CME review article

Clinical use of immunoassays in assessing exposure to fungi and potential health effects related to fungal exposure

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Objective: To review and summarize current evidence regarding the proper role of immunoassays in clinical assessments of exposure to fungi and health effects related to fungal exposure.

Data Sources: We reviewed relevant scientific investigations and previously published reviews concerning this topic.

Study Selection: The authors' clinical, laboratory, and public health experiences were used to evaluate relevant data for scientific merit.

Results: Testing to determine the presence of IgE to specific fungi may be a useful component of a complete clinical evaluation in the diagnosis of illnesses that can be caused by immediate hypersensitivity such as allergic rhinitis and asthma. Detection of IgG to specific fungi has been used as a marker of exposure to agents that may cause illnesses such as hypersensitivity pneumonitis. However, the ubiquitous nature of many fungi and the lack of specificity of fungal antigens limit the usefulness of these types of tests in the evaluation of potential building-related illness and fungal exposure. Specific serologic tests (such as tests for cryptococcal antigen, coccidioidal antibody, and *Histoplasma* antigen) have been shown to be useful in the diagnosis of some fungal infections, but these are the exception not the rule.

Conclusions: There is currently not enough scientific evidence to support the routine clinical use of immunoassays as a primary means of assessing environmental fungal exposure or health effects related to fungal exposure. Health care providers who care for persons expressing concerns about the relationship of symptoms to potential exposure to fungi are advised to use immunoassay results with care and only as an adjunct to a comprehensive approach to patient care.

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INTRODUCTION

Fungi are eukaryotic microorganisms that are widespread in nature and found in nearly every habitat, both indoors and outdoors. They are characterized by a rigid cell wall and the absence of chlorophyll; examples include mushrooms, toad-stools, yeasts, molds, mildews, smuts, and rusts. Molds can be defined as filamentous fungi that colonize their environment with long filaments called hyphae. Hyphae usually branch and grow together, forming an intertwined network known as a mycelium, which may be readily visible by the naked eye. Conidia (commonly known as fungal spores) grow from aerial branches of the mycelium and function in the dispersal of the fungus to new environments. Although the kingdom Fungi recognizes molds, yeasts, and mushrooms as its 3 major groups, the press and the public tend to use the terms *mold* and *fungi* interchangeably, with *mold* often used to describe any visible fungal growth. Fungi have been estimated to comprise 25% of the biomass of the earth[1]; consequently, human exposure to fungi is ubiquitous. Human exposure may occur via inhalation of airborne spores and hyphal fragments, ingestion of contaminated food products, and skin or eye contact after handling of contaminated material. Reports of human and animal diseases have involved only a very small percentage of the more than 1,000,000 existing fungal species.[2,3]

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Fungi are known to produce adverse health effects by 4 distinct mechanisms, including (1) immunologic hypersensitivity, (2) infection, (3) toxic reactions, and (4) irritant reactions. Each mechanism has associated with it well defined, scientifically validated, pathophysiologic processes. Immunologic hypersensitivity is the most commonly occurring mechanism of adverse health effects, most often manifested as rhinitis, conjunctivitis, and asthma and less often as allergic bronchopulmonary mycoses, allergic fungal sinusitis, and hypersensitivity pneumonitis (HP).[2,3] Superficial fungal infections, such as tinea and *Candida*, are common but are not generally related to the mold-contaminated indoor environments. Deeper fungal infections occur less frequently and often signal some form of immunodeficiency. Ingestion of large quantities of mycotoxin-contaminated foodstuffs has been shown to cause toxic clinical illness in humans and animals.[4,5]

Due in part to increasing and continued attention to this topic in the medical literature and the lay press, concerns about exposure to fungi, particularly *Stachybotrys chartarum*, have become widespread.[6,7] Many are seeking care from their physicians either for symptoms (often nonspecific symptoms such as fatigue and headache) that they attribute to fungal exposure or because they discover fungi in their home or work environment and are concerned about possible adverse health effects. Because fungi are known to produce antigenic proteins that can lead to hypersensitivity (immunologic) responses among some people,[8] medical evaluations of people concerned about exposure to fungi may include laboratory immunologic evaluations. A number of different immunoassays are being used in the clinical evaluation of these patients.

