

Multiple sclerosis and herpesvirus interaction

Esclerose múltipla e interação com os herpesvirus

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ABSTRACT

Multiple sclerosis is the most common autoimmune inflammatory demyelinating disease of the central nervous system, and its etiology is believed to have both genetic and environmental components. Several viruses have already been implicated as triggers and there are several studies that implicate members of the *Herpesviridae* family in the pathogenesis of MS. The most important characteristic of these viruses is that they have periods of latency and exacerbations within their biological sanctuary, the central nervous system. The Epstein-Barr, cytomegalovirus, human herpesvirus 6 and human herpesvirus 7 viruses are the members that are most studied as being possible triggers of multiple sclerosis. According to evidence in the literature, the herpesvirus family is strongly involved in the pathogenesis of this disease, but it is unlikely that they are the only component responsible for its development. There are probably multiple triggers and more studies are necessary to investigate and define these interactions.

Keywords: demyelinating disease, multiple sclerosis, herpesvirus.

RESUMO

A esclerose múltipla é a doença inflamatória auto-imune mais comum do sistema nervoso central. Sua etiologia já foi creditada apresentar tanto causas genéticas quanto ambientais. Vários vírus já foram implicados como desencadeadores desta doença e existem inúmeros trabalhos fazendo correlação entre a família *Herpesviridae* e a patogênese da esclerose múltipla. As características mais importantes dos *Herpesviridae* são as de apresentarem períodos de latência e exacerbação e terem como seu principal santuário biológico o sistema nervoso central. O vírus Epstein-Barr, o citomegalovírus, o herpesvírus tipo 6 e herpesvírus tipo 7 são os membros mais estudados como desencadeadores da esclerose múltipla. Conforme as evidências que a literatura apresenta a família *Herpesviridae* está fortemente envolvida na patogênese da esclerose múltipla, porém é pouco provável que sejam os únicos responsáveis pelo seu início. É provável que esta doença apresente inúmeros desencadeadores e mais estudos são necessários para determinar estas interações.

Palavras-Chave: doença desmielinizante, esclerose múltipla, herpesvírus.

The etiology of multiple sclerosis

Multiple sclerosis (MS) is the most common autoimmune inflammatory disease of the central nervous system (CNS). Even though the underlying mechanisms responsible for the myelin targeted autoimmune attacks on the CNS have been the goal of many studies, a precise etiological agent has yet to be defined. MS has many characteristics of an etiologically complex disease, including heterogeneity, incomplete penetrance, temporal changes, polygenic inheritance, environmental risk factors and genetic predisposition¹.

There are two main theories that try to explain MS: the genetic and the environmental theories. They interact among each other and are very susceptible to individual variations.

The first theory relies on inherited immune response abnormalities while the second discusses exposure to antigens, trauma, viral infections, and nutritional disorders, among others.

Two hypotheses seek to justify viral causes as the triggers for MS. The first hypothesis suggests that a virus can infect an adult, subsequently increasing his or her chance to develop MS, which wouldn't happen had the infection taken place during childhood. The second hypothesis affirms that trigger pathogens would have higher prevalence in areas with higher MS prevalence. The common point between the two theories is that the age during the first infection determines the population's susceptibility².

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In addition, there are other observations that point to a viral etiology in MS: 1) many viruses are associated with encephalomyelitis and other demyelinating processes; 2) axonal damage, usually associated with CNS viral infections, can precede MS lesions; 3) the T CD8 lymphocytes, involved in viral immunity, are the predominating lymphocytes in the active demyelinating plaques; 4) cortical and axonal damage in the absence of lesions, which are features observed in viral demyelinating processes, are commonly seen in MS; 5) some viruses, including the herpesvirus, have periods of latency and reactivation that resemble the relapsing remitting MS pattern³.

Members of the *Herpesviridae* family, like the Epstein-Barr Virus (EBV), Human Herpes Virus 6 and 7 (HHV-6 and HHV-7) and Cytomegalovirus (CMV) are among the plausible viral agents. Endogenous retroviruses should also be considered as important viral agents in MS.

The Herpesviridae family

The *Herpesviridae* family is composed of 8 types of agents, each divided into 3 subfamilies (alpha, beta and gamma). All herpesviruses have 3 basic structural elements: 1- a symmetric nucleocapsid with a diameter of 90 to 110 nm in the form of an icosahedron which contains the viral DNA, 2- an envelope embedding the viral glycoproteins and 3- the viral tegument consisting of a mix of proteins that occupy the space between the nucleocapsid and the envelope⁴.

