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Review

Fungal toxins and multiple sclerosis: A compelling connection

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ABSTRACT

Multiple sclerosis occurs as a consequence of central nervous system neuronal demyelination. Decades of research suggest that the primary suspects (e.g., viruses, genes, immune system) are associative rather than causative agents, but a surprisingly coherent relationship can be made between multiple sclerosis and fungal toxins. Specifically, certain pathogenic fungi sequester in non-neuronal tissue and release toxins that target and destroy CNS astrocytes and oligodendrocytes. Without these glial support cells, myelin degrades triggering the onset of multiple sclerosis and its associated symptoms. We propose here that fungal toxins are the underlying cause of multiple sclerosis and thus may offer an avenue towards an effective cure.

Multiple sclerosis (MS) occurs as a consequence of CNS (central nervous system) neuronal demyelination, which can lead to optic neuritis, tremors, muscle weakness, paralysis and sometimes death. More than 25,000 people are diagnosed with MS each year in the United States and worldwide cases number over two million. Since the first detailed description of MS in the late-1800s [8], debate has continued over its cause with no clear answer emerging. Most would agree that MS is a complex disease, determined by a combination of genetic, immunologic, environmental and/or other unknown factors. A critical evaluation of research over the past few decades, however, suggests that the underlying cause of this debilitating disease may be less complex than is currently thought.

The most accepted component of MS etiology is that activated T-cells cross the blood–brain barrier and initiate an inflammatory response to myelin [12]. Scars or plaques then form in damaged areas resulting in faulty nerve conduction. Initial demyelination of CNS axons triggers catastrophic events resulting in further immune activation and inflammatory injury to axons [9]. Partial remyelination of axons can occur but if remyelination fails, sodium channels at nodes of Ranvier redistribute along the axon in an effort to maintain nerve conduction [9,25]. Relocated channels generate a persistent current within demyelinated axons leading to ion imbalances that cause calpain-mediated axon digestion, nitric oxide and free radical production, mitochondrial dysfunction and ultimately neurodegeneration [27].

Most also agree that genetic factors explain part of an individual's susceptibility to MS. The risk of developing MS is several times higher for people with a blood relative having the disease; however, a monozygotic twin has only a ~30% chance of developing MS if the other twin has the disease [24]. Specific genes may also influence MS susceptibility. A variant of gene KIF1B, which mediates nerve cell function; and GPC5, associated with nerve fiber regeneration, have been linked with MS [2,3]. Additionally, individuals who lack HLA-DRB5, or down-regulate DRB1 due to vitamin D deficiencies, also display higher risks [6,22]. Significantly, none of these genetic anomalies occur in all MS patients, and genes associated with MS are frequently found in the general population. Possibly, some genes increase susceptibility to MS or work in concert with environmental factor(s) required for disease initiation.

Viral infections have also been associated with MS. Almost all (>99%) MS patients are seropositive for Epstein-Barr virus (though 90% of the general population is also positive), and those never infected with the virus have a reduced risk of developing MS [16,20]. Human Herpes virus (HHV-6) has been detected near brain lesions in a subset of patients with relapsing-remitting MS (RRMS) but the virus does not play an active role in secondary-progressive MS (SPMS); thus no obvious mechanistic link can be made between viral infection and MS disease progression [1].

Collectively, the role(s) of the immune system, genetic factors and viral infection in MS etiology appear to be associative or consequential, rather than causative. Indeed, a piece of the puzzle seems to be missing—or is it? Over the past few decades, a number of

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startling correlations have been made between MS and fungal toxins, leading to a surprisingly coherent story of MS development. And while no definitive link has been made, many researchers remain open to an infectious micro-organism as the causatve agent of MS [18]. In our proposed scenario, certain pathogenic fungi (e.g., species of *Aspergillus* and *Candida*) – masked from the immune system by their mannan coats – sequester in non-neuronal tissue, steadily releasing toxins (e.g., gliotoxin) into the bloodstream. Once across the blood–brain barrier, these toxins target CNS astrocytes, which are integral for maintaining this barrier; and oligodendrocytes, which provide nutritive support for myelin. Without proper glial support, the blood–brain barrier weakens and myelin degrades, generating myelin debris that triggers a full scale immune response in the CNS. Thereafter, the characteristic progression of MS ensues: further demyelination, conduction failure, redistribution of sodium channels, ion imbalances, anoxia, mitochondrial depletion and axon degeneration.

