

# Chronic Bacterial and Viral Infections in Neurodegenerative and Neurobehavioral Diseases

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## Abstract

Often patients with neurodegenerative or neurobehavioral diseases have chronic, neuropathic infections that could be important in disease inception, progression or increasing the types/severities of signs/symptoms. Although controversial, the majority of patients with various neurodegenerative or neurobehavioral conditions, such as amyotrophic lateral sclerosis, multiple sclerosis, Alzheimer's disease, Parkinson's disease and autistic spectrum disorders, show evidence of central nervous system and/or systemic bacterial and viral infections. For example, using serology or polymerase chain reaction evidence of *Chlamydia pneumoniae*, *Borrelia burgdorferi*, *Mycoplasma* species, human herpesvirus-1 and -6 and other bacterial and viral infections revealed high infection rates that were not found in control subjects. Although chronic infections were not found in some studies and the specific role of chronic infections in neurological disease pathogenesis has not been determined or is inconclusive, the data suggest that chronic bacterial and/or viral infections could be common features of progressive neurodegenerative and neurobehavioral diseases.

## Introduction

Neurodegenerative diseases are chronic degenerative diseases of the central nervous system (CNS) that cause dementia. For the most part, the causes of these brain diseases remain largely unknown.<sup>1</sup> They are characterized by molecular and genetic changes in nerve cells that result in nerve cell degeneration and ultimately nerve dysfunction and death, resulting in neurological signs and symptoms and dementia.<sup>1,2</sup> In addition to neurodegenerative diseases, there are also neurobehavioral diseases that mainly but not exclusively appear in the young, such as autistic spectrum disorders (ASD) that encompass autism, attention deficit disorder, Asperger's syndrome and other disorders.<sup>3</sup>

There appear to be genetic links to neurodegenerative and neurobehavioral diseases, but the genetic changes that occur and the changes in gene expression that have been found in these diseases are complex and not directly related to simple genetic alterations.<sup>1,4</sup> In addition, it is thought that nutritional deficiencies, environmental toxins, heavy metals, chronic bacterial and viral infections, autoimmune immunological responses, vascular diseases, head trauma and accumulation of fluid in the brain, changes in neurotransmitter concentrations, among others, are involved in the pathogenesis of various neurodegenerative and neurobehavioral diseases.<sup>1-3,5-8</sup> One of the biochemical changes found in essentially all neurological, neurodegenerative and neurobehavioral diseases is the over-expression of oxidative free radical compounds (oxidative stress) that cause lipid, protein and genetic structural changes.<sup>5-9</sup>

Oxidative stress can be caused by a variety of environmental toxic insults, and when combined with genetic factors pathogenic processes could result.<sup>10</sup> An attractive hypothesis for the causation or promotion of neu-

rological disease involves chronic bacterial and/or viral toxic products, which result in the presence of excess reactive oxygen species and culminate in pathologic changes.<sup>11,12</sup>

Infectious agents may enter the CNS within infected migratory macrophages, they may gain access by transcytosis across the blood-brain-barrier or enter by intraneuronal transfer from peripheral nerves.<sup>11</sup> Cell wall-deficient bacteria, principally species of *Chlamydia* (*Chlamydophila*), *Borrelia*, *Brucella*, among others, bacteria without cell walls, such as *Mycoplasma* species, and various viruses are candidate infectious agents that may play important roles in neurodegenerative and neurobehavioral diseases.<sup>12-14</sup> Since they are usually systemic, such infections can affect the immune system and other organ systems, resulting in a variety of systemic signs and symptoms.<sup>15-18</sup>

## Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is an adult-onset, idiopathic, progressive neurodegenerative 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.<sup>19,20</sup> The overall clinical picture of ALS can vary, depending on the location and progression of pathological changes found in nervous tissue.<sup>21</sup>

In ALS the role of chronic infections has attracted attention with the finding of enterovirus sequences in a majority of spinal cord samples by polymerase chain reaction (PCR).<sup>22,23</sup> This finding is not without controversy, since others failed to detect enterovirus sequences in spinal cord samples from patients with or without ALS.<sup>24,25</sup> Nonetheless, infectious agent(s) that penetrate the CNS may play a role in the etiology of ALS, although evidence for a transmission of an infectious agent or transfer of an ALS-like disease from man-to-man or man-to-animals has not been demonstrated.<sup>26</sup>

