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Review Article

Multiple sclerosis and environmental factors: the role of vitamin D, parasites, and Epstein–Barr virus infection

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Pathogenic mechanisms underlying multiple sclerosis development have yet to be clearly identified, but considerable evidence indicates that autoimmunity plays an important role in the etiology of the disease. It is generally accepted that autoimmune diseases like MS arise from complex interactions between genetic susceptibility and environmental factors. Although environmental factors unequivocally influencing MS development have yet to be established, accumulating evidence singles out several candidates, including sunlight–UV exposure or vitamin D deficiency, viral infections, hygiene, and cigarette smoking. Vitamin D deficiency has been associated with different autoimmune diseases. Several investigations indicate 125 $(OH)_2$ vitamin D plays a critical role in shaping T-cell response and inducing T cells with immunosuppressive properties. Likewise, helminth infections represent another potential environmental factor exerting immunomodulatory properties. Both epidemiological and experimental data provide evidence to support autoimmune down-regulation secondary to parasite infections in patients with MS, through regulatory T- and B-cell action, with effects extending beyond simple response to an infectious agent. Finally, different epidemiological studies have demonstrated that Epstein–Barr virus infection confers added risk of developing MS. Proposed mechanisms responsible for this association include activation and expansion of self-reactive T and B cells, lower threshold for self-tolerance breakdown, and enhanced autoreactive B-cell survival, all to be discussed in this review. Understanding environmental factors influencing propensity to MS will lead to new and more effective approaches to prevent and treat the disease.

J. Correale, M. I. Gaitán

Department of Neurology, Institute for Neurological Research Dr. Raúl Carrea, FLENI, Buenos Aires, Argentina

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J. Correale, Raúl Carrea Institute for Neurological Research, FLENI, Montañeses 2325, (1428) Buenos Aires, Argentina
Tel.: 54 11 5777 3200, ext.: 2704/2706/2708
Fax: 54 11 5777 3209
e-mails: jcorreale@fleni.org.ar; jorge.correale@gmail.com

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Introduction

Multiple sclerosis (MS) is an inflammatory disorder causing demyelination and axon injury within the central nervous system (CNS). Pathogenic mechanisms underlying its development have yet to be clearly identified, but considerable evidence indicates that autoimmunity plays an important role in MS etiology (1). It is generally accepted that autoimmune diseases like MS arise from complex interactions between individual genetic susceptibility and environmental factors. Several genomewide association studies (GWASs) have been performed to discover which genetic factors generate susceptibility. In Caucasian populations, the strongest genetic association with MS has been with MHC haplotypes, especially those containing HLA-DRB1*15.01. As a result of this known link, other GWASs have identified several different associations, many acting as cooperative networks. Individual genes, however, appear to contribute little to overall risk, and MHC remains the key susceptibility locus for MS (2, 3). Discordance observed between monozygotic twins also suggests that additional factors such as environmental modulators are likely to be involved (4).

One of the most striking illustrations of the importance of the environment in MS pathogenesis results from the particular geographical distribution of the disease. Prevalence rates are increased in high-latitude regions, and uncommon near the equator. Additionally, population migration studies indicate that individuals moving from areas of low to areas of high risk, particularly before the age of 15, show similar incidence to host country populations, suggesting the presence of either a protective factor in the region of origin or, alternatively, a harmful factor in the adopted region (5). Space–time cluster analyses performed both in Norway and in Sardinia have shown clustering between 13 and 20, or 1 and 3 years of age, respectively (6, 7). The hypothesis analyzed in these studies suggests that if disease prevalence in individuals living close to one another during the same time period is higher than expected, this may have resulted from exposure to putative common environmental risk factor(s) prior to disease onset, considering the cause to be probably an infection acquired either in adolescence or in early childhood, by individuals not protected by previous infection, depending on population-specific susceptibility. Furthermore, serial cross-sectional comparisons of MS epidemiology from various continents provide compelling evidence for a significant rise in MS incidence and prevalence in recent decades (8). Given the short duration over which these population changes have occurred, genetic factors seem an unlikely cause, whereas epidemiological studies appear to indicate MS risk is influenced by the environment. Identifying these environmental factors and elucidating how they increase autoimmune disease risk would help develop new strategies to treat the disease. Candidates likely to be responsible for this effect, alone or in combination, include sunlight–UV exposure/or vitamin D deficiency most notably, as well as viral infections, hygiene, and cigarette smoking (5, 9–11), which may not only influence disease onset at any time in the life of an individual, but also affect relapse rates in patients presenting relapsing–remitting forms of MS.

