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THE ROLE OF EPSTEIN-BARR VIRUS AND HUMAN ENDOGENOUS RETROVIRUSES IN THE PATHOGENESIS OF MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system (CNS), the development of which is associated with the action of a large number of pathogenetic factors which role can vary significantly at different stages of the disease. In recent years, ample evidence supports the hypothesis of the pathogenetic role of the Epstein-Barr virus (EBV) and some retroviruses [1-4].

The Epstein-Barr virus (EBV) is a ubiquitous human gamma-herpesvirus causing lifelong infection of more than 90% of the world's population and has a unique ability to infect, activate, and latently persist within B lymphocytes during human life. A number of studies have shown an increase in antibody titers (IgG) against EBV antigens (mainly EBNA1 or EBNA-complex) in the blood serum several years before the onset of clinical manifestations of MS [5-7], which may indicate the involvement of EBV in the early stages of development of MS [8]. Patients with a clinically isolated syndrome preceding MS exhibit marked humoral and cellular immune responses to EBNA1, but not to other EBV antigens. EBNA1 was the only viral antigen to which elevated serum IgG levels correlated with an increase in the number of active inflammatory demyelinating lesions on MRI (T2-lesions) and with subsequent disease progression assessed by clinical parameters (EDSS) and MRI [9-12].

In the last decade, convincing data have been obtained on the probable relationship between acute inflammation, EBV reactivation and cytotoxicity toward EBV-infected B cells in the central nervous system in patients with MS. Thus, in the study germianlSerafini B. et al. it was shown that a significant portion of B cells and plasma cells in postmortem brain sections of 21 out of 22 patients with MS were infected with EBV, while in the brain sections of patients with other inflammatory diseases of the CNS no infected B cells were detected [8]. The main sites of EBV persistence in brain samples of patients with secondary-progressive MS were meningeal structures resembling B cell lymphoid follicles with germinal centers. CD8+ T cells infiltrated all the sites in the CNS where infected B cells were located, including ectopic B-cell follicles in cases with progressive clinical course, and the number of CD8+ T cells strikingly correlated with the number of EBV infected B cells. Prominent accumulation of CD8+ T cells was also observed in active lesions of the two acute MS cases, matching the distribution of EBV-infected B cells. In acute foci of demyelination and meningeal B cell follicles, a significant number of B cells expressing BFRF1, the EBV lytic cycle-associated protein, was detected.

At the same time, not all studies have succeeded in detecting EBV in the central nervous system of patients with multiple sclerosis [13-15], which has been the subject of active discussion in recent years. Possible explanations for the discrepancy between these findings are differences in the methods and design of research. Features of tissue processing and fixation can significantly affect the safety of EBV-encoded small RNAs (EBER). The number and selection of brain tissue specimens for analysis, the degree of CNS infiltration by B cells, the sensitivity and specificity of the methods used, and the interpretation of the results of studies are also different [16-18]. Results confirming the findings of Serafini B. et al. were obtained in studies using similar research methods [18-20]. Simultaneous use of the most sensitive and specific methods for the detection of EBV in the central nervous system (PCR, EBER-ISH and immunohistochemistry) in another work [21] made it possible to establish the presence of EBV in brain samples in 90% of MS cases analyzed.

In meningeal inflammatory infiltrates from postmortem brain samples of patients with MS, CD8+CD57+ T cells capable of eliminating EBV-infected B cells were detected [22]. This not only confirms the presence of EBV in the CNS, but also indicates the association of of this T cells subpopulation with the pathological process in MS. Effector CD8+ T cells isolated from white matter lesions in brain samples of patients with MS were found to be highly reactive towards autologous EBV-infected B cells [23].

