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THE ROLE OF THE EPSTEIN–BARR VIRUS IN THE PATHOGENESIS OF SOME AUTOIMMUNE DISORDERS – SIMILARITIES AND DIFFERENCES

G. Füst*

Research Laboratory, 3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary

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After a brief summary on the properties of the Epstein–Barr virus (EBV), the course and latency stages of the infection, the characteristics of infectious mononucleosis (IM), and other disorders caused by this virus, as well as the course of the serological responses to EBV, the current paper focuses on the role of EBV in two autoimmune disorders: multiple sclerosis (MS), and systemic lupus erythematosus (SLE). Diverse evidence suggests that infection by EBV during late childhood or young adulthood may have a role in the pathogenesis of MS. These include the similarity between the geographical distribution of IM and MS, the high risk of contracting MS by individuals who have recovered from IM, the elevation of the titers of IgG antibodies against EBV nuclear antigens occurring years before the initial manifestations of MS, and the extremely rare occurrence of MS in individuals seronegative for EBV. However, the data on the mechanism underlying the relationship between EBV and MS are controversial. Moreover, many observations indicate that EBV contributes also to the pathomechanism of SLE. However, this contribution differs from the relationship between EBV and MS, as shown by the lack of any increase in the risk of SLE after IM. In SLE, EBV serology is quantitatively and qualitatively different from the normal response – that is, EBV viral load is higher and a strong cross-reaction can be detected between certain EBV antigens and autoantigens of pathological importance. These observations, along with the findings pointing to a possible role of EBV in rheumatoid arthritis and myasthenia gravis indicate that infection by EBV may be one of the environmental factors, which can facilitate the development of some autoimmune disorders in genetically susceptible individuals.

Keywords: EBV, multiple sclerosis, SLE, infectious mononucleosis

Introduction

This review article focuses on the evidence for – as well as on the mechanisms of – the relationship of infection by the Epstein–Barr virus (EBV) and two autoimmune disorders, multiple sclerosis (MS) and systemic lupus erythematosus (SLE). These subjects are neglected in the literature on EBV, as most papers discuss the mechanism of oncogenesis by the virus. Readers interested in these topics should consult the review article published recently by Niller et al. [1].

EBV, an ubiquitous microorganism, replication in healthy humans, and oncogenesis caused by the disturbed replication of EBV

The properties of the Epstein–Barr virus

The EBV is a ubiquitous microorganism present in practically every adult human being. It is a DNA virus belonging to the family of *Herpesviridae* and the subfamily of gamma herpesviruses – it is also referred to as the human herpesvirus type 4 (HHV-4). The structure of EBV is similar to that of other herpesviruses: the linear, double-stranded DNA spiral is enclosed by a nucleocapsid of 100–120-nm diameter and icosahedral structure, consisting of very many tiny parts. The nucleocapsid is engulfed by an amorphous substance (the tegument); the outer layer of the virus consists of the envelope carrying several viral proteins necessary for binding of the EBV to its receptor. The most important of these is GP350, a glycoprotein of 350 kD molecular weight. The genome of the virus encodes several proteins of antigenic nature that are expressed on the surface of infected cells only during specific stages of the infection (see later). These comprise nuclear antigens (EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, EBNA-LP), transmembrane proteins (LMP-1, LMP-2A, LMP-2B), and early antigens (EBV-EA) produced exclusively during the lytic cycle (see later), membrane antigens (EBV-MA), the viral capsid antigen (EBV-VCA). Additionally, cells infected by EBV are characterized by the expression of small-molecular-weight RNA (EBER1 and EBER2), BHRF, and many viral micro-RNAs processed from transcripts of BHRF1 and BART genes.

*E-mail: gyfust@gmail.com

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The life cycle of EBV in the human body

EBV can infect and activate B lymphocytes, as well as it is capable of persisting lifelong in these cells *(Table 1).* It also infects the epithelial cells in the oropharynx. The life cycle of EBV within the organism is rather complicated. In the tonsils – the subjects of the initial infection – the epithelial cells pass on the virus to B lymphocytes. The virus-specific receptor of B lymphocytes is the type 2 complement receptor (CR2, CD21); however, MHC II antigens are also necessary for viral penetration. This receptor is present on resting B lymphocytes and therefore, EBV infects these cells, predominantly. According to the most widely accepted, current scenario, EBV activates resting lymphocytes, which transform into lymphoblasts. Then – similar to the normal, antigen-triggered differentiation processes of B lymphocytes – these blasts develop into B memory cells with latent EBV infection. In the meantime, the virus can launch several "latency programs" in infected B cells:

