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REVIEW

Recent advances in understanding the pathogenesis and biological treatment of multiple sclerosis

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ABSTRACT
Multiple sclerosis is the most common demyelinating disease that develops in genetically predisposed individuals through various immunopathological mechanisms induced by environmental factors, especially viral infections. Th1, Th17, γδ T cells, activated macrophages, MAIT cells, and proinflammatory cytokines, particularly IFN-γ, TNF, IL-17, and GM-CSF, are the principal pathological players whose activities cause damage to the white matter. Furthermore, a recently identified subset of CD4+ T cells has been found to migrate directly to the brain cortex and cause damage to neurons. In 2022, a new mechanism was discovered in addition to these processes. It was shown that molecular mimicry between the EBNA-1 antigen of the Epstein-Barr virus and the GlialCAM molecule of glial cells forms the basis that triggers the entire pathological process. EBV is a highly B cell-tropic human herpesvirus that placed B cells at the centre of our attention. As a result, we must down-regulate their numbers using anti-CD20 monoclonal antibodies to treat such patients *(Tab. 1, Fig. 1, Ref. 37)*. Text in PDF *www.elis.sk*
KEY WORDS: multiple sclerosis, GlialCAM, HLA-DR15, T-, B-, MAIT-cells, EBV, monoclonal antibodies.

Multiple sclerosis (MS) is the most common neurological disease, affecting approximately 2.8 million people worldwide. It is an autoimmune disease resulting from a complex interplay of several fundamental factors, including genetic predisposition, environmental factors (infections, obesity, smoking, insufficient vitamin D intake), and dysregulation of immune processes (1, 2). As with many autoimmune diseases, the prevalence of MS is higher in women, with a noticeable decrease in disease activity during pregnancy. Regarding genetic determination, more than 200 genes have been identified as factors contributing to MS susceptibility, with the human leukocyte antigen (HLA) complex being particularly significant. Alleles belonging to HLA class II (*HLA-DRB1*15:01*, *-DRB1*13:03*, *-DRB1*03:01*, *-DRB1*08:01* and *-DQB1*03:02*) represent the most significant genetic risk factors, while alleles of HLA class I genes (*HLA-B*38:01*, *-A*02:01*, *-B*44:02* and *-B*55:01*) have a protective effect (3, 4). As αβ T cells recognize antigens presented by HLA molecules through their T cell receptors (TCRs), they are the key to understanding the pathogenesis of the disease. However, somewhat surprisingly, a study in which patients were specifically depleted of T helper cells did not yield clinically beneficial results (5). This could be due to the monoclonal antibodies used not being able to eliminate all CD4+ T lymphocytes or the HLA complex antigens playing a role elsewhere in the pathogenesis of MS. Alternatively, during the disease course, the HLA complex may become less essential, and other cell types could assume pathogenic roles. Indeed, the role of γδ T cells, which produce proinflammatory interferon (IFN)-γ upon recognizing myelin glycosyl sphingolipid sulfatide in the groove of CD1c or CD1d molecules, has also been confirmed (6).

Th1 and Th17 cells recognize antigens such as myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), or proteolipid protein (PLP). They primarily exert their immunopathological effects in the white matter of the brain and are responsible for the relapsing-remitting (RR) form of the disease (7). However, recent discoveries have identified CD4+ T cells that specifically recognize β-synuclein. These lymphocytes are of particular interest as they directly migrate to the grey cortex of the brain and damage neurons. They are responsible for the secondary progressive (SP) or primary progressive (PP) forms of MS (8).

Likewise, mucosal-associated invariant T (MAIT) cells from MS patients show increased interleukin (IL)-17 production and the expression of its receptor. They also exhibit high levels of CCR5, CCR6, CXCR6 and VLA4, especially during clinical exacerbation, implying their propensity to migrate to the central nervous system (CNS). This is consistent with their higher frequency in cerebrospinal fluid (CSF) compared to blood (9).

When considering the cells that reach the brain, the question arises as to how they accomplish this. In the case of Th1 cells, this question was resolved more than ten years ago when it was discovered that they utilize their VLA4 molecules (integrins α4β1) to bind to VCAM-1 on endothelial cells. This led to the development of monoclonal antibodies such as natalizumab, which are currently

