AUTOIMMUNE NEUROLOGICAL AND RHEUMATIC DISEASES: ROLE OF CHRONIC INFECTIONS IN MORBIDITY AND PROGRESSION

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Autoimmune neurological and rheumatic diseases unfortunately do not have known causes, and in most cases, effective treatments. Although there has been tremendous progress in the last few years on these diseases, they still remain among the most difficult diseases to manage. The Institute for Molecular Medicine has been working with its affiliated institutions to develop new diagnostic and therapeutic approaches to treating autoimmune diseases.

MICROORGANISMS CAUSE MORBIDITY IN MANY CHRONIC ILLNESS PATIENTS

Although the causes of autoimmune neurological diseases are for the most part unknown, the complex signs and symptoms that evolve in most patients may be due, in part, to systemic chronic infections (bacteria, viruses, fungi) in patients with an appropriate genetic background. Such infections can follow acute or chronic chemical or other insults (heavy metal, viral, environmental, trauma, etc.) that have the potential to suppress the immune system [1]. Thus these illnesses probably evolve over time as a multistep process that may require multiple toxic exposures, including infections that can be causative for the illness in some patients, cofactors for the illness (not causative but important to morbidity) in others or opportunistic in immune-compromised patients. Chronic infections that are usually held in check by our immune systems can take hold if they can avoid immune surveillance and penetrate and hide in various tissues and organs, including cells of the Central Nervous System (CNS) and Peripheral Nervous System (PNS). When such infections occur, they can cause complex signs and symptoms, including immune dysfunction. Changes in environmental responses as well as increased titers to various endogenous viruses that are commonly found to be expressed in these patients as well as bacterial *(Mycoplasma, Chlamydia, Borrelia, Brucella,* etc.) infections have now been commonly seen in autoimmune neurological similar to other autoimmune disease patients, such as Rheumatoid Arthritis [2, 3].

It is proposed that autoimmune signs and symptoms are caused when intracellular pathogens, such as mycoplasmas and other bacteria, escape from cellular compartments. Microorganisms like mycoplasmas can incorporate into their own structures pieces of host cell membranes that contain important host membrane antigens that can trigger autoimmune responses, and they can also mimic host cell antigen structures [2, 3]. Thus patients with such infections may respond immunologically to microorganism antigens as well as their own membrane antigens, producing unusual autoimmune signs and symptoms.

MULTIPLE SCLEROSIS (MS)

Multiple Sclerosis (MS) is a disease associated with demyelation with relative preservation of nerve cells. Nerve cell loss does occur (~20%) in typical lesions in the spinal cord. MS is an inflammatory disease in which there is damage to the blood brain barrier, focal edema and swelling and release of plasma proteins into the CNS and inflammatory responses. The disease runs an intermittent or cyclic course, and the initial diagnosis is usually made after several attacks of disability associated with damage to the CNS. Although there is a poor correlation between a MS patient's disability and the

pathology found in the brain after death, small lesions imaged in the brainstem and spinal cord are usually associated with clinical signs and symptoms [4].

In the case of MS, there is evidence for an association of the disease with chronic bacterial and viral infections. Challoner et al. [5] found MS plaque-associated expression of the herpes virus HHV-6B in >70% of MS cases. More recently Friedman et al. [6] used PCR and serology to follow HHV-6 infections in MS (plaques) and non-MS brain sections and found that ~40% of MS patients expressed HHV-6, whereas HHV-6 protein was expressed in only 13% of non-MS brain sections and 0% of normal controls. Also, antibodies to HHV-6 were found in 80% of MS patients but only 16% of non-MS patients. Evidence for a systemic infection have been noted by Kim et al. [7] and Akhyani et al. [8] who found HHV-6 in the peripheral leukocytes of MS patients but not in control subjects. Extending these observations Knox et al. [9] found that 73% of patients with MS had HHV-6 infections in tissues sections showing active demyelination, whereas only 13% of sections free of active disease were positive. Blood samples from these patients showed that 54% were positive for HHV-6 in peripheral leukocytes compared to 0/61 controls.

