# FUNGAL EXPOSURE: TOXICITY & IMMUNOLOGY

There are four mechanisms by which fungal toxins cause pathology. One mechanism involves the direct toxic effects of fungi, molds, and yeast. The other three are exaggerated immune system reactions to fungal antigens.

1. The direct toxic effects of ochratoxins, aflatoxins, and tricothecenes.

    - neurotoxic
    - nephrotoxic
    - hepatotoxic
    - carcinogenic

2. Eosinophilic Fungal Rhinosinusitis

3. Mixed Mold Mycotoxicosis

4. Fungal Exposure Endocrinopathy

## FUNGAL TOXINS

Molds secrete over 400 different mycotoxins, all of which are toxic to all humans. Everyone in a mycotoxin-contaminated environment will have fatigue and other systemic symptoms, but may write them off to other causes. Fatigue from mycotoxin exposure indicates multisystem systemic toxicity. Even individuals with no exaggerated immune response to fungal/mold/yeast antigens, and therefore showing no major acute symptoms, are being slowly poisoned every minute spent in a mold-contaminated environment.

Mold spores have the highest concentrations of mycotoxins, although the vegetative portion of the mold, the mycelium, also contains significant amounts of poison. Viability of spores is not essential to toxicity, so the spore as a dead particle can still be a source of toxin. In fact, old, dead spores and mycelia dry out and become airborne much more easily than viable organisms, and thus can be an even greater source of mycotoxicity.

Most mycotoxins impair utilization of dietary protein, and the effectiveness of protein supplements in overcoming mycotoxicosis depends on the mycotoxin in question.

Chronic subtoxic doses of mycotoxins = tumor-evoking effect

Ergot alkaloids bind with alpha-adrenoreceptors and evoke an inhibition of beta-adrenoreceptors = vasoconstriction, contraction of the uterus, inhibition of prolactin secretion

The Fusarium mycotoxin zearalenone increases membrane permeability, and promotes uterine synthesis of RNA, DNA, and protein.

Zearalenon = estrogen effect, and, influences transcription in the cell nuclei (Note the positive feedback loop. Estrogen is well known to increase fungal/yeast infections, yet certain fungal toxins are estrogenic.)

Tricothecenes, even in a very low dose, decrease the formation of coagulation factors and decrease immunoglobulins, while inhibiting protein synthesis and DNA synthesis.

Tricothecenes = inhibit protein synthesis, DNA synthesis, and mitochondrial protein synthesis, and even in a very low dose decrease the formation of coagulation factors and decrease immunoglobulins

Tricothecenes disrupt normal protein metabolism by inactivating the ribosomal cycle. Protein supplements have little effect.

Tricothecene toxins can persist for years at room temperature (long after there are no longer viable spores or mycelia).

Tricothecene mycotoxins grow well at low temperatures and frequently contaminate grain and other foodstuffs. If a sufficient quantity of tricothecene mycotoxins are ingested, there will be alimentary toxic aleukia, the symptoms of which resemble a severe radiation injury. Of course, milder symptomatic responses (colitis, irritable bowel) are more common with quantitatively lower exposure. The tricothecene mycotoxins are extremely stable to heat and to ultraviolet light inactivation. They retain their bioactivity even when autoclaved; heating to 1500° Fahrenheit for 30 minutes as required for inactivation. Clorox does not effectively inactivate the toxins.

Tricothecenes = immuno-suppression, allowing opportunistic bacterial infections

Ochratoxins = interfere with gene transcription, are nephrotoxic, teratogenic, and carcinogenic.

Ochratoxin A is a mycotoxin produced by molds of the Aspergillus and Penicillium genera, and contaminates animal feeds and human foods.

Ochratoxin A toxicity can also occur via inhalation of mold spores. The metabolism of ochratoxin A and its toxicity are different from organ to organ. It is via oxidative pathways that ochratoxin leads to genotoxic compounds. Ochratoxin is activated to genotoxic metabolites by co-oxidation via the prostaglandin synthase route, which is dependent upon the enzyme prostaglandin H synthase.

Aspirin and indomethacin inhibit this enzyme, and thus have a favorable effect on ochratoxin toxicity. Aspirin decreases genotoxicity 90% in the kidney and 30% in the liver; indomethacin decreases genotoxicity 90% in the kidney and 80% in the liver. Pretreatment of mice by vitamin E induced a decrease in genotoxicity by 80% in the kidney and by 55% in the liver. Vitamin A and vitamin C decreased genotoxicity levels by 70% in the kidney. In the liver, genotoxicity was decreased by vitamin C by 90%, while vitamin A decreased the genotoxicity by only 25%.