The presence of systemic mycoplasmal infections in ALS patients has been investigated with PCR methods.<sup>27,28</sup> Our studies indicated that 100% of Gulf War veterans diagnosed with ALS (N=8 from three different nations) had systemic mycoplasmal infections.<sup>27</sup> All but one patient had *M. fermentans*, and one veteran from Australia had a systemic *M. genitalium* infection. In approximately 80% of nonmilitary (unrelated to military patients) ALS patients from the USA, Canada and Great Britain (N=28) blood mycoplasmal infections were also found.<sup>27</sup> Of the mycoplasma-positive civilian patients who were further tested for *M. penetrans*, *M. fermentans*, *M. hominis* and *M. pneumoniae*, most were positive for *M. fermentans* (59%), but other *Mycoplasma* species, such as *M. hominis* (31%) and *M. pneumoniae* infections (9%), were also found. Some of the civilian ALS patients had multiple mycoplasmal infections; however, multiple mycoplasmal infections were not found in the military patients with ALS.<sup>27</sup> In another study in Mexico 10 of 20 ALS patients showed evidence of systemic *Mycoplasma* species by analysis of their blood by PCR.<sup>28</sup>

Another chronic infection that is commonly found in ALS patients who live in certain areas is *Borrelia burgdorferi*, the principal etiologic agent of Lyme disease (LD). For example, ALS patients who live in New York, a LD-intense area, were examined for *B. burgdorferi* infections, and over one-half were found to be seropositive for Lyme *Borrelia* compared to 10% of matched controls.<sup>29</sup> In addition, some patients diagnosed with ALS were subsequently diagnosed with neuroborreliosis.<sup>30</sup> A survey of the literature indicates that spirochetal forms have been observed for some time in the CNS tissue of ALS and other neurodegenerative diseases.<sup>31</sup> Thus a byproduct of LD may be progression to ALS, but this is probably only possible in some LD patients who have the genetic susceptibility genes for the neurodegenerative disease and who have other toxic exposures.<sup>32,33</sup>

ALS patients also have other chronic infections, including human herpes virus-6 (HHV6), *Chlamydia pneumoniae* and other infections.<sup>34,35</sup> A suggestion that retroviruses might be involved in ALS and other motor neuron diseases<sup>36</sup> prompted McCormick et al.<sup>37</sup> to look for reverse transcriptase activity in serum and cerebrospinal fluid (CSF) of ALS and non-ALS patients. They found reverse transcriptase serum activity in one-half of ALS cases but in only 7% of controls ( $p < 0.008$ ). Interestingly, only one of 25 ALS CSF samples contained reverse transcriptase activity.<sup>37</sup>

The exact role that infections play in the pathogenesis or progression of ALS is not known. They could be cofactors in ALS pathogenesis, or they could simply be opportunistic infections that cause morbidity in ALS patients, such as the respiratory and rheumatic symptoms and other problems often found in ALS patients. They could also be involved in the progression of ALS rather than in its inception. Although the exact 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).<sup>22-24,27-29,31-34</sup> None of these hypotheses is exclusive, and ALS may have a complex pathogenesis involving multiple factors.<sup>34</sup> Future studies should determine more precisely the role of chronic bacterial and viral infections in the pathogenesis and progression of ALS.

## Multiple Sclerosis

Multiple sclerosis (MS) is the most common demyelinating disease of the CNS, and it can occur in young as well as older people. In MS, inflammation and the presence of autoimmune antibodies against myelin and other nerve cell antigens are thought to cause the myelin sheath to break down, resulting in decrease or loss of electrical impulses along the nerves.<sup>38,39</sup> In the progressive subset of MS neurological damage occurs additionally by the deposition of plaques on the nerve cells to the point where nerve cell death occurs. In addition, breakdown of the blood-brain barrier in MS is associated with local inflammation caused by glial cells.<sup>38,39</sup> The clinical manifestations of demyelination, plaque damage and blood-brain barrier disruptions are variable but usually include impaired vision, alterations in motor, sensory and coordination systems and cognitive dysfunction. Often these are cyclic (relapsing-remitting subset) over time, but a substantial MS subset progresses without remitting.<sup>39</sup>

There is strong evidence for a genetic component in MS.<sup>40,41</sup> Although it has been established that there is a genetic susceptibility component to MS, epidemiological and twin studies suggest that MS is an acquired, rather than an inherited, disease.<sup>42</sup>

The possibility that MS is linked to chronic infection(s) has attracted attention.<sup>43,44</sup> In fact, MS patients show immunological and cytokine elevations consistent with chronic infections.<sup>44-46</sup> A possible infectious cause for MS has been under investigation for approximately the last decade, and patients have been examined for various viral and bacterial infections.<sup>44,47</sup> One of the most common findings in MS patients is the presence of antibodies and DNA of *C. pneumoniae* in their CSF.<sup>47-49</sup> For example, Sriram et al.<sup>48</sup> examined relapsing-remitting (N=17) and progressive (N=20) MS patients for the presence of *C. pneumoniae* in CSF by culture, PCR and immunoglobulin reactivity with *C. pneumoniae* elementary body antigens. They were able to isolate *C. pneumoniae* from 64% of MS patients' CSF versus 11% of patients with other neurological diseases. High rates of PCR-positive (MOMP gene) patients (97% MS positive versus 18% of other neurological diseases) as well as serology-positive patients (86% MS positive, confirmed by enzyme-linked immunosorbent assays [ELISA] and Western blot analysis) were found in MS.<sup>48</sup> Further examination of MS patients for oligoclonal antibodies against *C. pneumoniae* revealed that 14 of 17 patients were positive, whereas none of the control non-MS patients had antibodies that were absorbed by *C. pneumoniae* elemental body antigens.<sup>49</sup>