1. During the initial infection of resting B cells, EBV initializes the so-called "latency III" or "growth" program. All forms of EBNA, along with LMP proteins are expressed while this program is active.
2. The lymphoblasts, which have transformed from B cells during the previous stage, migrate into the germinal centers of the tonsils. Here, the expression of several proteins ceases and only that of EBNA-1, as well as of LMP-1 and LMP-2 continues. This is known as the "latency II" or "default" program. The latter enables B lymphoblast to transform into memory B cells in the germinal centers, as well as to persist there or to return into the blood circulation.
3. In the circulation, EBV-infected B cells typically lack protein expression ("latency I stage"), except during division, when they express EBNA-1 on their surface. EBNA-1 makes it possible to transfer the viral genome also into filial cells.

The B-cells with persisting EBV infection and controlled by the latency I program can transform into plasma cells. In these, the virus initiates the lytic, replicative program, which leads to the production of complete, infective virions. The latter can infect either new, resting B cells, or epithelial cells of the oropharyngeal mucosa, and thereby complete the cycle of EBV infection within the organism.

Table 1. The most important properties of the Epstein–Barr virus

- It belongs to the family of herpesviruses and to the subfamily of gamma-herpesviruses (HHV-4)
- Structure: globular, diameter 150–200 nm, capsid membrane, membrane proteins
- Genome: double-stranded, circular DNA
- Primary target cells: B lymphocytes, epithelial cells
- Receptor on B-1 lymphocytes: CD21, latent infection, episome formation
- Multiple forms (III, II, I) of virus latency, lytic cycle, replication cycle in B lymphocytes and in epithelial cells
- Immortalization of B cells
- EBV genes

Transmission of the EBV and differences by age at infection

The virus spreads from humans to humans with the saliva, primarily *(Table 2).* Additionally, it can be transmitted by cough or infected food earlier during childhood. When it occurs during this period – as it is the case in the developing world and in the poor populations of developed countries – the infection is subclinical, or it is accompanied by mild and uncharacteristic symptoms. During adolescence and young adulthood, kissing is the primary route for viral transmission, and the infection often results in infectious mononucleosis (IM) causing severe symptoms. Accordingly, IM is also known as the "kissing disease," the leading symptoms of which include: shivering, shaking chills, fever, fatigue, pain in the extremities, photophobia, headache, pharyngitis, and the enlargement of the spleen and lymph nodes. In the early stage of the infection, when the cellular immune response has not yet launched, the infection, activation, and lytic cycle of B cells are extremely active processes. During this period, the proportion of cells with latent EBV infection can be as high as 50% of all circulating memory B lymphocytes. In the later stage of the disease, an intense response by cytotoxic T cells destroys these cells – this is responsible for the majority of clinical manifestations. Upon the resolution of symptoms, both the frequency of cells with latent infection and the number of cytotoxic T cells drop to a constant and low level.

In addition to IM, EBV can cause other diseases resulting from the malignant transformation of infected cells, but fortunately, this occurs much less frequently. In such instances, the infected cells are either controlled by the normal latency programs mentioned above or by some oth-

Table 2. The characteristics of EBV infection and the disorders caused by the virus

- EBV infects the majority of the population; it can be detected in almost every adult
- The most common route of transmission is with the saliva ("kissing disease")
- The infection occurs mainly during childhood. It is often followed by infectious mononucleosis, the incidence of which increases linearly with age at infection
- EBV is the unique or the most common causative agent of several malignancies. These include endemic Burkitt's lymphoma, nasopharyngeal cancer, Hodgkin's disease, non-Hodgkin lymphoma, (rarely) gastric cancer, and lymphomas caused by immunosuppressive drugs or AIDS
- It may contribute to the pathogenesis of several autoimmune disorders

ers. These conditions include Burkitt's lymphoma – a disease endemic in Africa, and endemic nasopharyngeal carcinoma (NPC) known to occur in South-East Asia. The anaplastic form of NPC is almost invariably associated with EBV. Moreover, the DNA of EBV can be detected in as high as 50% of Hodgin's lymphomas, and in 10% of gastric cancers.