This review examines how vitamin D deficiency as well as parasitic and Epstein–Barr virus (EBV) infections influences MS development and immune responses in particular.

Vitamin D deficiency. Its impact on the immunological system

It has by now been well established that the physiological importance of vitamin D status extends far beyond calcium homeostasis regulation. Among its manifold functions, vitamin D plays an important role in immune function regulation and reduction of inflammation. Accordingly, several autoimmune diseases including rheumatoid arthritis, LED, type I diabetes, inflammatory bowel disease and MS have been associated with vitamin D deficiency (12, 13). A protective effect of vitamin D on MS is supported by the reduced risk associated with sunlight exposure and use of vitamin D supplements (14–16). MS risk is 40% lower among women reporting regular intake of at least 400 IU/day of vitamin D supplements (15). Moreover, high circulating levels of vitamin D have been associated with lower risk of MS, and recent studies in patients with MS found that higher serum 25-hydroxy-vitamin D (25 (OH)D) levels (>40 ng/ml) correlated with fewer MRI lesions and relapses (17, 18). Furthermore, serum concentrations of 25(OH)D are lower during MS relapses than during remission (19) and correlate inversely with disease severity (20). Importantly, 25(OH)D levels between 30 and 100 ng/ml were considered 'normal', when established as basic for bone health (e.g., suppression of PTH levels), without taking into account 'extracalcemic' vitamin D action, although there is no clear evidence to indicate whether higher or lower values within this range are optimal for all vitamin D actions.

Genetic data also point to the influence of vitamin D on immune regulation. The CYP27B1 gene encodes the enzyme converting 25(OH)D to biologically active calcitriol. Non-functional variants of this gene causing type I vitamin D-dependent rickets in homozygous subjects have been identified and associated with significantly increased risk of MS (21). Likewise, MS risk is increased in heterozygous carriers of mutations in this gene. Vitamin D also specifically interacts with HLA-DRB1*1501, the stronger genetic risk factor in MS, inducing its expression (22).

These findings overlap with experimental evidence indicating a protective effect of vitamin D in animal models of MS. Experimental autoimmune encephalomyelitis (EAE), an animal model of MS, can be prevented by administering the active metabolite 1,25 $(OH)_2$ vitamin D prior to immunization and ameliorated when given after disease onset (23, 24). Conversely, vitamin D deficiency decreases time to EAE onset and increases severity (24). These evidences support vitamin D immune modulation.

We demonstrated that relapsing–remitting patients with MS (RR MS) have reduced serum levels of 25(OH)D, and 1,25 $(OH)_2$ vitamin D compared to healthy subjects, particularly during exacerbations, suggesting vitamin D-dependent T-cell regulation may play an important role in maintaining T-cell homeostasis in this group of

patients. Conversely, in primary progressive patients with MS, serum levels of 25(OH)D and 1,25 $(OH)_2$ vitamin D are similar to those observed in healthy individuals (19). An explanation for this difference between patient groups has yet to be found.