This article will discuss basic principles related to in vitro immunoassays and their utility in evaluating persons exposed to fungi. The distinction between clinical tools as measures of exposure and measures of health effects is also discussed. We reviewed relevant scientific investigations and previously published reviews concerning this topic and used our clinical, laboratory, and public health experiences to evaluate relevant data for scientific merit. Two brief clinical vignettes are included to illustrate common misuses of immunoassays in the clinical evaluation of persons with health effects potentially related to fungi.

REVIEW OF IMMUNE RESPONSE, ANTIBODIES, AND ANTIGENS

Antigens are substances that are foreign to the body and induce an immune response. Bacteria, viruses, and fungi are a common source of environmental antigens encountered in daily life. Exposure to fungal antigens can elicit both humoral and cellular immune responses. During a humoral immune response, most antigens activate many different B lymphocytes, which then proliferate and differentiate into individual clones of antibody-secreting plasma cells; such an immune response can be referred to as *polyclonal*. That portion of the antigen where the antibody physically binds it is called the *antigenic determinant* or *epitope*. Research with fungal

spores and monoclonal antibodies has shown that many phylogenetically related (as well as nonrelated) fungi share many common epitopes and that immune responses to fungi may be dominated by cross-reactivity (ie, many antibodies produced against fungi are cross-reactive with many fungal species). This cross-reactivity needs to be taken into account when interpreting the results of any antibody-based assay.

IMMUNOASSAYS

Immunoassays are based on the formation and detection of binding between antigens and antibodies of varying specificity. Radioimmunoassays use radiolabeled [eg, iodine 125 (^{125}I)] reagents to detect the reaction between antigens and antibodies; the presence of antigen-antibody reactions are measured using a gamma counter. Most radioimmunoassays have been replaced by enzyme-linked immunosorbent assays (ELISAs), sometimes referred to as enzyme immunoassays. In ELISAs, the solid support (microtiter plate) binding of a reactant allows for separation of bound vs unbound reactants by simple washing. The detector system in ELISAs is usually an enzyme (eg, horseradish peroxidase, alkaline phosphatase) bound to a reactant, usually an antibody. The antibodies used in an ELISA can be polyclonal or monoclonal. Polyclonal antibodies are usually prepared by injecting animals (usually rabbits) with antigen and adjuvant (a mixture that stimulates the immune response) and collecting serum from the animals. Polyclonal antibodies may be further purified and isolated, yielding essentially monospecific polyclonal antibodies. Monoclonal antibodies are produced by fusing tumor cells with antibody-producing B cells isolated from immunized animals (usually mice). The fused cells, called hybridomas, produce antibody to essentially one epitope, hence the term *monoclonal*.

ELISAs can be performed as competitive or noncompetitive assays in direct or indirect formats. In competitive assays, a nonlabeled sample analyte competes with a labeled target analyte. In direct assays, specific primary antibodies are labeled with an enzyme; indirect assays involve labeled secondary antibodies, which are used to reveal bound but unlabeled specific primary antibodies. The 2 most popular assays for the detection of specific serum antibodies are indirect ELISAs in which isotype-specific labeled antiglobulins are used to detect specific antibodies against surface-adsorbed or antibody-captured antigens.

Some commercial ELISAs are in vitro diagnostic devices cleared or approved by the Food and Drug Administration (FDA) through a process known as premarket notification (510k program) based on the Medical Device Amendments of 1976.[9,10] (It should be pointed out that FDA clearance does not guarantee diagnostic performance.) Many ELISAs are 510k cleared as being essentially equivalent to previously cleared assays. In 1996, the FDA introduced a new in vitro diagnostic device classification category called *analyte-specific reagents* (ASRs).[11] The FDA defines ASRs as "antibodies, both polyclonal and monoclonal, specific receptor proteins, ligands, nucleic acid sequences, and similar reagents

which, through specific binding or chemical reaction with substances in a specimen, are intended for use in a diagnostic application for identification and quantification of an individual chemical substance or ligand in biological specimens." In essence, the FDA recognized ASRs as the active ingredients of in-house tests, which when used in combination with general purpose reagents (such as buffers or reactive materials without specific intended uses) and general purpose laboratory instruments could be the basis for an assay developed and used by a single laboratory. In addition to those tests to which the regulatory oversight of the FDA applies, laboratories may develop and use in-house tests that are not regulated. Such tests may be useful as a tool in the diagnosis of disease; the responsibility for the validation of the test becomes that of the laboratory developing the test. There are no "rules" for validation of these tests; however, at a minimum such validation should address evaluation of solid phase binding of antigen or antibody, primary and secondary incubation antibody times, the effect of interfering substances, and matrix effects.