The genotoxic effects of ochratoxin have been demonstrated in liver, kidney, brain, heart, testicles, spleen, and urinary bladder. Ochratoxin is a genotoxic carcinogen by induction of oxidative DNA lesions coupled with direct DNA adducts via quinone formation.

The most pronounced adverse effect of ochratoxin is hepatonephrotoxicity. In ochratoxin-treated rats, the levels of lipid peroxidation product in serum, in liver, and in kidney were significantly increased. The levels of glutathione and enzyme activities of super oxide dismutase, catalase, glutathione peroxidase, and glutathione reductase in both liver and kidney were significantly decreased. When ochratoxin-exposed rats were supplemented with melatonin, the changes in lipid peroxidation product in serum and in liver and in kidney were no different than in controls. Concomitantly, the levels of glutathione peroxidase, glutathione reductase, and glutathione transferase in both liver and kidney tissues were significantly increased in comparison with controls. In conclusion, oxidative stress is a major mechanism for the toxicity of ochratoxin, and melatonin has a protective effect against ochratoxin toxicity through inhibition of oxidative damage and stimulation of glutathione activities.

Ochratoxin also increases the level of malondialdehyde and hydroxyproline in liver and kidney, while glutathione peroxidase is decreased. Melatonin decreases the malondialdehyde and hydroxyproline in ochratoxin toxicity, and increases glutathione peroxidase in both liver and kidney. Coenzyme Q10 decreases the malondialdehyde and hydroxyproline levels and increases glutathione peroxidase in ochratoxin toxicity of the liver, but has no beneficial effect on the kidneys.

Ochratoxin damages both the myocardium and the lung. The myocardial tissue of rats treated with ochratoxin showed extensive cytoplasmic vacuole formation, necrosis of the myocytes, dissolution of the nucleus, clumped fibers, fibrillolysis, swollen myocardial fibers, small hemorrhagic areas and hyperemic vessels. The lungs of rats treated with ochratoxin showed alveolar congestion, alveolar cell hyperplasia, prominent alveolar septal vessels, variable intensity loss of alveolar architecture, intraparenchymal inflammatory infiltration, intraparenchymal hyperemic vessels, respiratory epithelial proliferation, perivascular and peribronchial inflammation, pneumonic infiltration, distorted appearance of lung parenchyma, and emphysematous areas.

Supplementation with melatonin ameliorated the lung parameters of alveolar cell hyperplasia, prominent alveolar septal vessels, variable intensity loss of alveolar architecture, intraparenchymal inflammatory infiltration, perivascular inflammatory infiltration, distorted appearance of lung parenchyma, and focal emphysematous areas. Melatonin supplementation also significantly reduced myocardial damage in most of the parameters studied. Despite the protective effects on heart and lung exposed to ochratoxin, melatonin did not lower the degree of damage in lung and heart to the level of control rats, except for the parameters of the interstitial edema and small hemorrhagic areas only in myocardial tissue.

In rats exposed to ochratoxin, the total antioxidant power and the total thiol molecules were significantly reduced. The lower antioxidant capacity was accompanied by testicular degeneration, seminiferous tubule atrophy, dissociation of germinative epithelium, vasodilation with vascular thrombosis, perivascular immune cell infiltration, hypertrophied Leydig cells, giant cell formation, and negative tubular differentiation index. Supplementation with either melatonin or Glycyrrhiza extract enhanced the serum total antioxidant power and thiol molecules, and exerted a protective effect on the ochratoxin-induced damages. In summary, ochratoxin contamination of animal feeds and human foods causes reproductive abnormalities. The damage by ochratoxin is mediated by interference in the oxidative stress system, and the oxidative damage is prevented by melatonin and Glycyrrhiza.

Ochratoxin A accumulates in the brain. [Land, et al. Tremorgenic Mycotoxins in Conidia of Aspergillus fumigatus. 1994.] Ochratoxin A has a particular effect on NMDA receptors in the hippocampus. Concentration of NMDA receptor subunits was significantly lower in animals treated with ochratoxin A, but was increased when melatonin was administered concomitantly. Since hippocampal NMDA receptors are involved in the memory and learning processes, their damage, and their protection by melatonin, are noteworthy in view of the cognitive dysfunction associated with mycotoxicosis.

Phenobarbital increases genotoxicity from ochratoxin A.

Aflatoxins = inhibit synthesis of RNA in the liver = liver necrosis.