1,25 $(OH)_2$ vitamin D effects are mediated primarily through interaction with the intracellular vitamin D receptor (VDR) (25), although recent reports have highlighted rapid non-genomic effects of 1,25 $(OH)_2$ vitamin D (26). VDR is present in different tissues including immune system cells such as monocytes, DCs, and B cells (27–29). We demonstrated that VDR mRNA is constitutively expressed in CD4+ T cells and up-regulated following activation and 1,25 $(OH)_2$ vitamin D exposure (19). Allelic variations within the VDR gene might influence vitamin D effects on the immune system, affecting MS risk directly, or modifying vitamin D effects. However, little is known about the role of vitamin D-related genes, or specific genetic interactions with vitamin D in determining MS risk, effects which have been described for other autoimmune endocrine diseases (30). Similarly, 25(OH)D 1-α hydroxylase, found mainly in renal tubule cells, has recently been described in a wide variety of tissues (31), and although the enzyme present in immune cells is identical to the renal form, its regulation seems to be under a different control system, mediated mainly by immune signals, such as antigen stimulators or inflammatory mediators like IFN-γ (32, 33). Interestingly, 1α-hydroxylase mRNA is also expressed in resting CD4+ T cells at low levels, an expression which increases significantly after stimulation, but not following 1,25 $(OH)_2$ vitamin D exposure. Notably, vitamin D can be metabolized to 1,25 $(OH)_2$ vitamin D by CD4+ T cells, generating local production of active vitamin D, as has been previously demonstrated in macrophages, DCs, and thyroid tissue.

Activation of the VDR is known to alter transcription, proliferation, and differentiation of immune cells (25, 34, 35). Activated vitamin D has different immunoregulatory effects on CD4+ T cells. Namely it (i) inhibits CD4+ T-cell proliferation, (ii) enhances IL-10-secreting cell numbers, and inhibits IL-6 and IL-17-producing cells and (iii) induces CD4+CD25+ FoxP3+ regulatory T cells through an indoleamine 2,3-dioxygenase (IDO)-mediated pathway (19).

Inhibition of cell proliferation by 1,25 $(OH)_2$ vitamin D has been reported in lymphocytes and in different cancer cell lines and linked to different mechanisms, such as inducing gene transcription of cell cycle inhibitors p21 and p27, which can in turn inhibit cell cycle progression (29, 36). Alternatively, 1,25 $(OH)_2$ vitamin D may help limit uncontrolled proliferation, acting directly on the VDR in T lymphocytes, enhancing their responsiveness to apoptotic signals (37). APCs and in particular DCs are key targets of 1,25 $(OH)_2$ vitamin D both *in vitro* and *in vivo*. *In vitro* differentiation of DCs from monocytes or bone marrow-derived precursors is inhibited by 1,25$(OH)_2$ vitamin D (38). Moreover, the Ag-presenting function of monocytes and DCs is profoundly inhibited by down-regulation of the co-stimulatory molecules: CD40, CD80, and CD86. This in turn markedly decreases IL-12 and enhances IL-10 production, thus favoring induction of DCs with tolerogenic properties (38, 39). These effects can be reproduced in models of allograft rejection, in which tolerogenic DCs induced by short treatments with 1,25$(OH)_2$ vitamin D enhance CD4+ CD25+ regulatory T-cell numbers, mediating transplantation tolerance (40) and inhibiting recurrence of autoimmune disease (41).

Although 1,25 $(OH)_2$ vitamin D modulates DC function as described, shaping T-cell activation and development, it can also have direct effect on T cells. Active vitamin D is known to inhibit different transcription factors involved in cytokine gene regulation (42). CD4+ T cells from patients with MS cultured in the presence of 1,25 $(OH)_2$ vitamin D enhance IL-10-producing cell numbers (19). Notably, IL-10 is a positive autocrine factor that acts directly on T cells, exerting synergistic action on signaling pathways induced by 1,25 $(OH)_2$ vitamin D (19). These observations are supported by experiments in the EAE model, where 1,25 $(OH)_2$ vitamin D significantly inhibits disease development in wild-type animals, but not in IL-10 or IL-10 receptor knockout strains, indicating that the IL-10/IL-10 receptor pathway is essential for 1,25 $(OH)_2$ vitamin D-mediated EAE inhibition (43). Aside from up-regulating IL-10 production, vitamin D induces significant down-regulation of IL-6, IL-12, IL-17, and IFN-γ. Investigators have found that TGF-β and IL-6 are critical factors for Th17 development. These results are in agreement with the known inhibition of IL-17 production by VDR agonists during the course of experimental autoimmune prostatitis (44). Overall, these findings support the notion that 1,25 $(OH)_2$ vitamin D plays an important role in shaping T-cell responses, inducing T cells with immunosuppressive properties. These effects are partly attributable to direct targeting of T cells, but modulation of DC function also plays an important role in directing T-cell responses.

