^2 = .305$, $p < 0.02$). In all of these cases, Factor 1 had a significant *negative* beta weight, whereas Factor 5 had a significant *positive* beta weight. Increases in Factor 1 were correlated with decreases in the attention quotient scores, whereas increases in Fac-

Table 3.—Quantitative Electroencephalogram (QEEG) Predictors of Neuropsychological Test Performance

Test	*n*	QEEG variables in model	Significant β weights*	Adjusted multiple R^2	*p*
D-KEFS Trail Making Test: Number Sequencing	65	Mean frequency theta, absolute alpha, mean frequency beta	Mean frequency theta = 0.571 Absolute alpha = –0.44	.220	0.0004
D-KEFS Trail Making Test: Letter Sequencing	65	Mean frequency theta, absolute alpha, mean frequency beta	Mean frequency theta = 0.503 Absolute alpha = –0.43	.169	0.002
D-KEFS Trail Making Test: Number-Letter Switching	65	Absolute delta, mean frequency theta, mean frequency beta, relative alpha, absolute theta, absolute alpha, relative theta	Mean frequency theta = 0.502 Relative alpha = –1.1 Absolute theta = –1.9 Absolute alpha = 1.83 Relative theta = 0.886	.193	0.007
D-KEFS Trail Making Test: Motor Speed	65	Absolute delta, relative theta, mean frequency delta	Absolute delta = –0.31 Relative theta = 0.271	.110	0.017
D-KEFS Color-Word Inhibition	65	Mean frequency theta, absolute theta, relative theta, absolute delta, a bsolute alpha, relative alpha	Mean frequency theta = 0.560 Absolute theta = –3.2 Relative theta = 1.46 Absolute delta = 0.471 Absolute alpha = 2.91 Relative alpha = –1.6	.348	0.00002
IVA-CPT Visual Attention Quotient	98	Absolute alpha, relative alpha, absolute theta, relative theta, mean frequency theta, mean frequency delta, absolute delta	Mean frequency theta = 0.419 Absolute theta = –2.0 Relative theta = 0.904 Absolute alpha = 1.62 Relative alpha = –0.82	.137	0.005

*$p < 0.05$.

tor 5 were correlated with increases in the attention score. The sample size available for each analysis (n = 19) made these findings tentative at best.

SCL-90-R and QEEG. We also explored the effect of stress on cortical electrical activity. A significant relationship was found between only 2 of the 12 QEEG summary variables and SCL-90-R factors that accounted for small amounts of the variance on item analysis.

A positive beta weight was observed between relative power theta and Factor 6 (Cognitive Complaints), whereas a negative beta weight was seen between relative power theta and Factor 5 (Anxious Depression); adjusted multiple $R^2 = .107$, $p < 0.025$. That is, patients' reports of concern regarding cognitive abilities (Factor 6) were related positively to increasing relative power theta—often an indication of decreased cognitive arousal or efficiency. This can be interpreted as consistent with the negative relationship observed for Factor 5, in which symptoms of increased arousal, tension, and anxiety can indicate increased cortical arousal and vigilance and, therefore, decreased relative power theta.

Significant correlation (adjusted multiple $R^2 = .112$, $p < 0.052$) was also found between the mean frequency beta summary score—a marker associated with cognitive efficiency (faster = greater efficiency; slower = lesser efficiency)—and SCL-90-R Factor 4 (Affective Loss of Control) and Factor 5 (Anxious Depression). Factor 3 (Cognitive Distortion) and Factor 6 (Cognitive Complaints) showed negative relationships with this cortical indicator.

Our results suggested that, to a limited extent, increased frontal cortical arousal (as indicated by decreased relative power theta and increased mean frequency beta) was associated with increased anxiety and less complaints of cognitive deficits. Furthermore, decreased frontal cortical arousal, as indicated by increased relative power theta and decreased mean frequency beta, was associated with increased cognitive complaints and decreased anxiety.

Discussion

Psychological distress. Patients—including multiple family members—exposed to toxic molds reported moderate to severe levels of psychological distress related to the development of a wide range of physical, cognitive, and emotional symptoms. Problems included the frustration of trying to find knowledgeable and appropriate medical care, interference with social and work life, temporary or permanent abandonment of homes and possessions, financial stress, and anxiety and helplessness as a result of continuing poor health.

Most of these patients, in absence of any significant premorbid psychiatric problems, could be diagnosed as suffering from acute stress, adjustment disorder, or post-traumatic stress. *Only 3.8% of our sample population reported significant premorbid psychiatric or neuropsychiatric problems* (e.g., history of major depression, post-traumatic stress disorder, seizure disorder, closed head injury). Individuals with significant historical problems were eliminated from the data analysis in order to minimize the effect of such problems in this patient population.

The patients in this study showed a significant level of psychological stress, with depression being the only factor to account for a substantial amount of variance in the reported symptoms. There were limited significant relationships between some of the SCL-90-R factors and QEEG findings. Although a strong relationship was found between the average number of CFUs present in the environment and the subjectively reported cognitive difficulties on 2 of the SCL-90-R factors, it must be remembered that these 2 factors accounted for only 8.9% of the total variance on the item analysis. The process of differential diagnosis supports the conclusion that the individuals in this study suffered severe psychological distress resulting from a combination of overwhelming personal stress and poor health, with the mitigating influence of organically based central nervous system deregulation of emotions as a result of toxic or metabolic encephalopathy.

At this point, it is difficult to say what the cost of these deficits might be in terms of productivity or personal relationships, although it would clearly be significant, to a reasonable medical and scientific certainty, if the distress experienced by the patients in this sample is any indicator.

One limitation in our study was that patient reports could not be validated independently. We considered the reports to be credible, however, given that the patients were interviewed individually and completed a questionnaire; there was little motive to deceive in this clinical situation. Another measure of credibility is the fact that patients who were involved in litigation did not report more symptoms than nonlitigants.[19]

Neuropsychological testing. The results of the neuropsychological testing produced positive findings for impaired cognitive functioning on a wide variety of tasks, when compared with premorbid estimates of intelligence. The pattern and severity of results are similar to, and corroborate, the results obtained by other researchers who have conducted preliminary studies of cognitive functioning in toxic-mold–exposed individuals.[18,20–22] This pattern is also similar to that for individuals diagnosed with mild traumatic brain injury.[21]

Prior researchers have reported that symptoms of depression can impair neuropsychological test performance.[21,35,36] Baldo[21] assessed cognitive functioning and depression in a small group of 10 mold-exposed patients and found a significant relationship between cognitive impairment and depression. In the current study, in agreement with previous research, both anxiety and

depression were indicative of stress, but only depression had a detrimental effect on attention. One interpretation may be that symptoms of anxiety are associated with higher levels of vigilance, whereas symptoms of melancholic depression are associated with lower arousal and mental sluggishness. However, this preliminary and limited analysis does not offer conclusive evidence regarding the effects of psychological distress on neuropsychological test performance in this sample. The limitation of incomplete data sets for all of the neuropsychological tests prevents a clear interpretation at this time.

We acknowledge the complex relationships among toxic mold exposure, impaired cognition, psychosocial stressors, poor physical health, and emotional factors. Our overall findings, however, lend support to the hypothesis that patients' cognitive deficits are frequently related to underlying organic deficits caused by toxic mold exposure. Most critical for this study was the significant and consistent effect of mold-exposure measures on the results of cognitive testing. Also, abnormalities in cortical electrical activity—primarily in the frontal and prefrontal lobes—were significantly and consistently related to deficits in cognitive functioning and mold-exposure measures. An additional factor that suggests that the observed cognitive deficits were of organic origin is the lack of variability of deficits between tests usually most influenced by psychological factors (e.g., measures of attention or working memory) and tests not usually influenced by psychological factors (e.g., color naming or word reading). Corroboration was also found in a study that examined the influence of personality traits on neuropsychological test performance in toxic encephalopathy cases vs. healthy referent cases. Persson et al.[37] concluded that the neuropsychological performance decrements in toxic encephalopathy cases were not related to elevated mental stress, but were dominated by the effects of organic brain impairment.

It is important to address the fact that most of the patients in this study subjectively reported moderate to severe ratings of cognitive impairment, rather than mild to moderate as measured by the testing. The pattern of deficits commonly seen in mild traumatic brain injury is very similar to that found in mold-exposed individuals.[20,21,27] This phenomenon—clinically referred to as "brain fog"—is also common in individuals who suffer from multiple chemical sensitivity.[38] Patients reported a loss of their sense of self, of their usual ways of doing things, and even of their personality. They were painfully aware of their deficits and were constantly frustrated by their loss of cognitive efficiency and frequent mistakes. This can be understood as a disturbance or dysfunction of the frontal cortical areas, as implicated in the QEEG findings and the relationship of exposure data to test performance in this study. In humans, the sense of self is organized in the frontal brain areas.[39] For these reasons, we recommend that studies or clinical evaluations of cognitive functioning in mold-exposed patients employ functional imaging techniques to assess organic dysfunction.

QEEG. The results of the QEEG data recorded from mold-exposed patients indicate 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 and theta) and decreased (slowed) higher frequencies (beta). These changes indicate a collapse toward the middle of the frequency spectrum. Such findings, coupled with the increased levels of absolute and relative power theta and alpha in the frontal sites, indicate a hypoactivation of the frontal cortex. The latter may result from brainstem involvement and may indicate insufficient excitatory input from the reticular activating system anatomically seated in the midbrain. Deviant QEEG findings of this magnitude should not have been observed in frontal lobe functioning without some insult to the functioning of the neural systems that depend on integrative coordination from the frontal lobes. These findings are consistent with other functional imaging studies mentioned earlier.[1,2,3,24,25]

Measures of toxic mold exposure were related significantly to QEEG findings, and both measures of exposure and QEEG measures were related significantly to cognitive test performance. Psychological factors appeared to have only a limited relationship to QEEG results, reflecting the arousal level of the frontal lobes. This finding supports the conclusion that exposure to toxic mold results in central nervous system dysfunction, as measured by QEEG.

The use of QEEG and neurometrics in research and clinical practice has been the subject of some controversy, although recent opinions and evidence describe more strengths than weaknesses.[32,40–43] Our decision to use QEEG and neurometrics included the facts that they are noninvasive, relatively inexpensive, free from cultural and ethnic factors, have good reliability and validity, and are highly sensitive for detecting dementias and encephalopathy.[44,45]

The most significant limitation of the NxLink database is its exclusive reliance on banded EEG. Findings restricted to narrow frequencies are obscured with the use of relatively wide bands normed in the database. Plans for future investigations include examination of other indices of QEEG activity, such as coherence and phase lag relationships, and examination of the data using other databases that allow single-frequency analysis.

Summary and Conclusions

Patients exposed to toxic molds reported high levels of physical, cognitive, and emotional symptoms. Rat-

ings on the SCL-90-R were moderate to severe, with a factor reflecting depression accounting for most of the variance. Most patients could be diagnosed as suffering from problems of acute stress, adjustment disorder, or post-traumatic stress. Impaired cognitive functioning was observed on multiple cognitive tasks, compared with premorbid estimates of intelligence, in a pattern of impairment similar to that for mild traumatic brain injury. The QEEG findings indicated a hypoactivation of the frontal cortex, suggesting brainstem involvement and insufficient excitatory input from the reticular activating system. QEEG measures were correlated with neuropsychological test results.

