Acinetobacter Immune Responses in Multiple Sclerosis
Etiopathogenetic Role and Its Possible Use as a Diagnostic Marker

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Multiple sclerosis (MS) is the most common cause of neurologic disability among young people. The etiology of MS is controversial, but immune responses are considered to somehow be involved. The diagnosis of MS depends on a combination of various clinical and laboratory features, but apart from some myelin-neuronal autoantibody profiles or oligoclonal bands in the cerebrospinal fluid no other serologic diagnostic test or marker has yet been discovered. However, the presence of antibodies to Acinetobacter species in MS patients opens the possibility of developing a composite laboratory diagnostic marker, the myelin-Acinetobacter-neurofilament index. Whether Acinetobacter is the triggering agent of MS remains to be determined, but the measurement of anti-Acinetobacter antibodies could be used as a marker of disease activity. To evaluate this, prospective randomized controlled studies should be performed with MS patients, especially in the early stages of the disease.

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Multiple sclerosis (MS) is a common demyelinating disease of the central nervous system, usually affecting young people and characterized pathologically by scattered areas of inflammation with or without axonal damage, involving particularly the white matter. Multiple sclerosis has a worldwide distribution, and as many as 1 million people are affected by the disease. The prevalence of MS shows a latitudinal effect in that it is higher in Scandinavian countries than in the tropics.1 The reverse is observed in the Southern hemisphere, where MS is 7 times more common in Tasmania and Southern New Zealand than in tropical Queensland2 in populations coming predominantly from Anglo-Celtic stock. This latitudinal effect could be linked to greater prevalence of respiratory infections during autumn and winter.

ETIOPATHOGENESIS
Evidence of Genetic Factors

In a recent review,3 none of the 3 large screening studies performed in outbred populations of Northern European origin was successful in identifying genetic markers that show statistically significant linkage with the disease. It has been suggested that many different chromosomal regions are likely to be involved in MS.4

Evidence of Environmental Factors

Various studies have supported the role of environmental or microbial factors in the pathogenesis of MS: (1) a relatively low concordance rate for monozygotic twins (approximately 25%), dizygotic twins (approximately 5%), and siblings (approximately 3%) of patients with MS; (2) reports of an outbreak of MS in the Faroe Islands, which occurred after the start of World War II; (3) increase in the probability of developing MS with migration rate from low- to high-risk areas, especially among those in younger age groups; and (4) the occurrence of remissions and exacerbations in MS as a common hallmark of the disease and a possible result of fluctuations in the exposure to certain triggering environmental factors.
Microorganisms Possibly Involved in the Etiopathogenesis of MS

Various microbial agents have been implicated in the etiopathogenesis of MS. The role of viruses has been extensively studied, but no clear consensus has emerged from these investigations. Viruses such as Epstein-Barr virus and human herpesvirus 6, which have been implicated in MS, are ubiquitous in the environment, but others have failed to confirm a link between human herpesvirus 6 and MS. Some researchers have found a link between the gram-negative *Chlamydia pneumoniae* bacteria and MS, but others have failed to confirm these associations. The role of other bacteria, such as *Acinetobacter* species and *Pseudomonas aeruginosa*, has only been studied recently but with some encouraging results.

*Acinetobacter* and MS

Myelin sequences known to produce experimental allergic encephalomyelitis in guinea pigs, an animal model of MS, were examined using the GenBank (NCBI, Bethesda, Md) and SwissProt (TrEMBL, Geneva, Switzerland) databases for molecular mimicry between brain tissues and microbes. The ubiquitous saprophytic microbe, *Acinetobacter*, was found to possess such a sequence.

Antibody levels against 5 strains of *Acinetobacter* sp, *P aeruginosa*, and *Escherichia coli* were investigated in 26 English patients with MS and compared with 20 patients with cerebrovascular accidents and 25 healthy controls. There were significant elevations in the levels of antibodies of IgM, IgG, and IgA classes against all bacterial agents except *E coli* in patients with MS when compared with those with cerebrovascular accidents and healthy controls. The elevations in these antibodies were found to be more prominent against *Acinetobacter calcoaceticus*, *Acinetobacter 11171*, and *Acinetobacter Iwoffii* strains, and in some cases their levels were reaching titers of up to 1:6400.