In addition to HHV-6, bacterial infections have been found in MS patients. There are strong analogies between neuroborreliosis (*Borrelia bergdorferi*) found in Lyme Disease patients and MS. Chmielewska-Badora et al. [10] found that 38.5% of MS patients showed evidence of *Borrelia* antigens in their blood, whereas other neurological patients carried these antigens in blood at a lower prevalence (19%). *Chlamydia pneumoniae* has also been found in a subgroup of MS patients [11]. By examining cerebral spinal fluid (CSF) these authors found that 10% of MS patients and 18% of patients with probable MS had *Chlamydia* infections but none of 56 control patients with other neurological disease were positive [11]. Other authors, however, could not confirm this finding [12]. We have found a high incidence of mycoplasmal infections in MS patients, mostly as coinfections with other bacteria and viruses. Greenlee and Rose [4] have discussed the possible association of various bacterial infections caused by *Rickettsia, Mycoplasma, Borrelia, Chlamydia* and possibly other bacteria and viral infections caused by HHV-6 in various neurological diseases, including MS.

Recently we established a new treatment protocol for MS based on the detection of chronic bacterial and viral infections in MS patients. Hyperbaric oxygen therapy (HBOT) has been shown to be of benefit in many disorders, and it has been documented to be beneficial in MS [13]. Thus there is an existing rationale for using HBOT to treat MS patients, especially those MS patients with evidence of chronic infections. The chronic infections that have been found in some MS patients are borderline anaerobic infections and should respond to varying degrees to HBOT. The current treatment for chronic infections, such as those caused by bacterial infections, is long-term antibiotics plus nutritional support. The antimicrobial effects of antibiotics are often thought to be potentiated by the use of HBOT. In one part of the study we will evaluate the effects of using HBOT to treat MS with evidence of a chronic infection using signs and symptoms, sequential imaging, and determination of infections and immune parameters to monitor effect of therapy. It is anticipated that 50 subjects will be enrolled in this study.

AMYOTROPHIC LATERIAL SCLEROSIS (ALS)

Amyotrophic Lateral Sclerosis (ALS) is an adult-onset progressive degenerative disease affecting both central and peripheral motor neurons. Patients with ALS show gradual progressive weakness and paralysis of muscles due to destruction of upper motor neurons in the motor cortex and lower motor neurons in the brain stem and spinal cord, ultimately resulting in death, usually by respiratory failure

[14,15]. The overall clinical picture of ALS can vary, depending on the location and progression of pathological changes found in nervous tissue [16].

Although the cause of ALS remains unknown, there are several hypotheses on its pathogenesis: (a) accumulation of glutamate causing excitotoxicity; (b) autoimmune reactions against motor neurons; (c) deficiency of nerve growth factor; (d) dysfunction of superoxide dismutase due to mutations; and (e) chronic infection(s) [15,16]. Of these hypotheses, the role of chronic infections has attracted our attention with the finding of enterovirus sequences in 15 of 17 spinal cord samples from ALS patients by Polymerase Chain Reaction (PCR) [17]. Although others failed to detect enterovirus sequences in spinal cord samples from patients with or without ALS [18], more recent findings suggest that infectious agent(s), such as enterovirus, may play a role in the etiology of ALS. With the discovery of enterovirus RNA sequences in a high proportion (~88%) of formaldehyde-fixed spinal cord samples and at lower frequency in in other patients with neurological diagnoses or in control subjects, it is now likely that an enterovirus may be important in ALS [19]. Similarly, we have found a high proportion (~85%) of ALS patients have evidence of blood mycoplasmal infections, and this suggests that certain bacterial infections could also be important in ALS [20]. Enteroviruses have been found in the central nervous system (CNS) of ALS patients [19,21], and *M. fermentans* has been found in the CNS of patients with lethal mycoplasmal infections [22]. Similar to the possible role of enteroviruses in the pathogenesis of ALS, the exact role that mycoplasmal infections play in the pathogenesis or progression of ALS is not known.