Aflatoxicosis with aflatoxin $B_1$ is hepatotoxic and causes liver apoptosis, as indicated by elevated caspase-3 enzyme activity. The liver apoptosis resulting from aflatoxicosis is associated with degenerative and necrotic changes in the hepatocytes. Hepatocyte necrosis is accompanied by elevated levels of malondialdehyde and nitric oxide, while the levels of glutathione, zinc, and enzyme activities of glutathione peroxidase and glutathione reductase are significantly decreased. Supplementation with melatonin decreases the elevated levels of malondialdehyde and nitric oxide while increasing glutathione, zinc, glutathione peroxidase, glutathione reductase, and glutathione-S-transferase activities. Melatonin reduces the apoptotic rate and necrotic changes in the liver via its antioxidant effects.

Aflatoxins = decreased growth in animals, fatty infiltration of the liver, but in poultry, swine, and monkeys, supplements of dietary protein beyond normal requirements overcome these effects. However, in rats, high protein diets promote hepatoma characteristic of chronic aflatoxicosis.

Aflatoxin interferes with utilization of dietary protein by inhibiting synthesis of DNA, RNA, and protein. High protein diets promote the metabolism of aflatoxin by the hepatic microsomal drug-metabolizing enzyme system.

Aflatoxins destroy proteins of membranes, gap junctions, and the intercellular matrix, thus disturbing cell-to-cell communication, and leading to tumorigenesis.

EOSINOPHILIC FUNGAL RHINOSINUSITIS (EFR)

- Chronic Sinusitis, allergic fungal sinusitis (AFS), and systemic fungal symptoms = Genetic Defect of the variable beta chain helper T-cell receptor (TCR V beta) site. That T-cell defect requires the presence of a fungal antigen to be activated.

- (MAYO CLINIC) = AFS = nearly all cases of chronic sinusitis

    - Fungi are present in almost everyone's nose, but in those who suffer AFS, eosinophilic clusters are found simultaneously with fungal elements. = Eosinophilic Fungal Rhinosinusitis (EFR)
    - IgE-mediated hypersensitivity to fungal allergens is not evident in most patients; EFR is not a type 1 hypersensitivity. Eosinophils are present without IgE reaction. Therefore, this condition is not a mold allergy, and → AFS is more appropriately called EFR.
    - Cytokines are produced in lymphocytes of EFR patients but not in controls. → EFR is therefore a systemic disease
      Is intranasal anti-fungal treatment a successful option? Yes, such treatment is essential, but it will not control the immune pathology entirely.
    - Alternaria = airborne fungi present in most cases of EFR
    - EFR involves lower as well as upper respiratory tract (asthma)
    - Fungal-specific IgG3 (& not IgE) distinguishes patients from those with systemic IgE fungal allergy or with fungus within eosinophilic mucous
    - IgG3 + CD8 + T cells + Th2 IL-5

- Eosinophilia is frequently but not exclusively caused by IgE-mediated hypersensitivity and is dominated by the associated cytokine milieu of Th2 inflammation. Thus, allergic (IgE-mediated) rhinitis or allergy is a subcategory and not synonymous with Eosinophilic Fungal Rhinosinusitis.

  → Subcategories of mechanisms that promote eosinophilic infiltration: (Probable that some patients have overlapping mechanisms)

    - SUPERANTIGEN-induced eosinophilic chronic rhinosinusitis
    - allergic fungal sinusitis (AFS)
    - non-allergic fungal eosinophilic chronic rhinosinusitis
    - aspirin-exacerbated eosinophilic chronic rhinosinusitis

- (MAYO CLINIC) 16-20% of the population shows a SUPERANTIGEN systemic immune reaction to fungi. This means that 1 fungal spore from the air will cause more than 3,000 times the number of T cells (white

blood cells), which is fully 30% of the body's total T cells, in contrast to a normal person who responds with less than 0.01% of T cells.

  - eosinophilic inflammation
  - Th1 cytokine, IFN-g
  - Th2 cytokines IL-5 and IL-13
  - IgG specific antibodies to Alternaria and Cladosporium, but less than 30% have IgE specific antibodies to Alternaria and Cladosporium
  - With 3 cytokines, IFN-g, IL-5, and IL-13, responding with 3,000 times the ordinary response to fungal antigens, the overall response is 9,000 times the normal systemic inflammation and 9,000 times the normal IgG antifungal antibodies to airborne fungi. The overproduction of fungal antibodies can cause a decrease in normal antibody synthesis, which can cause immune suppression.
  - The elevated IgG correlates with the elevated IL-5.
  - Only 1/3 of patients with severe IgG delayed mold reactions have immediate IgE mold allergy. So, routine allergy skin tests will miss most immune reactions to fungi/yeast.