According to some studies, B-lymphocytes and plasma cells are an integral part of inflammatory infiltrates in white matter and meninges in MS [24-26], especially in patients with a progressive type of disease with severe inflammatory and neurodegenerative processes [27-29]. The high-throughput sequencing of the TCR-β gene repertoires revealed increased levels of TCR-β sequences of EBV-reactive CD8+ T cells in the cerebrospinal fluid of patients with MS, which were not observed in the control group [30]. Anti-CD20 therapy in MS significantly limits new focal inflammatory brain lesions and disease relapses in relapsing-remitting MS, and at later/progressive stages of the disease may limit worsening of disability [31]. The results of clinical trials of these drugs indicate abnormal pro-inflammatory properties of B-cells in MS, as well as their ability to influence the activity of the disease regardless of the production of antibodies.

Thus, despite the evidence of the presence of EBV-infected B cells in the CNS of patients with MS, these data require verification in the framework of additional independent studies.

The EBV bystander damage hypothesis suggested on the basis of these data does not explain the autoimmune nature of MS, although secondary autoimmune responses could develop as a result of sensitization to CNS antigens released after a cytotoxic response directed to EBV elimination causing bystander neuronal damage. It also does not explain why the EBV-targeted T cell immune response sufficient to cause bystander CNS damage does not eliminate EBV-infected B cells from the CNS.

An increasing amount of data points to a link between the possible pathogenetic role of EBV in MS with the

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features of the biology of EBV and its modification of B cells. Immediately after primary infection with EBV, two homologs of the anti-apoptotic protein Bcl-2 are expressed for survival of the infected B cells [32]. Depending on the localization and the state of differentiation, EBV-infected B cells are able to implement four transcription programs - one directed at the production of an infectious-active virus, the other three are associated with a latent infection. Latently infected memory B-cells show the properties of classical antigen-specific B memory cells, such as somatic hypermutation and class-switch recombination of their immunoglobulin genes. In classical B cell differentiation, naive B lymphocytes activated by antigen via B cell receptor (BCR) and by T cells through CD40, proliferate and pass through reactions in the germinal center. A feature of memory B cells latently infected with EBV is also the ability to express latent membrane proteins LMP2A and LMP1, which mimic BCR and CD40, respectively, and allow these cells to pass through the reactions of the germinal center independently of T cells. The ability of LMP2A to mimic and replace BCR in a BCR-mediated signaling, thereby maintaining apoptosis-resistant B lymphocytes in an active, proliferating state was demonstrated in vitro. In transgenic mice, LMP1 can also act as an active CD40 receptor that completely replaces CD40 in a CD40-mediated signaling, resulting in the normal development of B cells, their activation and the ability to switch classes of immunoglobulins, germinal center formation and somatic hypermutation [33].

Immune control of EBV infection in healthy organisms is realized through humoral and cellular mechanisms – EBV virions are destroyed by neutralizing antibodies, and proliferating and lytically active EBV-infected B cells are the targets of specific CD8+ T cells [34, 35]. At the same time, EBV remains latent for most of the life of the infected individual, expressing a single gene (EBNA1) within memory B cells. EBNA1 protein is not well recognized by CD8+ T cells, allowing infected memory B cells to avoid detection [36]. Serafini B. et al. have shown that the patterns of expression of EBV proteins in the analyzed brain samples in most cases indicated "latent phase III" (EBNA2, LMP1) and "latent phase II" (LMP1) of latent EBV infection [8]. Since cells expressing EBNA2 and LMP1 are usually absent in the blood, these data indicate complete disruption of EBV regulation, for example due to the inability of CD8+ T cells to eliminate latently infected B cells in the CNS [8; 37-39]. In MS patients subpopulations of EBV-specific CD8+ T cells show signs of depletion, which allows EBV-infected B cells to accumulate in the CNS and leads to the formation of a vicious circle, in which the initially defective T cell response is aggravated by depletion of T cells as a result of a constant high viral load in CNS [40, 22].

