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Role of Pathogens in Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is an inflammatory demyelinating autoimmune disease of the central nervous system (CNS). Although the etiology of MS is unknown, genetic and environmental factors play a role. Infectious pathogens are the likely environmental factors involved in the development of MS. Pathogens associated with the development or exacerbation of MS include bacteria, such as *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*, the *Staphylococcus aureus*-produced enterotoxins that function as superantigens, viruses of the herpes virus (Epstein-Barr virus and human herpesvirus 6) and human endogenous retrovirus (HERV) families and the protozoa *Acanthamoeba castellanii*. Evidence, from studies with humans and animal models, supporting the association of these various pathogens with the development and/or exacerbation of MS will be discussed along with the potential mechanisms including molecular mimicry, epitope spreading and bystander activation. In contrast, infection with certain parasites such as helminthes (*Schistosoma mansoni*, *Fasciola hepatica*, *Hymenolepis nana*, *Trichuris trichiura*, *Ascaris lumbricoides*, *Strongyloides stercolaris*, *Enterobius vermicularis*) appears to protect against the development or exacerbation of MS. Evidence supporting the ability of parasitic infections to protect against disease will be discussed along with a brief summary of a recent Phase I clinical trial testing the ability of *Trichuris suis* ova treatment to improve the clinical course of MS. A complex interaction between the CNS (including the blood-brain barrier), multiple infections with various infectious agents (occurring in the periphery or within the CNS), and the immune response to those various infections may have to be deciphered before the etiology of MS can be fully understood.

Keywords

bacteria; parasites; protozoa; superantigens; viruses

INTRODUCTION

Multiple sclerosis (MS), originally described by Charcot almost 150 years ago [1], is the most common inflammatory demyelinating autoimmune disease of the central nervous system (CNS) of young adults with onset usually occurring between the ages of 20 and 50

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Declaration of Interest

The authors declare that there are no conflicts of interest.

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[2]. Approximately, 2.1 million persons are affected by MS worldwide and it affects women more often than men in a ratio of 2:1 [2]. The onset and progression of MS are variable; the clinical course of MS is most commonly (85% of cases) of a relapsing and remitting (RR) nature but may be primarily (PP) or secondarily (SP) progressive [3]. Sensory and motor disturbances, alterations in vision and cognitive impairment are common clinical features of MS. Oligodendrocytes, the myelin-forming cells of the CNS, are thought to be the main target for attack by a $CD4^+$ T helper (Th) 1-mediated autoimmune response. However, the predominant lymphocyte found in focal inflammatory demyelinating plaque lesions, which may form within the periventricular white matter of the brain, brain stem, spinal cord and optic nerve, are $CD8^+$ T cells [4], which are emerging as important effector cells in MS (reviewed in [5]). In addition to the inflammation, destruction of oligodendrocytes and demyelination, axonal loss occurs from disease onset and ultimately results in the development of irreversible neurological disability in the affected individual (reviewed in [6, 7]).

Although the etiology of MS is currently unknown, factors that play a role in the development of MS include genetics and environmental insults. Genetic risk factors for MS include the human leukocyte antigen (HLA) loci (HLA-DR and HLA-DQ alleles), which have been known for decades, and currently more than 50 non-HLA genomic regions (reviewed in [8]). Examples of the non-HLA gene loci that have been recently associated with MS include the interleukin (IL)-2 receptor α gene [9], the IL-7 receptor α gene [9–11], both of which are involved in the immune response, and many more loci that are located either near or within genes encoding immune system-related molecules [12]. Even more susceptibility genes are suspected and currently being sought as a means of fully accounting for the disease heritability (reviewed in [8]). The discovery that a large number of immune system-related molecules are genetic risk factors for MS [12], in combination with (1) the low concordance rate of 25% for MS in identical (homozygotic) twins [13], (2) concordance studies carried out on non-twin sibling pairs [14] and (3) the presence of elevated levels of immunoglobulin (Ig) Gand oligoclonal bands in the cerebrospinal fluid (CSF) of MS patients, both of which are characteristic of CNS disorders of infectious origin [15], all support a role for environmental insults in the development of MS.

Epidemiological studies suggest that infectious agents are the likely environmental insults involved in the development of MS ([16]; reviewed in [17]). Both migration studies, examining age (prepubescent vs. postpubescent) of migration between low (close to the equator) and high (far from the equator) risk areas for MS [16], and studies of isolated populations (Faroe Islands), demonstrating MS epidemics following exposure to North Americans or Europeans [16, 18, 19], support the infectious nature of the environmental insult. The hygiene hypothesis has been proposed to explain how an infectious agent could account for the epidemiology of MS ([20–23]; reviewed in [17]). The basic premise of this hypothesis is that exposure early in life to multiple, widespread, relatively harmless, infectious agents protects against MS whereas exposure later in life increases the risk of MS. In support of this hypothesis, there is evidence for a recent (three decade) steady rise in the incidence of MS in developing countries (reviewed in [24]). This increase has coincided with a decrease in the incidence of many infectious diseases, due to antibiotics, vaccinations and improved hygiene and socio-economic conditions (reviewed in [24]). According to this

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hypothesis, MS would develop in genetically susceptible young adults as a result of multiple infections, not one specific etiological agent, triggering an immune-mediated reaction. In addition to contributing to the development of MS, common infections of the respiratory, gastrointestinal or urogenital tract, and their concomitant systemic inflammations, have been associated with exacerbations of MS (reviewed in [17, 25, 26]). Infectious agents that have been explored as possible etiological agents of MS include bacteria and bacterial superantigens, viruses, as well as protozoa that infect humans (Table 1). On the flip side, infection with some parasites may protect against MS (Table 1).