- Occasional fungi in the nasal mucous are harmless. But in 16-20% of people, fungi stimulate an eosinophilic inflammatory response. EOS leave the blood vessels, entering the nasal and sinus tissue, and ultimately the nasal airway mucous. In the nasal mucous the EOS attack and destroy the fungi by the release of a toxic substance called major basic protein (MBP) from the granules in the EOS. MBP not only destroys the fungi, but produces collateral damage to the nasal and sinus mucosa. The injury to the mucosa makes it <u>susceptible to secondary bacterial infection</u>. Antibiotics should <u>not</u> be used. Topical irrigation with AMPHOTERICIN B --- 20 mL of a 100ug/mL 2 times daily, or with VORICONIZAL.

Much controversy exists regarding the role of viruses, bacteria, and fungi in sinusitis. Until recently, it was not really known that the sinuses take part in the infectious process of the common cold (viral rhinitis). In the vast majority of otherwise healthy volunteers with the common cold, and without previous history of sinusitis, the sinuses are involved, too. A viral rhinitis alone, however, does not elicit a "clinical" acute sinusitis. Bacteria determine the clinical picture and outcome of acute sinusitis. S. pneumonia, H. influenza, and M. catarrhalis are the most frequently involved bacteria. However, though bacteria are very important in acute sinusitis, their role in chronic sinusitis is minimal, the <u>bacteria being merely opportunistic colonizers</u>.

In patients with Eosinophilic Fungal Rhinosinusitis, eosinophils form clusters in the mucus where they release MBP, which is diffusely deposited <u>on</u> the epithelium, a process not observed <u>in</u> the tissue. Estimated MBP levels far

exceed those needed to damage epithelium from the luminal side and predispose patients with EFR to secondary bacterial infections.

Allard, et al. TH2 allergic immune response to inhaled fungal antigens is modulated by TLR-4-independent bacterial products. EUR J Immunol 2009.

> Allergic airway disease is characterized by eosinophilic inflammation, mucus hypersecretion, and increased airway resistance. Fungal antigens are ubiquitous within the environment and are well-known triggers of allergic disease. Bacterial products are also frequently encountered within the environment and may alter the immune response to certain antigens. The consequence of simultaneous exposure to bacterial and fungal products on the lung adaptative immune response has not been explored.
>
> In this study we show that oropharyngeal aspiration of fungal lysates (Candida albicans, Aspergillus fumigatus) promotes airway eosinophilia, secretion of Th2 cytokines, and mucus cell metaplasia. In contrast, oropharyngeal exposure to bacterial lysates (Pseudomonas aeruginosa) promotes airway inflammation characterized by neutrophils, TH1 cytokine secretion, and no mucus production. Administration of bacterial lysates simultaneously with fungal lysates deviates the adaptive immune response to a Th1 type associated with neutrophilia and diminished mucus production. The immunomodulatory effect that bacterial lysates have on the response to fungi is TLR4 independent but MyD88 dependent. Thus, different types of microbial products within the airway can alter the host's adaptive immune response and potentially impact the development of allergic airway disease to environmental fungal antigens.

Fungal extracts of Alternaria and Cladosporium stimulate higher levels of IL-5 from monocytes in EFR patients than in healthy controls. A skewed TH2 response to fungal antigen exposure is confirmed by an elevated IL-5/IFN-g ratio in EFR subjects. Healthy controls express an inhibitory cytokine IL-10 response to fungal antigens, possibly serving as a protective response.

The pro-inflammatory cytokines IL-1 beta, IL-6, and especially the neutrophil-chemoattractant IL-8, play a dominant role in acute sinusitis of viral and allergic etiology. In contrast, IL-3 protein dominates the cytokine profile in chronic sinusitis, giving support to a variety of inflammatory cells. The most striking finding is the increased synthesis of IL-5 in bilateral nasal polyposis, whereas IL-5 is not found in healthy controls. As the cytokine IL-5 is known to enhance eosinophil activation and survival, IL-5 is a key protein in the pathogenesis of EFR.

However, immunologic response to fungus is not universally associated with EFR. IL-5 responses to Alternaria are not necessarily predictive of EFR presence. Systemic symptoms associated with mycotoxicosis can therefore occur even in those without sinusitis symptoms. (See MIXED MOLD MYCOTOXICOSIS below.)

- Resolution of sinusitis in 93% who decrease mold count to ≤ 4 colonies

The histopathologic findings of asthma, namely heterogeneous eosinophilic inflammation and features of airway remodeling, are also present in EFR. These findings, coupled with the common clinical coexistence of both diseases, suggest that the same pathologic disease process is manifest as EFR in the sinonasal tissue and as asthma in the lower airway.