Other studies have also found evidence for the presence of *C. pneumoniae* in MS patients but at lower incidence. Fainardi et al.<sup>50</sup> used ELISA techniques and found that high-affinity antibodies against *C. pneumoniae* were present in the CSF of 17% of 71 MS cases compared to 2% of 52 patients with non-inflammatory neurological disorders. They found that the majority of the progressive forms of MS were positive compared to patients with relapsing-remitting MS. The presence of *C. pneumoniae* antibodies was also found in other inflammatory neurological disorders (N=51), and thus it was not specific to MS.<sup>50</sup> Using immunohistochemistry Sriram et al.<sup>51</sup> performed a study of formalin-fixed CNS tissue from MS and non-MS neurological disease controls and found that in a subset (7 of 20) of MS patients chlamydial antigens were localized to ependymal surfaces and paraventricular regions. Staining was not found in 17 CNS tissue samples from other neurological diseases. Frozen tissues were available in some of these MS cases, and PCR amplification of *C. pneumoniae*

genes was accomplished in 5 of 8 CNS tissue samples from MS patients but none in 17 frozen CNS tissues from other neurological diseases. In addition, they examined CSF sediment by immuno-gold-labeled staining for chlamydial antigens and found by electron microscopy that the electron dense bodies resembling bacterial structures correlated with PCR-positive results in 10 of 11 MS cases.<sup>51</sup> The same group also used different nested PCR methods to examine additional *C. pneumoniae* gene sequences in the CSF of 72 MS patients and linked these results to MRI evidence of MS-associated lesions.<sup>52</sup> Similarly, Grimaldi et al.<sup>53</sup> linked the presence of *C. pneumoniae* infection with abnormal MRI results in 23 of 107 MS patients with more progressive disease. In addition, a higher rate of *C. pneumoniae* transcription was found by Dong-Si et al.<sup>54</sup> in the CSF of 84 MS patients. The above, among other data,<sup>55-57</sup> support the presence of *C. pneumoniae* in the CNS of MS patients, at least in a subset of more progressed patients that are most likely the progressive forms of MS.

Not all studies have obtained evidence, however, for the presence of *C. pneumoniae*<sup>58,59</sup> or other bacteria<sup>60</sup> in the CNS of MS patients. Hammerschlag et al.<sup>61</sup> used nested PCR and culture to examine 12 frozen brain samples from MS patients but could not find *C. pneumoniae* in any of the tissue samples. Alternatively, in one study *C. pneumoniae* was found at similar incidence in MS and other neurological diseases, but only MS patients had *C. pneumoniae* in their CSF.<sup>59</sup> Swanborg et al.<sup>62</sup> have reviewed the evidence linking *C. pneumoniae* infection with MS and has concluded that they are equivocal due to negative reports, and they also speculated that specific genetic changes may be necessary to fulfill the role of such infections in the etiology of MS.

Another possible reason for the equivocal evidence linking MS etiology with infection, such as *C. pneumoniae*, is that multiple co-infections could be involved. In addition to *C. pneumoniae* found in most studies, MS patients could also have *Mycoplasma* species, *B. burgdorferi* and other bacterial infections as well as viral infections.<sup>63</sup> When multiple infections are considered, it is likely that >80% of MS patients have obligate intracellular bacterial infections caused by *Chlamydia (Chlamydochlamydia)* or other bacteria that can be intracellular, such as *Mycoplasma*, *Borrelia* and other infections. These infections were found only singly and at very low incidence in age-matched subjects.<sup>63</sup> In spite of these findings, others did not find evidence of *Mycoplasma* species in brain tissue (N=30), CSF or peripheral blood (N=57) of MS patients.<sup>64</sup>

Viruses have also been associated with MS. Certain viruses have been found in MS patients, such as HHV6, but these viruses have also been found at lower incidence in control samples.<sup>62</sup> Sanders et al.<sup>65</sup> used PCR to examine postmortem brain tissue (N=37) and controls (N=61) for the presence of neurotrophic viruses. They found that 57% of MS cases and 43% of non-MS neurological disease controls were positive for HHV6, whereas 37% and 28%, respectively, were positive for herpes simplex virus (HSV1 and HSV2) and 43% and 32%, respectively, were positive for varicella zoster virus. However, these differences did not achieve significance, and the authors concluded that “an etiologic association to the MS disease process [is] uncertain.” They also found that 32% of the MS active plaques and 17% of the inactive plaque areas were positive for HHV6.<sup>65</sup> Challoner et al.<sup>66</sup> used sequence difference analysis to search for pathogens in 86 MS brain specimens. Using PCR they found that >70% of the MS specimens were positive. They also used immunocytochemistry and found staining around MS plaques more frequently than around white matter; nuclear staining of oligodendrocytes was also seen in MS samples but not in controls.<sup>66</sup> Using immunofluorescent and PCR methods HHV6 DNA has also been found in peripheral leukocytes in the systemic circulation of MS patients.<sup>67,68</sup> However, using PCR methods, others did not find herpesviruses in the peripheral blood or CSF of MS patients.<sup>69,70</sup>