Another important effect of 1,25 $(OH)_2$ vitamin D is to trigger induction of T cells with regulatory properties (25). Regulatory T cells are induced by modulation of APCs, particularly DCs (45). CD4+CD25+FoxP3+ percentage is significantly increased when PBMCs are cultured in the presence of 1,25 $(OH)_2$ vitamin D. Our group has demonstrated that this process is mediated by an IDO-dependent pathway, explained through different mechanisms: (i) expansion of CD4+CD25+FoxP3+ cells, (ii) increased apoptosis of CD4+CD25− over CD4+CD25+ T cells, and (iii) conversion of CD4+CD25− into CD4+CD25+ T cells (19). 1,25 $(OH)_2$ vitamin D not only favors CD4+CD25+FoxP3+ regulatory T-cell induction, but can also promote recruitment at inflammatory sites, a process mediated by increased DC production of the CCR4 agonist, CCL22 (46, 47).

Alongside this overall pattern of broad autoimmune response suppression, vitamin D has also been shown to decrease plasma cell conversion and antibody production by B cells. It should be noted, however, that most of these *in vitro* effects were observed at pharmacological vitamin D dosing. Further randomized clinical trials should be performed in patients with MS administered vitamin D supplements, to better characterize immunological changes under these conditions.

We recently demonstrated that immunomodulatory effects of 1,25 $(OH)_2$ vitamin D are significantly stronger in females than in males (48). Parallel to these findings, female subjects have fewer CYP24A1 transcripts coding the 1,24 dihydroxy vitamin D-inactivating enzyme, as well as greater binding and internalization of vitamin D-binding protein (a transporter for vitamin D and its metabolites). These gender-based disparities lead to accumulation of vitamin D and its metabolites in target cells in female subjects, resulting in more potent anti-inflammatory effect. Interestingly, 17-β estradiol reproduces these actions on self-reactive T cells from male subjects, suggesting functional synergy between 1,25 $(OH)_2$ vitamin D and 17-β estradiol, mediated through estrogen receptor α. These findings converge with experimental evidence indicating that 1,25 $(OH)_2$ vitamin D significantly inhibits EAE in female mice, but not in male mice, and that ovariectomy abrogates the protective effect. Furthermore, estradiol implants mimicking hormone estrus levels restore vitamin D-mediated EAE inhibition in ovariectomized female mice, suggesting a link between female sex hormones and 1,25 $(OH)_2$ vitamin D metabolism (49).

Overall, 1,25 $(OH)_2$ vitamin D affects the immune system at different levels through several mechanisms, conferring mostly an immunosuppressive effect. Correction of 1,25 $(OH)_2$ vitamin D deficiency may be useful in suppressing autoimmune disorders such as MS. Nevertheless, before conclusions can be drawn, further investigations are needed to determine optimal doses and compare efficacy and safety of vitamin D analogs with less hypercalcemic effect and improved immune function regulation.

The hygiene hypothesis and the influence of parasite infections on MS course

An ongoing debate persists as to whether infections prevent or precipitate autoimmune disease. Several studies implicate infectious environmental factors present during childhood and young adulthood as strong determinants of MS risk. Microbial infections have also been identified as triggers inducing autoimmunity, resulting in clinical disease manifestations in genetically predisposed individuals. Alternatively, infections might accelerate subclinical autoimmune processes (50, 51).