M. Pender has proposed an improved hypothesis about the pathogenetic role of EBV in the development of MS. According to this hypothesis, genetically determined CD8+ T cells deficiency leads to an increase in the number of EBV-infected memory B cells, including autoreactive B cells that penetrate the CNS and produce oligoclonal IgG in the cerebrospinal fluid. The presence of MS-associated polymorphic variants of class II HLA, common systemic infections can lead to the activation of CD4+ T cells that are able to cross-react with CNS antigens. After migration to the CNS, CD4+ T cells are reactivated by EBV-infected B cells that present CNS antigens. These EBV-infected B cells provide costimulatory signals that promote T cell survival thus inhibiting activation-induced apoptosis of T cells, which normally occurs when autoreactive T cells infiltrate the CNS. The autoreactive T cells activated in this way organize an immune attack on the CNS by recruiting macrophages and B cells. The release of an increasing number of CNS antigens as a result of this attack leads to the spread of an immune response to other CNS antigens. Repeated attacks of T cells on the CNS, supported by local EBV-infected B cells, lead to the development of meningeal B-cell follicles with germinal centers that generate CNS-reactive B cells that produce autoantibodies that cause demyelination and damage to neurons in the cerebral cortex and the cerebellum, which leads to a progressive phase of MS. In addition, activated in the CNS CD4+ T cells, in turn, can complement the BCR and CD40 receptor signaling on the surface of the EBV-infected B cells, which is already provided by LMP2A and LMP1, respectively, that is, provide "double signaling". This can lead to a vicious circle in which activated EBV-infected autoreactive B cells promote autoimmunity, which in turn promotes infection of the CNS with EBV [33].

Based on the understanding of the pathogenetic role of EBV in the development of MS, theoretically, the delay between seroconversion in the EBV-positive status and the onset of MS in young people would be short enough because the EBV load is 10,000 times higher during primary infection compared to established EBV infections, in which a transition from a high viral load to a low occurs over approximately one year [36]. However, in fact, this delay lasts for several years, which reflects the existence of additional factors in the virus-mediated pathogenetic mechanisms of MS.

A significant number of studies devoted to the research and verification of infectious factors involved in the development of MS indicate the possible pathogenetic role of human endogenous retroviruses (HERV). In some ways, HERV is an intermediate link between exogenous viruses and genes, as they are the remnants of ancient retroviral infections endogenously transmitted by a multitude of generations over tens of millions of years. They represent almost 8% of human DNA. The association with MS was established for two members of the HERV-W family: MS-associated retroviruses (MSRV), and ERVW-1, an element expressing only env protein called Syncytin-1 [3,4]. It was shown that in vitro binding of the EBV gp350 protein caused activation of MSRV / HERV-W in peripheral blood mononuclear cells and in astrocytes [41]. Furthermore, in patients with infectious mononucleosis, increased expression of MSRV / HERV-W in peripheral blood mononuclear cells was observed, and a direct correlation was found between IgG levels to EBNA-1 and the levels of MSRV-specific mRNA expression [42]. Data obtained with the immunohistochemical method using double staining [21] indicate that not only B cells, but also astrocytes and microglia in brain sections of patients with MS (10-15% of the total the number of cells detected as EBER+ in brain samples) are infected with EBV. How

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EBV enters astrocytes and microglial cells, at the moment remains unknown.

Activation of MSRV / HERV-W has also been established in inflammatory and neuropathogenic processes in MS. In the peripheral blood mononuclear cells culture of MSRV-positive individuals, expression of MSRV was activated by the action of pro-inflammatory cytokines such as TNF-α, IL-6, and IFN-γ (and significantly decreased by IFN-β). These pro-inflammatory cytokines, in turn, were overproduced in response to MSRV / HERV-Wenv by cells from MS patients, and correlated with MS severity [4]. MSRVenv can also induce phenotypic and functional maturation of dendritic cells and enables them to support the development of Th1-like effector T lymphocytes [43]. At the brain level, HERV-Wenv activates Toll-like receptors (TLR4) of oligodendroglial precursor cells, which results in the production of pro-inflammatory cytokines as well as inducible nitric oxide synthase, and a decrease in myelin protein expression [44]. Within chronic brain lesions in MS, HERV-Wenv was detected in microglia / macrophages near TLR4-positive oligodendroglial precursor cells. Immunohistochemical detection of HERV-Wenv protein in postmortem brain samples of MS patients (age interval 37-65 years) showed its elevated levels only in active lesions in astrocytes and microglia, and the intensity of staining correlated with the degree of active demyelination and inflammation [45]. These results are consistent with studies of brain samples of patients with MS [46, 47], showing the relative accumulation of HERV-Wenv RNA and protein in brain samples of MS patients.