Findings of a dose-response relationship between measures of exposure and the outcome of neuropsychological tests and QEEG measures suggest that evaluation of neuropsychological and neurobehavioral deficits in mold-exposed patients should consider degree of exposure, organic-based central nervous system dysfunction, and psychological variables. Differential diagnosis supported an etiology of organic-based dysregulation of emotions and cognitive functioning as a result of toxic or metabolic encephalopathy, with some degree of mitigation by psychological variables, especially depression.

Additional work is needed to examine the effect of the length of time since last exposure to toxic mold on outcome measures. Patients have reported some reduction in symptoms when they have been removed from continued exposure. How much of a reduction, under what circumstances, and for which patients have not been fully determined.

Limitations of this study included lack of a comparison group that underwent the same testing as the mold patients, and small sample size. The lack of a comparison group was mitigated by the fact that the measures used in this study were all compared with the published normative databases developed for each of the tests. With respect to sample size, the fact that the analyses were performed on data gathered from chart review resulted in inconsistent sample sizes and the variable sample sizes reported in some of the stepwise regression analyses and descriptive statistics. For this reason, the relative contribution to the variance accounted for by any given variable can be compared only with the relative contributions of variables for which a similar number of cases were available.

Future research will include expanded use of neuropsychological testing, QEEG measures, exposure measures, correlations with immune parameters, an increase in sample size, and more complete data analysis. The use of causal modeling and path analysis may improve the interpretability of the results, allowing the multidirectional relationships that exist within this complex topic to be modeled in several different ways.

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Neural Autoantibodies and Neurophysiologic Abnormalities in Patients Exposed to Molds in Water-Damaged Buildings

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ABSTRACT. Adverse health effects of fungal bioaerosols on occupants of water-damaged homes and other buildings have been reported. Recently, it has been suggested that mold exposure causes neurological injury. The authors investigated neurological antibodies and neurophysiological abnormalities in patients exposed to molds at home who developed symptoms of peripheral neuropathy (i.e., numbness, tingling, tremors, and muscle weakness in the extremities). Serum samples were collected and analyzed with the enzyme-linked immunosorbent assay (ELISA) technique for antibodies to myelin basic protein, myelin-associated glycoprotein, ganglioside GM_1, sulfatide, myelin oligodendrocyte glycoprotein, α-B-crystallin, chondroitin sulfate, tubulin, and neurofilament. Antibodies to molds and mycotoxins were also determined with ELISA, as reported previously. Neurophysiologic evaluations for latency, amplitude, and velocity were performed on 4 motor nerves (median, ulnar, peroneal, and tibial), and for latency and amplitude on 3 sensory nerves (median, ulnar, and sural). Patients with documented, measured exposure to molds had elevated titers of antibodies (immunoglobulin [Ig]A, IgM, and IgG) to neural-specific antigens. Nerve conduction studies revealed 4 patient groupings: (1) mixed sensory–motor polyneuropathy (n = 55, abnormal), (2) motor neuropathy (n = 17, abnormal), (3) sensory neuropathy (n = 27, abnormal), and (4) those with symptoms but no neurophysiological abnormalities (n = 20, normal controls). All groups showed significantly increased autoantibody titers for all isotypes (IgA, IgM, and IgG) of antibodies to neural antigens when compared with 500 healthy controls. Groups 1 through 3 also exhibited abnormal neurophysiologic findings. The authors concluded that exposure to molds in water-damaged buildings increased the risk for development of neural autoantibodies, peripheral neuropathy, and neurophysiologic abnormalities in exposed individuals.
<Key words: mold exposure, mycotoxins, neural antibodies, neuropathy, neurophysiology>

WATER INTRUSION into houses and office buildings leads to the growth of molds and bacteria, which are known to produce toxic byproducts that include endotoxins (lipopolysaccharides), β-D-glucans, and mycotoxins (e.g., trichothecenes, ochratoxins, and aflatoxins, tremorgens), as well as volatile organic compounds.[1–8] These compounds have been found in water-damaged buildings and homes, and in artificially infested building materials.[7–11] Indoor air can be contaminated with mold spores and hyphae fragments.[12] In addition, mycotoxins have been identified in ventilation duct particulate matter or dust, and in the air of buildings in which occupants and pets have experienced adverse health effects related to mold exposure.[13–23]

Molds and mycotoxins affect the respiratory tract, kidneys, liver, and skin, as well as the immune and nervous systems.[24–45] Neurotoxic mycotoxins include ergot alkaloids, trichothecenes, citreoviridin, patulin, fumonisins, and tremorgens.[46–54] Tremorgens affect the brainstem[46] and stellate ganglion, and the basket and Pur-

kinje cells of the cerebellum.[47] Mycotoxins affect neuroreceptor sites (e.g., gamma-aminobutyric acid [GABA] receptor site[48] and inositol 1,4,5-trisphophate receptor[49]), inhibit acetylcholinesterase,[50] release excitatory neurotransmitters (e.g., glutamate, aspartate, GABA, and serotonin),[51,52] and block biosynthesis of complex sphingolipids through inhibition of ceramide synthase.[53,54] They are also mitochondrial toxins and apoptotic agents.[43,44,53]

The symptoms and health problems associated with mold-infested, water-damaged buildings involve multiple organs, including the upper and lower respiratory tracts, gastrointestinal tract, circulatory system, and the central nervous system (CNS) and peripheral nervous system (PNS).[2,5,24–37] Recent studies have shown that mold exposure has adverse effects on the nervous system. Some mycotoxins have been shown to be tremorgenic and are suspected as causative agents in woodtrimmer's disease[20] and tremorgenic encephalopathy;[21] mycotoxins present in household environments have been found to affect dogs.[22,23]

Two patterns of neurobehavioral impairment attributable to mold exposure have been described. Kilburn[26] reported on 10 individuals who had impaired balance, reaction time, color discrimination, visual fields, cognition, verbal recall, and trail making. A different group of 10 subjects exhibited impairments in all but measures of color discrimination and visual fields. Abnormalities in electroencephalograph (EEG) theta and delta activity, visual evoked potentials, and brainstem evoked potentials have been reported in children exposed to molds.[27] The EEG changes in the children were specific to the frontotemporal area of the brain, suggesting a metabolic encephalopathy. Six individuals had abnormal nerve conduction. In addition, abnormal brainstem auditory evoked potentials have been described in 4 children with suspected mycotic neuromas who were exposed to mixed molds, including *Stachybotrys chartarum* and *Aspergillus species*.[57] Moreover, both neurobehavioral[31,57] and correlated quantitative EEG[31] changes indicative of right frontal lobe involvement have been reported in patients with chronic exposure to mold in water-damaged buildings. Mold exposure has also been implicated in optic neuritis[57] and multifocal choroiditis.[58] Finally, demyelination of the CNS has been reported following exposure to ibotenic acid,[59] abuse of "magic mushrooms" (Psilocybe),[60] and gliotoxin.[61] Because stachylysin has been found in human serum following exposure to *S. chartarum*,[62] and mycotoxins are present in indoor air and bioaerosols,[13–19] it is imperative that health complaints of occupants exposed to molds in water-damaged buildings be taken seriously and be investigated with appropriate diagnostic testing.

This communication describes 119 mold-exposed patients who had multiorgan symptoms and peripheral neuropathy. Complaints included severe fatigue, decreased muscle strength, sleep disturbances, numbness and tingling of extremities (with and without tremors of the fingers and hands), and severe headache. Patients had abnormal neurological examinations. Ninety-nine of these individuals had abnormal nerve conduction velocities (NCVs) in association with autoantibodies against 9 neural antigens, whereas 20 had normal test results. We present data on motor neuropathy, sensory neuropathy, and mixed sensory–motor polyneuropathy, as well as increased antibodies to neural antigens.

Materials and Method

Patients. The study population consisted of 119 patients (79 females and 40 males; mean age ± standard deviation [*SD*] = 41.3 ± 12.9 yr). The patients had health complaints and proven environmental exposure to molds in their homes and/or workplaces. Mold exposure was documented by Aerotech Laboratories (Phoenix, Arizona). All patients were interviewed one-on-one by the principal author (AWC) regarding exposure history, as well as health problems and symptoms for each organ system (e.g., CNS, PNS, respiratory, skin, musculoskeletal). Mold-specific serum antibody tests for *S. chartarum, Penicillium, Aspergillus, Cladosporium, Alternaria,* and *Chaetomium,* performed on each patient by Immunosciences Lab., Inc. (Beverly Hills, California), verified exposure to molds. Some of these data have been reported previously.[63–65]

We studied patients who had symptoms of peripheral neuropathy (e.g., tingling, tremors, loss of sensation in extremities). Blood was drawn for serology testing for neural antigens. NCV tests were performed at or near the time of initial presentation as follows: all 119 patients were tested at 10.8 ± 41 days; patients with abnormal NCVs (n = 99) were tested at 11.5 ± 44 days; and patients with normal NCVs (n = 20, controls) were tested at 7.5 ± 18 days.

Blood samples. Peripheral venous blood was collected and shipped at ambient temperature to Immunosciences Lab., Inc. (Beverly Hills, California). Autoantibodies (immunoglobulin IgG, IgM, and IgA) against 9 neural antigens were assessed for each patient.

Neural antigens. Myelin basic protein (MBP), myelin-associated glycoprotein (MAG), ganglioside (GM_1), chondroitin sulfate ($CONSO_4$), α-B-crystallin (crystallin), and tubulin were purchased from Sigma-Aldrich (St. Louis, Missouri). Neurofilament antigen (NAF) was purchased from Boehringer Mannheim Roche (Indianapolis, Indiana). MBP peptides 87-206 and myelin oligodendroctye glycoprotein (MOG) peptides 21-4-, 61-80 were synthesized by Research Genetics (Huntsville, Alabama).

Controls for neural antigens. The controls for determination of the mean ± *SD* and 95% confidence intervals (CIs) for the neural antigens consisted of 500

healthy adult blood donor volunteers. The controls were of similar age and sex distribution as the 119 patients.

ELISA testing. We used enzyme-linked immunosorbent assay (ELISA) to test for antibodies against 9 different neural-specific antigens, as reported previously.[66–68] Briefly, antigens were dissolved in methanol at a concentration of 1.0 mg/ml and then diluted 1:100 in 0.1 M carbonate-bicarbonate buffer (pH 9.5). Then, 50 µl of the mixture was added to each well of a polystyrene flat-bottom ELISA plate. Plates were incubated overnight at 4 °C and then washed 3 times with 20 mM Tris-buffered saline (TBS) containing 0.05% Tween 20 (pH 7.4). The nonspecific binding of immunoglobulins was prevented by adding a mixture of 1.5% bovine serum albumin (BSA) and 1.5% gelatin in TBS and then incubating for 2 hr at room temperature, followed by incubation overnight at 4 °C. Plates were washed as described above, and serum samples diluted 1:100 in 1% BSA-TBS were added to duplicate wells and incubated for 2 hr at room temperature. Sera from patients with multiple sclerosis (MS), polyneuropathies, and other neurological disorders with known high titers of IgG, IgM, and IgA against different neurological antigens were used to rule out nonspecific antibody activities of inter-assay and intra-assay variability. Plates were washed, and peroxidase-conjugated goat antihuman IgG, IgM, or IgA antiserum (KPI [Gaithersburg, Maryland]), diluted 1:400 in 1% BSA-TBS, was added to each well. The plates were incubated for an additional 2 hr at room temperature. After washing 5 times with TBS-Tween buffer, the enzyme reaction was started by the addition of 100 µl of *o*-phenylenediamine in citrate-phosphate buffer containing hydrogen peroxide diluted to 1:10,000 (pH 5.0). After 45 min, the reaction was stopped with 50 µl of 2N sulfuric acid. The optical density was read at 492 nm with a microtiter reader (Dynex Laboratories [Chantilly, Virginia]). Several control wells containing all reagents except human serum were used to detect nonspecific binding.