All immunoassays of any format should be accurate, precise, and well controlled. Limits of detection, linearity on dilution of analyte, methods for data reduction, and standards analyses quality control should all be rigorously examined. Units should be reported as concentrations (wt/vol), although absorbance or other units may be applicable in some assays. All reported units should be identified. One of the most common problems associated with ELISAs for fungi is the absence of standardized fungal extracts, which precludes the establishment of universal calibrator reagents and standard curves for the quantification and extrapolation of antigen concentrations in environmental samples. The implications are such that immunoassay-based monitoring results for fungi obtained with different extracts in the same or a different laboratory may not be comparable (ie, the repeatability and the reproducibility of the assay may be compromised).

When developing an assay to determine whether elevated concentrations of an analyte (such as a fungi-specific protein or product) are present in a cohort compared with a comparison population, great care must be exercised in selection of an appropriate comparison group. Appropriate comparison groups would generally consist of persons without identifiable excessive exposure to the fungi of concern among the "exposed" group. By evaluating clinical samples among the test ("exposed") and comparison ("unexposed") groups, it may be possible to determine whether a statistically elevated concentration of the analyte is present in the samples from the test group.[12] Additionally, methods have been described in which the diagnostic sensitivity and specificity of a newly developed immunoassay have been estimated in a relatively short time.[13]

The National Committee for Clinical Laboratory Standards (NCCLS) is an international, interdisciplinary, nonprofit, standards-developing, and educational organization that promotes the development and use of voluntary consensus standards and guidelines within the health care community. The NCCLS has published a guideline[14] that should be useful to anyone performing or evaluating immunoassays. The responsibility for evaluating the usefulness of assays performed only by in-house laboratories lies with both the laboratorians and the clinicians who are interpreting the tests. Evaluation of the steps used to validate the tests is an essential component of this responsibility.

IMMUNOASSAYS IN THE DIAGNOSIS OF FUNGAL INFECTION

Fungal infections of the skin and mucous membranes are called *superficial mycoses*. These are common infections and include athlete's foot, ringworm, and thrush. Their occurrence has not been associated with fungal growth on building materials or furnishings indoors, except for "athlete's foot" caused by commonly moist flooring (eg, in locker rooms). The diagnosis is made based on visual appearance and confirmed by obtaining skin scrapings for potassium hydroxide preparation and/or culture. Serologic analysis has no role in the diagnosis and evaluation of fungal skin infections.[15]

Systemic mycoses are acquired mainly by inhalation and are disseminated via the lymphohematogenous route to one or more organs. Examples include histoplasmosis, coccidioidomycosis, and blastomycosis. In healthy individuals, these are usually mild or asymptomatic pulmonary infections that heal without sequelae. Severe pulmonary infection or spread to other organs is less common. Although most other fungi are opportunistic pathogens, usually infecting individuals with compromised immune systems, some can cause infections of deeper tissue (such as sinusitis), even in persons with normal immune function. Fungal cultures and histopathologic studies of infected body fluids and tissues are the principal means of diagnosing systemic fungal infections.[16,17] Skin testing may be useful in epidemiologic studies for documenting past fungal infection but is not useful in individual diagnosis. Serologic analysis is of limited value because of poor sensitivity, specificity, and inconsistent standardization of reagents and methods.[18] Furthermore, serologic analysis is unable to distinguish between infection and colonization and might not be a reliable measure in immunodeficient patients who do not readily produce antibodies.[19–21] In some situations, serologic analysis can be used as an initial rapid and less invasive means of diagnosing fungal infections[22] and monitoring the progress of infection and the patient's response to therapy.[17] Serologic tests that have been found clinically useful include latex agglutination of cryptococcal antigen and coccidioidal antibody in serum or cerebrospinal fluid as indicators of infection and detection of *Histoplasma* antigen in urine or serum as a means of assisting in the diagnosis and follow-up of disseminated histoplasmosis.[23]