Conversely, however, certain epidemiological and experimental studies support the hygiene hypothesis, which considers infections protect rather than induce or accelerate autoimmune diseases like MS (52). In line with this concept, in 1966, Leibowitz and coworkers suggested that greater MS prevalence correlated with higher sanitation levels in childhood environments (53). Support for this hypothesis came from epidemiological data demonstrating an inverse relation between infections and allergies as well as autoimmune diseases in the developed world during the last five decades, even after adjusting for improvements in access to medical attention and diagnostic capabilities (52). Also, epidemiological investigations demonstrated an inverse correlation between global distribution of MS and that of the parasite *Trichuris trichiura,* a common human pathogen. MS prevalence appears to fall steeply once a critical threshold of *T. trichiura* prevalence (about 10%) is exceeded in any given population (54). Thus, dichotomous distribution of MS and *T. trichiura* infection would hint at helminth-induced protection against MS development. Indeed, regions of the world where poor sanitary conditions generate endemic areas of parasitoses show lower prevalence of allergic and autoimmune diseases. Longitudinal and migratory studies evaluating MS prevalence, such as those conducted in the French West Indies between

1978 and 1994, show increased MS incidence in the region in association with significant reduction of parasite infections during the same time period, in line with the hygiene hypothesis (55). Additionally, interventional studies report that individuals whose infections clear after antihelminth drug administration show increased skin reactivity to different allergens as well as increased MS activity, indicating once again that helminths appear to directly suppress allergic reactions (56, 57). Overall, these observations suggest that removing regulatory effects resulting from microorganism and parasite infection from populations adapted to live with these infections tends to cause immune system imbalance and increase immune-mediated disease incidence. Consequently, the question arises as to whether helminthes should be considered harmful pathogens or beneficial commensals (58).

Investigations in animal models validate studies in humans described above. Pre-established infection with the helminth *Schistosoma mansoni* or pretreatment of mice with *S. mansoni* ova significantly reduces incidence, attenuates clinical course, and delays onset of EAE in mice. It is also associated with decreased IFN-γ, TNF-α, IL-12, and nitric oxide production by splenocytes, as well as with increased production of IL-10 and TGF-β (59, 60). Moreover, IL-12p40 transcript levels are dramatically reduced in the spinal cord of *S. mansoni*-infected animals, in correlation with decreased brain and spinal cord macrophage infiltrates. Likewise, infection with *Fasciola hepatica* exerts bystander suppression of immune responses to autoantigens and attenuates clinical signs of EAE. Protection is associated with autoantigen IFN-γ and IL-17 production suppression (61).

Immunoregulatory mechanisms induced by helminths in patients with MS

The long life span of helminths is evidence enough of just how accomplished these organisms are at immune evasion. Several studies in humans and in animal models have shown that chronic helminth infections trigger prominent anti-inflammatory network development, leading to attenuation of Ag-specific immune responses against both the parasite and unrelated pathogens (11, 59–61). These findings suggest parasites not only suppress host immune responses directed against them, but also exert bystander suppression against third-party Ags. Helminths and their excretory–secretory molecules are endowed with the ability to act through a broad array of cellular mediators, to temper host immune responses. Following this premise, we demonstrated that parasite-infected MS patients showed significantly lower number of relapses and presented minimal changes on disability scores and significantly less disease activity on MRI compared to uninfected MS individuals. Parasite-driven protection was associated with induction of regulatory T cells secreting suppressive cytokines IL-10 and TGF-β, as well as CD4+CD25+FoxP3+ T cells displaying significant suppressive functions (11). Evidence of regulatory T cells present during parasite infections is now emerging, offering a potential explanation for the mechanism through which infected hosts exhibit altered immune responses to bystander Ag. Thus, parasites may increase regulatory T-cell numbers or activity, either by generating new cells, or by activating or expanding existing ones.