Mycoplasmas like *M. fermentans* are particularly interesting because they have the capacity, like enteroviruses to penetrate the CNS, and they possess the potential to cause persistent neurological signs and symptoms [22, 23]. Our results on mycoplasmal infections in ALS patients suggest that coinfections with certain persistent viruses and bacteria might generate the particular signs and symptoms seen in ALS patients [20]. We also found that ALS patients have some of the signs and symptoms seen in a variety of chronic illness patients, consistent with their having mycoplasmal infections that are also found in these patients. Similar to chronic mycoplasmal infections [2, 3, 23], enteroviruses have also been found in patients with chronic myocarditis [24] and chronic fatigue [25]. It is interesting that both enteroviruses and mycoplasmas have the capacity to cause slow, persistent infections that can eventually result in cellular dysfunction and eventually cell death [19, 23], and mycoplasmal infections have been implicated in infectious neurological diseases and autoimmune diseases [23].

RHEUMATOID ARTHRITIS (RA)

Rheumatoid Arthritis (RA) is typically characterized by joint pain, swelling and tenderness, loss of joint mobility and joint destruction due to autoimmune inflammation, but there are also a number of other less specific chronic signs and symptoms in RA patients. Among these are fatigue, muscle pain and tenderness, headaches, gastrointestinal and heart problems and sometimes intermittent low-grade fevers and other signs and symptoms. In many patients the diagnosis of RA is accompanied by other secondary diagnoses, such as Chronic Fatigue Syndrome (CFS), Fibromyalgia Syndrome (FMS), Inflammatory Bowel Diseases, osteoporosis and other conditions, which can also present with overlapping signs and symptoms. This suggests that although the exact causes of these illnesses are not known and may be different in each patient, there are similarities in these conditions that may be important in causing patient morbidity (sickness) or in illness progression [1].

Instead of concentrating on possible initial causes of RA, we have been interested in the progression of

chronic illnesses like RA and other autoimmune diseases and in the potential role that chronic infections may play in patient morbidity. The complex signs and symptoms that evolve in many FMS, CFS and RA patients may be due, in part, to systemic chronic infections (bacteria, viruses, fungi), and this is why these patients can respond to antibiotics [26], as pioneered by Dr. Brown and the *Road Back Foundation* for RA. Such infections can follow acute or chronic chemical, environmental or other insults (trauma, chemical exposures, acute viral illness, etc.) that have the potential to suppress the immune system and cause metabolic imbalances [1].

PROGRESSION OF ILLNESS IN RA PATIENTS

Illnesses like RA evolve over time, probably in a multistep process that may require multiple toxic exposures, including infections that could be causative for the illness in some patients, cofactors for the illness (not causative but important in patient morbidity) in others, or opportunistic infections that cause morbidity and eventually become the major source of sickness. Thus infections need not be the actual trigger or cause of illness or be present at the onset of illness to be important. Because of their dysfunctional metabolic and immune systems, many RA patients may be particularly susceptible to secondary chronic infections that worsen their illness. Of course, illness progression in RA patients could also be caused by other factors, psychological stress, physical trauma, other illnesses and many other factors that impair the immune system and stimulate autoimmunity [1, 27].

Chronic infections are usually held in check by our immune systems, but they can take hold and cause problems if they can avoid immune surveillance and penetrate and hide in various tissues and organs, including synovial cells in joints and nerve cells. When such "stealth" viral and bacterial infections occur, they can cause many of the complex signs and symptoms seen in FMS, CFS and RA, including enhanced immune dysfunction and metabolic imbalances [1, 2]. Changes in environmental responses as well as increased titers to various endogenous viruses (some are commonly found in these patients) have been seen in RA, MS, ALS, FMS and CFS patients. If infectious agents are involved, few can produce the complex chronic signs and symptoms found in these patients. One that can is represented by a small, primitive class of bacteria called mycoplasmas [1, 23, 27]. Mycoplasmas and other bacteria (*Chlamydia, Coxiella, Brucella, Borrelia*, etc.), although not as well known as other agents in causing various diseases, are now considered important emerging pathogens in various chronic illnesses, including RA. For example, *Borrelia* species (Lyme Disease) infections can result in RA signs and symptoms.