In a more recent study performed on serum samples taken from the same group of patients and controls included in the previous study, antibody levels against mimicking peptide from *Acinetobacter* (*P* < .001), *Pseudomonas* (*P* < .001), myelin basic protein (MBP) (*P* < .001), and myelin oligodendrocyte glycoprotein (MOG) (*P* < .001) were found to be elevated in patients with MS when compared with those with cerebrovascular accidents or healthy controls. Antiserum raised in mice against *Acinetobacter* mimicking peptides were found to be significantly inhibited by peptides from the MBP or *Pseudomonas* microbe. Furthermore, MOG peptides were found to inhibit antibodies against the mimicking sequences present in *Acinetobacter*, but no inhibition was observed when human papillomavirus peptides were used as controls.

In another study, serum samples from Austrian patients with MS or other neurologic diseases and healthy controls were screened against 3 strains of *Acinetobacter* spp. There were significant elevations of total antibodies against *A calcoaceticus*, *A Iwoffii*, and A 11171 bacterial strains in patients with MS and those with some other neurologic diseases, such as sporadic Creutzfeldt-Jakob disease, but not in patients with Alzheimer disease and dementia, when compared with controls.

**UPPER RESPIRATORY TRACT AND PARANASAL SINUSES AS THE SOURCE OF INFECTIONS IN MS**

The most likely source for the entry of any triggering microbial factor in MS is through the upper respiratory tract (URT). Several lines of evidence support this possibility. First, sinusitis is present in many MS patients, and the rate of MS exacerbations during the sinusitis attacks was found to be doubled. Furthermore, using magnetic resonance imaging of the nasal sinuses, 33% of MS patients had evidence of sinusitis. Second, in one study, the main causative agents involved in sinusitis were found to be *Acinetobacter*, *Pseudomonas*, and *Staphylococcus aureus*. In a subsequent study involving antral tap and endoscopically directed tissue culture performed on acute sinusitis patients, 35% and 33% of the isolates from the antral tap and the endoscopically directed tissue culture, respectively, were *Acinetobacter* bacteria. Third, in another retrospective study, more than 50% of MS patients had a history of repeated respiratory tract infections during their childhood. Fourth, clinically manifest infections predominantly of the URT were observed to be followed by more attacks of exacerbations in patients with MS.

These results suggest that MS could be triggered and perpetuated following repeated attacks of subclinical or overt infections in the URT or paranasal sinuses. Furthermore, viral infections of the URT and paranasal sinuses, which occur more frequently in autumn and winter, could provide a suitable biological milieu for the secondary growth of a saprophytic microbe such as *Acinetobacter*.

**MOLECULAR MIMICRY AND MS**

Molecular mimicry or cross-reactivity has been proposed as the main pathogenetic mechanism for development of autoimmune diseases, such as rheumatic fever, Sydenham chorea, rheumatoid arthritis, and ankylosing spondylitis. The molecular mimicry hypothesis is based on the demonstration of autoimmunity, involving molecular and/or immunologic cross-reactivity between the putatively causative microbes and autoantigens, increased antibodies against these microbial and autoantigenic molecules, and the binding of these microbial cross-reactive antibodies to the targeted tissues with resultant immune-mediated cytotoxic tissue damage.

**Evidence of Autoimmunity in MS**

Autoantibodies to many myelin-neuronal antigens have been found in patients with MS. For example, the levels of antibodies to MBP, MOG, and neurofilament were found to be elevated in patients with MS when compared with control groups. Some of these antibodies were even found to be predictive of the development of clinically definite MS after a first demyelinating event. Furthermore, levels of anti-MBP or anti-MOG antibodies were reported to be elevated in the cerebrospinal fluid and/or isolated from the plaque lesions in the central nervous system of MS patients. In studies that involved anti-
Evidence of Molecular Similarities Between Acinetobacter and Pseudomonas Bacteria and Brain Antigens

Acinetobacter and Pseudomonas enzymes show molecular similarities to certain brain antigens (Figure 1). The enzyme 4-carboxy-muconolactone decarboxylase possesses an amino acid sequence that is similar to MBP,13 and protocatechuate 3,4-dioxygenase has a sequence homologous to neurofilament.32 The 3-oxoadipate coenzyme A transferase enzyme of the same bacteria possesses amino acid sequences that are similar to those present in the MOG brain tissue antigens.15 In this latter study, it was also shown that the enzyme γ-carboxy-muconolactone decarboxylase of the Pseudomonas microorganisms possesses amino acid residues that are homologous to those present in the MBP molecules.