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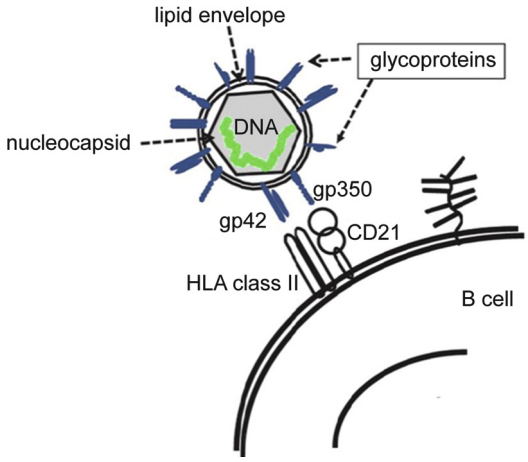

Fig. 1. Interaction between Epstein-Barr virus and B cell. Epstein–Barr virus expresses various glycoproteins in its membrane. Among them, gp42 and gp350 interact with class II HLA and CD21 molecules in the membrane of the B cell, respectively. These interactions enable the virus to infect the target cell and replicate there.

used in the treatment of RR and active SP forms of MS. Th17 cells, on the other hand, employ the adhesive molecule LFA-1 for their transition from the blood to the CNS, while those that recognize β-synuclein utilize VLA4 (10). However, in 2015, it was proven that the concept of the blood-brain barrier (BBB) does not hold, challenging the model of the brain's immune privileged position. That year also marked the discovery of the lymphatic drainage of the brain. Prior to this, it was impossible to prove the existence of lymphatic vessels and lymph-containing cells in the CNS. However, works published in Nature (11) and Trends Immunology (12) demonstrated that these lymphatic vessels exist within the subdural space. Their network originates in the areas of both eyes, traverses through the subdural space, and eventually enters the nasal lymphatic vessels within the space of the olfactory bone, terminating in the deep cervical nodes. This discovery indicates that the primary stimulation of lymphocytes could also occur in the mentioned nodes, not solely in the periphery, and that lymphocytes could simply reach the brain parenchyma via the lymphatic system.

The responsibility of maintaining peripheral tolerance to autoantigens lies with regulatory cell populations, particularly CD4+CD25+FOXP3+Treg cells. In patients with MS, Treg cells appear to be unchanged in frequency compared with controls, but they exhibit impaired suppressive function (13, 14).

In the previous part, we primarily focused on different populations of T cells while omitting B cells. However, current research suggests that B cells may play a more significant role and could be involved in triggering the entire cascade of immunopathological processes. As mentioned earlier, activating B cells requires a combination of environmental factors and a genetic predisposition to the disease. Viruses are often implicated as triggering factors due to their ability to induce immunopathological processes through molecular mimicry mechanisms. In fact, numerous viruses contain proteins that have a similar structure to the autoantigens present in affected individuals. Infections caused by viruses such as the measles virus, Epstein-Barr virus (EBV), hepatitis B virus (HBV), herpes simplex virus (HSV), human herpesvirus 6 or 7, coronaviruses, and others have been variably linked to MS. Furthermore, the connection between viruses and MS is also supported by evidence that viral infections often precede disease attacks (15). The only exception is cytomegalovirus (CMV) infection, as it appears to reduce the risk of developing MS (16). Despite these findings, it has remained unclear which virus could be identified as the primary causative factor in triggering the entire cascade of immunopathological processes. Recent studies published in Science in 2020 (17) and Nature (18) in 2022 seem to have resolved this issue. The authors provided evidence establishing a relationship between EBV infection and the induction of MS.

EBV is an enveloped virus with a double-stranded DNA genome that encodes approximately 85 proteins. Among them is EBV nuclear antigen 1 (EBNA-1), responsible for the latent phase of infection. Molecular mimicry between EBNA-1 (specifically epitope AA386 - 405) and the GlialCAM molecule of glial cells has been demonstrated. GlialCAM is a component of the chloride channel and is primarily expressed by oligodendrocytes and astrocytes. In MS, GlialCAM has been detected in chronically active plaques. Furthermore, sera samples from MS patients have shown reactivity with EBNA-1 proteins, and antibodies in the CSF have also reacted with EBNA-1 (18).

Neurofilaments (NF) are present in the cytoplasm of neurons and, along with microtubules and microfilaments, form the neuronal cytoskeleton. When detected in the serum of patients, they serve as a biomarker of ongoing neuroaxonal degeneration. To elucidate the temporal relationship between EBV infection and MS, Bjornevik et al. found that NF levels increase as early as six years before the clinical onset of MS (19).

EBV enters the body through saliva and infects the epithelial cells of the oropharynx. Additionally, it infects B cells located in the tonsils. EBV entry into the cell is facilitated by the specific interaction of its gp42 molecules with HLA class II molecules (DR15), and gp350 with CD21 in B-lymphocyte membranes, respectively (20, 21) (Fig. 1). Activated B-lymphocytes infected with EBV can cross the BBB, which becomes more permeable due to higher levels of GM-CSF in both the periphery and the brain itself (22).