Extracellular HERV-W / MSRV and MSRV-specific mRNA sequences were repeatedly detected in blood, cerebrospinal fluid and brain samples of MS patients, and MRSV presence/load was found to strikingly correlate with the stages and active/remission phases of MS [Mameli G. et al., 2013]. In secondary-progressive multiple sclerosis a higher expression of MSRV / HERV-Wenv RNA was detected compared to relapsing-remitting sclerosis, and patients with an elevated number of copies of MSRV / HERV-Wenv DNA had a higher extended disability status scale (EDSS) score [48].

In interferon-β therapy, accompanied by lowering in disease activity, there is also a significant decrease in the reactivity of antibodies to HERV-Wenv and HERV-Henv [4]. In a long-term study of effects of interferon-β therapy in MS patients, it was found that in cases of efficient therapy MSRV viremia rapidly decreased to levels below detection limits (the earliest effect was detected 48 hours after the first administration of the drug).

Thus, EBV infection and activation of retroviruses are considered as important factors in the pathogenesis of MS. Primary EBV infection in young adults up to 20-25 years [49] may be the initiating trigger of a pathological process leading to the development of multiple sclerosis, after which HERV-W / MSRV act as active cofactors of MS neuropathogenesis accompanying the course of the disease [4]. Within the framework of the "viral hypothesis," the most important tasks are the verification of data indicating the possible etiological role of EBV, the study of the pathogenetic mechanisms associated with MSRV / HERV-W at different stages of MS development, and the identification of immunological and genetic factors associated with defective control of EBV-infected B cells and, as a result, their migration and accumulation in the CNS. The moments that also require clarification are the pathogenetic context and mechanisms for the migration of EBV-infected B cells to the CNS leading to the development of MS, and whether the presence of these cells in the CNS is a pathogenetic mechanism for all types of MS. In addition, the question remains what type of CD8+ T cells is dominant in MS lesions - specific to EBV, specific to myelin proteins, or both types of cells.

The latest data on the association of EBV and HERV / MSRV with neuropathogenic processes in the central nervous system are an important basis for the development and improvement of treatment strategies for multiple sclerosis, such as personalized therapy with interferon-β, directed depletion of B cells by anti-CD20 monoclonal antibodies, and therapy with the use of allogeneic CD8+ T lymphocytes specific to EBV-infected B cells.

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Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system (CNS), the development of which is associated with the action of a large number of pathogenetic factors which role can vary significantly at different stages of the disease. Although the etiology of MS still remains unclear, in recent years the hypothesis of the pathogenetic role of Epstein-Barr virus (EBV) and human endogenous retroviruses, such as MSRV / HERV-W, is actively considered. EBV has a unique ability to infect, activate, and latently persist within B lymphocytes during human life. Immune control of EBV infection in healthy organisms is realized through humoral and cellular mechanisms – EBV virions are destroyed by neutralizing antibodies, and proliferating and lytically active EBV-infected B cells are the targets of specific CD8+ T cells. At the same time, EBV remains latent for most of the life of the infected individual, expressing a single gene (EBNA1) within memory B cells. EBNA1 protein is not well recognized by CD8+ T cells, allowing infected memory B cells to avoid detection. In addition to epidemiological data, association of EBV with MS is indicated by a significant increase in IgG titres to EBV antigens, mainly to EBNA1, in serum of patients a few years before the onset of clinical manifestations of the disease. Although the data on the presence of EBV in the CNS remain controversial due to a number of methodological difficulties, a number of studies have shown the presence of EBV-infected B cells in the CNS, as well as effector CD8+ T cells specific for them in meningeal inflammatory infiltrates and white matter lesions in brain samples of MS patients. At the same time, the EBV bystander damage hypothesis which considers CNS damage in multiple sclerosis as a result of EBV-targeted cytotoxic reactions of CD8+ T cells, does not explain the autoimmune nature of MS, although secondary autoimmune responses could develop as a result of sensitization to CNS antigens released after a cytotoxic response directed to EBV elimination causing bystander neuronal damage. It also does not explain why the EBV-targeted T cell immune response sufficient to cause bystander CNS damage does not eliminate EBV-infected B cells from the CNS. It was found that