The pathogenic role of Th2 cells has been clearly demonstrated in allergic diseases such as asthma. Such disorders are characterized by recruitment of mixed leukocyte inflammatory infiltrate, including a predominant eosinophil component. The development of this inflammatory response is dependent on accumulation of Th2 cells in the affected tissues. What is the mechanism that recruits Th2 cells to the airway? Th2 cells are on their own, poorly competent for antigen-induced recruitment. In contrast, Th1 cells are avidly recruited to the lungs in response to airway antigen challenge. Recruitment of Th1 cells results in an enhanced subsequent recruitment of Th2 cells. The increased Th1 cell-induced recruitment of Th2 cells is associated with upregulation of endothelial vascular cell adhesion molecule-1 expression in airway-associated endothelial cells, and can be largely blocked by systemic treatment with monoclonal anti-VCAM-1 antibody. Systemic blocking of TNF also blunts the airway inflammatory response.

The prominent roles of TNF and VCAM-1 in recruitment of Th2 cells suggest that an inflammatory microenvironment is essential for Th2 cell recruitment. In fact, recruitment of Th2 cells to the airway can be induced in an antigen-independent fashion by proinflammatory stimuli such as intranasal instillation of endotoxin. This antigen non-specificity of the Th2 cell recruitment suggests a model in which Th2 cell recruitment is in response to general inflammatory signals rather than to an antigen itself. This model explains the clinical observation that bacterial or viral respiratory tract infections are associated with disease exacerbations in allergic asthmatics. Th2 cells, like other leukocytes, are recruited efficiently to sites of tissue inflammation.

In a study of 297 consecutive general medical outpatients, 65 (22%) had unexplained chronic fatigue, 33 (11%) had unexplained bodily pain (fibromyalgia), and 26 (9%) had both chronic fatigue and bodily pain. In the 22% of the sample population with unexplained chronic fatigue, there was 22 times the incidence of rhinosinusitis symptoms relative to those who did not have chronic fatigue. There was a similar predominance of rhinosinusitis

symptoms in those with bodily pain and in those with both bodily pain and chronic fatigue. There was no increased prevalence of pollen allergy in those with chronic fatigue or unexplained bodily pain. Rhinosinusitis symptoms are at least as common as gastrointestinal complaints, sleep disturbance, and psychiatric problems (previously well-documented complaints associated with unexplained chronic fatigue and bodily pain).

Recent studies challenge the belief that most facial pain is caused by sinusitis and that chronic purulent sinusitis causes facial pain. Most facial pain is associated not with sinusitis, but with a neurological cause, which in most cases is a version of tension-type headache that affects the midface. Chronic infective sinusitis (which makes up only a small portion of patients with chronic rhinosinusitis) usually causes pain only when there is an acute exacerbation. It is psychological and neurochemical factors that cause facial pain in most cases, and recent studies correlate this pain with unexplained chronic fatigue, irritable bowel, multiple symptoms, and a maternal history of chronic pain.

MIXED MOLD MYCOTOXICOSIS

Glucans or fungal cell wall components (also known as beta-(13)-D-Glucans) are small pieces of the cell walls of molds and yeasts, which may cause inflammatory lung and airway reactions or inflammatory GI reactions. These glucans always affect the immune system when inhaled or ingested.

- Glucans are released into the systemic circulation of patients with fungal infections. Glucans specifically stimulate various aspects of innate immunity via interaction with membrane receptors on immune-competent cells

    - Glucan-specific receptors exist on cells outside the immune system. Glucan binding sites exist on pituitary F3 cell membranes.

      → In response, the anterior pituitary secretes prolactin (a hormone that plays an important role in the response to infection), and there is an ↑ expression of TLR4 recognition of fungal pathogens and CD14 genes.

- Fungal glucan: the cell wall of fungi/molds/Candida is composed of glucan. = stimulates fibroblast NF-Kappa B nuclear binding activity and IL-6 = 1st demonstration of pattern recognition receptors for glucan on fibroblasts, and the 1st recognition of glucan binding sites on cells other than leukocytes

- Fungal Glucans stimulate macrophages, neutrophils, and natural killer cells of the immune system, but also stimulate FIBROBLASTS' NF Kappa B, and IL-6.

- Chronic low-dose mycotoxins can cause immune suppression with low white blood cell count (toxic aleukia).

- Fungal antigens = activate IL-13 (Th2), IFN-g (Th1), and IL-5 eosinophils. All contribute to the production of fungal-specific IgG antibodies.