In addition to the development of regulatory T cells, helminth infections also induce regulatory B cells in patients with MS, capable of dampening the immune response through production of IL-10 (62). Existence of immune-regulatory B cells, exerting a key role in immune regulation via production of IL-10, has already been demonstrated in animal models of collagen-induced arthritis and EAE (63). IL-10 is essential for regulatory function development in this subset of B cells, and B cells isolated from IL-10 knockout mice fail to show this protective function (63). In agreement with these findings, recent observations indicate that B cells from patients with MS exhibit relative deficiency in their capacity to produce IL-10. Interestingly, production of IL-10 by B cells is restricted to helminth-infected individuals exclusively. B cells from patients with infections caused by other parasites such as *Trypanosoma cruzi* exhibit IL-10 production levels similar to those observed in uninfected MS patients, indicating intracellular parasites are unable to down-modulate harmful autoimmune responses in the same way helminths do (62). Likewise, B cells from *Paracoccidioides brasiliensis*-infected patients, although expressing Th2 immune response after Ag stimulation of peripheral blood mononuclear cells, exhibit B-cell IL-10 production levels similar to those observed in uninfected MS patients, suggesting increased production of IL-10 by B cells found in helminth-infected MS patients is not determined by the Th2 profile present in these individuals (62). IL-10-producing B cells isolated from helminth-infected MS patients express MHC class Ib molecule CD1d, which aside from Ag presentation is also involved in immune regulation. Glycolipids presented by

CD1d cells binding to T cells expressing the non-variant T-cell receptor Vα14 (in mice), or Vα24 (in humans), activate natural killer T cells, a mechanism found to prevent autoimmune responses in different animal models (64). Furthermore, the cytoplasmic tail of CD1d is linked to signaling cascades associated with IL-10 transcription, suggesting yet another immune regulation mechanism (65). Overall, these findings provide evidence to support autoimmune down-regulation secondary to parasite infections in patients with MS, through regulatory T- and B-cell action, with effects extending beyond simple response to an infectious agent.

Another potentially relevant environmental factor showing immunomodulatory properties is vitamin A, which can influence the course and severity of MS. Both immunological and neurotrophic effects of vitamin A metabolites (66), particularly retinoic acid (RA), make a causal relationship between vitamin A levels and MS disease activity biologically plausible. Reduction in the retinol level has already been reported in patients with MS, compared to levels found in healthy controls or patients with non-inflammatory neurological diseases (67). Furthermore, a recent study described inverse association between increased s-retinol levels and MRI lesion activity in patients with MS, finding s-retinol levels predicted MRI outcome during follow-up (68).

We recently demonstrated that RA serum levels were significantly higher in helminth-infected MS patients than in uninfected MS subjects or healthy controls, suggesting RA produced during parasite infections might be responsible for the protective effects observed in helminth-infected MS patients (69). Genes involved in RA biosynthesis and metabolism, such as *Adh*1 and *Raldh*2, as well as RA receptors and IL-10 were induced in DCs via TLR2-dependent ERK signaling. This programmed DCs to induce Foxp3+ Treg cells and suppress pro-inflammatory cytokine production (IL-6, IL-12, IL-23, and TNF-α) via induction of SOCS3, an effect mediated by soluble egg antigen (SEA) obtained from *S. mansoni*, and by RA. SEA-activated DCs also inhibited IL-17 and IFN-γ production through autoreactive T cells (69). These inhibitory effects were abrogated when SOCS3 gene expression was silenced, indicating that SEA-mediated signaling inhibited production of these cytokines by T cells, through a SOCS3-dependent pathway. Overall, RA-dependent pathways modulate immune response in patients with MS through two different mechanisms: (i) induction of IL-10 and FoxP3+ Treg cells and (ii) suppression of pro-inflammatory cytokine production mediated by SOCS3.