As chronic illnesses, such as RA, FMS and CFS, progress there are a number of accompanying clinical problems as well as increases in multi-organ autoimmune signs and symptoms. This may occur when cell wall-less microorganisms like mycoplasmas bleb from cells and are released while carrying host cell antigens on their membrane surface [1]. This can set up what is termed a concomitant host immune response, which are immune responses against the mycoplasma antigens as well as the host antigens carried by the mycoplasmas. Mycoplasmas and other microorganisms can also express antigens that mimic host cell antigens [23], and these may directly stimulate autoimmune responses. Although it is certainly not proven, this pattern is consistent with certain chronic infections, such as mycoplasmal infections, that penetrate into nerve cells, synovial cells in joints, muscle cells and other cell types. Thus patients with such infections may respond immunologically to microorganism antigens as well as their own membrane antigens, producing unusual autoimmune signs and symptoms. This could explain why such patients often do not have all of the clinical characteristics expected for their autoimmune disease.

MICROORGANISMS CAUSE MORBIDITY IN RA

Microorganisms like Mycoplasmas are not considered important human pathogens when they are found at superficial sites, such as the oral cavity or gut, but some species, such as *M. fermentans, M. penetrans, M. pneumoniae, M. genitalium, M. pirum* and *M. hominis*, among others, have the capacity to penetrate into the blood circulation and colonize various tissues, and these cell-penetrating microorganisms have been closely associated with various human diseases [1, 23, 27]. Do such infectious agents actually cause FMS, CFS or RA? Probably not on their own, but some bacteria and viruses appear to be an important element in causing chronic illness progression, patient morbidity, or exacerbating the major signs and symptoms seen in patients with chronic illnesses.

We have found that ~70% of FMS, ~60% of CFS [3, 28] and ~50% of RA [2] and Gulf War Illness [29] patients have mycoplasmal blood infections that can explain many of the chronic signs and symptoms found in these patients. In the majority of RA, FMS and CFS patients we have found multiple pathogenic mycoplasmas in their white blood cells but these infections are only found in 0-9% of controls [1, 28, 29]. Interestingly, the majority of RA, CFS and FMS patients had multiple mycoplasmal infections but none were found in controls [2, 28]; however, single infections are found in some nonsymptomatic subjects (0-9%). The tests that we use to identify mycoplasmal infections, Forensic Polymerase Chain Reaction, are very sensitive and highly specific. These tests are a dramatic improvement over the relatively insensitive serum antibody and other tests that have been used to assay for systemic infections. Other laboratories have found and published similar findings [30].

NEW TREATMENTS FOR RA, CFS, FMS AND OTHER CHRONIC ILLNESSES

Many patients with chronic illnesses are treated as psychiatric patients. However, when microorganism infections are identified in the blood, these patients should be treated as medical not psychiatric patients, just like any other patients with blood infections. This does not mean that psychological or psychiatric problems are not important in chronic illness patients. But if such infections are important in these disorders, then appropriate treatments with antibiotics or other medications that suppress chronic infections should result in improvement and even recovery. This is exactly what has been found [1, 26, 29]. The majority of patients with confirmed pathogenic mycoplasmal infections eventually recover from 50-100% of their premorbid health on therapies that are directed specifically against their chronic infections not against possible psychological problems [26].