The *herpesviridae* are capable of staying dormant in the host's organism after first infection due to a latency state in which there is no viral replication or in a state of chronic low level replication. It's important to note that each of these replication states will occur in different sites in the body of the host. While the salivary glands and brain tissue are considered sites of chronic replication, monocytes and bone marrow are considered sites where there is virus latency. It is a very important fact that one of the *herpesviridae* biological sanctuaries is the CNS³.

Epstein Barr virus and MS

Certainly the most important herpesvirus related to MS is the EBV. The relation between positive serology to EBV and MS development has been extensively studied. In addition, the effects of EBV within the immune system are undeniable because it maintains a persistent immune response and immortalizes lymphocytes (to a certain point). There have also been a few studies that correlate EBV expression with major MS activity. Finally, the interaction between EBV and endogenous retrovirus has been a recent fascinating field of study.

The EBV was the first virus from the *Herpesviridae* to be implicated as a possible trigger for MS, in 1971. By that time EBV was a candidate due to some factors: is widely distributed in the nature, establishes a long quiescent infection with continuous viral production, with long periods of reactivation and is capable of modulating the human immune system.

EBV serology and MS development

The major difficulty in associating MS to the EBV is proving that the infection comes prior to the autoimmune disease. Studying MS pediatric populations was a way to reduce this bias. Pohl *et al.* presented a study with 147 pediatric MS patients in Europe where the serum prevalence of EBV was of 99%, in comparison with 72% in the control group ($p=0.001$)⁵.

Levin *et al.* estimated the viral infection time through measures of antibody titers of EBV in serial serum samples collected before MS onset among cases, and on matched dates among controls. They had access to the medical history of approximately 8 million USA Army employees whose blood samples were stored. The case-control study had 305 people that came to develop MS comprising the study group and 610 people comprising the paired control group. Ten individuals from the study group and 32 from the control group were EBV-negative at the beginning. Everyone from the EBV-negative cases of the study group (100% of the cases) became EBV-positive before the onset of MS in comparison to only 35.7% in the control group ($p=0.0008$)⁶.

Other research projects also tried to evaluate the relationship between EBV antibodies serum titers and the onset of MS. According to the work of DeLorenze *et al.*, patients with MS already showed a significant elevation of EBV antibodies 20 years before the onset of the first symptoms. It is possible to explain this data in two ways: by the previous existence of an infection that alters the balance of the EBV related T memory cells or by a re-infection caused by a different strain of EBV⁷.

The EBV targeted T lymphocyte response sets up and maintains MS

EBV can infect the B lymphocytes in a very persistent and highly immunogenic way. The T lymphocyte specific antigens are continually produced in increased amounts in response to the first infection and to the chronically high levels of the virus. This autoimmune control is essential to prevent EBV related neoplasm. In its modulating action it is capable of recruiting infected B lymphocytes via expression of latent antigens and helps their differentiation into B memory cells, where the virus persists⁸.

In chronic EBV infections there are strong stimuli to EBV-specific T lymphocytes. When grown *in vitro* these cells suppress the production of immunoglobulins by the B cells. Some studies show that patients with autoimmune diseases might have defects in the EBV-specific immunity, such as having a tendency to produce ten times more lysate stimulating interferon.

However it has not been proven if the increase in EBV-specific T CD4 cells is a response to an increase in stimulation by specific myelin antigens identification. The EBV viral load in MS is not very high and the specific T cells for this virus do not seem to be differentiated from the same cells in hosts without MS⁹.

EBV expression is correlated with major MS activity

During the MS reactivation period there is an increased viral replication period in comparison to the remission period. Patients with early antibody response to EBV antigen are more susceptible to show disease activity, measured through gadolinium nuclear magnetic resonance, than those patients without humoral response. Because of this the disease activity may be related to a latent EBV infection and also is associated with the progression of the disease¹⁰.

EBV and endogenous retroviruses as emergent associated viruses contributing to MS pathogenesis

About 8% of the human genome is constituted by endogenous retroviruses and those have been implicated in many diseases. The activity increase of these viruses may be observed in MS patients. The endogenous retroviruses of the W family was isolated in the leptomeninges, choroid plexus and cultures of monocytes/macrophages in patients with multiple sclerosis and their origin is still under investigation¹¹⁻¹⁴. Our group has recently published the first Brazilian study about the association between MS and Endogenous retrovirus with similar results¹⁵. The oligodendrocytes are responsible for the myelin production of the central nervous system and can be more sensitive to the endogenous retrovirus than the astrocytes on an animal model that mimics MS¹⁶.