BACTERIA

Mycoplasmas are the smallest of the free-living, self-replicating bacteria and many species have been associated with numerous diseases in humans [27]. In particular, *Mycoplasma pneumoniae* has been shown to invade the CNS [28] and is known to induce demyelination, at least in the periphery [29]. However, PCR and real-time PCR examination, targeting the *16S* rRNA mycoplasma gene, on DNA extracted from brain, serum and CSF of MS patients at various stages of the disease failed to detect mycoplasma DNA (102 mycoplasma species examined) [27]. A second group using a similar approach also failed to detect mycoplasma DNA in the CSF of MS patients [30]. Although these results were negative, the authors insist that these results do not preclude the possibility that mycoplasma infection in the periphery in MS patients could induce CNS-directed autoimmunity [27]. In support of this, another study showed that, although mycoplasma seropositivity was not significantly different between MS patients and controls overall, female MS patients in remission had significantly higher amounts of *Mycoplasma pneumoniae*-specific IgG in their serum compared to controls, suggesting a possible role for mycoplasma infection and/or the host immune response to mycoplasma infection in the development or progression of MS in female patients [31].

Another bacterial infection, again associated with numerous diseases in humans, that has been associated with MS is infection with *Chlamydiapneumoniae*, an obligate intracellular gram-negative bacterium [15]. In one study, it was found that *Chlamydia pneumoniae* could be grown in culture and detected by PCR from CSF samples in a much higher percentage of MS patients at various stages of the disease than controls [32]. In addition, a large percentage of MS patients had significantly elevated levels of CSF antibodies specific for *Chlamydia pneumoniae* antigens compared to controls [32]. Recently, oligoclonal bands in the CSF of progressive MS patients have been shown to be specific for *Chlamydia pneumoniae* [33]. A meta-analysis of 26 studies examining the association of *Chlamydia pneumoniae* with MS demonstrated a significant association between MS and the detection of *Chlamydia pneumoniae* DNA in the CSF by PCR [34]. Additionally, in a study of pediatric MS, *Chlamydia pneumoniae*-specific serum IgM levels were significantly higher in pediatric MS patients compared to controls [35]. However, more recently, PCR, targeting the bacterial *16S* rRNA gene, on CSF of MS patients at various stages of the disease failed to detect chlamydia DNA in the CSF of MS patients [30].

Other bacterial groups that have been examined recently for an association with MS include spirochetes, *Campylobacter*, *Bartonella*, *Mycobacteria* and *Streptococcus* [30]. PCR,

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targeting the bacterial *16S* rRNA gene, on CSF of MS patients at various stages of the disease failed to detect DNA from any of these bacterial groups in the CSF of MS patients [30]. Therefore, further investigation as to what types of bacteria may possible be involved in MS is warranted.

In addition to looking for an association between bacteria and MS directly, a common autoimmune animal model of MS, experimental autoimmune encephalomyelitis (EAE) has also been employed to examine the effects of bacteria on disease. EAE is a demyelinating disease that is induced either actively through the injection of encephalitogenic peptides (epitopes) or whole CNS proteins in adjuvant, or passively through the adoptive transfer of CNS antigen-sensitized lymphocytes (reviewed in [36]). Experiments have shown that infection with certain bacterial pathogens exacerbates EAE ([37, 38]; reviewed in [25]). Systemic infection of mice with *Chlamydia pneumoniae* after either active (with multiple antigens) or passive induction of EAE resulted in dissemination of the organism to the CNS and in an increase in the severity of the disease [37]. Likewise, systemic infection of mice with *Streptococcus pneumoniae*, a gram-positive bacterium, after active induction of EAE resulted in an increase in the severity of the disease, most likely due to an elevation of proinflammatory cytokines and activation of dendritic cells in the systemic circulation [38].