Activated B cells in the CNS carry out three tasks. They synthesize cross-reacting antibodies that, in cooperation with complement, destroy glial cells, they act as antigen-presenting cells and activate T cells, especially cytotoxic ones that also recognize GlialCAM. By utilizing their granzymes, they contribute to the destruction of glial cells. Memory B cells from patients possessing *HLA-DRB1*15:01* can also activate helper T cells in the absence of exogenous peptides; this phenomenon is known as autoproliferation of T cells. This is possible because B cells can present their peptides, including RAS guanyl-releasing protein 2 (RASGRP2) and peptides originating from molecules determined by *HLA-DRB1*15:01* and *HLA-DRB5*01:01* alleles (23).

During the interaction between activated B and T cells, a phenomenon known as trogocytosis is observed. In this biological process, CD20 molecules are transferred from B cell membranes

Tab. 1. Currently available medicaments for the treatment of multiple sclerosis.

Drug	Mechanism of action
IFN-β	It suppresses the expression of proinflammatory cytokines and promotes the expression of anti-inflammatory cytokines, inhibiting the proliferation of inflammatory cells.
Glatiramer-acetate	It inhibits APCs by competing with myelin antigens presented by HLA-DR molecules.
Fingolimod Ozanimod Ponesimod Siponimod	They prevent the egress of CD4+ & CD8+ T cells and B cells from secondary lymphoid tissues.
Natalizumab	A humanized mAb against the cell adhesion molecule α4-integrin. It blocks the transmigration of autoreactive T cells into the CNS.
Alemtuzumab	A humanized mAb against CD52. It induces the depletion of CD4+ & CD8+ T cells, B cells, NK cells, and monocytes.
Rituximab Ocrelizumab Ofatumumab Ublituximab	Anti-CD20 monoclonal antibodies. They induce the depletion of B cells.
Cladribine	It impairs DNA synthesis and repair and thereby promotes cell death.
Mitoxantrone	It intercalates with DNA causing cross-links and strand breaks.
Monomethyl fumarate Dimethyl fumarate Diroximel fumarate	They induce the downregulation of aerobic glycolysis.

to T cell membranes. The result is an increase in their immunopathological activities through increased synthesis of IL-17, IFN-γ and tumour necrosis factor (TNF). This phenomenon has been confirmed in experimental allergic encephalitis (EAE) of mice, an animal model of MS, where the adaptive transfer of CD20+ T cells worsened the course of the disease, both clinically and immunohistologically (24).

Based on our understanding of immunopathological processes, efforts have been made to develop appropriate therapies to halt the development and progress of MS. Following the initial treatment with interferon beta, several drugs have been developed (for overview, see 4 and 25), including biological agents, particularly monoclonal antibodies (mAb), which are available in clinical practice. The choice of therapy depends on the nature of the disease and the severity of its course, with patients being treated accordingly (Tab. 1). Given the fundamental role of EBV-infected B cells in the immunopathological processes of MS, it is logical to prioritize interventions to reduce their numbers. This is achieved through the use of monoclonal antibodies (mAbs). Since CD20 is expressed in virtually all stages of B cell differentiation, including B memory cells that serve as the main reservoir of EBV, mAbs targeting CD20 are utilized to eliminate them. Currently, there are four available mAbs: rituximab, ublituximab, ocrelizumab, and ofatumumab. Among these, ofatumumab is preferred due to its higher affinity for CD20, enabling more effective destruction of B cells by employing a potent antibody-dependent cell-mediated cytotoxicity (ADCC) reaction in addition to complement activation. Ofatumumab has demonstrated both high efficacy and safety. It has received official approval from the United States Food and Drug Administration for the treatment of RR and active SP forms of MS. It is administered subcutaneously once a month, and patients have the option to self-administer it at home (26, 27).

Concluding remarks

Multiple sclerosis is a chronic autoimmune disease that affects the central nervous system. While the exact cause of MS is not yet fully understood, it is believed to involve a combination of genetic and environmental factors. One factor that has been extensively studied in relation to MS is the Epstein-Barr virus. Research has shown that molecular mimicry is a principal factor that triggers the pathological processes associated with MS. However, it is essential to note that while there is a strong link between EBV and MS, not all individuals with EBV infection develop MS, and not all individuals with MS show evidence of prior EBV infection. The relationship between EBV and MS is complex and involves multiple factors, including genetic predisposition and other environmental triggers. Further research is needed to fully understand the mechanisms underlying the association between EBV, B cells, and MS.

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