We calculated coefficients of intra-assay variation by running 5 samples 8 times in 1 assay. Coefficients of inter-assay variation were determined by measuring the same samples in 6 consecutive assays. This replicate testing established the validity of the ELISAs, determined the appropriate dilutions with minimal background, and detected IgG, IgM, and IgA against different antigens. Sera from 500 asymptomatic blood donors in southern California were used to calculate expected ranges at 95% CI.

Neurophysiological tests. Bilateral peripheral nerve studies involving nerve conduction and central response (F wave) were performed on the 119 patients in accordance with accepted techniques of the American Society of Electroneurodiagnostic Technologists (Kansas City, Missouri) and the American Neurological Association (Minneapolis, Minnesota).[69–71] The testing was performed under the direct supervision of, and interpreted by, a board-certified neurologist. Onset latency (ms), amplitude (µV), and velocity (m/sec) were recorded for 4 motor nerves (median, ulnar, peroneal, and tibial). The peak latency (ms) and amplitude (µV) were recorded for 3 sensory nerves (median, ulnar, and sural). F wave and H reflex were recorded for the median, ulnar, peroneal, and tibial nerves. The studies were conducted with a TECA Synergy Multimedia Electromyograph with multisync color SVGA monitor and Delux stimulator probe (TECASyngery, Synergy Version 8.2 [Oxford Instruments {Surry, U.K.}]). The motor axons of peripheral nerves that innervate somatic muscle were evaluated by recording the response following electrical stimulation.

Statistical analysis. We performed critical 2-tailed *t* tests on neural autoantibodies. Odds ratios (ORs) were calculated for the data to determine the percentage of individuals with antibody titers that exceeded the maximum expected laboratory range (95% CI) for each neural autoantibody. For this calculation, data for patients with abnormal and normal NCVs were combined.

Results

Neural autoantibodies. The mean ± *SD* of autoantibodies for each isotype (IgA, IgM, and IgG) against each neural antigen for patients with abnormal NCVs (n = 99), normal NCVs (n = 20), and asymptomatic blood donor controls (n = 500) are given in Table 1. Rather than repeating the data for each antineural antigen isotype, the salient features will be outlined briefly. In general, the highest isotype titers detected were MBP, MAG, tubulin, and NAF. These were followed by the other 5 neural antigens: GM_1, sulfatide, MOG, crystallin, and $CONSO_4$.

We performed critical 2-tailed *t* tests for each isotype titer against neural antigens, comparing abnormal vs. normal patients, abnormal patients vs. controls, and normal patients vs. controls for each isotype (statistical data not shown). With respect to IgG titers, the only significant difference between abnormal and normal patients was NAF ($p < 0.01$). IgG titers for all isotypes for abnormal and normal patients differed significantly from controls ($p < 0.001$). IgM titers for neural antigens were significantly different between abnormal and normal patients for glutamate receptor ($p < 0.01$), tubulin ($p < 0.01$), NAF ($p < 0.01$), and $CONSO_4$ ($p < 0.05$). IgM titers against each neural antigen for abnormal patients vs. controls ($p < 0.001$) and for normal patients vs. controls ($p < 0.001$) were significantly different. The only significant difference between patients with abnormal vs. normal IgA titers was NAF ($p < 0.05$). Comparison of abnormal and normal patients vs. controls revealed IgA titers for each neural antigen which were

significantly different ($p < 0.01$), except for normal crystallin titers ($p < 0.05$).

The percentages of individuals with autoantibodies for each isotype that exceeded the laboratory expected range at 95% CI against the neural antigens are presented in Table 2. IgG titers for abnormal patients exceeded the 95% CI for sulfatide (17.2%), MOG (10.1%), crystallin (10.1%), glutamate receptor (11.1%), tubulin (57.6%), $CONSO_4$ (27.3%), and NAF (6.1%); MBP (4%), MAG (4%), and GM_1 (0%) did not exceed their expected ranges. Similar observations for normal patients were made for sulfatide (20%), MOG (20%), crystallin (20%), tubulin (30%), and $CONSO_4$ (20%), except for MBP (0%), GM_1 (0%), glutamate receptor (0%), and NAF (0%). IgM titers for abnormal patients exceeded those of controls for all neural antigens (range = 17.2%–42.4%) except GM_1 (0%). In normal patients, IgM titers did not exceed control values for GM_1 (0%), sulfatide (5%), MOG (5%), glutamate receptor (0%), and tubulin (5%), whereas MBP (20%), MAG (30%), crystallin (25%), $CONSO_4$ (20%), and NAF (20%) exceeded control values. IgA autoantibodies in abnormal patients for each neural antigen exceeded control values for MBP (20.3%), MAG (23.2%), crystallin (8.1%), glutamate receptor (7.1%), tubulin (8.1%), $CONSO_4$ (10.5%), and NAF (10%). With respect to normal patients, only MAG (15%), tubulin (10%), and NAF (10%) exceeded values for controls.

The percentage of patients who had ORs that exceeded the 95% CI are given in Table 3. The ORs for IgG were not significant for MBP (0.66) and MAG (1.05), whereas those for all other neural autoantibodies were significant as follows: sulfatide (3.36), crystallin (6.53), glutamate receptor (10.08), tubulin (55.1),

Table 1.—Autoantibody Titers against 9 Neural Antigens in Patients with Abnormal Nerve Conduction Velocities (NCVs) ($n = 119$) and Those with Normal NCVs ($n = 20$), vs. Asymptomatic Controls ($N = 500$), for Each Isotype

	IgG						IgM						IgA					
	Abnormal		Normal		Controls		Abnormal		Normal		Controls		Abnormal		Normal		Controls	
Neural autoantibody	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*
MBP	63.3	21.6	54.7	18.3	27.0	12.2	50.8	24.3	47.0	9.2	25.1	13.2	18.5	6.0	16.8	4.7	7.2	3.7
MAG	63.6	18.8	69.0	18.2	26.0	7.3	51.0	16.1	48.1	12.4	24.5	8.1	19.4	7.6	17.5	7.4	8.3	2.4
GM_1	15.8	6.7	16.4	7.0	11.1	2.8	16.4	6.3	15.6	4.5	10.4	3.3	12.3	6.2	12.6	4.4	11.5	3.6
Sulfatide	16.7	6.2	17.5	6.7	12.2	3.4	17.2	6.3	15.1	4.3	11.3	3.7	13.3	9.1	12.4	4.0	9.4	3.1
MOG	15.9	5.9	17.5	6.6	8.9	5.5	18.2	6.3	16.8	4.7	8.4	5.5	12.0	3.5	11.4	2.3	7.9	4.6
Crystallin	16.1	5.8	16.5	6.7	11.9	2.5	18.7	6.9	19.0	8.2	12.1	2.9	13.6	7.5	13.2	4.7	11.1	4.3
Glutamate	10.2	3.4	9.2	1.5	7.0	2.8	10.6	3.7	9.3	1.2	7.6	2.0	9.5	3.7	8.5	2.1	8.1	2.1
Tubulin	64.1	16.8	58.7	11.9	23.7	9.2	41.9	18.5	33.0	7.8	18.1	7.9	17.9	16.1	14.7	3.7	9.8	2.6
$CONSO_4$	11.5	4.7	10.2	2.1	7.7	2.2	11.8	4.9	10.4	2.0	5.6	2.1	11.0	7.7	9.2	1.4	7.0	2.7
NAF	61.6	23.9	53.3	10.7	26.4	11.3	53.1	20.7	44.1	12.0	24.7	10.3	18.2	8.2	16.4	5.4	8.7	3.4

Notes: Ig = immunoglobulin, $\bar{x}$ = mean, *SD* = standard deviation, MBP = myelin basic protein, MAG = myelin-associated glycoprotein, GM_1 = ganglioside, MOG = myelin oligodendroctye glycoprotein, $CONSO_4$ = chondroitin sulfate, and NAF = neurofilament antigen.

Table 2.—Percentages of Individuals with Autoantibody Titers that Exceeded Expected Ranges (95% Confidence Intervals), for Patients with Abnormal Nerve Conduction Velocities (NCVs) and Those with Normal NCVs, vs. Asymptomatic Controls, for Each Isotype

	IgG			IgM			IgA		
Neural autoantibody	Abnormal (%)	Normal (%)	Controls (%)	Abnormal (%)	Normal (%)	Controls (%)	Abnormal (%)	Normal (%)	Controls (%)
MBP	4.0	0	5	34.3	20	2	20.2	5	1
MAG	4.0	5	4	42.4	30	3	23.2	15	2
GM_1	0.0	0	2	0.0	0	4	4.0	5	5
Sulfatide	17.2	20	6	17.2	5	5	3.0	5	2
MOG	10.1	20	5	25.3	5	4	1,0	0	3
Crystallin	10.1	20	2	27.3	25	4	8.1	5	3
Glutamate	11.1	0	1	15.2	0	0	7.1	0	2
Tubulin	57.6	30	2	39.4	5	2	8.1	10	1
$CONSO_4$	27.3	20	2	33.3	20	1	10.5	5	2
NAF	6.1	0	3	41.4	20	2	18.2	10	1

Notes: Ig = immunoglobulin, MBP = myelin basic protein, MAG = myelin-associated glycoprotein, GM_1 = ganglioside, MOG = myelin oligodendroctye glycoprotein, $CONSO_4$ = chondroitin sulfate, and NAF = neurofilament antigen.

$CONSO_4$ (17.26), and NAF (5.15). The OR for GM_1 could not be calculated because of the 0 value for the patients. The ORs for IgM neural autoantibodies were significant for all antigens, and ranged from 3.39 to 44.6. The ORs for GM_1 and glutamate receptor were not calculated because of 0 values for the controls. With respect to IgA autoantibodies, the ORs for sulfatide (1.7) and MOG (0.82) were not significant, whereas those for the other neural antigens were significant as follows: MBP (21.2), MAG (13.7), crystallin (2.65), glutamate receptor (3.03), tubulin (9.08), $CONSO_4$ (4.99), and NAF (2). GM_1 could not be calculated because of 0 values; the 95% CI for MOG was not calculated because of the value of 1 in controls.