BACTERIAL SUPERANTIGENS

Superantigens are proteins produce by bacteria (or viruses) that potently activate $CD4^+$ T cells, inducing massive cell proliferation and cytokine production, of predominantly IL-2 and interferon (IFN)-γ (reviewed in [39]). Superantigens function by directly binding to major histocompatibility complex (MHC) class II molecules on the surface of antigen presenting cells and the superantigen/MHC complex then interacts with the T cell receptor β-chain variable region resulting in T cell activation. The effects of superantigens on the immune system can be both acute and long-term, and long-term effects include deregulation of the immune response resulting in proliferation of autoreactive T cells and the development and/or exacerbation of chronic autoimmune diseases. The first superantigens to be extensively characterized as to T cell activation were the *Staphylococcus aureus* enterotoxins (A, B, C, D and E) and exotoxin (toxic shock syndrome toxin), and it is these same enterotoxins (A and B) that have been implicated in MS (reviewed in [39]). The association of superantigens with MS was initially based on experiments using the EAE model. Immunization of PL/J mice with rat myelin basic protein (MBP) causes acute EAE which resolves without the occurrence of relapses [40]. However, administration of *Staphylococcus aureus* enterotoxins A or B following resolution of EAE in rat MBP-immunized PL/J mice induced reactivation of the disease and multiple administrations resulted in relapses of EAE over an extended period of time [41, 42]. Additionally, superantigens initiated disease in immunized but asymptomatic animals. These results demonstrate that superantigens can reactivate autoreactive T cells and thus may play a role in the development or progression of autoimmune diseases such as MS [41, 42]. A possible mechanism for the development of clinical disease following superantigen administration in asymptomatic and recovered immunized animals is epitope spreading [43]. The pathogenic process of epitope spreading is a cascade of new autoreactivity that occurs during autoimmune-mediated tissue damage that shifts the T cell autoreactivity from the primary

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initiating self-epitope to secondary self-epitopes (reviewed in [44]). Epitope spreading may be intramolecular, to other epitopes within the same self-protein, or intermolecular, to epitopes within other self-proteins within the same affected organ or system. During the course of EAE, and most likely MS, new epitopes are recognized in a consistent, predictable, sequential order and epitope spreading correlates with clinical relapse/progression (reviewed in [44]). In the rat MBP-immunized PL/J mouse model, *Staphylococcus aureus* enterotoxin A administration following resolution of EAE induced intramolecular epitope spreading after EAE reactivation [43].

In addition to the animal experiments described above, a study in MS patients examining the association of bacterial superantigen with MS has recently been carried out [45]. This study examined the potential association of nasal colonization with superantigen-producing *Staphylococcus aureus* and MS exacerbations. Although there were no differences in the numbers of patients that tested positive for *Staphylococcus aureus* colonization between non-MS patients, MS stable patients (no relapse within past six months) and MS exacerbation patients (relapse within 30 days), among the *Staphylococcus aureus* positive patients, a significantly greater number of MS exacerbation patients tested positive for *Staphylococcus aureus* enterotoxin A compared to non-MS patients. Therefore, colonization of the nose with enterotoxin A-producing *Staphylococcus aureus* may play a role in triggering $CD4^+$ T cell activation and MS exacerbations [45].

VIRUSES

Viruses have long been considered to be possible etiological agents of MS. Mechanisms of viral-induced demyelination include: (1) direct lysis of virus-infected oligodendrocytes by the virus, (2) direct lysis of virus-infected oligodendrocytes by the host immune response to the virus, (3) lysis of uninfected oligodendrocytes by a self-reactive immune response triggered by the virus and (4) lysis of oligodendrocytes by a nonspecific bystander immune response triggered by the virus (reviewed in [46]). Both epidemiological and neuropathological studies have been performed as a means of detecting an association between particular viruses and MS. As far back as 1946, rabies virus was the first virus to be considered as having an association with MS (reviewed in [47–50]). Through the years other viruses have also been considered to have an association with MS to include such diverse viruses as: varicella zoster virus, parainfluenza virus type 1, measles virus, simian virus-5, chimpanzee cytomegalovirus, coronavirus, tick-borne encephalitis flavivirus and human T cell lymphotrophic virus type 1, among others (reviewed in [47–50]). Currently, the viruses considered to be most promising as etiological agents of MS include herpes viruses and human endogenous retroviruses (HERVs) (reviewed in [47]).

Herpes Viruses

Herpes viruses are large, enveloped, double-stranded DNA viruses which commonly persist in infected hosts in a latent state (reviewed in [17, 51]). Reactivation of latent virus results in recurrent infections. Two herpes viruses that have been associated with MS are Epstein-Barr virus (EBV), a lymphotropic γ -herpesvirus, and human herpesvirus 6 (HHV-6), a lymphotropic and neurotropic β-herpesvirus (reviewed in [17, 49, 51]).