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subpopulations of EBV-specific CD8+ T cells in MS patients show signs of depletion, increasing with the duration of the disease, which apparently allows EBV-infected B cells to accumulate in the CNS and leads to the formation of a vicious circle, in which the initially defective T cell response is aggravated by depletion of T cells as a result of a constant high viral load in CNS. M. Pender has proposed a hypothesis of the pathogenesis of MS according to which MS is caused by the accumulation in the CNS of autoreactive EBV-infected B cells that are capable of self-sustaining proliferation, production of pathogenic antibodies in the CNS, and providing costimulatory and survival-promoting signals to autoreactive CD4+ T cells. But it remains unclear what type of CD8+ T cells is dominant in CNS lesions in patients with MS - specific to EBV, specific to myelin proteins, or both types of cells.

However, the delay between seroconversion in the EBV-positive status in late EBV infection and the development of MS may indicate the presence of additional factors in the development of the disease. In recent years, a number of studies indicate a possible pathogenetic role of endogenous human retroviruses (HERV) in MS. In infectious mononucleosis, the increased expression of MSRV/HERV-W in peripheral blood mononuclear cells has been observed, moreover, a direct correlation has been found between levels of IgG to EBNA-1 and levels of MSRV-specific mRNA expression. Binding of the EBV caused activation of MSRV / HERV-W in peripheral blood mononuclear cells and in astrocytes. Activation of MSRV/HERV-W was also revealed in inflammatory context and in neuropathogenic processes in MS. In the peripheral blood mononuclear cells culture of MSRV-positive individuals, expression of MSRV was activated by the action of pro-inflammatory cytokines such as TNF-α, IL-6, and IFN-γ, and significantly decreased by IFN-β. At the brain level, HERV-Wenv activates Toll-like receptors (TLR4) of oligodendroglial precursor cells, which results in the production of pro-inflammatory cytokines as well as inducible nitric oxide synthase (iNOS), and a decrease in myelin protein expression. Within chronic brain lesions in MS, HERV-Wenv was detected in microglia / macrophages near TLR4-positive oligodendroglial precursor cells. Immunohistochemical detection of HERV-Wenv protein in postmortem brain samples of MS patients showed its elevated levels only in active lesions in astrocytes and microglia, and the intensity of staining correlated with the degree of active demyelination and inflammation. Thus, EBV infection and activation of retroviruses are considered as important elements in the pathogenesis of MS. Within the framework of the "viral hypothesis", the most important tasks are the verification of data indicating the possible etiological role of EBV, the study of the pathogenetic mechanisms associated with MSRV/HERV-W at different stages of MS development, as well as the identification of immunological and genetic factors associated with the defective control of EBV-infected B cells and, as a result, their migration and accumulation in the CNS. Thus, EBV infection and activation of retroviruses are considered as important factors in the pathogenesis of MS. Late EBV infection may be the initiating trigger of the pathological process leading to the development of MS years later, and HERV-W / MSRV affect as active cofactors of the neuropathogenesis of the MS accompanying the course of the disease. **The aim of the review** was to consider the latest evidence of possible mechanisms of the involvement of EBV and human endogenous retroviruses in the pathogenesis of multiple sclerosis. **Keywords:** multiple sclerosis, Epstein-Barr virus, human endogenous retroviruses, immunopathogenesis.

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