Possible Pathogenetic Pathway in MS Involving Acinetobacter Species

It is proposed that antibodies against Acinetobacter and possibly Pseudomonas microorganisms are produced as the result of URT or paranasal sinus infections by these microbes (Figure 2). In view of the existing molecular similarities between microbes and brain antigens, these cross-reactive antibodies, which are mostly of the IgG isotype, could cross the blood-brain barrier. The binding of these antibodies to the myelin-neuronal antigens, such as MBP, MOG, and neurofilaments, when present in high titers would activate complement and other inflammatory cascades, thereby producing demyelination through the process of antibody-dependent, cell-mediated cytotoxicity in the same way that has been shown in patients with Sydenham (rheumatic) chorea.33 These events could eventually result in multiple sites of demyelinations with or without axonal degenerations.

PROPOSAL FOR A NEW LABORATORY DIAGNOSTIC MARKER IN MS

Apart from the criteria for the diagnosis of MS by Poser et al.,34 other diagnostic criteria, which have been recommended by neurologists from the United States and Europe, are all based on a combination of clinical and paraclinical features.35 The main clinical evidence is based on the objective findings of dissemination in time (relapses) and space (different locality) of the clinical presentations typical of MS, whereas the nonclinical criteria are mainly based on the following: (1) magnetic resonance imaging evidence of multiple neurologic lesions of different sizes and locations; (2) identification of IgG oligoclonal bands in the cerebrospinal fluid; and (3) delayed but well-preserved visual evoked potentials.

These paraclinical criteria are helpful in aiding the diagnosis of clinically probable cases of MS, but they require expensive facilities. Furthermore, these diagnostic criteria cannot identify all cases of MS because of the clinical variability of the disease. Hence, the search for the development of a less invasive and reproducible test is of crucial importance.

We propose that a combination of elevated titers of antibodies against Acinetobacter, MBP, and neurofilament could be used for such a diagnostic test. The myelin-Acinetobacter-neurofilament (MAN) index, which has been described previously,14 was calculated using the optical density (OD) readings from the IgG antibody determined by enzyme-linked immunosorbent assay. The for-
mula used was as follows: MAN Index = (MBP OD × 10) (Acinetobacter OD × 10) (Neurofilament OD × 10).

The MAN index is calibrated by determining scores in MS patients and healthy controls. For an active MS patient, the MAN index is expected to be above the 95% confidence intervals of the healthy controls. Further research is needed to elaborate and modify this particular diagnostic marker. For example, instead of using whole bacteria, Acinetobacter peptides, which carry the cross-reactive brain antigens, may give a clearer separation when compared with controls.15

**MONITORING TREATMENT IN MS**

The current therapeutic strategy in the management of MS involves the use of immunomodulatory and immunosuppressive drugs. These modalities have been found to be effective mainly in cases of relapsing-remitting forms but less so in the progressive forms of MS.36 The evidence of the bacterial (Acinetobacter and Pseudomonas) involvement in the etiopathogenesis of MS suggests the possibility of using antimicrobial therapy, and its use should be evaluated in prospective, longitudinal randomized controlled studies.

In conclusion, the demonstration of the cross-reactivity between Acinetobacter and Pseudomonas bacteria and brain antigens has raised the possibility of developing a disease marker, the MAN index, which could predict the occurrence of relapses in patients with MS and/or monitor response to therapy. However, further studies need to be performed on greater numbers of MS patients to establish the sensitivity and specificity profiles and the degree of the reproducibility of the MAN index.

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