The percentages of individuals with autoantibody titers that exceeded the maximum 95% CI for expected laboratory ranges, along with ORs, are presented in Table 4. We calculated these data for abnormal, normal, and control patients as follows: If an individual had only 1 isotype against a neural antigen (i.e., IgG), that person was given the same score as an individual with 2 or more isotypes (i.e., IgG + IgM + IgA). The percentages of individuals with autoantibodies against each neural antigen were highest among the abnormal

Table 3.—Odds Ratios (ORs) and 95% Confidence Intervals (CIs) for Autoantibodies for Each Isotype Presented in Table 1

	IgG		IgM		IgA	
Neural autoantibody	OR	95% CI	OR	95% CI	OR	95% CI
MBP	0.66	1.92, 0.22	22.98	47.9, 11.0	21.2	57.4, 7.8
MAG	1.05	3.86, 0.39	21.86	40.9, 11.59	13.7	6.2, 28.8
GM_1	—*	—*		—*		
Sulfatide	3.36	6.1, 1.84	3.39	5.6, 2.05	1.7	0.52, 5.5
MOG	2.53	4.95, 1.25	6.7	12.2, 3.5	0.82	—†
Crystallin	6.53	15.2, 2.83	8.83	16.4, 4.9	2.65	1.13, 6.17
Glutamate	10.08	29.4, 3.3	—*		3.06	8.17, 1.14
Tubulin	55.1	112.4, 27.1	24.8	50.9, 11.8	9.08	38.02, 26.8
$CONSO_4$	17.26	36.6, 8.2	44.6	116.7, 16.4	4.99	2.05, 11.9
NAF	5.15	13.06, 1.88	29.8	62.2, 14.9	20.0	7.26, 54.6

Notes: Ig = immunoglobulin, MBP = myelin basic protein, MAG = myelin-associated glycoprotein, GM_1 = ganglioside, MOG = myelin oligodendroctye glycoprotein, $CONSO_4$ = chondroitin sulfate, and NAF = neurofilament antigen. For these calculations, patients with abnormal nerve conduction velocities (NCVs) were grouped with those with normal NCVs and compared vs. controls.
*Not calculated because of zero values (refer to Table 2).
†Not calculated because of the value of 1 in a cell (refer to Table 2).

Table 4.—Percentages of Individuals with 1 or More Isotypes for Each Neural Antigen that Exceeded the Maximum 95% Confidence Interval (CI) Titer

Neural autoantibody	Abnormal (n = 119) (%)	Normal (n = 20) (%)	Controls (n = 500) (%)	OR	95% CI
MBP	47.5	20	8	8.63	11.5, 6.4
MAG	54.5	45	9	11.4	16.4, 7.8
GM_1	4.0	5	11	0.91	1.7, 0.48
Sulfatide	30.3	25	13	2.8	4.4, 1.7
MOG	32.3	25	12	3.3	4.85, 2.2
Crystallin	35.4	35	9	5.5	8.0, 2.7
Glutamate	26.3	0	3	9.0	17.6, 4.6
Tubulin	69.7	35	5	33.58	56.8, 19.1
$CONSO_4$	46.5	30	5	14.75	25.3, 8.58
NAF	50.5	25	5	16.3	16.3, 12.4

Notes: MBP = myelin basic protein, MAG = myelin-associated glycoprotein, GM_1 = ganglioside, MOG = myelin oligodendroctye glycoprotein, $CONSO_4$ = chondroitin sulfate, and NAF = neurofilament antigen. The percentages were determined as follows: If a patient had 1 isotype (e.g., immunoglobulin [Ig]G, IgM, or IgA), that patient was given the same score as a patient with 2 or more isotypes. Thus, the total percentages for patients with abnormal nerve conduction velocities (NCVs), normal NCVs, and controls were less than the totals for each isotype as presented in Table 1. For simplicity, the data for patients with abnormal and normal NCVs were combined to obtain the odds ratios (ORs).

patients (range = 26.3%–69.0%) when compared with controls (range = 3%–13%), with the exception of GM_1 (4%). Similarly, the normal patients had an increased percentage of individuals with higher titers (range = 20%–45%) when compared with the controls, with the exception of GM_1 (5%) and glutamate receptor (0%). The ORs were significant (95% CI) for all neural antigens, except for GM_1 (0.91). The ORs were highest for tubulin (33.58) and decreased, in descending order, for NAF (16.3), $CONSO_4$ (14.75), MAG (11.4), glutamate receptor (9.0), MBP (8.63), crystallin (5.5), MOG (3.3), and sulfatide (3.3).

NCV testing. No changes or abnormalities were observed for F and H waves in the abnormal or normal patients (data not shown).

The data obtained from the NCV studies for motor nerves and sensory nerves are summarized in Tables 5 and 6. Patients with abnormal findings comprised 3 groups, as follows: (1) mixed sensory–motor polyneuropathy ($n = 55$), (2) motor neuropathy ($n = 17$), and (3) sensory neuropathy ($n = 27$). There were 20 patients with no abnormalities (controls). We compared the data obtained for the 20 controls with data for the 3 groups of abnormal patients (mixed, motor, and sensory neuropathies), for statistical purposes.

Results for the mixed polyneuropathy group differed significantly from controls. Latencies for the median (4.2 ± 1.2 ms, $p < 0.001$), ulnar (3.13 ± 1.1 ms, $p < 0.05$), peroneal (5.1 ± 1.4 ms, $p < 0.001$), and tibial motor nerves (5.5 ± 2.9 ms, $p < 0.001$) were signifi-

Table 5.—Neuropathies Experienced by Patients with Abnormal Nerve Conduction Velocity (NCV) Measurements ($n = 119$), vs. Controls with Normal Values ($n = 20$), by NCV Parameter Tested

	Neuropathy with abnormal NCV							
	Mixed ($n = 55$)		Motor ($n = 17$)		Sensory ($n = 27$)		Controls ($n = 20$)	
NCV parameter	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*
Median latency (ms)	4.2	1.2*	3.6	1.1	3.4	0.36	3.3	0.4
Median amplitude (μV)	9.5	4.3	7.6	3.3†	10.8	2.6	10	3.3
Median velocity (m/sec)	56.5	7.8‡	55.9	5.3‡	58.1	7.4	61.5	7.1
Ulnar latency (ms)	3.13	1.1†	3.7	2.05‡	2.7	0.38	2.56	0.53
Ulnar amplitude (μV)	9.9	3.7	9.9	2	9.9	1.8	10	3.13
Ulnar velocity (m/sec)	60.9	9.9§	60.4	7.4‡	65.1	6.6	66.2	5.4
Peroneal latency (ms)	5.1 ($n = 54$)	1.4*	5.5	1.2*	4.7 ($n = 26$)	0.52*	3.9	0.86
Peroneal amplitude (μV)	5.6 ($n = 54$)	3.2	4.2	1.9§	6.05 ($n = 26$)	2.2	6.6	3
Peroneal velocity (m/sec)	52.5 ($n = 54$)	12.7	47.5	13.3†	52.2 ($n = 26$)	5.8	58.2	13.8
Tibial latency (ms)	5.5 ($n = 53$)	2.9*	6.3	3§	4.2	0.55	4	1
Tibial amplitude (μV)	9.8 ($n = 53$)	6	8.1	4.8†	13.6	4.8	12.7	6.8
Tibial velocity (m/sec)	46.6 ($n = 53$)	11.3†	47.3	5.5†	46.6	4.4§	52.3	8.3
Median latency (ms)	4.2 ($n = 54$)	1.3*	3 ($n = 16$)	0.16	4.2	1.5*	3.08	0.17
Median amplitude (μV)	27.1 ($n = 54$)	15.9	40.3 ($n = 16$)	15.2	32.4	20.5	35.1	15.6
Ulnar latency (ms)	3.7 ($n = 54$)	1.41*	2.7 ($n = 16$)	0.2	4.6	2.3*	2.54	0.25
Ulnar amplitude (μV)	27 ($n = 54$)	18.5†	31.9 ($n = 16$)	14.9	28.1	18.6	35	15.3
Sural latency (ms)	4.9 ($n = 44$)	3.2‡	3.8 ($n = 11$)	0.28	4.4 ($n = 24$)	1.4†	3.8 ($n = 17$)	0.32
Sural amplitude (m/sec)	11 ($n = 44$)	8.4†	14.2 ($n = 11$)	5.9	11.2 ($n = 24$)	10.5†	21.7 ($n = 17$)	19.2

Notes: With respect to the 20 normal patients, no abnormal values were observed for each measurement except for the tibial motor nerve measurements, in which the velocity was slightly reduced (41 ms), with the cutoff for normal being > 41 ms. For cases in which the number of patients was not the same as that shown in the column heading, the actual number (n) is given within the table.

*$p < 0.001$.
†$p < 0.05$.
‡$p < 0.02$.
§$p < 0.01$.

Table 6.—Percentages of Individuals with Various Numbers (0–7) of Nerves Showing Abnormal Nerve Conduction Measurements (Latency Onset, Amplitude, or Velocity), for Mixed (Motor and Sensory), Motor, and Sensory Neuropathies

No. of nerves with abnormal measurements	Neuropathy		
	Mixed (n = 55) (%)	Motor (n = 17) (%)	Sensory (n = 27) (%)
0	0.0	0.0	11.1
1	1.8	41.2	33.3
2	38.2	47.1	33.7
3	23.6	5.9	18.5
4	21.8	5.9	—
5	10.9	—	—
6	1.8	—	—
7	1.8	—	—

Notes: Among the 20 controls, 1 individual had an abnormal measurement for the tibial motor nerve (velocity = 41 ms), resulting in an abnormal rate of 0.4% for all motor nerve measurements. No abnormalities were seen among the controls for the other 4 motor nerves or the 3 sensory nerves.

cantly increased vs. controls. Amplitudes for all motor nerves were not significantly different from controls. Velocities for the median (56.5 ± 7.8 m/sec, $p < 0.02$), ulnar (60.9 ± 9.9 m/sec, $p < 0.01$), and tibial (46.6 ± 11.3 m/sec, $p < 0.05$) motor nerves were significantly decreased vs. controls. Latencies for the median (4.2 ± 1.3 ms, $p < 0.001$), ulnar (3.7 ± 1.41 ms, $p < 0.02$), and sural (4.9 ± 3.2 ms, $p < 0.02$) sensory nerves were significantly increased vs. controls. Amplitudes for the ulnar (27 ± 18.5 μV, $p < 0.05$) and sural (11 ± 8.4 μV, $p < 0.5$) sensory nerves were significantly decreased compared with controls.

In patients with only motor nerve neuropathy, latencies for ulnar (3.7 ± 2.05 ms, $p < 0.02$), peroneal (5.5 ± 1.2 ms, $p < 0.001$), and tibial (6.2 ± 3 ms, $p < 0.01$) nerves were significantly increased; amplitudes for the median (7.6 ± 3.3 μV, $p < 0.05$), peroneal (4.2 ± 1.9 μV, $p < 0.01$), and tibial (8.1 ± 4.8 μV, $p < 0.5$) nerves were significantly decreased; and velocities for the median (55.9 ± 5.3 m/sec, $p < 0.01$), ulnar (60.4 ± 7.4 m/sec, $p < 0.02$), peroneal (47.5 ± 13.3 m/sec, $p < 0.05$), and tibial (47.3 ± 5.5 m/sec, $p < 0.05$) nerves were significantly slower than the controls. Latencies and amplitudes for the sensory nerves (median, ulnar, and tibial) were not significantly different from control values.