Epstein–Barr virus infection

Epstein–Barr virus is a double-stranded DNA virus of the herpes family infecting 90 percent of the general population in the first decades of life, persisting latently and for life in B-cell memory. Primary infection usually occurs in early childhood and is asymptomatic, but often causes acute febrile illness, known as infectious mononucleosis (IM) when it occurs in adolescence or adulthood. Evidence exists supporting EVB as a strong risk factor for MS, whereas EBV seronegative individuals show very low risk of developing MS. These findings contradict observations described above for parasite infections. Notably, abnormal regulation of the immune response induced by EBV may, in susceptible individuals, contribute to MS pathogenesis (70).

Evidence linking EBV to MS

There is a persuasive body of evidence linking EBV infections to MS risk. Infectious mononucleosis and MS have similar epidemiology. Both diseases affect young adults and are rare after 50 years of age; geographical distribution is similar, following a latitude gradient that increases to the north and south of the equator; both are less frequent in blacks, Asians, and Eskimos and show higher prevalence in high-income populations (9, 71, 72). Under poor hygiene conditions, most children are infected with EVB during early childhood, and less cases of IM are observed. In contrast, initial EVB infection in developed countries usually occurs in young adults and IM incidence is higher. MS risk is twofold to threefold higher than average among individuals with a history of IM (73).

In addition to these epidemiological findings, immunological, pathological, and radiological observations also support a link between EBV infection and MS risk. Several studies have shown that a significant number of patients with MS are EBV seropositive (74). Notably, the prevalence of high titers of anti-EBV antibodies in patients with MS is significantly greater compared to those found in age-matched healthy individuals infected with EVB. A recent meta-analysis of EBV infections showed strong association between MS and exposure to EBV, documented by anti-VCA IgG, anticomplex EBNA IgG, and anti-EBNA-1 IgG antibodies (75). Among healthy individuals infected with EBV, significant

increase in anti-EBV antibodies was documented over 5 years prior to disease development (76). In these subjects, increased EBV antibody titers indicated increased risk of MS, suggesting this was an early event in the disease process. Unlike EBV, evidence does not show increased risk of MS following exposure to other common viral infections including measles, rubella, mumps, HSV-1, or VZV (9, 77). An age-dependent relationship between EBV infection and MS development has also been described. Before the age of 20, anti-EBNA titers in presymptomatic MS patients were similar to those of controls, but after the age of 25, levels increased twofold to threefold (78).

Cellular immunity is also somewhat altered in patients with MS relative to healthy controls. Several studies have documented that EBV-specific CD8+ T-cell responses are significantly higher in patients with MS than in healthy volunteers, or in patients with other inflammatory neurological diseases (79). EBNA-1 CD4+ memory T-cell levels are elevated in patients with MS and show increased proliferative capacity, greater interferon-γ production, and broader specificity compared to demographic and HLA-DR-matched healthy controls (80). In a recent study, the frequency of CD8+ T cells, specific for EBV lytic and latent antigens, was higher in active and inactive MS patients, respectively (81).

Pathology studies also provide strong evidence linking EVB to MS. EBV-encoded small nuclear mRNA (EBER) transcripts were found in B cells and plasma cells infiltrating the brain of patients with MS, with maximal expression in ectopic follicles and in perivascular cuffs of acute and chronic active white matter lesions. Expression of latent viral proteins was observed in MS brains, whereas viral reactivation proteins were detected only in ectopic B-cell follicles and acute lesions. Activation of CD8+ T cells with signs of cytotoxicity against plasma cells was also noted at sites of greater EBV-infected cell density (82). In postmortem studies, brains from secondary progressive patients with MS showed B-cell follicles in meninges entering cerebral sulci, next to large subpial cortical lesions (83). Interestingly, these follicles expressed EBV-encoded small RNA and early lytic EBV proteins (BZLF1, BFRF1) in most of the intracortical perivascular cuffs examined, as well as cytotoxic activity directed toward EVB-infected plasma cells in cortical lesions (84). These postmortem findings have not, however, been replicated in follow-up studies by other groups.