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Humans are the exclusive natural host for EBV [52]. Evidence for the association of EBV with MS, an exclusively human disease, abounds (reviewed in [17, 48, 49, 51]). EBV, if acquired in early childhood, is asymptomatic, but it can cause acute symptomatic infectious mononucleosis, in approximately half of the infections, if acquired later in life (adolescence or young adulthood) (reviewed in [17, 48, 51]). Studies have shown that a clinical history of infectious mononucleosis is a risk factor for the development of MS; those who have had infectious mononucleosis have a >2-fold increased risk, over controls, of developing MS [53, 54]. In addition to the past history of infectious mononucleosis being an independent risk factor for MS, the co-occurrence of a past history of infectious mononucleosis with the presence of the HLA-DRB1*15 allele, also an independent risk factor for MS, in a given individual was found, using an additive scale, to substantially increase the risk of disease [55]. An extensive review of EBV antibody findings, in serum and CSF, and the detection of EBV DNA, in plasma, peripheral blood mononuclear cells (PBMCs), CSF and brain, in MS patients versus controls has been presented elsewhere [49], therefore, a brief summary of the pertinent points suggesting an association between EBV infection and MS will follow. Oligoclonal bands in the CSF of MS patients have been shown to be specific for two EBV proteins, one of which is EBV nuclear antigen (EBNA)-1 [56]. Also, abnormal accumulations of EBV-infected B/plasma cells in the cerebral meninges of the brain appears to be a common occurrence specific for MS and the presence of these EBV-infected B/plasma cells and/or an immune response toward these EBV-infected B/plasma cells may play a role in the development and/or exacerbation of MS [57]. A longitudinal study investigating the presence of EBV serum antibodies prior to the onset of MS determined that there was an age-dependent increase in the anti-EBNA complex IgG serum titer prior to disease onset; the titer was significantly higher at the age of 25 years and above in subjects who went on to develop MS compare to those who did not [58]. In addition to the antibody findings and the detection of DNA, EBNA-1-specific $CD4^+$ T cells were also isolated at a higher frequency from MS patients than from healthy EBV carriers and these cells had increased cytokine production and proliferative capacity [59]. Therefore, the immune response to EBV, and in particular EBNA-1, may play a role in MS. A possible mechanism for the development of clinical disease following EBV infection is molecular mimicry in that EBV-reactive $CD4^+$ T cells that have been isolated from the CSF of MS patients have been shown to cross-react with MBP [60]. Molecular mimicry could be the effective mechanism that bridges the gap between an infectious etiology and a resultant disease that demonstrates autoimmune pathology [52]. Finally, in addition to a role in the development of disease, another recent study demonstrated that EBV epidemics may be associated with disease exacerbations [61]. Using retrospective data for the years 1986–1995 and comparing the monthly occurrence of MS relapses among 407 MS patients to the occurrence of viral infection epidemics among a general population of 1million people in Southwestern Finland, it was demonstrated that a significant increase in MS relapse counts followed EBV epidemics [61].

Evidence for the association of HHV-6 with MS is also extensive (reviewed in [17, 49, 51]). Of the two subtypes of this virus, HHV-6A and HHV-6B, HHV-6A is the subtype primarily associated with MS [62]. As for EBV, an extensive review of HHV-6 antibody findings, in serum and CSF, and the detection of HHV-6 DNA, in serum, PBMCs, CSF and brain, in MS

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patients versus controls has been presented elsewhere [49], therefore, again, a brief summary of the pertinent points suggesting an association between HHV-6 infection and MS will follow. Oligoclonal bands in the CSF of MS patients have been shown to be specific for HHV-6A in 20% of MS patients [62, 63]. The detection of HHV-6 DNA in the brains of MS subjects in conjunction with the detection of HHV-6 antigen (in oligodendrocytes present in lesions) is suggestive of an active viral infection, whereas the detection of HHV-6 DNA in the brains of control subjects in the absence of HHV-6 antigen is indicative of a latent infection ([64]; reviewed in [65]). As with EBV, a possible mechanism for the development of clinical disease following HHV-6A infection is also molecular mimicry in that HHV-6-reactive $CD4^+$ T cells that have been isolated from MS patients have been shown to cross-react with MBP [66, 67]. In addition, increased levels of HHV-6 DNA in the serum, and particularly in PBMCs, was also found to correlate with exacerbations of RR-MS, suggesting that an active HHV-6 infection may contribute to exacerbations of RR-MS [68]. Finally, a recent prospective cohort study found a possible role for HHV-6, or the immune response to HHV-6, in both relapses and progressive disease [69]. First, a strong positive association was found between the serumanti-HHV-6 IgG titer at baseline (study entry) and the risk of subsequent relapse in RR-MS subjects. RR-MS subjects who reported a relapse during the study had a significantly higher anti-HHV-6 IgG baseline titer (1.7 times higher) than those subjects who did not report a relapse. Second, female progressive-course (SP-MS and PP-MS) subjects had significantly higher anti-HHV-6 IgG titers (2.8 times higher) at baseline than male progressive-course subjects. The absence of similar trends for anti-EBV IgG titers argues against the presence of nonspecific immune responses and supports the role for a specific anti-HHV-6 immune response in both relapses and progression [69].

HERVs

HERVs, or retroelements, are the result of the incorporation of human exogenous retroviral DNA into the genome of germ cells, resulting in Mendelian inheritance of the HERV and the loss of viral replication in most loci due to the accumulation of mutations (reviewed in [47, 48, 70–74]). HERVs, which are widely distributed in many copies throughout the genome and which can be grouped into numerous HERV families, comprise approximately 8% of every human genome ([75]; reviewed in [71]). Although replication incompetent, HERVs can express some of their genes as proteins, such as the capsid (gag), polymerase (pol) and envelope (env) proteins, and can demonstrate reverse transcriptase activity and release non-infectious viral particles (reviewed in [47, 48, 72, 74]). Arguments have been made for the association of HERVs with the development of MS. The founding member of the HERV-W family, the MS-associated retroviral agent (MSRV), has been repeatedly isolated from the CSF and blood of MS patients [76]. Isolated MSRV virions and the MSRV envelope protein were found to trigger an abnormal immune response characteristic of superantigens, producing polyclonal T cell activation [77]. MSRV envelope RNA expression and MSRV DNA copy numbers in PBMCs were significantly higher in MS patients, compared to healthy controls, and the DNA copy numbers were significantly higher in progressive MS (SP-MS and PP-MS), compared to RR-MS [78]. MSRV DNA copy numbers in PBMCs were also significantly higher in female MS patients than in male MS patients and the MSRV load correlated with clinical disease score [79]. In addition, HERV-W envelope antigen was detected in the serum in 79% of MS patients [78]. Indeed, it