In patients with only sensory neuropathy, latencies for the median (4.2 ± 1.5 ms, $p < 0.001$), ulnar (4.6 ± 2.3 ms, $p < 0.001$), and sural (4.4 ± 1.4 ms, $p < 0.05$) nerves were significantly increased vs. controls; amplitudes of the sural nerve (11.2 ± 10.5 μV, $p < 0.05$) were significantly decreased; and all neurophysiological measurements tended to differ from the control values. None of the measurements for motor nerves in this group—except for peroneal latency ($p < 0.001$) and amplitude ($p < 0.01$)—were different from the controls.

Table 6 summarizes the data for the percentages of patients with various numbers of nerves that demonstrated abnormal conduction. In those patients with mixed neuropathy, all nerves had abnormal measurements with a distribution as follows: 1 involved nerve (5.5%), 2 involved nerves (38.2%), 3 involved nerves (23.6%), 4 involved nerves (21.8%), and 5 or more involved nerves (14.5%). Of those patients who exhibited motor neuropathy, 41.2% had only 1 involved nerve, whereas 58.9% had 2 or more involved nerves. Patients with sensory neuropathy had the following distribution: 11.1% had nerves with no abnormal findings, 33.3% had only 1 nerve with abnormal measurements, and 52.2% had 2 or more nerves with abnormal measurements.

Discussion

All patients in this study had documented exposure to molds in their homes and/or workplaces. They also had significantly elevated antibodies to molds and to mycotoxins, which confirmed exposure.[63–65] In addition, multiple organ symptoms were present, as reported previously.[63] In this particular group of patients, additional health complaints consisted of symptoms of peripheral neuropathy (i.e., tingling, numbness, tremors, and muscle weakness in the extremities). Thus, we evaluated these patients for the presence of antibodies to 9 neural antigens, as well as for evidence of abnormalities in peripheral nerve conduction. All patients had significant increases in autoantibodies against neural antigens (Tables 1–4). Abnormalities in latencies, amplitudes, and velocities of selected peripheral nerves (Tables 5 and 6), and peripheral neuropathy, were observed in 99 patients, whereas 20 symptomatic patients had normal NCV measurements.

Examination of the patients' antibody titers revealed that IgG antibody titers to the neural antigens between patients with abnormal vs. normal NCVs were not significantly different, with the exception of NAF ($p < 0.01$). However, when compared with healthy controls, the difference between IgG titers for abnormal vs. normal patients was highly significant ($p < 0.001$). In contrast, IgM titers in abnormal patients were consistently elevated when compared with normal patients, with significant differences for $CONSO_4$ ($p < 0.05$), glutamate receptor ($p < 0.01$), tubulin ($p < 0.01$), and NAF ($p < 0.01$). Autoantibodies in both abnormal and normal patients showed significant elevations compared with controls ($p < 0.001$). With respect to IgA antibodies, NAF titers were significantly elevated in abnormal vs. normal patients. The titers for both abnormal and normal patients were significantly elevated compared with controls, with the exception of GM_1 and glutamate receptor. Thus, we concluded that exposure to molds,

and symptoms of peripheral neuropathy, are associated with autoantibodies to 9 different neural antigens. These data support and extend the observations of Gray et al.,[30] who demonstrated increased antibodies to myelin and NAF in mold-exposed individuals.

Autoantibodies to neural antigens have been reported for several neurological conditions.[66,72] Pestronk et al.[66] confirmed that elevated titers of MAG antibodies in patients are relatively specific for sensory and motor polyneuropathy syndromes with demyelination. NCVs were used to confirm the demyelinating changes in these patients. Of the patients studied, 92% with IgM antibodies to MAG had physiologic evidence of demyelination.[66] MS patients have shown antibodies to myelin, MOG, MBP, α-B-crystallin, and complement-mediated demyelination.[68,73] Anti-ganglioside, anti-glycolipid, anti-sulfatide, anti-MAG, anti-tubulin, and anti-$CONSO_4$ antibodies have been demonstrated in motor, sensory, and polyneuropathies with demyelination.[72–86] IgM isotypes to sulfatide,[74,75,77] ganglioside,[78] and MAG[83] are correlated with electrophysiological peripheral nerve abnormalities. Moreover, antigangliosides and galactocerebroside antibodies are associated with infections from *Campylobacter jejuni* and *Mycoplasma pneumoniae* in patients with Guillain-Barre syndrome.[81] In addition, IgM, anti-MAG, anti-glycolipids, and anti-NAF antibodies are present in individuals with chronic demyelinating conditions of the nervous system.[67,73,83,86] Thus, we suggest that the presence of autoantibodies to neural antigens in our patients is the result of exposure to toxic metabolites[13–23,60,61] of molds, or may result from an infectious process. The presence of abnormal T and B cell function of the immune system[30] in nasal,[41] pulmonary,[39,40] and neurologic[87,88] infections by molds supports this conclusion. Finally, we have observed increased T cell activation; C3 and C4 complements; and IgA, IgM, and IgG immune complexes in 33 patients who had chronic exposure to molds in a water-damaged building, which suggests an inflammatory process (manuscript forthcoming).

The ORs in Table 3 are also revealing. IgM isotypes against the neural antigens had ORs consistently greater (range = 3.39–44.6) than those for IgG isotypes (range = 0.66–17.26), with the exception of antitubulin (55.1 vs. 24.8). The ORs indicate an increased risk of developing antineural antibodies, but also may suggest that IgM isotypes are more consistent with symptomatic active or progressive neuropathy than are IgG isotypes, and may represent an ongoing acute or subacute process. As mentioned earlier, IgM antibodies to various neural antigens have been associated with neurophysiological and pathological changes characteristic of various neuropathies. Finally, the ORs in Table 4 for the percentages of individuals with 1 or more isotypes against the neural antigens show a relative increased risk (range = 2.8–33.58) of developing autoantibodies. The only exception was GM_1 autoantibodies (OR = 0.91). Thus, we concluded that individuals exposed to molds in a water-damaged building have an increased risk of developing antineural antibodies. Additional work is needed to determine at what point these processes become irreversible.

Our neurophysiological data revealed 3 different types of peripheral neuropathies: mixed sensory–motor polyneuropathy (55 abnormal patients), motor neuropathy (17 abnormal patients), and sensory neuropathy (27 abnormal patients), as well as patients who exhibited symptoms but had no abnormal electrophysiological findings (20 normal controls) (Table 5). The differences between the 20 normal patients and the 99 abnormal patients are likely attributable to the significant increase in IgG and IgM autoantibodies to NAF, tubulin, glutamate receptor, and $CONSO_4$ observed in the abnormal patients. The role that IgA antibodies play is unclear at this time. Additional observations are needed to clarify the role of each isotype (IgA, IgM, and IgG) and to determine which neural autoantibodies contribute to the observed neuropathies.

The increased latencies for motor and sensory nerves observed in the 55 patients with mixed neuropathy suggest a demyelinating process.[83] The increased latencies were accompanied by a decrease in velocities for the median, ulnar, peroneal, and tibial nerves. All three sensory nerves (median, ulnar, and sural) exhibited increased latencies and decreased amplitudes. Thus, the polyneuropathy observed in these patients appears to be a demyelinating process with decreased number and size of fibers (decreased amplitudes) and chronic involvement of the nerve (decreased velocities).[72,83] Those with motor neuropathies (17 patients) had decreases in latencies (ulnar, peroneal, and tibial nerves), decreased amplitudes (median, peroneal, and tibial nerves), and decreased velocities (median, ulnar, peroneal, and tibial nerves). This appears to be a diffuse neuropathy and may involve some demyelination.[89] Finally, those with sensory neuropathies (27 patients) had increased latencies for all 3 nerves, whereas the sural nerve had a decreased amplitude. The increased latencies and decreased amplitude of the these nerves suggest that demyelination is occurring.[90]

The severity of the neuropathies experienced by the patients in our study is implicit as a result of the involvement of several nerves (Table 6). With respect to the mixed-neuropathy patients, only 1.8% had abnormalities in only 1 nerve, whereas 38.2% had at least 2 nerves involved. The remaining patients (59.5%) had 3 or more nerves with abnormal neurophysiological recordings. Impairments in the patients with motor neuropathy were slightly less dramatic, with 41.2% having a single nerve involvement, and the remainder having 2 or more nerves involved. Finally, in those patients with sensory neuropathy, 33.3% had 1 nerve and 52.2% had

2 or more nerves involved. Thus, we concluded that the neuropathies in these patients were severe and in many cases involved several nerves.

In summary, 119 individuals exposed to mold colonies in water-damaged buildings were found to have autoantibodies directed against 9 different neural antigens. Neurophysiological recordings for latencies, amplitudes, and velocities on 4 motor nerves and 3 sensory nerves revealed peripheral neuropathies in 99 patients (83%). Three abnormal conditions were found: mixed sensory–motor polyneuropathy, motor neuropathy, and sensory neuropathy. We recommend that mold-exposed individuals with symptoms of neuropathy be evaluated for antibodies against neural antigens and for neurophysiological abnormalities. Additional work is needed to correlate and clarify the extent of the peripheral nerve pathology and demyelination, as well as the role of neural autoantibodies in this process.

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Antibodies to Molds and Satratoxin in Individuals Exposed in Water-Damaged Buildings

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ABSTRACT. Immunoglobulin (Ig)A, IgM, and IgG antibodies against *Penicillium notatum, Aspergillus niger, Stachybotrys chartarum,* and satratoxin H were determined in the blood of 500 healthy blood donor controls, 500 random patients, and 500 patients with known exposure to molds. The patients were referred to the immunological testing laboratory for health reasons other than mold exposure, or for measurement of mold antibody levels. Levels of IgA, IgM, and IgG antibodies against molds were significantly greater in the patients ($p < 0.001$ for all measurements) than in the controls. However, in mold-exposed patients, levels of these antibodies against satratoxin differed significantly for IgG only ($p < 0.001$), but not for IgM or IgA. These differences in the levels of mold antibodies among the 3 groups were confirmed by calculation of *z* score and by Scheffé's significant difference tests. A general linear model was applied in the majority of cases, and 3 different subsets were formed, meaning that the healthy control groups were different from the random patients and from the mold-exposed patients. These findings indicated that mold exposure was more common in patients who were referred for immunological evaluation than it was in healthy blood donors. The detection of antibodies to molds and satratoxin H likely resulted from antigenic stimulation of the immune system and the reaction of serum with specially prepared mold antigens. These antigens, which had high protein content, were developed in this laboratory and used in the enzyme-linked immunosorbent assay (ELISA) procedure. The authors concluded that the antibodies studied are specific to mold antigens and mycotoxins, and therefore could be useful in epidemiological and other studies of humans exposed to molds and mycotoxins.
<Key words: antibody, *Aspergillus,* ELISA, molds, mycotoxins, *Penicillium, Stachybotrys*>

WATER INTRUSION into houses and office buildings leads to the amplification of molds and bacteria.[1–6] These organisms are known to produce toxic byproducts that include endotoxins (lipopolysaccharides), β-D-glucans, and mycotoxins (e.g., trichothecenes, ochratoxins, and aflatoxins), as well as solvents. These compounds have been demonstrated in water-damaged buildings and from artificially contaminated building

materials.[7–11] Molds can be aerosolized, resulting in indoor air contaminated with spores and hyphae fragments.[11] In addition, mycotoxins have been identified in ventilation duct dust and in the air in buildings in which occupants have experienced adverse health effects related to mold exposure.[12–18]