Although significant information (reviewed in<sup>43,44,70</sup>) points to an infectious process in MS, this remains a controversial concept. As evidence emerges of new possible pathogens in MS, such as a new putative retrovirus,<sup>71</sup> these reports must be intensively examined and further studies initiated. Since most studies have found that the progressive form of MS rather than relapsing-remitting forms of MS were associated with chronic infections, infections might be more important in MS progression than in its inception. Various infections may also nonspecifically stimulate the immune system.<sup>43</sup> As in other neurodegenerative diseases, multiple factors appear to be involved in the pathogenesis of MS. Thus like ALS, MS progression may turn out to be more likely linked to chronic infections, rather than its inception.

## Alzheimer's Disease

Alzheimer's Disease (AD), the most common cause of dementia, is a collection of brain disorders usually found in aged patients. The disease is characterized by slow, progressive loss of brain function, especially notable by lapses in memory, disorientation, confusion, mood swings, changes in personality, language problems, such as difficulty in finding the right words for everyday objects, loss of behavioral inhibitions, loss of motivation, and paranoia. The prognosis and course of AD varies widely, and the duration of illness can range from a few years to over 20 years. During this time the parts of the brain that control memory and thinking are among the first affected, followed by other brain changes that ultimately result in brain cell death.<sup>72</sup>

AD is characterized by distinct neuropathological changes in the brain. Among the most notable are the appearance of plaques and tangles of neurofibrils within brain nerves that affect nerve synapses and nerve-nerve cell communication. Both of these structural alterations involve the deposition of altered amyloid (A $\beta$ ) proteins.<sup>73,74</sup> Although the cause of AD is not known with any certainty, the formation of the amyloid plaques and neurofiber tangles may be due to genetic defects and resulting changes in the structure of A $\beta$  proteins, neurotoxicity caused by chemicals or other toxic events, inflammatory responses, oxidative stress and increases in reactive oxygen species, loss of nerve trophic factors important in nerve physiology and loss of nerve cell transmission.<sup>73-77</sup>

Brain infections in AD have only recently become an important topic.<sup>78-80</sup> One pathogen that has attracted considerable attention is *C. pneumoniae*.<sup>81,82</sup> As mentioned above, this intracellular bacterium has a tropism for neural tissue,<sup>81</sup> and it has been found at high incidence in the brains of AD patients (17 of 19 patients in brain areas of typical AD-related pathology) by PCR and immunohistochemistry methods.<sup>82</sup> *C. pneumoniae* has also been found in nerve cells in close proximity to neurofibrillary tangles.<sup>82,83</sup> This microorganism can invade endothelial cells and promote the transmigration of monocytes through human brain endothelial cells into the brain parenchyma.<sup>84</sup> Although *C. pneumoniae* has been found in the brains of most AD patients studied,<sup>77,81</sup> and this infection results in amyloid beta (A $\beta$ ) plaque formation in mice injected with *C. pneumoniae*,<sup>85</sup> some investigators have not found an association of *C. pneumoniae* infection with AD using PCR or immunohistochemistry.<sup>86,87</sup>

In addition to *C. pneumoniae*, evidence has been forthcoming that AD patients also have other bacterial infections, such as *B. burgdorferi*.<sup>88</sup> This infection has been examined in AD cases by serology, culture, Western blot and immunofluorescence.<sup>89,90</sup> However, others could not find evidence of *B. burgdorferi* in AD patients.<sup>91,92</sup> The presence of intracellular infections like *B. burgdorferi* in AD patients has been hypothesized to be a primary event in the formation of AD amyloid plaques by forming "congophilic cores" that attract amyloid materials.<sup>93</sup> Multiple reports show that AD nerve cells are often positive for *B. burgdorferi*.<sup>88-90,93,94</sup>

In addition to the hypothesis that intracellular microorganisms may provide "cores" for the attraction of amyloid materials, the induction of reactive oxygen species, lipid peroxidation and the breakdown of the lysosomal membranes releasing lysosomal hydrolases are also thought to be important in amyloid deposition.<sup>94</sup> Although the possibility that infections may be important in AD pathogenesis is attractive, some negative reports where investigators have not confirmed the presence of infections, such as *B. burgdorferi*, in AD patients, indicate that this is still controversial (reviewed in<sup>91,94</sup>).