The recommended treatment for confirmed mycoplasmal blood infections is long-term antibiotic therapy, usually multiple 6-week cycles of doxycycline (200-300 mg/day), ciprofloxacin or Cipro (1,500 mg/day), azithromycin or Zithromax (500 mg/day) or clarithromycin or Biaxin (750-1,000 mg/day). Some physicians treat every other day with antibiotics. Multiple cycles are required, because few patients recover after only a few cycles, possibly because of the intracellular locations of the infections, the slow-growing nature of these microorganisms and their inherent insensitivity to antibiotics [31, 32]. We now recommend that patients who have been diagnosed with blood infections receive oral antibiotics for at least 6 months before using the 6-week cycles of treatment. Although patients starting such therapy usually have Herxheimer reactions and feel initially worse due to die-off or release of toxic materials from damaged microorganisms, they eventually stabilize within days to a few weeks and then slowly begin to recover. Unfortunately, the treatment requires long-term therapy,

and recovery is usually very slow. Patients that have been sick for many years are unlikely to recover within a year of therapy [31, 32].

The clinical responses that are seen are not due to placebo effects, because administration of some antibiotics, such as penicillins, resulted in patients becoming more not less symptomatic. In addition, they are not due to immunosuppressive effects of some of the antibiotics, because other antibiotics that do not cause immune suppression are also effective but only if they suppress the chronic infections. If they don't have these infections, then antibiotics should not work [26]. Some patients recover to a certain point and then fail to continue to respond to the recommended antibiotics, suggesting that other problems, such as viral infections, environmental exposures and other toxic events, and even stress, also play an important role in these illnesses, and may even play a predominant role in some patients. Other considerations include the removal of refined sugars, alcohol, certain fats and allergy-prone foods from your diet and the appropriate support for your endocrine and immune systems [8, 9].

What results have been obtained with antibiotics for chronic illnesses like RA, CFS and FMS? Although a majority of patients diagnosed with chronic blood infections appear to benefit from antibiotic therapy, many patients respond and have some alleviation of most signs and symptoms but do not fully recover. A 3-year follow-up of antibiotic therapy in Northern California indicates that a majority (~80%) of FMS/CFS patients from Shasta County that were confirmed with mycoplasmal infections recovered from 50-100% of their pre-illness health within this time period, and even some patients who did not test positive showed benefit from antibiotics, suggesting other bacterial infections. Similar to other therapies for chronic illnesses, not every patient benefited from antibiotic therapy, and the time required for recovery was quite variable in different patients [26]. In RA patients blinded clinical trials have shown the effectiveness of antibiotic therapy with minocycline [33, 34]. In addition to antibiotics, patients must take vitamins, minerals, immune enhancement and other supplements to help boost immunity. For example, these patients are often depleted in vitamins B complex, C and E, among others, and certain minerals [31, 32]. Amino acids, fish oils, probiotic bacteria (*Lactobacillus acidophillus*) and a number of natural remedies have proven useful during therapy [31, 32]. In some patients oxygen therapy has proved useful. Oxygen suppresses the borderline anaerobic chronic infections like *Mycoplasmas* and *Chlamydias* [32].

COMPLEX CAUSES OF CHRONIC ILLNESSES

Do chronic infections explain some of the pathogenesis of autoimmune illnesses? It is unlikely that there is only one or even a few explanations for complex chronic illnesses like MS, ALS, RA, FMS or CFS. Rather, these illnesses are probably due to a combination of multiple toxic exposures, chemical and biological, in combination with genetic susceptibility (immune systems and/or detoxification systems, cellular metabolism, etc.) that determine whether a person becomes chronically ill. These considerations probably also play an important role in determining who will recover to various extents on different types of therapy. In addition, recovery can be complicated by patients' over-dependence on drugs, such as certain antidepressants or other drugs that can suppress portions of the immune system [31]. Interestingly, those patients that slowly recover after several cycles of antibiotics are generally less environmentally sensitive, suggesting that their immune systems may be returning to pre-illness states. If such patients had illnesses that were solely caused by psychological or psychiatric problems or by environmental or chemical exposures, they should not respond to the recommended antibiotics and recover their health. In addition, if such treatments were just reducing the autoimmune responses, then patients should not maintain recovery after the treatments are discontinued.

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