  IL-5 → activates B-cells for differentiation into IgG-secreting cells
  IL-13 → causes IG Isotype switching to IgE and IgG1
  IFN-g → ↑ fungal-specific IgG production

    - Lymphocytes of normal patients do not secrete IL-5, IL-13, and IFN-g in the presence of fungi

- Patients have a genetic defect in the T-cell receptor VBeta chain → respond to fungal antigens as a superantigen → activates up to 30% of the body's total T-cells (in contrast to normal < 0.01% T-cell response). → ∴, with a response 3000 x the normal T-cell response per fungal antigen, and 3 inflammatory cytokines (IL-5, IL-13, and IFN-g) per T-cell responding to fungal antigen = up to 3x 3000 = 9000 x the systemic inflammation of a normal person = systemic symptoms in these fungal-exposed patients.

- Mold Exposure = ↑ Natural Killer Cell activity (* = conflict below)
  ↑ NKC = headache, general debilitating pains, nose bleeding, fevers, cough, memory loss, depression, mood swings, sleep disturbances, anxiety, chronic fatigue, vertigo/dizziness, (occasionally) seizures

  - Sleep disturbances = mycotoxins disrupt circadian rhythm

Field exposures of animals to molds (in contrast to controlled laboratory exposures) show effects on the immune system as the lowest observed adverse effect. In other words, abnormal immune responses and/or immune suppression are the first manifestations of mycotoxicosis. Such immune effects are manifest in animals as increased susceptibility to infectious diseases. Virtually all mycotoxins have an immunosuppressive effect, although the exact target within the immune system may differ from one mycotoxin to another. Many mycotoxins are also cytotoxic, so they have route of entry effects that may be damaging to the gut, the skin, or the lung.

Toxins are not the same as spores. An opinion piece appearing in The Mold Source by Dr. Gary Ordog explains that, "We have documented many cases of mycotoxicosis when the spore counts are zero, but the mycotoxins are high. Our general rule, which we have seen clinically many times over, is that there are enough mycotoxins in 1 piece of contaminated paper to make someone sick for 3 days…Currently, we can measure and identify spores, but it is difficult to measure mycotoxins." The problem with mycotoxins in the environment in the absence of measurable spores becomes even worse when the mold dries. As the mold dries, the mycotoxin production increases up to 40,000 times. [Many mold remediation "specialists" will reassure property owners that, "This mold is dead, so we will remove it, but it is really not doing any harm."]

Gray, et al. Mixed Mold Mycotoxicosis: Immunological changes in humans following exposure in water-damaged buildings. Arch Environ Health, 2003.

Chronic exposure (approximately 40 months) to mixed molds in a water-damaged building:

Patients had a preponderance of neurological and immune-related conditions:

- 100% with both CNS and peripheral nervous system complaints
- Severe fatigue (75%)
- Shortness of breath and chest tightness (75%)
- Recurrent flu-like illness (61%)
- Sensory neuropathy (43%)
- Sensory motor polyneuropathy (30%)
- Brain stem auditory evoked response (BAER) abnormalities (55%)
- Optic nerve dysfunction (10%)
- Abnormal EEG (10%)
- Auto antibodies against neural antigens myelin basic protein (MBP), ganglioside GMI, and sulfatide
- Increased C3 and C4 complements and immune complexes IgG, IgM, and IgA, compatible with inflammatory conditions
- Increased T and B cell markers and increased helper/suppressor ratio, indicating a relative lymphocytosis and immune activation
- Elevated mitogenesis to PHA, ConA, PWM, and LPS
- Natural killer cell activity suppressed (42%) (* = conflict above)

Abnormally high levels of antinuclear antibodies, autoantibodies against smooth muscles, and CNS myelin antibodies were found, and odds ratios for each were significant at 95% confidence intervals, showing a dramatic increased risk for autoimmunity.

Common autoimmune diseases that can be associated with mycotoxicosis include:

- Thyroiditis (Hashimoto's Disease or Grave's Disease)
- Type I Diabetes
- Rheumatoid Arthritis
- Crohn's Disease and most Ulcerative Colitis
- Lupus
- Polymyositis or Dermatomyositis
- Sjogren's Syndrome
- Scleroderma
- Psoriasis
- Multiple Sclerosis
- Myasthenia Gravis
- Narcolepsy
- Guillain-Barré Syndrome

The percentage of exposed individuals with increased lymphocyte phenotypes were: B cells CD20+ 76%, CD5+CD25+ 69%, CD3+CD26+ 91%, CD8+HLR- DR+ 62% and CD8+CD38+ 57%, whereas other

phenotypes were decreased: CD8+CD1 1B+ 16% and CD3-16-CD56+ 39%. Mitogenesis to phytohemagglutinin was decreased in 26% of the exposed patients.

It is concluded that these patients with an ongoing exposure to molds had developed neurological dysfunction and pathology, while at the same time undergoing continuous antigenic stimulation resulting in the above immune findings.