HSV infections have also been found in AD, and an interesting relationship has developed between the presence of HSV1 in AD.<sup>95</sup> It had been noted previously that HSV1 but not a related neurotrophic virus, varicella zoster virus, was found often in AD brains and may be linked to patients who have the AD risk factor ApoE e4 allele.<sup>96,97</sup> In AD HSV1 is thought to be involved in the abnormal aggregation of beta amyloid or A $\beta$  fragments within the brain by reducing the amount of full length amyloid precursor protein and increasing the amount of the A $\beta$  fragment from this precursor.<sup>98</sup> Recently Wozniak et al.<sup>99</sup> showed that HSV1 infection of cultured glial and neuronal cells results in a dramatic increase in the intracellular levels of beta amyloid forms, whereas the levels of native amyloid precursor protein decreased. This is similar to what has been found in mice infected with HSV1, indicating that HSV1 is probably involved directly in the development of senile-associated plaques. Another herpesvirus, HHV6, has also been found in AD patients, but it is thought that this

virus is not directly involved in AD pathogenesis, but it may exacerbate the effects of HSV1 in ApoE e4 carriers.<sup>100</sup>

In spite of the evidence that AD has been associated with, for example, *C. pneumoniae*, HSV1 or other infections, Robinson et al.<sup>101</sup> have stressed caution in concluding that infections act as a trigger or co-factor in AD. In particular, there is a paucity of experimental evidence that pathogens can elicit the neuropathological changes and cognitive deficits that characterize AD. They also stress that there is a need for consideration of systemic infections as potential contributors to the pathogenesis of AD.

## Parkinson's Disease

Parkinson's disease (PD) is characterized by akinesia, muscular rigidity and resting tremor. Also present are autonomic dysfunction, olfactory disturbances, depression, sensory and sleep disturbances and frequently dementia.<sup>102</sup> PD pathology indicates a progressive loss of the dopamine neurons of the substantia nigra together with the presence of Lewy bodies and  $\alpha$ -synuclein. More extensive brain degeneration also occurs, from the medulla oblongata to the cerebral cortex.<sup>103,104</sup>

Although there is consensus among investigators that age-related inclusion bodies and protein aggregations or defects in their degradation occur in PD, their cause and role in PD pathogenesis remains a subject of intense research.<sup>103,104</sup> Some evidence suggests a relationship between PD and specific genetic changes, such as changes in the genes affecting mitochondria, protein degradation, organelle trafficking and vesicular fusion, and in proteins involved in oxidative stress or antioxidant function.<sup>102</sup> Inflammation has also been associated with PD pathology.<sup>105</sup>

Similar to other neurodegenerative diseases, the pathogenesis of PD has been proposed to be due to multiple genetic and neurotoxic hits that produce oxidative damage and cell death. However, in the case of PD the relevant targets of toxic events are neuromelanin-containing dopaminergic neurons of the substantia nigra.<sup>104,106</sup> A case-control study in Italy indicated that multiple environmental factors and genetic background were statistically related risk factors for PD.<sup>107</sup> Among these, toxic exposures (over many years) and trauma early in life may be important in the pathogenesis of PD.<sup>108</sup> For example, early life exposure to brain injury, chemicals and/or infections may initiate a cyclic inflammatory process involving oxidative damage, excitotoxicity, mitochondrial dysfunction and altered proteolysis that later in life results in substantia nigra neuron death by apoptosis.<sup>109-111</sup>

The possible role of chronic infections in PD pathogenesis has been proposed for a number of years.<sup>109</sup> One infection found in PD that has aroused considerable interest is the presence of chronic gastrointestinal *Helicobacter pylori*, as treatment of this infection offered some relief to late stage cachexia in PD patients who were receiving L-dopa.<sup>112</sup> Indeed, Pierantozzi et al.<sup>113</sup> found that *Helicobacter pylori*-infected PD patients had reduced L-dopa absorption and increased clinical disability, while treating this infection reduced L-dopa absorption and increased clinical disability.<sup>114</sup> *H. pylori* may not be directly involved in PD, but its systemic presence could affect the progression and treatment of PD, probably by stimulating inflammation and autoimmunity.<sup>115</sup>

Inflammation and autoimmune responses have also been attributed to other chronic infection(s) found in PD.<sup>115-117</sup> Indeed, experimental models of PD have been developed using neurological viral or bacterial infections to initiate the pathogenic process.<sup>118,119</sup> In humans, spirochetes have been found in Lewy bodies of PD patients.<sup>29</sup> Other infections, such as viral encephalitis,<sup>120</sup> AIDS-associated opportunistic infections of the basal ganglia,<sup>121</sup> coronavirus,<sup>122</sup> and other infections<sup>63,123,124</sup> have been found in PD and could be important in stimulating inflammation and autoimmune responses. Richy and Mégraud<sup>125</sup> have stressed, however, that more rigorous investigations will be required to establish whether a causal link exists between infections and PD.