IMMUNOASSAYS IN DIAGNOSIS OF HYPERSENSITIVITY DISEASES

Type 1 (Immediate) Hypersensitivity
Immediate hypersensitivity (Gell and Coombs type 1) involves the release of mediators from mast cells or basophils

caused by the bridging of IgE antibodies on the surface of these cells. Clinical manifestations of this type of reaction include rhinitis, asthma, and eczema. A widely accepted clinical method of determining the presence of specific IgE to a given substance is skin prick testing (SPT),[24] which provides a biologically relevant immediate-type hypersensitivity response in the skin.[25] SPT is simple, provides results within minutes, and is inexpensive. Determination of specific IgE antibodies to known allergens by skin testing (or in vitro tests) is an important component of an appropriate systematic clinical evaluation for many common medical conditions, including, for example, rhinitis, sinusitis, and reactions related to food allergies.[26,27] Contraindications to SPT include generalized skin disease or the inability to discontinue antihistamine use; in these cases, in vitro assays for specific IgE may be useful. Although highly sensitive, SPT is not 100% sensitive or specific; SPTs can produce false-positive or false-negative results.[28] The American Academy of Allergy, Asthma, and Immunology states that some patients who have a strong history of systemic reactions may have negative skin test results for IgE to suspected allergens.[29] Conversely, up to 60% of positive SPT results to foods and up to 50% of positive SPT results to latex do not reflect symptomatic allergy.[30,31]

Clinical tests can identify relevant allergens related to causation. However, any interpretation of the clinical relevance of positive test results must consider the potential for a high frequency of false-positive test results, particularly when inappropriately high concentrations of allergen extracts have been used. In addition, fungal allergens and food allergens are more likely than other types of allergens to produce false-positive results by this method.

Challenge testing (eg, bronchoprovocation, nasal provocation) may be the most clinically relevant test available to demonstrate causation by a specific allergen. However, its use is problematic due to the clinical and ethical issues raised by such testing and questions raised about the clinical relevance of nonnatural exposure protocols.

Development of serologic methods for detection of fungi-specific antibodies is especially problematic because of the lack of standardized fungal extracts.[32,33] Any fungal species can make several allergens, which are produced variably, depending on the strain cultured and the conditions of growth (including the substrate).[34] There is also substantial cross-reactivity among allergens of various fungal species and genera.[35–38] This extensive cross-reactivity has been confirmed with monoclonal antibodies produced against *Aspergillus versicolor* and *Penicillium brevicompactum*.[39] Furthermore, an isotype-specific ELISA for measuring IgE, IgG, and IgA to *S chartarum* has been developed but is known to cross-react with antibodies to 2 common outdoor fungi, *Aspergillus fumigatus* and *Alternaria alternata*.[40]

There is no single test that serves as a "gold" standard for the diagnosis of fungal allergy or allergy in general. If symptoms consistent with established pathophysiological mechanisms of allergy, such as those occurring with rhinitis or asthma, occur on suspected exposure to a specific substance or substances, then a search (using in vivo or in vitro testing) for evidence of sensitization should be undertaken. Finding specific IgE by either type of testing only indicates sensitization (the presence of a humoral immune response leading to increased amounts of specific antibody in the serum). Nonspecific symptoms, such as fatigue, malaise, or headache, may be caused by allergy when other symptoms or signs known to be associated with allergy are also present. However, in the absence of allergy-related symptoms, nonspecific symptoms are unlikely caused by allergy. The diagnosis of allergy (immediate hypersensitivity) requires a clinical history (including exposure history) consistent with any positive in vivo or in vitro test results (with established clinical relevance) if one is to attribute causation.

Other Hypersensitivity Diseases
Immunoassays have been helpful in diagnosing illnesses such as HP that can be caused by bioaerosol exposure.[41–43] However, the results of immunoassays should be considered along with other clinical information when performing diagnostic evaluations of individual patients. Although skin testing and radioallergosorbent tests are commonly used to identify the presence of specific IgE for potential allergens, immunoassays can also measure minute amounts of specific IgG in the serum of patients. However, just as with specific IgE, the significance of elevated levels of specific IgG will depend on the entire clinical evaluation. In general, IgG testing to identify causative agents for uncomplicated asthma has been found to have no clinical utility[44] and is not used in the diagnosis of asthma.[45] The mere presence of specific IgG, even in increased concentrations, may only indicate exposure not causation.[46] There are only a few uncommon disorders in which the presence of increased specific IgG has clinical significance (diagnostic predictive value), such as HP, allergic bronchopulmonary mycosis, and possibly allergic fungal sinusitis. Consequently, the measurement of specific IgG only has utility in the presence of a clinical picture of a disorder where this test has established predictive value.