Experimental evidence for this putative fungal-based etiology is limited but strongly suggestive. Fungi are recognized by the human immune system due to polymeric beta-glucan in their cell walls [29]. Some fungal pathogens, including clinically important dimorphic fungi, are able to mask themselves with a mannoprotein coat that evades the host's immune system [29], thus enabling them to colonize certain areas of the body (e.g., gastrointestinal tract) while their cytotoxic metabolites create the neurological havoc associated with MS.

Different mycotoxins may in fact be responsible for different forms of MS. Fumonisin B(1), isolated from species of *Fusarium*, which contaminates both animal feed and human food, disrupts the biosynthesis of sphingolipids [26], which are characteristically lost from the white matter of MS patients [30]. Fumonisin B(1) is cytotoxic to murine microglia and primary astrocytes as well [26]. Penitrem A from *Penicillium crustosum* causes neurological problems in laboratory animals including sustained tremors, nystagmus, ataxia, pseudoparalysis, mitochondrial swelling and severe neurologic dysfunction [7].

Gliotoxin, a heat-stable, secondary metabolite produced by various species of *Aspergillus* and *Candida* [15], belongs to the immunosuppressive epipolythiodioxopiperzine class (ETP) of toxins that have been associated with various mycotoxicoses. Intracellular gliotoxin levels can reach >1000 times that of extracellular concentrations due to a unique glutathione-dependent redox mechanism, enabling gliotoxin to act in a pseudocatalytic manner [5]. Gliotoxin destroys CNS astrocytes following intraperitoneal injections in rats [32], and heat-treated cerebrospinal fluid (CSF) from MS patients (containing functional gliotoxin at ng/ml concentration) causes apopotic death of astrocytes and oligodendrocytes, but not fibroblasts, endothelial cells or Schwann cells [19,21]. Importantly, CSF from patients suffering from other inflammatory or non-inflammatory neurological diseases displays no toxicity to these same cell types [19]. Further, intraventricular injection of CSF gliotoxic factor increases blood–brain barrier permeability in rats, which becomes leaky to immmunoglobulins [23]; partially purified CSF gliotoxic fragment injected directly into rat CSF causes astrocyte and oligodendrocyte death *in vivo* after 10 days, and extensive oligodendrocyte death and demyelination after three months [4]; and serum samples from MS patients in exacerbation contain a factor that interferes with myelin synthesis [10]. Finally, a protein with identical biochemical characteristics to gliotoxin was identified in the urine of MS patients, and 32 of 35 patients had urine that caused glial cell apoptosis (the three negative samples being from patients with little discernable MS symptomotology) [17]. Note that the origins of the gliotoxic fragments in the aforementioned studies [4,10,17,19,21,23] have not been determined. As proposed by Rieger et al., an alternate method of inducing MS in an animal model may be intraventricular injections of gliotoxin into rat [23], in contrast with the experimental autoimmune encephalomyelitis (EAE) animal model currently in use (see Ref. [14]).

The diagnostic immune response in MS patients is predominantly an elevation of immunoglobulin G (IgG) kappa bands in CSF [13]. Interestingly, patients suffering from systemic *Candida albicans* infections also display high IgG antibody levels, but do not mount an immune response to the fungi's mannan coat [14]. (Note that caspofungin, an anti-fungal drug capable of unmasking *C. albicans* by disrupting cell wall formation at subinhibitory concentrations in mice [31], offers clear potential for exposing sequestered fungal infections to the host immune system and could lead the way towards a therapeutic treatment of MS.)

The correlation between MS and pathogenic fungi extends beyond experimental investigation. Geographically, temperate areas where MS is more prevalent (i.e. above the 39th parallel in the northern hemisphere) correlate with high corn and wheat production, both of which are particularly susceptible to fungal contamination by *Fusarium* and *Aspergillus* [28,33]. Based on a standard grain-based diet, daily consumption of the deadly aflatoxin from *Aspergillus* sp., which is found in wheat, corn and all corn-based products, can reach 0.15–0.5 mg; the acute lethal dose in human is ~10 mg [11]. Thus, continuous exposure to grain-based fungi and their airborne spores in northern regions may constitute a key environmental component of MS, and contribute to the perplexing distribution of MS cases worldwide.

Could fungal metabolites be the missing pathogenic factor in MS? Are toxic metabolites targeting astrocytes and oligodendrocytes while also impairing myelin synthesis and blood–brain barrier integrity? As the scientific community struggles to understand the complex nature of this disease, and clinicians attempt to ameliorate symptoms with drugs whose side effects can be worse than the disease itself, perhaps it is time to determine the definitive role that fungal toxins play in MS etiology.

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