[This Mixed Mold Mycotoxicosis may exist independently of, or in addition to, the other severe fungal-derived pathologies --- Eosinophilic Fungal Rhinosinusitis and Fungal Exposure Endocrinopathy.]

- Patients with symptoms following mold exposure: Elevated B-cells: CD20+ = 76%, CD5+CD25 = 69%, CD3+CD26 = 91%, CD8+HCR-DR+ = 62%, and CD8+CD38 = +56, and, decreased B-cells CD8+CD11 = 16%, and CD3-CD16 + CD56 = 38%.

↑ autoimmune antibodies for IgA, IgM, and IgG isotypes

Neurological antibodies = numbness, tingling, tremors, muscle weakness

- Mycotoxin (rats) = ↓ body weight, loose stools, ↓ growth, ↓ WBC, ↓ platelets, ↓ RBC in males, ↓ hemoglobin concentration in females, thymic atrophy, hypocellular bone marrow, hypertrophy of basophilic cells, ↑ castration cells in anterior pituitary, ↑ ovarian atretic follicles

Depending upon available oxygen, fungi may engage in either aerobic or anaerobic metabolism. They may produce alcohols or aldehydes and acidic molecules. Such compounds in low but significant aggregate concentration can irritate the mucus membranes of the eyes and respiratory system. In addition to mucus membrane irritation, fungal volatile compounds may impact the "common chemical sense," which senses pungency and responds to it. This sense is primarily associated with the trigeminal nerve (and to a lesser extent the vagus nerve). This mixed sensory and motor nerve response to pungency, not odor, initiates avoidance reactions including breath holding, discomfort, paresthesias, and odd sensations such as itching, burning, and skin crawling. Changes in sensation, swelling of mucus membranes, constriction of respiratory smooth muscle, or dilation of surface blood vessels may be part of fight or flight reactions in response to trigeminal nerve stimulation. Decreased attention, disorientation, diminished reflex time, dizziness, and other effects can also result from such exposure (Otto, et al. Neurobehavioral and Sensory effects of controlled exposure to a complex mixture of volatile organic compound. Neurotoxicology and Teratology 12:649-652. 1989.).

A mold-contaminated building may have a significant contribution derived from its fungal contaminants, which are added to those volatile organic compounds (VOCs) emitted by building materials such as paints, plastics, cleaners, and plywood. A study by Miller in 1988 measured total VOC concentration in typical indoor environments, which approached the levels at which Otto et al found trigeminal nerve effects.

Patients exposed to molds and mycotoxins and those exposed to chemicals and heavy metals have many similar symptoms of eye, nose, and throat irritation, poor memory and concentration, and other neurobehavioral dysfunctions. Both those exposed to molds and to chemicals show:

- Decreased balance
- Slower reaction time
- Longer blink reflex latency
- Color discrimination errors
- Decreased visual field performance
- Decreased grip strength
- Decreased cognition
- Decreased memory
- Dizziness
- Poor digit symbol substitution
- Decreased immediate verbal recall
- Decreased delayed verbal recall
- Decreased picture completion and information
- Slower peg placement and trail making
- Altered Mood State scores
- Decreased vital capacity
- Asthma

- Candida albicans = secretes aspartic proteinases, which are a key factor that ↑ virulence in causing mucosal and disseminated infections.

    - Glucan in the cell wall of Candida causes some people to become allergic to Candida growth in the gut, which causes inflammation and swelling in the gut, making larger spaces between gut cells, allowing larger food particles to become absorbed into the blood, which causes food sensitivities even to the point of Universal Reactor Syndrome. This immune sensitivity to Candida glucan is something other than, and in addition to, an actual Candida infection.
    - Candida yeast infections of the vagina and in the mouth (thrush) can also elicit this immune sensitivity reaction.
    - 3 very common places for Candida colonization are at the base of the esophagus, just proximal to the cardiac sphincter of the

stomach, at the base of the tongue, deep in the throat, and in the sinuses.

- Candida albicans glucan has a similar structure to gluten; so, about 30% of patients with an immune reaction to fungi, molds, Candida, also have a sensitivity to the gluten grains wheat, oats, barley, and rye.
- The Candida population will multiply, and the Candida-provoked immune response will recur with both GI and systemic inflammatory symptoms in response to:
  - antibiotics
  - drinking or cooking with chlorinated tap water (including coffee and tea in restaurants)
  - excessive consumption of sugar and refined carbohydrates
  - exposure to an environment with a high mold count
- According to Dr. Donald Dennis, a small amount of chlorine will cause yeast overgrowth and defeat all other treatment.