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was found that peripheral blood B cells and monocytes from patients with active MS had significantly higher surface expression of HERV-Hand -W envelope proteins, compared to patients with stable MS and controls [80]. Also, significantly elevated specific antibody reactivity to the HERV-H and -W envelope proteins was found in the majority (>65%) of MS patient sera and the higher antibody levels correlated with higher MS disease activity [80, 81]. Immunohistology by several groups demonstrated the presence of the HERV-W envelope protein in infiltrated perivascular macrophages and activated microglia in early active MS lesions, using three anti-HERV-W envelope-specific monoclonal antibodies [78], as well as in astrocytes in acute MS lesions, using an antibody specific for syncytin-1, a HERV-W envelope glycoprotein specifically encoded by the replication-incompetent chromosome 7q21 copy of HERV-W [82]. Syncytin-1, which shares 88% homology with the MSRV envelope protein sequence (reviewed in [47, 74]), can be induced by the proinflammatory cytokine tumor necrosis factorα and may cause neuroinflammation and oligodendrocyte injury leading to neurodegeneration via both direct and indirect mechanisms explored in depth elsewhere ([82]; reviewed in [74]). Interestingly, by treating HERVs as inheritable loci and by analyzing DNA single nucleotide polymorphisms (SNPs) near HERV loci, a highly significant association with MS was detected for HERV-Fc1, which is located on the X chromosome, is closely related to HERV-H and for which the *gag* and *env* genes appear intact [83]. Further study showed that the extracellular HERV-Fc1 RNA (*gag* sequence specific) viral load in plasma was 4-fold higher in MS patients with active MS, compared to healthy controls [84]. Additionally, a genome-wide association study examining SNPs in PP-MS patients, compared to healthy controls, found suggestive evidence of an association with a HERV element located on chromosome 7 which belongs to the HERV16 family [85]. Taken together, these results support an association of HERVs, possibly from various HERV families, with the development and/or progression of MS, and the association of HERVs with MS could provide a genetic rationale for the gender bias observed in MS due to the presence of multiple HERV copies, to include multiple copies of HERV-W, on the X chromosome (reviewed in [70, 72]).

PROTOZOA

Acanthamoeba castellanii is a free-living, protozoan frequently found in soil, dust and fresh water [86]. This ameba infects humans causing diseases such as amebic keratitis, cutaneous amebiasis and, in chronically ill or immunocompromized individuals, *Acanthamoeba* granulomatous encephalitis, a chronic progressive disease of the CNS resulting from the presence of infectious organisms in the brain [86]. Although *Acanthamoeba castellanii* has never been directly associated with MS in humans, two peptides from this organism have been demonstrated to induce EAE [86, 87]. One novel peptide, termed $ACA_{83–95}$, which corresponds to those amino acids within the *Acanthamoeba castellanii* rhodanese-related sulfurtransferase protein, induced clinical and histological signs of EAE in SJL/J mice by both active immunization with peptide and through adoptive transfer of T cells generated in response to $ACA_{83–95}$ [86]. This peptide was shown to be a disease-producing microbial mimic of the myelin proteolipid protein (PLP) peptide, $PLP_{139–151}$, capable of stimulating the expansion of $PLP_{139–151}$-reactive $CD4^+$ T cells, through cross-reactivity, and inducing CNS autoimmunity [86]. A second novel peptide derived from the amebic nicotinamide

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adenine dinucleotide dehydrogenase subunit 2 (NAD) protein, $NAD_{108–120}$, again induced EAE in SJL/J mice through both active immunization and adoptive transfer [87]. This peptide was shown to be a disease-producing microbial mimic of the MBP peptide, $MBP_{89–101}$, capable of stimulating the expansion of $MBP_{89–101}$-reactive $CD4^+$ T cells through cross-reactivity [87]. In the case of both peptides, it was found that the stimulated cross-reactive $CD4^+$ T cells produced Th1 (IL-2 and IFN-γ) and Th17 (IL-17A, IL-17F, IL-22) cytokines that favor CNS autoimmunity [86, 87]. In addition, through examination of the peptide sequence and homology modeling, it was proposed that the $NAD_{108–120}$ peptide, which contained the three amino acid sequence critical for MBP-specific auto-antibody recognition, could bind to and activate MBP-specific B cells [87]. Therefore, of the three candidate autoantigens identified in MS – PLP, MBP and myelin oligodendrocyte glycoprotein – for which MS patients demonstrate varying degrees of T cell and antibody reactivity ([88, 89]; reviewed in [90, 91]), *Acanthamoeba castellanii* has been found to contain microbial mimics for two of these myelin antigens potentially resulting in the induction of multiple myelin-reactive T cells and possibly B cells [87].