## Autism Spectrum Disorders

ASD, such as autism, attention deficit disorder, Asperger's syndrome, etc., are neurobehavioral diseases of primarily the young where patients generally suffer from an inability to properly communicate, form rela-

tionships with others and respond appropriately to their environment. Such patients do not all share the same signs and symptoms but tend to share certain social, communication, motor and sensory problems that affect their behavior in predictable ways. These mostly children often display repetitive actions and develop troublesome fixations with specific objects, and they are often painfully sensitive to certain sounds, tastes and smells.<sup>3,126,127</sup>

The causes of ASD are unknown but appear to include genetic defects, heavy metal, chemical and biological exposures, which are probably different in each patient.<sup>3,4,126-128</sup> Moreover, in ASD patients there may be similarities in genetic defects and environmental exposures that are important in patient morbidity or in illness progression.

Chronic infections appear to be an important element in the development of ASD.<sup>14,129</sup> In some patients there are a number of nonspecific chronic signs and symptoms, such as fatigue, headaches, gastrointestinal and vision problems and occasional intermittent low-grade fevers and other signs and symptoms that are generally excluded in the diagnosis of ASD.<sup>130</sup> Although nonspecific and not always related to infection, these signs and symptoms suggest that ASD patients could suffer from bacterial or viral infections.<sup>14,130</sup> Indeed, increased titers to various viruses as well as bacterial and fungal infections have been commonly seen in ASD patients,<sup>14,129-131</sup> although epidemiological evidence for an association of childhood infections in the first two years of life and ASD is lacking.<sup>132</sup> In addition, environmental exposures to chemicals and heavy metals also appear to be important in the development of ASD.<sup>126,127,133</sup>

Although controversial, the relationship between ASD and heavy metals may involve the role of multiple vaccines in ASD pathogenesis.<sup>126,127</sup> ASD patients often show their first signs and symptoms after multiple childhood immunizations.<sup>126</sup> Rimland<sup>126</sup> noted that the sharp rise in Autism rates occurred only after the multiple vaccine MMR came into widespread use, and now in some states in the U.S. children receive as many as 33 vaccines before they can enroll in school. Such vaccines often contain mercury and other preservatives,<sup>127</sup> and some may also contain contaminating bacteria, as found in veterinary vaccines.<sup>134</sup>

Previously we found that 42 veterans of the Gulf War with chronic fatiguing illnesses (Gulf War illness) exhibited multiple nonspecific signs and symptoms similar to Chronic Fatigue Syndrome/Myalgic Encephalomyopathy (CFS).<sup>135,136</sup> Interestingly, their symptomatic children (N=35) were often diagnosed with autism or attention deficit disorder, two disorders that fall under ASD.<sup>137,138</sup> In our study ~42% of Gulf War illness patients had mycoplasmal infections, and almost all of these (~82%) were single infections, usually *M. fermentans*. When the few multiple infection cases were examined, most were found to have combinations of *M. fermentans* plus either *M. pneumoniae*, *M. hominis* or *M. genitalium*. In contrast, in healthy control subjects (N=70) only 8.5% were positive for any mycoplasmal infection, and all of these were single infections of various types.<sup>137,138</sup>

When we examined the immediate family members (N=107) of veterans with Gulf War illness, we found that >53% had positive tests for mycoplasmal infections and showed symptoms of CFS. Among the CFS-symptomatic family members, most (>70%) had mycoplasmal infections compared to the few non-symptomatic family members who had similar infections. When the incidence of mycoplasmal infections was compared within families, the CFS-positive family members were more likely to have mycoplasmal infections compared to non-symptomatic family members ( $p<0.001$ ).<sup>137</sup> Symptomatic children (mostly ASD, N=35) were also infected with *M. fermentans*, and this was not seen in aged-matched control subjects. Although some non-symptomatic family members did have mycoplasmal infections (~10%), this was not significantly different from the incidence of mycoplasmal infections in healthy control subjects (8.5%).<sup>137,138</sup>

Examining ASD patients (N=28) who were not in military families for systemic mycoplasmal infections showed that the majority (~54%) were positive for mycoplasmal infections. However, in contrast to the children of Gulf War illness patients who for the most part had only one type of mycoplasmal infection, *M. fermentans*, the civilian children tested positive for a variety of *Mycoplasma* species. We also tested a few siblings without apparent signs and symptoms, and for the most part few had these infections.<sup>138</sup> In another study we examined the blood of ASD patients (N=48) from Central and Southern California and found that a large subset (>58%) of patients showed evidence of *Mycoplasma* infections compared to age-matched control subjects (Odds Ra-

tio=13.8,  $p<0.001$ ).<sup>14</sup> ASD patients were also examined for *C. pneumoniae* (8.3% positive, Odds Ratio=5.6,  $p<0.01$ ) and HHV6 (29.2% positive, Odds Ratio=4.5,  $p<0.01$ ). The results indicated that a large subset of ASD patients display evidence of bacterial and/or viral infections (Odds Ratio=16.5,  $p<0.001$ ).<sup>14</sup>

Recently ASD patients have been examined for *B. burgdorferi* infections. Various studies revealed that 22-30% of ASD patients (N=76) have *Borrelia* infections.<sup>139</sup> The incidence of *Borrelia* infections in ASD patients may be related to Lyme disease (LD) distribution, with some LD-intense areas having high prevalence, whereas other areas have a low prevalence.