A recent study conducted in Sassary, Italy has shown that, *in vitro*, EBV stimulates the expression of HERV-W in cells deriving from blood and brain. They suggest that in MS pathogenesis, a possible model could include EBV as the initial trigger of future MS, years later, and HERV-W as an actual contributor to MS pathogenicity, in striking parallelism with disease behavior¹⁷.

Cytomegalovirus and multiple sclerosis

Studies that correlate levels of antibodies against CMV and MS are controversial. Studies show high rates of CMV infectivity in patients with MS but also a more benign presentation of the disease. Sanadgol et al. collected serum, plasma, peripheral blood mononuclear cells (PBMCs), saliva and urine from MS patients (n=78) and healthy subjects (n=123) and screened for the presence of anti-CMV antibodies and CMV-DNA by nephelometric and PCR methods. Positive results for CMV antibodies were found in 98% of MS patients compared with 52% in the control group (p<0.001)¹⁸. Zivadinov demonstrated that patients who had antibody against CMV had later age of onset, lower rates of reactivation of the disease, and fewer signs of cerebral atrophy on MRI. Those who had higher titers of these antibodies had less brain atrophy and fewer lesions on imaging studies, when compared with patients who had lower titers¹⁹. Pirko et al., using an animal model to mimic the patterns of MS, demonstrated that animals that had CMV infection before a

demyelinating induced disease had milder clinical presentations than those not infected²⁰. These studies suggest a beneficial effect of CMV infection by immune response modulation during MS.

Herpes virus type 6 and 7 and multiple sclerosis

HHV-6 and HHV-7 viruses are closely related and have a similar biologic pattern. These viruses can infect cells of the immune system and modulate their functions. The work described by Nora-Krukke et al. attempted to investigate whether there were associations between HHV-6 and HHV-7 in MS by analyzing the peripheral blood of patients. Patients were randomly selected and divided into two groups: 14 with relapsing remitting multiple sclerosis (RR-MS) and 14 with secondary progressive multiple sclerosis (SP-MS). Among the 28 patients, 25 had latent HHV-6 and/or HHV-7. HHV-6 has been found in 9 patients with RR-MS and 9 with the SP-MS. HHV-7 was found in 10 patients with RR-MS and 14 with the SP-MS²¹.

Opsahl and Kennedy, through the technique of *in situ* fluorescence hybridization, examined tissues from human cadavers to assess the presence of recent and old HHV-6 viral gene expression in normal brain white matter of MS patients, injured tissue of patients with MS and brain samples of a control group without the disease. The gene transcription of HHV-6 was found in all samples analyzed and was restricted to oligodendrocytes. Quantitative analysis of viral RNA expression showed that in both groups of patients with MS (no brain tissue with abnormal white matter), samples had significantly higher levels of expression of this virus. Furthermore, the tissue lesions presented higher levels of expression of the viral gene, a fact that contributes to the theory that HHV-6 is implicated in the pathogenesis of MS²². The same study methodology using HHV-7 showed no difference between groups²³. Using all these virus research methods repeatedly, conflicting results can be observed. In fact, when the technologies used were unable to distinguish an active from a latent HHV-6 infection (via analysis of blood leukocytes by PCR and cell liquor containing central nervous system tissue), no difference is found among the samples from patients and control groups. However, when diagnostic technologies are used specifically for detecting the activity of HHV-6 (PCR analysis of acellular specimens, detection of specific IgM antibodies to HHV-6 or immunohistochemical analysis of tissues of the central nervous system), a strong correlation between HHV-6 and pathogenesis of MS is observed²⁴. However, it is important emphasize that HHV-7 has little evidence supporting its correlation MS.

Conclusion

Undoubtedly, MS is an extremely complex disease. The heterogeneity of symptoms, its multiple presentations and

its patterns of symptoms are highly variable from person to person. This enormous variation is the reason why some patients are already much debilitated in the beginning of the disease while others keep living their lives normally.

Apart from its vast spectrum of symptoms this disease is of utter importance due to its immense socioeconomic impact. The etiology of MS has been thoroughly studied yet not one single etiology has been defined. A viral trigger was suggested

over 100 years ago and, since then, several viruses have been appointed as possible candidates.

The main hypothesis is that there is no isolated factor as a cause for MS but a myriad of them acting together and in different ways on each individual, associated with genetic susceptibility. More studies are needed in an attempt to confirm this hypothesis and permit possible interventions that may minimize the effects of such interactions.

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