PARASITES

As described earlier, infection with some parasites may protect against MS. Helminths are worm-like extracellular parasites, to include flukes, tapeworms and roundworms, that infect humans [92]. Helminths often infect immunocompetent hosts and these infections are often long-lived and asymptomatic [93]. The effects of helminthic infection on EAE have been examined [94–96]. Studies found that both the incidence and the severity of actively induced EAE in mice, as well as the amount of cellular infiltration into the CNS, were reduced and the onset of disease was delayed by either prior infection with live *Schistosoma mansoni* or prior exposure to *Schistosoma mansoni* ova (eggs) [94, 95]. Exposure to this helminth was associated with a decrease in IFN-γ and IL-12 and an increase in IL-10 and transforming growth factor (TGF)-β [94, 95]. Another study found that infection with the live helminthic parasite *Fasciola hepatica* also reduced the severity and delayed the onset of actively induced EAE in mice [96]. Protection was associated with regulatory cell-mediated bystander suppression of self-antigen-specific Th1 (IFN-γ) and Th17 (IL-17) responses which could be abrogated *in vivo* via neutralization of TGF-β [96].

In humans, studies have been carried out examining the association of parasitic exposure with the occurrence of MS. One study found that the prevalence of MS dropped precipitously once a 10% critical threshold of infection with *Trichuris trichiura*, a common human helminth with world-wide distribution, was reached and exceeded [97]. Another study found that a highly significant reduction in intestinal parasitic infections between 1978 and 1994 (70% to 8% in the 5–15 age group) in Martinique, part of the French West Indies, could possibly be associated with the emergence of MS in the nonmigrant population of this island [98]. Both of these findings support the hygiene hypothesis.

The effect of chronic helminthic infection on the clinical and radiological disease activities in MS patients has been recently studied ([99–101]; reviewed in [102]). Those MS patients with helminthic infections (*Hymenolepis nana*, *Trichuris trichiura*, *Ascaris lumbricoides*, *Strongyloides stercolaris*, *Enterobius vermicularis*) were found to have significantly fewer

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exacerbations, significantly lower magnetic resonance imaging (MRI) activity (newor enlargingT2 lesions; number of gadolinium-enhancing lesions) and minimal changes in disability score (EDSS – extended disability status scale), compared to MS patients without helminthic infection [99]. Upon examination of the immune responses in these subjects, it was found that the MBP-specific responses in MS patients with helminthic infections were characterized by increased IL-10 and TGF-β and decreased IL-12 and IFN-γ secreting cells, compared to MS patients without helminthic infection. There was also an increase in $CD4^+CD25^+FoxP3^+$ regulatory T cell development in MS patients with helminthic infections, compared to MS patients without helminthic infection [99]. B cells isolated from MS patients with helminthic infections were found to secrete high level of IL-10, brain-derived neurotrophic factor and nerve growth factor, compared to MS patients without helminthic infection [100]. Intervention studies with anti-helminthic therapy resulted in a significant increase in the number of exacerbations, the EDSS and the MRI activity, compared to untreated controls [101]. Anti-helminthic therapy also resulted in a significant decrease in IL-10 and TGF-β secreting cells, an increase in IL-12 and IFN-γ secreting cells and a decrease in $CD4^+CD25^+FoxP3^+$ regulatory T cell development, compared to untreated controls. This study, by providing evidence for a direct suppressive role of helminthic infections on the autoimmune response, thus establishes a direct link between parasites and the autoimmune response observed in MS patients with helminthic infections [101]. The characteristic induction of a Th2 response profile, in conjunction with diminished Th1 responses, along with the induction of regulatory T and B cells, upon infection with extracellular parasites is likely responsible for the parasite-driven autoimmune downregulation secondary to parasite infection in MS patients [99, 100].

The possibility that chronic helminthic infection may improve the course of MS has been recently examined in a small, short Phase I clinical trial [103]. MRI activity was compared with newly diagnosed, treatment-naive RR-MS patients at baseline, at the end of probiotic (live, nonpathogenic microbes incorporated into food) treatment with 2500 *Trichuris suis* ova every two weeks for three months and at two months after discontinuation of treatment. The number of new gadolinium-enhancing MRI lesions decreased by 70% at the end of the three-month treatment, compared to baseline, however, the numbers returned to baseline by the two-month posttreatment follow-up. In addition, most of the subjects developed increased serum levels of IL-4 and IL-10 at the end of the three-month treatment, compared to baseline. Therefore, although the results from this clinical trial appear promising, further studies are warranted to better assess the efficacy and safety of this treatment [103].