Exposure to airborne molds can result in immunologic-mediated illnesses, as well as pathological and inflammatory processes. Immunologic health effects include upper and lower respiratory allergy, asthma, and hypersensitivity pneumonitis.[19–25] Immunoglobulin (Ig)E antibodies to a variety of molds are demonstrable in individuals with suspected allergic sensitivity to molds.[26–29] By using *Stachybotrys* extracts in enzyme-linked immunosorbent assay (ELISA) and radioallergosorbent (RAST) tests, researchers have measured mold-specific IgG and IgE in the blood of individuals exposed to toxigenic fungi.[5] These investigators found no elevated levels of IgG or IgM antibodies to *Stachybotrys chartarum* that had a statistically significant association with health outcome.[5] In a mouse study, an increase in IgA production and IgA nephropathy were reported after injection of trichothecene or vomitoxin.[19] Similar findings were reported when IgE, IgG, and IgA antibodies against *S. chartarum* were identified in patients with asthmatic or mycotoxicosis symptoms.[30] Mycelium extract was used in the ELISA assay, and the *Stachybotrys*-specific IgG and IgA were detected in the group of exposed subjects, but not in control groups. IgA levels were significantly higher ($p < 0.01$) and IgG levels less high ($p < 0.05$) in the patient group than in the control groups. IgE levels did not differ between groups. It has been suggested that exposure to *Stachybotrys* does not cause IgE-mediated allergy in humans and that the IgA response better reflects exposure to the fungus than does the IgG response.[30]

Other investigators have reported that serum IgA level was a more specific factor than IgG level for indicating Farmer's Lung, a disease associated with fungal exposure. They reached the same conclusion concerning antibodies against *Aspergillus fumigatus*.[31,32] When specific IgA was detected in patients' sera, the IgA concentrations in bronchoalveolar lavage were high. This suggests that the concentration of serum IgA may depend on the magnitude of respiratory exposure to fungi.[33] In contrast, IgG antibodies to 8 different molds in subjects with Farmer's Lung disease, compared with healthy farmer controls, were significantly elevated above their healthy counterparts. The Farmer's Lung group had positive titers against several species of mold, whereas the control farmers usually had a positive titer against 1 or 2 microbes. A positive association between IgG antibody levels and chronic bronchitis and diffusing capacity was observed in the Farmer's Lung patients, but not in the control farmers.[34]

Pathological and inflammatory conditions are often caused by molds. Molds and yeast invade tissues in both immunocompromised and normal individuals, establishing an infectious state that is difficult to manage clinically.[35–50] With respect to human infants, *Stachybotrys chartarum* has been associated with pulmonary hemosidersosis.[51–54] The organism *S. chartarum*, isolated from the lungs of an infant, produced a hemolysin (stachylysin), siderophore, and stachyrase A. Apparently, different strains of *S. chartarum* produce different quantities and types of various highly toxic materials, including trichothecenes.[55–61] In an earthworm model, stachylysin increased the permeability of blood vessels, causing leakage through the blood vessel endothelium and walls.[59] Additionally, pathology may result from interference with lung surfactant synthesis by *S. chartarum* spores and isosatratoxin-F in juvenile mice.[62] Ultrastructural changes in Type II alveolar cells (condensed mitochondria, increased cytoplasmic rarefaction, and distended lamellar bodies with irregular shapes) have been observed following exposure to *S. chartarum*.[62–65] Thus, hemolysins, siderophores, and proteases appear to have an important role in the pathogenesis of mold infections.[55–69]

Numerous species of molds, including some found indoors in contaminated buildings, produce mycotoxins.[11,69,70] Controlled laboratory exposure of animals to mycotoxins has pointed to several potential toxic effects on humans (e.g. cytotoxicity, immunosuppression, mitochondrial toxicity, carcinogenicity, nephrotoxicity, and deoxyribonucleic acid [DNA] adducts).[71–87] The adverse health effects from exposure to a mixture of molds and their metabolites are different from those related to a single mycotoxin under controlled conditions. Thus, humans express symptoms that include the central and peripheral nervous systems; neurocognitive deficiencies; problems with skin, upper and lower respiratory tracts, gastrointestinal tract, and skeletal-muscular system; chronic fatigue; and flu-like illnesses.[5,17,23,88–94]

Inasmuch as the respiratory tract is the major route of human exposure to fungi and mycotoxins in water-damaged buildings, we conducted this study to measure specific IgG, IgM, and IgA antibodies to 3 mold species commonly found in such environments. An ELISA procedure to detect antibodies to satratoxin H was also undertaken. Simultaneous detection of antibodies against toxigenic molds and their mycotoxins is considered a biomarker of exposure to molds and their metabolites, and may explain clinical disease processes resulting from mold exposure in the workplace and other environments.[93–95]

Materials and Method

Study subjects. The study population consisted of 500 patients exposed to molds in water-damaged build-

ings, from 3 different states—143 from California (64 males, 79 females); 159 from Arizona (72 males, 87 females), and 198 from Texas (83 males, 115 females)—who were referred to Immunosciences Lab, Inc. (Beverly Hills, California) by 3 of the authors (MRG, GH, AWC). Patients' ages ranged from 22 to 76 yr. Environmental engineering firms tested approximately 60 damaged buildings by swab samples, tape transfer, and viable microbial activities and reported *Aspergillus, Penicillium,* and *Stachybotrys* at a level of > 2,000 colony-forming units per m^3. Patients had lived in these buildings for periods ranging from several weeks to more than 2 yr and had reported unpleasant musty odors to their referring physicians.

The patients had the following symptoms in common: neurological and behavioral symptoms (memory loss, blurred vision, migraine, loss of balance, cognitive deficit), fatigue, nausea, rhinitis, sinusitis, rashes, and allergies. Blood samples were drawn from the patients and sent to Immunosciences Lab (Beverly Hills, California) to test for antibodies against the 3 most common molds detected in their respective buildings. Antibodies to satratoxin H were also determined. For comparison, we used 500 blood samples obtained from healthy, asymptomatic blood donors from California of similar age and sex. An additional 500 individuals, whose blood had been sent to the laboratory for health reasons other than mold exposure, were selected randomly from our patient population. Blood samples from the mold-exposed patients, randomly selected patients, and controls were tested with ELISA for levels of IgG, IgM, and IgA against *Aspergillus niger, Pencillium notatum,* and *Stachybotrys chartarum,* and for satratoxin H antigens.

Preparation of fungal antigen. The protocol we followed for preparation of optimal fungal antigen extracts was based on procedures reported previously.[96–99] Molds (*Stachybotrys chartarum, Penicillium notatum,* and *Aspergillus niger*) were obtained from the American Type Culture Collection (Rockville, Maryland). The molds were first cultured in 2% malt extract agar for 8 days at 25 °C, after which spore suspensions were prepared in 0.1 *M* phosphate-buffered saline (PBS) pH 7.4 containing 0.05% Tween 20 (Sigma Aldrich [St. Louis, Missouri]). One milliliter of each spore suspension was inoculated into 100 ml of 2% malt extract broth (for *Stachybotrys*) or cellulose broth (for the other molds) in glass bottles; the cultures were incubated for 10 days at 25 °C. The mycelium was separated from the broth by centrifugation at 2,000 *g* for 20 min, dried in a vacuum dryer, and stored at –70 °C. Dried mycelium containing spores was suspended at 50 mg/ml in 0.1 *M* PBS pH 7.5 containing 0.02% phenylmethylsulfonyl flouride (PSF) and 0.02% sodium azide. Mycelium suspension was sonicated for 5 sec at an output of 70% using a Virsonic 50 cell disruptor (Virtis Co. [Gardiner, New York]). The sonication step was repeated 10 times for maximum cell disruption, and thereafter the suspension was kept on a shaker for 24 hr at 4 °C. After centrifugation at 4,000 *g*, the supernatant was dialyzed at molecular cut-off 2kD against PBS at 4 °C for 24 hr, lyophilized, and stored at –70 °C. For quality control and reproduction of antigenic preparation of these mold extracts, 20 mg of each was dissolved in 1 ml of 0.01 *M* PBS, the protein content was determined,[28] and the sample's components were analyzed by 15% sodium dodecyl sulfate (SDS) gel electrophoresis. The protein content of our preparations was compared with allergenic extracts for the same molds obtained from Antigen Laboratories, Inc. (Liberty, Missouri). Many more protein bands were present in our preparations than in the commercial antigens; however, a few bands were present in the allergenic extracts but not in our preparations. Consequently, we mixed our antigenic preparations with the Antigen Laboratories extracts, in a ratio of 1:1, and used the combined extracts in our ELISA assays.

Preparation of satratoxin H. Satratoxin H was prepared in accordance with the method of Johanning et al.,[5] with modification in our laboratory. One hundred mg of dried mycelium was extracted with 2 ml of 20% methanol in chloroform at 40 °C, with repeated sonication for 30 min. The extract was passed through a silica gel Column Whatman LPS-1 (Whatman [Clifton, New Jersey]) and washed with 10 ml of 8% methanol in dichloromethane. The eluent was evaporated under a stream of dry nitrogen, and the remaining oily material was dissolved in 1 ml of ethanol and analyzed by reversed-phase high-performance liquid chromatography with a Model 5600 CoulArray Detector with solvent delivery pump Model 580 and an analytical cell that makes use of the 2 porous graphite electrodes (ESA, Inc. [Chelmsford, Massachusetts]). The column was C-18 rainin, 5 µm, 4.6 × 250 mm, with a 15-min gradient of 60–75% methanol in water, flow of 1 ml/min, and monitoring at 260 nm. Two peaks—one at 10.6 and the other at 12.2 min, corresponding in retention time to satratoxin H and other trichothecenes—were obtained. The total satratoxin H and trichothecene obtained in 100 mg of sample were estimated to be about 1.5 µg and 1.7 µg, respectively. Satratoxin H was also obtained from the Department of Microbiology, Texas Tech University, Health Sciences Center (Lubbock, Texas).

Binding of satratoxin to human serum albumin (HSA). Satratoxin was coupled to the carrier protein-HSA with 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide-metho-4-toluolsufonate (CCMT) and succinic anhydride. One hundred µg of satratoxin in 100 µl pyridine was reacted with 2 mg of succinic anhydride in a 45 °C water bath with off-and-on vortexing. The reaction mixture was then evaporated to dryness. The satratoxin-hemisuccinate was dissolved in 200 µl of dimethyl formamide and added dropwise to a 1 ml solution of 0.1

M carbonate buffer pH 9.5 containing 10 mg of HSA and 2 mg of CCMT. The mixture was kept on the stirrer for 4 hr, followed by the addition of 1 mg CCMT and adjustment of the pH to 7.5 using 1 *M* hydrochloric acid, followed by an additional incubation of 4 hr. Finally, after the addition of another 1 mg of CCMT, the pH was adjusted to 5.5 and the mixture was kept on the stirrer for 4 hr at room temperature. Uncoupled residues of the reagents and derivatives were removed by dialysis at a cutoff of 2,000 against 0.1 *M* PBS pH 7.2 for 48 hr. After centrifugation at 10,000 *g*, binding of satratoxin to HSA was examined by SDS gel electrophoresis. A shift in the location of the HSA band after the addition of mycotoxin was used as evidence for the binding capacity of satratoxin to the carrier protein.