## Chronic Fatigue Syndrome

CFS is characterized by unexplained, persistent long-term disabling fatigue plus additional signs and symptoms, including neurophysiological symptoms.<sup>140</sup> Brain imaging studies showed that CFS patients are dysfunction in their ventral anterior cingulate cortex function, and they also have other brain MRI abnormalities.<sup>141,142</sup> Most CFS patients also have immunological abnormalities (reviewed in<sup>143,144</sup>).

A large subset of CFS patients can be characterized by the presence of chronic bacterial and viral infections,<sup>16,17,144-148</sup> although this has not been seen in all studies.<sup>149</sup> For example, using the blood of CFS patients (N=100) and PCR procedures an overwhelming majority of patients showed evidence of multiple, systemic bacterial and viral infections (Odds Ratio=18.0,  $p<0.001$ ).<sup>17</sup> CFS patients had a high prevalence (51%) of one of four *Mycoplasma* species (Odds Ratio=13.8,  $p<0.001$ ) and often showed evidence of co-infections with different *Mycoplasma* species, *C. pneumoniae* (8%, Odds Ratio=8.6,  $p<0.01$ ) and active HHV6 (30%, Odds Ratio=4.5,  $p<0.001$ ).<sup>17</sup> In a separate study the presence of these infections has also been related to the number and severity of signs/symptoms in 200 CFS patients.<sup>146</sup>

A sizable percentage of CFS/ME patients are also infected with *B. burgdorferi*.<sup>148,150,151</sup> Other infections are also found in CFS patients, such as cytomegalovirus, enteroviruses and human herpesvirus-7 (reviewed in<sup>43</sup>).

## Lyme Disease

LD is the most common tick-borne disease in North America. It is caused by a tick bite and the entry of the spiral-shaped spirochete *B. burgdorferi* and other co-infections.<sup>152</sup> After incubation for a few days to a month, the *Borrelia* spirochete and co-infections migrate through the subcutaneous tissues into the lymph and blood where they can travel to near and distant host sites.<sup>153</sup> Transplacental transmission of *B. burgdorferi* and co-infections can occur in pregnant animals, including humans, and blood-borne transmission to humans by blood transfusion is likely but unproven. The tick-borne LD co-infections can and usually do appear clinically at the same time.<sup>154</sup>

Since the signs and symptoms of LD overlap with other chronic conditions, LD patients are often diagnosed with other illnesses, such as CFS or chronic arthritis.<sup>150,151,153</sup> About one-third of LD cases start with the appearance of a round, red, bulls-eye skin rash (*erythema migrans*) at the site of the tick bite, usually within 3-30 days.<sup>154</sup> Within days to weeks mild flu-like symptoms can occur that include shaking chills, intermittent fevers and local lymph node swelling. After this localized phase, which can last weeks to months, the infection(s) can spread to other sites (disseminated disease), and patients then show malaise, fatigue, fever and chills, headaches, stiff neck, facial nerve palsies (Bell's palsy) and muscle and joint pain and other signs/symptoms.<sup>154</sup>

LD can eventually become persistent or chronic and involve the CNS and peripheral nervous system as well as ophthalmic, cardiac, musculoskeletal and internal organ invasion. At this late chronic stage arthritis, neurological impairment with memory and cognitive loss, cardiac problems (myocarditis, endocarditis causing palpitations, pain, bradycardia, etc.) and severe chronic fatigue are often apparent.<sup>154,155</sup> The signs and symptoms of the late chronic phase of the disease usually overlap with other chronic conditions, such as CFS, chronic arthritis, among others, as well as neurodegenerative diseases, such as AD, PD, ALS, etc., causing confusion in the diagnosis and treatment of the chronic phase in LD patients.<sup>29,30,88-90,141,156-158</sup> These late stage neurobor-

reliosis patients exhibit neuropathologic and neuropsychiatric disease similar to neurodegenerative diseases discussed above.<sup>31,89,128,156-159</sup>

The involvement of co-infections in LD has not been carefully investigated; however, such infections on their own have been shown to often produce comparable signs/symptoms. Diagnostic laboratory testing for LD at various clinical stages is, unfortunately, not fool-proof, and experts often use a checklist of signs and symptoms and potential exposures, along with multiple laboratory tests to diagnose LD.<sup>156</sup>