POTENTIAL THERAPEUTICS

Treatments currently available for MS (IFN-β, glatiramer acetate, monoclonal antibodies) are immunomodulatory: anti-inflammatory and immunosuppressive (reviewed in [26, 104]). They are capable of slowing the self-reactive immune process that underlies the disease. However, none of the available drugs either targets possible underlying initiating or potentiating pathogens or promotes remyelination. One drug that may meet these needs is intravenous Ig (IVIg) (reviewed in [104]). IVIg is prepared from pooled blood plasma of healthy individuals and it contains polyclonal antibodies against ubiquitous infectious agents [105, 106]. IVIg preparations of human origin may contain superantigen-neutralizing

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antibodies as it has been shown to reduce the toxin activity of *Staphylococcus aureus* enterotoxin A *in vitro* [105]. Additionally, pooled Ig of mouse origin has been shown to be effective in enhancing remyelination in the Theiler's murine encephalomyelitis virus model of demyelination [107]. Also, human IVIg has been shown to be effective as a prophylactic treatment in reducing the clinical symptoms and underlying pathology of EAE induced with spinal cord homogenate in rats ([108]; reviewed in [106]). Finally, IVIg has been the subject of multiple clinical trials for the treatment of MS patients (reviewed in [104, 106, 109, 110]). A reduction in the relapse-rate and/or lesion activity detected with MRI was seen in clinical studies involving RR-MS patients (reviewed in [104, 106, 109, 110]). Therefore, IVIg, with its multiple and overlapping anti-inflammatory and immunomodulatory mechanisms of action at both the cellular and humoral levels, may be found to be an effective treatment for MS once effective dose and dosing intervals have been established or in combination with other existing drug therapies for MS (reviewed in [106, 110]).

CONCLUSIONS

Although many infectious agents have been explored as possible etiological agents of MS, one must keep in mind that the mere association of a pathogen with a disease does not constitute causality [47, 49]. Indeed, most of the infectious agents that have shown association with MS over the years have failed to withstand further scrutiny [49, 52]. Additionally, most of the infectious agents that are putatively associated with MS are ubiquitous and infect most humans at some point in their lives [111]. With regards to the infectious agents discussed above, it was found that, in one study, >50% of the MS patients showed serological evidence of prior infection with *Mycoplasma pneumoniae* [31]. Also, *Chlamydia pneumoniae* antibodies occur in 70% of the adult population [112]. The seroprevalence to EBV is 90–95% in the general population and 100% in adults with MS ([113]; reviewed in [48, 51]). In addition, HHV-6 seroprevalence is >80% by age 2, although this is forHHV-6B; the seroprevalence to HHV-6A is unknown [49]. As discussed above, all humans carry HERVs as an integral part of their genome (reviewed in [47, 48]). Surveillance studies have shown that persistent *Staphylococcus aureus* nasal colonization occurs in 20% of the general healthy human population [114]. The seroprevalence to the protozoa, *Acanthamoeba castellanii*, has been found to be >85% in healthy individuals from diverse racial and ethnic backgrounds [115]. Finally, 24% of the world's population is infected with soil-transmitted helminths [116]. Therefore, to date, no single pathogen has been accepted as the causal agent of MS [47, 49]. Considering the heterogeneous phenotypes (RR, SP, PP) of the disease, the number of genetic risk factors (HLA, non-HLA) for the disease and other environmental influences [geographic (latitude-dependence, UV exposure), socio-economic, nutrition (vitamin D depletion, dairy product consumption), lifestyle (smoking tobacco)] that may have an effect on the disease, it is very difficult to identify any single infectious pathogen as the etiological agent of MS [47]. Indeed, two-hit theories for the induction of autoimmune diseases have been proposed. The ability of superantigens to induce disease and relapses in asymptomatic and recovered immunized animals, respectively, suggests that superantigens could be the second hit in a two-hit model [41]. The fertile field hypothesis was also proposed as a means of explaining why it has proven impossible to identify any virus as the cause of any human autoimmune disease,

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including MS [117]. This hypothesis proposes that an induction phase occurs early in life when any one of several different viral infections induces or expands, through either molecular mimicry or bystander activation, autoreactive T cell to a subclinical disease level (fertile field). Much later in life the transition to autoaggression and disease occurs following a new infection with any of several different pathogens which are not required to encode cross-reactive antigens. It is also possible that multiple subsequent infections are needed to induce the transition to autoaggression and disease [117]. Finally, evidence supports the possibility that interactions between multiple viruses may play a role in MS. A recent study examining the association of dual-infection with EBV types 1 and 2 with MS demonstrated that, among EBV-positive MS and control subjects, significantly more MS patients were dual-infected with EBV types 1 and 2 (90%) compared to controls (37.4%); whereas, again among positive subjects, more controls were singly infected with either EBV type 1 (controls: 32.5% versus MS: 8.6%) or EBV type 2 (controls: 30.1% versus MS: 1.4%) [118]. Other viral interactions that may play a role in MS are interactions between herpes viruses and HERVs. A study examining the levels of reverse transcriptase activity, an indicator of HERV activation, induced by herpes antigens showed that HHV-6 antigens induced significantly higher reverse transcriptase activity, and therefore HERV activation, in cells isolated from MS patients than healthy controls, independently of herpes virus replication [119]. Another study showed that EBV infection of astrocytes or exposure of astrocytes, blood derived B cells and monocytes or differentiated macrophages to the gp350 protein of EBV caused a significant increase in MSRV *env* and syncytic-1 transcription, suggesting that HERV-W activation can be induced by EBV in these cell types [120]. Finally, the allelic genotype of K18.3 of the HERV-K18 *env* gene, which encodes a superantigen that can be transactivated by EBV and HHV-6 [121, 122], was found to be significantly associated with the risk of developing MS [121]. Therefore, EBV and HHV-6 might activate HERVs which than may be involved in the pathogenesis of MS (reviewed in [70, 72]). HERVs could essentially form the bridge between genetic predisposition and environmental factors (reviewed in [71, 72]).