ELISA for detection of IgG, IgM, and IgA against fungal antigens and mycotoxins. The levels of IgG, IgM, and IgA antibodies against antigens of molds in human sera were analyzed by indirect ELISA. Microtiter plates were coated with 0.1 ml of either HSA (in duplicate to serve as controls) or mold extract and satratoxin at a protein concentration of 10 μg/ml. After incubation, washing, and blocking with 2% BSA blocking solution, 0.1 ml of human serum, at optimal dilution of 1:2 for IgE and 1:100 in serum diluent buffer (2% BSA in 0.1 ml PBS plus 0.01% Tween 20) for IgG, IgM, and IgA, were added into the quadruplicate wells of the plates. Plates were incubated at 37 °C for 2 hr and washed 3 times with PBS Tween 20. Then, 0.1 ml of affinity-purified goat anti-human IgG(γ), IgM(μ), or IgA(α) or light chain specific, conjugated with alkaline phosphatase at dilutions of 1:500, were added and incubated at 37 °C for 1 hr. Color development was measured after repeated washing and the addition of 0.1 ml of paranitrophenylphosphate substrate, incubation for 30 min, and stop solution. The intensity of color was measured spectro-photometrically at 405 nm. For each specimen, the ELISA background readings of wells coated with HSA were automatically subtracted from the ELISA readings of the wells coated with mold antigens. The background readings of wells coated with nonspecific antigen (HSA) reacted with the serum and all other reagents and was less than 12% of the absorbencies of the wells coated with mold antigens. Serially diluted sera from rabbits immunized with molds antigens, and from patients following immunotherapy, with assigned values of 400, 3,200, and 12,800 ELISA units against different molds, were used to construct a standard curve. To determine the ELISA values for unknowns, we plotted the mean absorbances obtained from duplicate wells from each calibrator against the antibody concentration, with the absorbance on the vertical axis and concentration on the horizontal axis.

Coefficients of intra-assay and inter-assay variations and optimal dilution of serum for mold and mycotoxin antibodies. Coefficients of intra-assay variations were calculated by running 5 samples 8 times in 1 assay. Coefficients of inter-assay variations were determined by measuring the same samples in 6 consecutive assays. This replicate testing established the validity of the ELISA assays, determined the appropriate dilution with minimal background, and detected serum IgG, IgM, and IgA against different antigens. Two sera from healthy controls and 2 from patients exposed to molds were used to construct control curves.

The optimal dilutions of sera were determined by diluting sera from 20 different controls, and patients sera, 1:25–1:400 in serum diluent buffer and adding them to duplicate wells coated with either HSA or mold antigens. Dilutions between 1:50 and 1:200 resulted in a good linearity; therefore, a serum dilution of 1:100 was used for all IgG, IgM, and IgA antibody assays.

Antibody-specificity testing by absorption of sera. Specificity of the ELISA assay for molds and satratoxin antibodies was confirmed by mold antigens competition. For this, 3 different sera with high levels of IgG, IgM, and IgA antibodies (optical density in ELISA > 0.8) against *Stachybotrys* were used in different test tubes. One ml of each serum was pre-incubated with 1 mg of either HSA, *Stachybotrys*, *Aspergillus*, *Penicillium*, or *Alternaria* antigens. After mixing, the tubes were kept for 1 hr in a 37 °C water bath, followed by 1 hr of incubation at 4 °C and centrifugation at 3,000 *g* for 10 min. The supernatant was used for measurement of IgG, IgM, and IgA antibody levels against *Stachybotrys* antigens. ELISA values of unabsorbed serum were compared with those for serum absorbed with HSA or with fungal antigens.

Statistical analysis. The main objective of our data analysis was to examine the differences between the levels of IgG, IgM, and IgA antibodies against different molds and a mycotoxin among 3 groups: (1) controls, (2) random patients, and (3) mold-exposed patients. The IgG, IgM, and IgA were considered as 3 dependent variables with 1 factor variable that divided our samples into the 3 groups mentioned above.

The General Linear Model (GLM) for Windows, version 11.5 (SPSS, Inc. [Chicago, Illinois]), with advanced option, was used in this study. The *z* scores and *p* values were obtained using Hotelling's Trace. In addition, for the post hoc tests, Scheffé's significant difference test was performed on 500 samples in each group. The GLM multivariate procedure provided analysis of variance for multiple dependent variables by 1 or more factor variables.

Results

Fungal contamination of buildings. We were in continuous personal contact with our patients' referring physicians.[93–95,100] Each of the suspect buildings was tested by a different independent environmental

firm for the presence of mold and mold spores. All buildings were contaminated with a variety of mold genera. Inasmuch as *Aspergillus* sp., *Pencillium* sp., and *Stachybotrys chartarum* were the most frequently identified molds, we selected *Aspergillus niger, Penicillium notatum,* and *Stachybotrys chartarum* as representative organisms. Satratoxin H was incorporated in our study as being representative of trichothecene because this mycotoxin is known to be produced by *S. chartarum.*

Detection of IgG, IgM, and IgA antibodies against molds and satratoxin H. Sera from controls, mold-exposed patients, and randomly selected patients were analyzed for the presence of IgG, IgM, and IgA antibodies to the 3 molds and satratoxin H. The mean titers ± standard deviations (*SD*s), as determined by ELISA for each isotype, along with *z* scores and *p* values, are given in Tables 1–4. The serum dilution used was 1:100, which was determined previously to be optimum.[100] IgG, IgM, and IgA titers against *P. notatum, A. niger,* and *S. chartarum* are presented in Tables 1–3, respectively. The *z* scores for all 3 isotypes against *Penicillium, Aspergillus,* and *Stachybotrys* were higher than 3.3. IgG, IgM, and IgA antibodies against satratoxin H are given in Table 4. For satratoxin IgG, the differences between the controls and the molds-exposed group were statistically significant ($p < 0.001$; $z = 11.3$).

To examine statistical differences among the 3 groups for the levels of molds and mycotoxins, the post hoc tests (Scheffé's significant difference tests) were performed and were classified under 3 different subsets, as presented in Table 5. Means for groups in homogenous subsets are based on Type III sum of squares (sample size = 1,500, with 500 subsets in each group). Means that are reported in the same subset are statistically similar. For example, means for IgA (satratoxin) for controls, random patients, and mold-exposed patients are 627, 784, and 759, respectively, which are statistically alike. Similarly, the means for *Aspergillus* and *Stachybotrys* IgA in controls and in random patients are classified under the same subset, whereas the means for IgA in mold-exposed groups are statistically different for both controls and random patients. For all other determinations, 3 different subsets are formed, meaning that IgG and IgM against *Penicillium, Aspergillus, Stachybotrys,* and satratoxin are statistically different in controls, random patients, and mold-exposed patients. For IgA against *Penicillium*—although formation of 3 subsets indicates statistical differences among the 3 groups—the IgA values for *Aspergillus* and *Stachybotrys* were reported in 2 subsets, meaning that the mold-exposed group was statistically different from both controls and random patients. And, finally, satratoxin IgA means are classified under the same subset, indicating that no dif-

Table 1.—Antibody Levels (in ELISA Units) in Response to *Penicillium notatum* in Controls, Randomly Selected Patients, and Mold-Exposed Patients, with *z* Scores and *p* Values

	Controls vs. mold-exposed						Random vs. mold-exposed					
	Controls (n = 500)		Exposed (n = 500)				Controls (n = 500)		Exposed (n = 500)			
Antibody	$\bar{x}$	*SD*	$\bar{x}$	*SD*	*z*	*p*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	*z*	*p*
IgG	620	535	2,159	2,458	13.7	< 0.001	1,383	1,839	2,159	2,458	5.6	< 0.001
IgM	692	551	1,692	2,442	8.9	< 0.001	1,241	1,530	1,692	2,442	3.5	< 0.001
IgA	640	572	1,256	2,163	6.1	< 0.001	853	1,070	1,256	2,163	3.7	< 0.001

Notes: $\bar{x}$ = mean, *SD* = standard deviation, and Ig = immunoglobulin.

Table 2.—Antibody Levels (in ELISA Units) in Response to *Aspergillus niger* in Controls, Randomly Selected Patients, and Mold-Exposed Patients, with *z* Scores and *p* Values

	Controls vs. mold-exposed						Random vs. mold-exposed					
	Controls (n = 500)		Exposed (n = 500)				Controls (n = 500)		Exposed (n = 500)			
Antibody	$\bar{x}$	*SD*	$\bar{x}$	*SD*	*z*	*p*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	*z*	*p*
IgG	618	426	1,795	2,316	11.1	< 0.001	1,349	1,417	1,795	2,316	3.7	< 0.001
IgM	782	420	1,725	2,449	8.5	< 0.001	1,177	1,302	1,725	2,449	4.4	< 0.001
IgA	732	595	1,346	2,456	5.4	< 0.001	849	938	1,346	2,456	4.2	< 0.001

Notes: $\bar{x}$ = mean, *SD* = standard deviation, and Ig = immunoglobulin.

Table 3.—Antibody Levels (in ELISA Units) in Response to *Stachybotrys chartarum* in Controls, Randomly Selected Patients, and Mold-Exposed Patients, with *z* Scores and *p* Values

	Controls vs. mold-exposed						Random vs. mold-exposed					
	Controls (n = 500)		Exposed (n = 500)				Controls (n = 500)		Exposed (n = 500)			
Antibody	$\bar{x}$	*SD*	$\bar{x}$	*SD*	*z*	*p*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	*z*	*p*
IgG	803	530	2,304	2,432	13.5	< 0.001	973	1,234	2,304	2,432	10.9	< 0.001
IgM	629	602	1,940	2,478	11.5	< 0.001	1,115	1,212	1,940	2,478	6.7	< 0.001
IgA	665	665	1,511	2,660	6.9	< 0.001	760	1,086	1,511	2,660	5.8	< 0.001

Notes: $\bar{x}$ = mean, *SD* = standard deviation, and Ig = immunoglobulin.

Table 4.—Antibody Levels (in ELISA Units) in Response to Satratoxin in Controls, Randomly Selected Patients, and Mold-Exposed Patients, with *z* Scores and *p* Values

	Controls vs. mold-exposed						Random vs. mold-exposed					
	Controls (n = 500)		Exposed (n = 500)				Controls (n = 500)		Exposed (n = 500)			
Antibody	$\bar{x}$	*SD*	$\bar{x}$	*SD*	*z*	*p*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	*z*	*p*
IgG	767	641	1,523	1,352	11.3	< 0.001	1,054	1,147	1,523	1,352	5.90	< 0.001
IgM	611	648	1,320	1,590	9.2	< 0.001	1,160	1,170	1,320	1,590	1.80	< 0.060
IgA	715	588	705	868	2.1	< 0.440	747	819	705	868	0.78	< 0.430

Notes: $\bar{x}$ = mean, *SD* = standard deviation, and Ig = immunoglobulin.

ferences were detected among the 3 groups. The statistical differences among the 3 groups for the levels of IgG, IgM, and IgA against molds and mycotoxins were further confirmed by the calculation of exact *z* scores and *p* values, which are provided in Tables 1–4. We considered *p* values < 0.05 and *z* scores > 3.3 to be statistically significant.