Among patients with clinical isolated syndrome (CIS), serum IgG responses to EBV-encoded VCA and EBNA-1 correlate with T2 lesion number as well as with number of Barkhof criteria present. In addition, anti-EBNA-1-specific antibody responses correlate with the presence of new T2 lesions and neurological disability at 1 and 5 years after disease onset. Interestingly, in this group of patients, elevated EBNA-1 immune responses predicted conversion to MS (85). Likewise, in patients with RRMS, PPMS, and CIS, gadolinium-enhancing lesions significantly correlated with EBNA-1 IgG and EBNA-1:VCA IgG ratio (86). Also, decline in overall brain parenchyma fraction over 3 years correlated with increase in anti-EBV VCA IgG titers in patients with MS (87).

Areas of involvement and possible mechanisms of EBV contribution to MS

Mechanistic support for an epidemiological association between MS and EBV infections is far from well understood, and several hypotheses should be considered. EVB could contribute to MS pathology outside the CNS. In this sense, a phenomenon of molecular mimicry between EBV and myelin antigens might exist. In susceptible individuals, immune response to EVB infection in circulation and lymphoid organs may cross-react with myelin antigens involving both T cells and B cells (88, 89). EBV could induce immortalization of B cells, leading to auto-antibody production and antigen presentation to pathogenic T cells (90). In favor of this hypothesis, one report indicates EBNA1-specific T cells recognize myelin antigens more frequently than other auto-antigens and that myelin cross-reactive T cells produce IFN-γ (91). Another possible scenario is that EBV-infected B cells may express superantigens (92) or transcriptionally activate an endogenous retrovirus with superantigen activity, inducing strong T-cell activation (93). The heat-shock protein hypothesis suggests exposure to infectious agents induces over-expression of αB-cristallin, generating a CD4+ T-cell response that attacks the protein expressed in the oligodendrocytes, inducing inflammatory demyelination (94). Another putative mechanism through which EBV infection promotes CNS inflammation suggests that after primary infection, B cells latently infected by EVB become resistant to apoptosis (95). In genetically susceptible individuals, these cells may seed the CNS, express their antigen, produce autoantibodies, and provide co-stimulatory survival signals, rescuing autoreactive T cells from apoptosis.

EVB could also directly injure the CNS. For example, EBV-infected B cells in meningeal

follicles and in perivascular spaces of blood vessels in the white or gray matter of MS brains may induce a cytotoxic T lymphocyte response with the consequent damage to surrounding tissues (82). Both latent and lytic EBV antigens may elicit strong recruitment of cytotoxic CD8+ T cells inducing bystander brain tissue damage. Additionally, EBV-infected cells may drive IFN-α and pro-inflammatory cytokine production, through stimulation of TLR3 receptors via the release of EBERs RNA (96).

Lastly, in patients with MS, T-cell hyperreactivity may delay virus-neutralizing antibody production, establishing viral sanctuaries within the brain (97). In this scenario, the immune system would be unable to completely clear the virus, thus facilitating loss of immune tolerance toward self-antigens.

Overall, the observations described above suggest EBV infection is a strong risk factor favoring MS development. Prevention of EVB infection may therefore reduce the risk of disease (98).

During the past decade, substantial progress has been made in understanding MS risk factors. In-depth elucidation of how these factors interfere with MS development would lead to new approaches for prevention and treatment of the disease.

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None.

Conflict of interest

Jorge Correale is a board member of Merck-Serono Argentina, Biogen-IdecLATAM, and Merck-Serono LATAM. Dr. Correale has received reimbursement for developing educational presentations for Merck-Serono Argentina, Merck-Serono LATAM, Biogen-Idec Argentina, and TEVA-Tuteur Argentina as well as professional travel/accommodations stipends. María I. Gaitán received speaker compensation for educational lectures and professional travel/accommodations stipends from Merck-Serono Argentina and honoraria for educational lectures from Genzyme Sanofi Argentina.

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