Hypersensitivity Pneumonitis
There are many agents, including microorganisms, capable of causing HP; exposure to specific fungi, in both the indoor and agricultural environments, has been associated with HP.[47,48] HP is thought to be the result of a combination of immune complex and cell-mediated responses to inhaled antigens.[49,50] Detection of antibody by precipitin testing is a commonly used means of confirming exposure to a suspected antigen source, but it is not clear if the antibody detected is related to the pathogenesis of the illness.[51] Precipitin assays are relatively insensitive, generally only detecting larger concentrations of specific IgG. Newer, more sensitive assays, such as IgG-specific ELISAs, can also be used to detect exposure to suspect antigens. Notably, antibodies to antigens suspected of playing causative roles in several types of HP are found in substantial percentages of asymptomatic farmers[52] and pigeon

breeders.[53] Diagnostic criteria for patients with suspected HP have been reviewed in the literature and include a variety of clinical findings.[41–43,54,55] Conversely, antibodies to suspected causative antigens may not be found in patients with clinically confirmed HP as a result of poorly standardized antigens used in the immunoassays, incorrect identification of the causative antigen, or low concentrations of IgG antibody.[56] Therefore, in the evaluation of an individual with HP, detection of antigen-specific IgG antibodies is thought to primarily reflect past exposure to *those antigens*, offering supporting evidence of causation in cases where environmental exposure to a specific causal antigen has been confirmed.

SCIENTIFIC CHALLENGES ASSOCIATED WITH THE INTERPRETATION OF IMMUNOASSAYS USED TO ASSESS EXPOSURE TO FUNGI

Although antigen-specific antibodies can serve as indicators of previous exposure to fungi (or fungal fragments), they do not indicate where or when the exposure took place. The immune system "remembers" past exposures by producing antibodies for months or years, and it is often impossible to determine exactly when an initial exposure took place. The kinetics and variable persistence of the IgG response make it difficult to determine the nature and duration of exposure. Another challenge in the interpretation of fungal immunoassays is the widespread immunological cross-reactivity between phylogenetically related and nonrelated fungi as detected with serum[57,58] and monoclonal antibodies.[39,59] In most cases, without additional evidence, the presence of serum antibodies against specific fungi (ie, sensitization) cannot be used to determine which specific fungi were the initial sensitizing fungi. Similarly, the presence of antibodies to specific fungi cannot be used to determine whether specific species of fungi found in a person's environment are the cause of health concerns a patient may be having. Although antibody reactivity is necessary to identify certain fungi as possible sources of sensitization, the presence of serum antibody against a specific fungus offers only supporting evidence of causation in cases where environmental exposure has been confirmed.

LITERATURE REVIEW

Many serologic studies have failed to demonstrate a correlation between specific antibody measurements and environmental fungal exposure in domestic and work environments. For example, Makkonen et al[60] compared the IgG and IgE reactivity to 8 different fungi from serum samples of 70 persons (the "exposed" group, 55 of whom had symptoms) to the antibody reactivity from the serum samples of 31 persons ("unexposed" comparison group) without a history of exposure to fungi. The fungi against which subjects were tested included *Stachybotrys atra, A versicolor, Cladosporium cladosporioides, Trichoderma viride, Penicillium* spp, *Chaetomium globosum, Aspergillus niger,* and *A fumigatus.* The authors reported that these fungi were chosen because they are typical "molds occurring on water-damaged house materials." However, limited information was provided concerning whether any fungi selected for testing were present in the home or work environments of any of the study subjects. Exposure to mold in the "exposed" group was confirmed by "expert technical investigations," which included visual inspection, moisture readings, detection by dogs, and microbiological investigation (which were not described). No such investigations to confirm absence of mold exposure were described for the unexposed group. The authors reported that 20 of the "exposed" subjects worked in an office where *Chaetomium* sp, *A versicolor*, *Cladosporium* spp, and *Penicillium* spp had been found. Detectable IgG to all 8 molds was present in most of both the "exposed" and the "unexposed" groups. IgG titers were not significantly different between groups with the exception of titers to *A niger*. There was no apparent correlation between clinical symptoms and IgG antibodies. Although IgE to 1 or more molds was found in 6 of 55 symptomatic subjects and in none of the asymptomatic subjects, the study does not allow for any conclusions to be made concerning the etiology and clinical significance of the detectable IgE.