Specificity and intra-assay and inter-assay precision. Specificity, and intra-assay and inter-assay precision, for each of the molds, and for several other genera and satratoxin H, have been determined previously.[100] In brief, the coefficients of intra-assay variation calculated for 8 replicates of ELISA assays were 5.7–10.2% for IgG, 5.8–9.2% for IgM, and 5.6–11.3% for IgA. Inter-assay precision was calculated for the same 5 samples assayed in 6 different runs. The inter-assay variations were 7.8–12.7%, 9.5–15.5%, and 10.6–15.3% for IgG, IgM, and IgA, respectively.

Absorption of *Stachybotrys* antibodies with HSA and mold antigens. Similar to our earlier study,[100] 3 different sera with high levels of IgG, IgM, and IgA against *Stachybotrys* were absorbed with nonspecific and specific antigens. Data summarized in Table 6 show that nonspecific proteins, such as HSA, did not change IgG, IgM, and IgA antibody levels against *Stachybotrys*, whereas *Stachybotrys* antigens absorbed the IgG antibody titer levels from 42.7–58%, IgM antibody levels from 21.4–38.5%, and IgA levels from 26.8–34.6%. Similar to IgG, but to a lesser degree, IgM and IgA antibodies were absorbed with *Stachybotrys* antigens. This significant absorption and inhibition of IgG, IgM, and IgA antibodies by fungal antigens is excellent evidence for the specificity of fungal antibodies. Furthermore, other molds antigens, such as *Alternaria*, were not capable of absorbing levels of *Stachybotrys* antibodies, whereas *Penicillium* and *Aspergillus* absorbed only IgG antibody against *Stachybotrys* from 14–20%. This absorption of anti-*Stachybotrys* antibody by *Penicillium* and *Aspergillus* indicates minor antigenic cross-reactivity between these molds.

Discussion

Adverse health effects from exposure to molds in water-damaged buildings can result in allergic reactions, asthma, hypersensitivity pneumonitis, pulmonary infections, and mucous membrane irritation and toxicity. However, despite this variety of adverse health effects, significant emphasis has been placed mainly on Type I allergy and asthma,[20,21,101–103] and not on the other immunopathologic mechanisms involved in the pathogenesis of Types II–IV allergy.[104]

Hundreds of molds, with thousands of antigens, can contaminate indoor air. In addition, some of these

Table 5.—Multiple Comparisons and Means for Groups in Homogenous Subsets Determined with Scheffé's Post Hoc Tests

Mold/mycotoxin and group	IgG subset			IgM subset			IgA subset		
	1	2	3	1	2	3	1	2	3
Penicillium									
Controls	689			748			679		
Random patients		1,547			1,386			944	
Mold-exposed			2,386			1,985			1,841
Aspergillus									
Controls	578			679			688		
Random patients		1,398			1,214		842		
Mold-exposed			2,035			2,147			1,756
Stachybotrys									
Controls	744			617			668		
Random patients		1,083			1,184		815		
Mold-eposed			2,398			2,086			2,153
Satratoxin									
Controls	725			636			627		
Random patients		1,109			1,163		784		
Mold-exposed			1,460		1,405		759		

Notes: Statistical analyses for examination of differences between the levels of immunoglobulin (Ig)G, IgM, and IgA antibodies against molds and a mycotoxin among 3 different groups using the General Linear Model for Windows, version 11.5 (SPSS, Inc. [Chicago, Illinois]). Means for classification of groups in different subsets were based on the sum of squares of sample size of 500 subjects in each of 3 groups. Means reported in the same subset are statistically similar; if they are classified under a different subset it means that controls differed from random patients, as well as from mold-exposed patients.

Table 6.—Optical Densities of Sera with High Levels of Immunoglobulin (Ig)G, IgM, and IgA Antibodies against *Stachybotrys*, before and after Absorption with Nonspecific and Specific Antigens

Absorption status	Sample 1			Sample 2			Sample 3		
	IgG	IgM	IgA	IgG	IgM	IgA	IgG	IgM	IgA
Before absorption									
Optical density	1.54	1.92	1.36	1.10	2.15	1.83	2.30	0.96	0.87
After absorption with:									
Human serum albumin									
Optical density	1.49	1.83	1.27	0.98	2.26	1.95	2.16	0.93	0.91
% inhibition	NS	NS	NS	NS	NS	NS	NS	NS	NS
Stachybotrys chartarum antigens									
Optical density	0.69	1.18	0.89	0.63	1.69	1.34	0.96	0.61	0.57
% inhibition	55.20	38.50	34.60	42.70	21.40	26.80	58.20	36.40	34.50
Aspergillus niger antigens									
Optical density	1.31	1.73	1.27	0.95	1.98	1.76	1.89	0.88	0.78
% inhibition	15.00	10.00	6.60	13.60	8.00	4.00	18.00	8.40	10.30
Penicillium notatum antigens									
Optical density	1.29	1.80	1.26	0.91	1.89	1.81	1.83	0.92	0.79
% inhibition	16.20	6.30	7.30	17.20	12.00	10.90	20.40	4.20	9.20
Alternaria alternata antigens									
Optical density	1.51	1.83	1.29	1.16	2.10	1.72	2.25	0.94	0.80
% inhibition	NS	NS	NS	NS	NS	NS	NS	NS	NS

Note: NS = Nonsignificant.

molds produce potentially toxic metabolites (e.g., mycotoxins and solvents)[3–17,69,70] and can invade tissues (causing aspergilliosis, cryptococcosis, coccidioidomycosis, or pulmonary hemosiderosis)[35–50] by producing proteases, hemolysins, and siderophores.[51–59,61,66–68] Moreover, the potential cytotoxic action of molds and their metabolites includes DNA adducts,[89,82,85,86] adverse effects on pulmonary surfactant synthesis in rodents,[62–65] mitochondrial toxicity,[77,78,83] apoptosis,[73–76] abnormalities of the human immune system,[93] neuro-

cognitive deficits with changes in electroencephalogram,[89,90,94] peripheral neuropathy,[90] autoimmunity,[93,95] and carcinogenesis.[79,81,87] Because it is becoming increasingly apparent that both atopic and nonatopic individuals experience adverse health reactions to mold exposure unrelated to IgE-mediated sensitivity,[89,90,93–95] biomarkers and clinical tests involving the immune system, the respiratory tree, and the central nervous system must be developed and implemented.[89,93–95,100]

Our data clearly show that exposure to molds in water-damaged buildings leads to the production of IgA, IgM, and IgG antibodies to antigens of *Aspergillus niger, Penicillium notatum, Stachybotrys chartarum,* and satratoxin H in subpopulations of patients, and corroborates previous observations on these and other molds by some of the authors.[100] Previous reports on IgG antibodies against *S. chartarum* and *Aspergillus* have produced equivocal results. In one study,[5] 4 of 48 individuals possibly exposed to *S. chartarum* had IgG antibodies to the organism; other researchers found statistically nonsignificant differences between exposed and control groups.[23,105] On the other hand, a recent cross-sectional comparison of water-damaged or mold-contaminated homes in Finland found fungal-specific IgG concentrations in the sera of patients living in houses both with and without mold, with other cases showing a tendency to exhibit higher antibody levels to most fungi than seen in the control groups.[106]

The differences between our study and others in regard to antibodies to molds might be accounted for by differences in the selection of controls and in the preparation of mold antigens. First, in addition to the controls and patients presented herein, we have tested for antibodies to molds in 500 randomly selected individuals with diagnoses other than mold exposure. The titers to molds IgG, IgM, and IgA, and satratoxin H IgA were intermediate to the controls and patients in this study, but significantly different than both the controls and patients presented herein (Tables 1–5). Some of the controls—who represented a healthy population—probably were exposed to molds. Such exposure likely would result in less statistical significance when the control group is compared with the patients. Therefore, if supposedly true controls (individuals not exposed to molds) could be used in this study, the mean ± *SD* would have been less (estimated at 10–20% lower) than reported in Tables 1–5. We anticipate that this would result in higher *z* scores and lower *p* values, or a greater statistical difference between controls and exposed groups. Moreover, in the selection of controls, one assumes they have a likelihood of exposure. Thus, it is apparent from these observations that selection of controls is critical and that it is probable that clinicians miss molds as a possible cause of the illness for which medical attention is being sought.

The second explanation for the differences between the observations presented herein and those of other investigators likely lies in the preparation of antigens. We prepared our fungal antigens similar to the methods described by others,[5,30] but with some modification.[100] Similar culture and suspension techniques were used, but were modified with repeated sonications during 24 hr of extractions, which resulted in higher yield of fungal antigens in suspension. Comparison of commercially prepared fungal antigens with our preparations showed differences in protein concentrations before and after sonications. Protein concentrations of commercial antigens ranged from 0.4 to 2.1 mg/ml, whereas the protein concentrations used in this study ranged from 0.8 to 3.5 mg/ml. After 10 sonications, our protein concentrations ranged from 5.8 to 16.5 mg/ml. In addition, SDS gel electrophoresis on an equal amount of protein (1 mg) revealed (depending on mold species) 9–22 bands in commercial antigens, 7–16 bands in our preparations before sonications, and 21–36 protein bands after repeated sonications. Therefore, for maximum efficacy, we mixed our mold antigens with commercially available allergenic mold extracts and used 2 µg/well of these mixtures on ELISA plates. With this mixture of mold antigens, we found that human sera diluted 1:100 for IgG, IgM, and IgA in serum diluent resulted in optimum ELISA optical densities. The coefficients of intra-assay and inter-assay variations were less than 16% for all isotypes. IgG antibodies to molds was the lowest (5.7–10.2%) and IgA the highest (5.6–11.3%).[100] Nonspecific proteins (HSA) did not change the levels of these antibodies; however, specific mold antigens extracted from *Stachybotrys* absorbed the IgG antibodies 43–58%, the IgM antibodies 21–38%, and the IgA antibodies 27–34%. Interestingly, when similar absorptions of sera were performed with other mold antigens, absorption of IgG, IgM, and IgA antibodies to *Stachybotrys* with *Alternaria* was insignificant, or less than 10%; however, reaction of the same sera with *Aspergillus* and *Penicillium* was capable of absorbing only the IgG antibody against *Stachybotrys* significantly (14–20%). This minor inhibition of IgG antibody against *Stachybotrys* with *Aspergillus niger* and *Penicillium notatum* antigens is an indication of antigenic cross-reactivity between these molds, which warrants further investigation. The significant inhibition of IgG, IgM, and IgA antibodies against *Stachybotrys* by specific molds and antigens (Table 6) provides further evidence for the specificity of these antibodies. Inhibition of these antibodies with mold antigens, along with the simultaneous presence of IgG, IgM, and IgA antibodies against different molds and satratoxin (Tables 1–4) leads us to conclude that these specific antibodies could be used in subpopulation studies and in epidemiologic investigations of mold and mycotoxin exposure. Furthermore, clinicians may want to consider possible mold exposure in patients who present with multiorgan system abnormalities.

Finally, the detection of IgG antibodies to satratoxin H reveals that the mycotoxin—or the spores and hyphae containing the mycotoxin—can behave as an antigen. This likely occurs by the combination of satratoxin H with carrier mold proteins, and their presentation to the cells involved in the immune system, resulting in subsequent antibody production. Similar observations have been reported for aflatoxin B1, patulin, and ochratoxin A used as a hapten.[107–112]

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