Additionally, although an infectious pathogen may not be causative, it may still influence the course of disease by potentiating the disease [29]. Disease potentiation could occur indirectly through nonspecific stimulation of immune responses, resulting in disease relapse or progression, or directly through pathogen-specific macrophage activation and cellular damage, resulting in additional damage at the sites of active lesions [29].

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TABLE 1

Summary of reported associations between pathogens and MS.

Pathogen type	Pathogen	Animal models	Refs.	MS patients	Refs.
Bacteria	*Mycoplasma pneumoniae*			(−) Failure to detect mycoplasma DNA	[27, 30]
				(+) Higher pathogen-specific serum IgG levels in females	[31]
	Chlamydia pneumoniae	(+) Systemic infection increased severity of EAE	[37]	(−) Failure to detect mycoplasma DNA	[30]
				(+) Pathogen grown from and DNA detected in CSF	[32]
				(+) Elevated levels of pathogen-specific CSF antibodies	[32]
				(+) Pathogen-specific oligoclonal bands	[33]
				(+) Meta-analysis association between detection of pathogen DNA and MS	[34]
				(+) Higher pathogen-specific serum IgM levels in pediatric MS	[35]
	Streptococcus pneumoniae	(+) Systemic infection increased severity of EAE	[38]		
Bacterial Superantigens	*Staphylococcus aureus* enterotoxins (A and B)	(+) Administration causes relapses of EAE	[41, 42]	(+) Higher positivity for enterotoxin A-producing pathogen in exacerbation patients	[45]
		(+) Administration-initiated EAE in immunized asymptomatic animals	[41, 42]		
Viruses	*Herpes viruses*			(+) Infectious mononucleosis is risk factor	[53, 54]
	Epstein–Barr virus			(+) Co-occurrence of infectious mononucleosis and the HLA-DRB1*15 allele increases risk	[55]
				(+) Pathogen-specific oligoclonal bands	[56]
				(+) Pathogen-infected B/plasma cells in the cerebral meninges	[57]
				(+) Age-dependent increase in pathogen-specific serum IgG levels prior to disease	[58]
				(+) Higher pathogen-specific $CD4^+$ T cells	[59]
				(+) Pathogen-specific $CD4^+$ T cells cross-react with MBP	[60]
				(+) Epidemics associated with exacerbation	[61]
	human herpesvirus 6			(+) Pathogen-specific oligoclonal bands	[62, 63]
				(+) Pathogen-specific $CD4^+$ T cells cross-react with MBP	[66, 67]
				(+) Correlation between serum pathogen DNA and exacerbations	[68]
				(+) Association between pathogen-specific serum IgG levels and risk of subsequent relapse	[69]

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Pathogen type	Pathogen	Animal models	Refs.	MS patients	Refs.
	human endogenous retroviruses (HERV)			(+) Repeated isolation of MSRV	[76]
				(+) Higher pathogen RNA expression and DNA copy number	[78]
				(+) MSRV load correlated with clinical disease score	[79]
				(+) Pathogen antigen detected in serum	[78]
				(+) Higher blood cell surface expression of pathogen proteins in active MS	[80]
				(+) Higher pathogen-specific serum antibody reactivity correlated with disease activity	[80, 81]
				(+) Pathogen antigen detected in active lesions	[78, 82]
				(+) Significant association between MS and HERV-Fc1	[83]
				(+) Higher plasma HERV-Fc1 RNA viral load	[84]
				(+) Genome-wide association study association with HERV16 family member	[85]
Protozoa	*Acanthamoeba castellanii*	(+) Two peptides induce EAE	[86, 87]		
Parasites	Helminths *Schistosoma mansoni*	(−) Exposure delayed onset and reduced the incidence and severity of EAE and the amount of cellular infiltration into the CNS	[94, 95]		
	Fasciola hepatica	(−) Exposure delayed onset and reduced the severity of EAE	[96]		
	Trichuris trichiura, {Trichuris trichiura, Hymenolepis nana, Ascaris lumbricoides, Strongyloides stercolaris, Enterobius vermicularis} Trichuris suis			(−) Prevalence of MS dropped with ≥10% threshold of infection	[97]
				(−) MS patients with infections had fewer exacerbations, lower MRI activity and minimal changes in disability score	[99]
				(−) Anti-helminthic therapy caused more exacerbations, higher MRI activity and increased disability score	[101]
				(−) Probiotic treatment with ova reduced new MRI lesions	[103]

(−) indicates negative association between pathogen and MS.
(+) indicated positive association between pathogen and MS.

MS, multiple sclerosis; Ig, immunoglobulin; EAE, experimental autoimmune encephalomyelitis; CSF, cerebrospinal fluid; MBP, myelin basic protein; MSRV, MS-associated retroviral agent; CNS, central nervous system; MRI, magnetic resonance imaging.

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