Several studies have evaluated persons working in water-damaged buildings. One study[61] reported findings from a study of workers exposed to *Stachybotrys* in a water-damaged office environment. No difference in *S chartarum*–specific serum IgE or IgG concentrations was found between 53 "exposed" workers and 21 workers with no exposure to the problem building. A second study[62] evaluated employees of 2 buildings with histories of water incursions to determine if there was an association of symptoms with exposure to fungi. Symptomatic employees (defined as those employees with 2 or more symptoms) from the problem building did not have significantly higher levels of IgG specific to fungi identified in the building than did individuals from an unexposed comparison group.

Researchers compared fungal-specific serum IgG and IgE concentrations in 47 office workers in an indoor environment heavily contaminated with *Penicillium* spp to antibody reactivity in the serum of 44 workers not working in the heavily contaminated areas.[63] Despite the environmental evaluation that found quantitative differences in fungal types and concentrations between the 2 work areas (especially differences in *Penicillium* spp), there were no detectable differences in fungal-specific IgG levels. Overall, 67% of all participants had IgG to *Penicillium* spp. With the exception of 3 workers (for whom no symptom information was provided) with elevated IgE levels to *Alternaria* spp, no one else demonstrated detectable IgE to any of the other molds evaluated, including *Penicillium* spp. The authors found increased serum levels of IgE specific to common tree, grass, and weed aeroallergens that clinically correlated with some of the reported symptoms.

A recent study[64] looked at specific IgG to 24 fungi in students at schools with identifiable fungal contamination and students at schools without observable fungal contamination. The study found no association between IgG to fungi and exposure to fungi in the school. In addition, there was no

association between fungal IgG levels and asthma, coughing, or wheezing. Another study[65] compared IgG levels with a variety of fungi between 93 students in schools with moisture problems and 33 students from a school without moisture problems. The authors found no relationship between IgG levels and "exposure" status or the presence of asthma among study participants. Finally, a study of serum antibodies among healthy blood donors (without known exposure to excessive amount of fungi) found that 13 (9%) of 139 samples contained IgE against *S chartarum* and 65 (49%) of 132 contained IgG against *S chartarum*[66]; this study suggests that the presence of antibodies to *S chartarum* is not unusual among the general population.

CLINICAL VIGNETTES

Vignette 1

The National Institute for Occupational Safety and Health (NIOSH) received a request for an evaluation to determine whether a school building was "safe" to occupy. The building occupant who requested the evaluation had experienced "severe allergies, asthma, and other lung problems" and was concerned that the symptoms were caused by exposure to "mold and fungi" in the building. The requestor had seen a physician who performed serologic testing for IgE and IgG antibodies to a variety of antigens. IgE concentrations against 8 specific antigen preparations were all reported to be negative (reported as <0.10 kU/L). IgG was detected in the patient's serum at concentrations above the laboratory reference range for 7 of the 8 antigen preparations tested by ELISA, including antigens from *Micropolyspora faeni*, *A alternata*, *A fumigatus*, *Aureobasidium pullulans*, *Penicillium notatum*, *Phoma herbarum*, and *T viride* (the 8 antigens tested were from a standard HP panel). Bulk samples of dust and building material were collected by building representatives and analyzed for bacterial and fungal content (total count and speciation) in specific areas where suspected microbial colonization was observed. Fungal concentrations in the bulk samples ranged from less than 980 CFU/g to more than 63,000 CFU/g. Predominant fungal species included *Penicillium*, *Aspergillus*, and *Cladosporium*. The NIOSH investigators inspected portions of the building where evidence of past water incursion (leaks) had been observed or reported; no visible widespread microbial contamination was present. The requestor was told by the treating physician that the test reports indicated "exposure from working in the building." Based on this testing, the treating physician was concerned that health problems being experienced were caused by exposure to fungi in the school building.

