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) $\bar{x}$	*SD*	% abnormal	Males (n = 83) $\bar{x}$	*SD*	% abnormal	Females (n = 123) $\bar{x}$	*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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