- <u>Vitamin B12 Deficiency</u> in chronic mold exposure

  - Mycotoxins interfere with the functions of vitamin B12 in one-carbon metabolism
  - B12: - source of coenzymes
    - intracellular recycling of methionine
    - methionine synthase reaction (conversion of homocysteine to methionine)
    - prevention of chromosome breakage
    - methylation
    - maintaining a one-carbon metabolic balance

A majority of patients with neurological disorders from chronic exposure to toxigenic molds and mycotoxins have vitamin B12 deficiency that is unrelated to dietary insufficiency. It is likely that mycotoxins interrupt the structure and function of vitamin B12 through reactive interference with the normal 1-carbon metabolism, leading to the observed clinical neurological manifestations such as nerve damage, demyelination, degeneration of the peripheral nervous system leading to paralysis, progressive peripheral neuropathy, and spinal degeneration.

FUNGAL EXPOSURE ENDOCRINOPATHY

Dennis – Robertson Syndrome – Fungal Exposure Endocrinopathy

- Patients with a history of indoor mold exposure, fatigue, and chronic sinusitis

    - 97% had positive serum IgG-specific antibodies to fungal antigens
    - Urinary trichothecenes, ochratoxin A, or aflatoxins
    - 51% GH deficient (but IGF-1 most often WNL)
    - 81% primary or secondary hypothyroid
    - 75% ACTH insufficiency

- Possible Mechanism: Fungal glucan receptors in the lenticulostellate cells of the anterior pituitary bind to fungal cell wall glucans, thus activating the immune system

    - The immune system activates macrophages that destroy the fungus but also the lenticulostellate tissue of the anterior pituitary.

- Fungal antigen stimulation of T-cell IL-5, IL-13, IFN-α, enhancing fungal-specific IgG is NOT a part of the pathology that causes GH deficiency in fungal-exposed patients. In other words, the anterior pituitary destruction is an immune reaction independent of the systemic eosinophilic reaction to fungal antigen.

Fungal Exposure Endocrinopathy = pituitary reaction to fungal cell wall glucans.

- GERD 73%, with IgG Candida allergy and visible Candida on endoscope of base of tongue

- Significant food sensitivities in 73% (30% gluten intolerant)

- The food sensitivities may be due to gut inflammation caused by Candida – since 8 weeks after gut yeast and environmental fungal load is reduced, most of the mild food sensitivities resolve.

- Folliculostellate cells (FS) = multifunctional, endocrine-inactive cell type of the anterior pituitary

    - Curcumin inhibits the proliferation of FS cells and stimulates apoptosis. Immune-like functions of FS cells were impaired since curcumin down-regulates Toll-like receptor 4, ↓ nuclear factor-Kappa B, and ↓ bacterial endotoxin-induced IL-6.

Thyroid hormone feedback on the pituitary involves a central role for FS cells in thyroid hormone activation. FS cells thus play an important role in the suppression of TSH secretion by circulating thyroxin. Pituitary Folliculostellate cells directly recognize and respond to fungal cell wall glucans, resulting in stimulation of pituitary cell TLR4 and CD14 gene expression. In addition, glucan stimulates secretion of prolactin, a hormone that plays an important role in the response to infection.

- IL-6 = mainly produced by monocytes and macrophages = influences secretion of anterior pituitary hormones.

- IL-6 is also produced within the pituitary by FS cells.

TREATMENT OF EFR, MMM, and/or FEE

1. A GOOD THYME via Grossan Hydro Pulse nasal irrigation 2-4x daily to remove fungal antigens and toxins
2. Anti-fungal nasal spray or nebulizer – Amphotericin-B, or Voriconazole (short term for severe invasive infection only)
3. Oral anti-fungals (short-term for severe invasive infection only):
   Candida = Diflucan 100 mg/day for 14-30 days;
   Nystatin 1, 2X daily
   Other Fungi = Itraconazole 200 mg 2 x daily for 30-60 days
   If CNS Fungi = Voriconazole 200 mg, 2x daily for 30-60 days
   Maintenance with some combination of 4, 5, and/or 6 below.
4. A GOOD THYME oral supplementation
5. BOOGEY BUSTER, 4 times daily
6. IMMUNO-SYNBIOTIC, 3, twice daily, before meals
7. ↓ Carbs; avoid food allergens
8. GO POWER and/or OXY POWER
9. Saturated FAs are anti-fungal: capric, lauric, myristic, palmitic, and myristoleic. Alpha Linolenic Acid if ANAEROBIC or PARASYMPATHETIC.
10. Daily sunlight on skin, but especially needed is unfiltered eye exposure (outdoors with no glasses or contacts)
11. Remediation of indoor mold (= No therapy will yield lasting benefits until mold is removed from the patient's home and/or workplace.)