Vignette 2

One of the authors (J.M.S.), reviewing medical records for a legal case involving mold exposure and alleged resulting personal injury to a 2-year-old boy, found that the following immunoassays had been ordered by a treating physician and performed at one commercial laboratory: IgG, IgA, IgM, and IgE against mycotoxins (satratoxins, aflatoxins, and trichoth-

ecenes) that can be produced by various molds and IgM, IgG, and IgA against anti–myelin-associated glycoprotein, myelin basic protein, oligodendrocyte, asialoganglioside, sulfatide, crystallin, neurofilament, tubulin, cerebellar neuronal proteins, and oxidized low-density lipoprotein. According to the interpretation text provided by the commercial laboratory, these tests were used to indicate the following:

1. IgE antimycotoxin: "atopic allergy to that fungus."
2. IgG antimycotoxin: "long-term exposure to that fungus or of prior desensitization. Assay should be repeated 3 months later to confirm successful desensitization or avoidance of the fungus."
3. IgG, IgM, and IgA against myelin and myelin protein and lipid components: "observed in a high percentage of patients with . . . multiple sclerosis, Guillain-Barré syndrome, motor neuron disease, . . . toxic chemical exposure, and silicone adjuvant disease."
4. IgG, IgM, and IgA against neurofilament, tubulin, cerebellar neuronal proteins: "markers of central nervous system damage," including that due to toxicity from "mercury and other metals."
5. Antioxidized low-density lipoprotein: used to detect and follow the progression of atherosclerosis.

At the request of the same treating physician, a second commercial laboratory performed an HP panel and an *S chartarum* serology panel (IgE, IgG, and IgA) on the same patient. Medical records revealed no evidence to suggest that this 2-year-old boy might have HP, central or peripheral nervous system damage, or atherosclerosis.

Discussion of Vignettes

Clinicians and public health practitioners are finding that situations similar to those represented by these vignettes are occurring commonly throughout the United States. Immunoassays are commonly being misused in the evaluation and treatment of symptoms reported by persons who present to health care providers with concerns about exposure to fungi as a cause of their symptoms. The first vignette illustrates the improper use and interpretation of a standard HP panel as a diagnostic tool when exposure to fungi and/or a bioaerosol is suspected as being related to reported symptoms. Standard HP panels typically include antigens that, in certain environments, have been associated with HP. The patient has detectable IgG to a number of agents on the panel of antigens; however, the presence of those antibodies allows for no determination of when or where the patient was exposed and, by itself, has no clinical relevance in relation to reported respiratory symptoms. The presence of *Penicillium*, *Aspergillus*, and *Cladosporium* fungal species in bulk samples of dust and building material from areas with previous water damage is not surprising given the ubiquitous nature of fungi and the fact that fungi grow in areas where an appropriate substrate and moisture are present. The second vignette describes the use of a variety of laboratory immunoassays in addressing health concerns related to a young boy. Although some of the tests described, such as the HP panel, may provide useful

clinical information when used in the appropriate clinical setting, the immunoassays used in this vignette have no scientific validity (excepting the HP panel) or clinical relevance to the 2-year-old boy on whom they were performed.

DIRECTIONS FOR FUTURE RESEARCH

To improve our ability to determine the potential health effects of human exposure to fungi and/or fungal products, scientifically validated and clinically relevant biomarkers of exposure must be developed. The simple detection of antigen-reactive antibodies is not an appropriate means by itself to attribute causation of health effects. An example of a new area of research involves characterization of potentially relevant fungal products as biomarkers, for example, a recently described hemolysin from *S chartarum*. Hemolysins are substances produced by microorganisms that have the ability to destroy red blood cells; hemolysins also likely have other biological activity and are considered important virulence factors.[67,68] A hemolysin produced by *S chartarum*, Stachylysin, has been suggested as a biomarker of exposure to *S chartarum*.[69] The sensitivity and specificity of this test need to be confirmed before it can be recommended for use. Development and standardization of similar assays for hemolysins (or other biomarkers) produced by other fungi need to be investigated and developed before such assays can be considered useful tests in assessing exposure to fungi.