Co-infections are common in LD, and we<sup>148,155</sup> and others<sup>160</sup> have found that the most common co-infection found with *B. burgdorferi* are various species of *Mycoplasma*, usually *M. fermentans*. In some cases multiple mycoplasmal infections are present in LD patients.<sup>148</sup> Other common LD co-infections include: *Ehrlichia* species, *Bartonella* species and *Babesia* species, and 10-40% of cases of LD show such co-infections.<sup>148-150</sup> *Ehrlichia* and *Bartonella* species are usually found along with *Mycoplasma* species in LD.<sup>155,161-183</sup> *Bartonella* species, such as *B. henselae*, which also causes cat-scratch disease,<sup>164</sup> are often found in neurological cases of Lyme Disease.<sup>154,164</sup> In addition, protozoan co-infections have been found with *B. burgdorferi*, such as intracellular *Babesia* species,<sup>165</sup> The combination of *Borrelia*, *Mycoplasma* and *Babesia* infections can be lethal in some patients, and ~7% of patients can have disseminated intravascular coagulation, acute respiratory distress syndrome and heart failure.<sup>165</sup>

## Future Directions

This review suggests that various infections could be directly or indirectly involved in the pathogenesis and/or progression of neurodegenerative and neurobehavioral diseases. Although the evidence is far from conclusive for their general involvement,<sup>43</sup> certain cases may eventually be explained by considering infections in the mix of multiple toxic events, such as head trauma, excitotoxicity, nutritional deficiencies, local inflammation and genetic susceptibilities. These toxic events occur over time and likely participate in pathogenic processes.<sup>2,5-7</sup>

An argument can be made that neurodegenerative and neurobehavioral disease progression are affected by various infections. Even if infections are not directly involved in the pathogenesis of neurodegenerative and neurobehavioral diseases, patients with these diseases are at risk for a variety of opportunistic infections that could result in co-morbid conditions or promote disease progression. Infections can complicate diagnosis and treatment, and late-stage patients with complex neurological manifestations, meningitis, encephalitis, peripheral neuropathy, psychiatric conditions or with other signs and symptoms could have infections that are not recognized or treated by their physicians.

Patients with neurodegenerative and neurobehavioral diseases are particularly difficult to treat using single modality approaches. This may be due, in part, to the multi-focal nature of their disease and to the fact that often treatments are given to suppress signs and symptoms, rather than treat causes of the disease or its progression. However, even if the causes of neurodegenerative and neurobehavioral diseases are known, by the time therapeutic intervention is undertaken, it may be entirely too late to use this approach. Moreover, if complex, chronic infections are ignored or left untreated, recovery may be difficult to achieve.<sup>139</sup>

CNS infections can stimulate glial responses, and the presence of viral and bacterial infections in nerve cells, in particular, may stimulate autoimmune responses against nerve cell antigens. In the case of MS some 20 different bacterial and viral infections have been found, but the link between these infections and the pathogenesis of MS is still being debated.<sup>43,70</sup> Perhaps this is the reason that one or even a few types of infections cannot be causally linked to MS—there are just too many possibilities. Eventually certain infections may eventually be linked, at least in a subset of MS patients, to the pathogenesis of this neurodegenerative disease. Further research may shed more light on this important subject.

Does the overall evidence suggest that chronic infections may be involved in the pathogenesis of neurodegenerative and neurobehavioral diseases? At the moment the evidence is inconclusive. Some individuals can harbor chronic infections without any observable signs or symptoms, although the incidence of infection in such individuals is usually very low, only a few percent (for example<sup>14-17</sup>). Animal models have provided some

additional insight,<sup>117-119</sup> but this area will require much more intensive investigation. Some information outside the area exists on the infection of non-human primates with neuropathologic microorganisms, such as *Mycoplasma fermentans*, which results in CNS infection and a fatal disease with neurological signs and symptoms.<sup>166</sup> Animal models that can be reproducibly infected with specific microorganisms to reproduce a similar disease will be an important resource. Future basic and clinical research may ultimately elucidate the involvement of chronic infections in the pathogenesis and progression of neurodegenerative and neurobehavioral diseases.

Finally, one problem that will have to be overcome is the disparity of results from different laboratories.<sup>43</sup> This may be due, in part, to differences in the sources of clinical materials, qualities of reagents and techniques used. Indeed, in some procedures, such as PCR, there are various problems that must be overcome in the handling of specimens, their stability, presence of interfering substances, contamination, sensitivity and specificity of the tests and interpretation of the results. Inter-laboratory variability will remain a problem unless laboratories work closely together to solve these problems. For example, a multi-center research study on the presence of *C. pneumoniae* in the CSF of clinically defined, mono-symptomatic MS patients was conducted by Sriram et al.<sup>167</sup> with good concordance of results. Such studies should eventually alleviate the discrepancies found in the data from different research groups.

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