Much of the recent concern about illness potentially related to exposure to fungi has been related to concerns over exposure to mycotoxins in the indoor environment. Recent reviews of the literature specifically addressing concerns about health effects related to mycotoxins have concluded that there is as yet no compelling scientific evidence linking exposure to fungi in residential or commercial indoor environments to toxin-induced adverse health effects.[4,5,70–75] However, it is also evident that inadequacies of current sampling techniques for mycotoxins and the lack of readily available immunoassays prevent the characterization of any potential causal relationship between mycotoxin exposure and health effects. Mycotoxin production is highly variable and depends on the species of fungus, the genetic pattern of a strain, growth time, substrate and water availability, temperature, light, and other parameters. It is clear that the presence of a given fungus that has the potential to produce mycotoxins in any particular environment does not necessarily imply exposure to a toxin.[4] Our inability to measure whether clinically relevant exposure to mycotoxins occurs in the indoor environment limits our ability to determine whether observed symptoms and/or health effects may be related to such exposure. A recent field study based on mycotoxin analysis with immunoassay technology failed to serologically differentiate workers who were known to be exposed in the workplace to mycotoxin-producing fungi from workers with no known exposure to such fungi.[76] Further research is needed to allow us to understand whether clinically relevant exposures to mycotoxins occur in nonindustrial indoor environments.

CONCLUSION

When indicated, testing to determine the presence of IgE to specific fungi is a useful component of a complete clinical evaluation in the diagnosis of health complaints that can be caused by immediate hypersensitivity (eg, allergic rhinitis and asthma). Detection of IgG to specific fungi has been used as a marker of exposure to potential causes of HP and allergic bronchopulmonary mycosis. However, the ubiquitous nature of many fungi and the lack of specificity of fungal antigens limit the usefulness of these types of tests in the evaluation of potential building-related illness and fungal exposure. Specific serologic tests (such as tests for cryptococcal antigen, coccidioidal antibody, and *Histoplasma* antigen) have been shown to be useful in the diagnosis of some fungal infections, but these are the exception not the rule. There is currently not enough scientific evidence to support the routine clinical use of immunoassays as a primary means of assessing environmental fungal exposure or health effects related to fungal exposure. Persistent problems include the absence of standardized procedures and reference reagents and difficulties in the interpretation of results. Furthermore, technical incompatibilities of current environmental sampling techniques with ELISA-based sample processing and difficulties associated with the collection of representative samples from a given target environment lead to ambiguous interpretations of environmental sampling results with regard to possible health effects. Health care providers who care for persons expressing concerns about the relationship of symptoms to potential exposure to fungi are advised to use immunoassay results with care and only as an adjunct to a comprehensive approach to patient care.[75,76]

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CME Examination

Self-Assessment Exam Questions

1. Fungi are known to produce adverse health effects by 4 distinct mechanisms. Which of the following mechanisms of adverse health effects related to fungal exposure is the most commonly occurring among immunocompetent individuals and of the greatest clinical relevance in most clinical settings?
   a. infection
   b. immunologic hypersensitivity
   c. toxic reactions
   d. irritant reactions
   e. a and c

2. Research concerning immune responses to fungi has revealed which of the following, which must be considered when interpreting results of antibody-based assays?
   a. immune responses to fungi are specific for individual fungal species
   b. immune responses to fungi may be dominated by cross-reactivity
   c. phylogenetically related and unrelated fungi share many common epitopes
   d. a and b
   e. b and c

3. Development of serologic methods for detection of fungi-specific antibodies is especially problematic because of the lack of standardized fungal extracts (true or false).
   a. true
   b. false

4. Which of the following are supportive of a diagnosis of fungal allergy?
   a. presence of symptoms consistent with established pathophysiological mechanisms of allergy
   b. evidence of sensitization
   c. presence of nonspecific symptoms (such as headache, fatigue, and malaise) in the absence of allergy-related symptoms
   d. history of exposure to source of fungal antigens
   e. a, b, and c
   f. a, b, and d
   g. a, b, c, and d

5. Which of the following is/are true:
   a. detection of IgG to specific fungi can be used as a marker of exposure to potential causes of illnesses such as hypersensitivity pneumonitis and allergic bronchopulmonary aspergillosis
   b. testing to determine the presence of IgE to specific fungi is often a useful component of a complete clinical evaluation in the diagnosis of health complaints that may be caused by immediate hypersensitivity
   c. the presence of antibodies against a mycotoxin-producing fungus is evidence that the patient has experienced a toxic reaction to that fungus
   d. antibody-based immunoassays can routinely be used by themselves as a primary means of determining the presence or absence of clinically relevant fungi-related health effect
   e. a, b, c, and d
   f. a and b
   g. a and c
   h. c and d