The characteristics of the immune response to EBV, and the serological properties of EBV

The antibodies produced against various antigens of the virus appear/disappear (if the latter occurs at all) in/from the blood of infected individuals at different times. Of these, IgM and IgG type antibodies to viral capsid antigens (VCA), as well as IgG antibodies produced against early D antigens (EA-D) and nuclear antigens (EBNA) are the most important. Non-specific, heterophilic antibodies (once referred to as the "Paul-Bunnell reaction") can be detected during the initial weeks of IM. The IgA antibody against the capsid antigen (EBV VCA-IgA), and an IgG type antibody to the early antigen of EBV (EA-R IgG) appear shortly after the infection.VCA-IgM also appears early, and begins to decrease 4–6 weeks later. Next, the VCA-IgG antibody becomes detectable; its titer peaks on the second to the fourth week and then, decreases slightly. Its reduction halts at a certain level and subsequently, this antibody remains detectable lifelong. The EA-D antibody also appears – relatively late – during the acute phase of the infection. Then, its titer decreases within 3–6 months, but in approx. 20% of infected individuals, it remains detectable for several years after the infection. Antibodies reactive to EBNA antigens usually appear later, after the acute infection has resolved – that is 2–4 months after the onset of the infection and then, remain detectable lifelong. The infection can be diagnosed using appropriate combinations of specific tests for the detection of individual anti-EBV antibodies. VCA-IgG (and occasionally of EBNA-1 IgG) is usually determined even in symptom-free persons, in order to ascertain whether they are susceptible to – or have previously had – an infection by EBV.

The potential role of EBV in the pathogenesis of multiple sclerosis

Epidemiological and seroepidemiological evidence

MS is an autoimmune disorder of the nervous system, which follows a lifelong course. Its pathomechanism is believed to be heterogeneous. The pathological cellular immune response, probably directed against the proteins (e.g. myelin basic protein – MBP) of the myelin sheath covering the axons of central nervous system (CNS) neurons, is likely to play an important role. Clinically, the autoimmune reaction often induces recurrent relapses of CNS inflammation, accompanied by the disruption of the blood–brain barrier, and eventually, destruction of the myelin sheath, and dysfunction of the involved nerves. The prevalence of MS is approx. twice higher among females than in males.

The incidence of MS varies by geographical regions of the Earth. In some of these, such as in Europe or the Northern United States, its prevalence exceeds 100/100,000, whereas this disorder is extremely uncommon in other regions, e.g. in tropical countries, and in Japan, or among the Inuit. Such an uneven distribution can result either from genetic or environmental causes (infectious agents, primarily) – alternatively, a combination of these may be responsible. The poliomyelitis hypothesis – a very remarkable, tentative explanation – has been suggested as early as in the sixties of the last century, from investigations into the characteristics of the poliomyelitis epidemic, which was extremely common during the fifties and at the beginning of the sixties. Before the advent of active immunization, the poliomyelitis virus infected virtually all children, but fortunately, paralysis was relatively uncommon. One of the most conspicuous features of the epidemic was that the risk of paralysis increased linearly with age, from 1:1000 in smaller children to 1:75 in adults. This prompted Poskanzer et al. to formulate their theory that MS results from a ubiquitous infection, which, however, manifests only in a proportion of infected individuals [2]. The question was, of course, who was going to get ill? The widely familiar "hygiene hypothesis" has been developed to answer this question [3]. Essentially, this hypothesis asserted that people living in poor hygienic conditions become exposed to a variety of infectious agents – parasites, viruses, and bacteria – very early during their lives. Recovery from the disease caused by these agents confers relative immunity to autoimmune and allergic disorders. A potential explanation for the relationship is that the inflammatory and autoimmune processes accompanying the infections contracted during early childhood are less intense, compared to those seen in adults with a fully mature immune system. For many long years, the hygiene hypothesis was the accepted explanation for the characteristic feature of MS that this disorder is less prevalent in developing than in developed countries, and as regards the latter, its prevalence is higher in northern countries with better hygienic conditions than in the south or in the southern states of the United States. Early, pertinent studies were published from Israel during the sixties of the last century [4]. Surveys conducted in the United States, and internationally on immigrants relocating from countries with a high risk to countries with a low risk of MS or *vice versa* yielded very interesting findings [5]. The results of earlier studies consistently showed that in individuals who have immigrated from a high-risk region to a country with a low risk during their childhood, the chance of contracting MS decreased substantially in comparison to their non-immigrant peers [6]. At the same time, while the incidence of MS did not increase among immigrants who had relocated – for example – from the Caribbean to the United Kingdom, their offspring contracted the disease just as frequently as the children of nat-

ural-born British subjects [7]. The findings from subsequent investigations into migration within the United States were less unequivocal, probably due to the improvement of hygienic conditions in the southern states.

In addition to the generic hygiene hypothesis, other explanations have been proposed on the relationship between infectious agents and MS. The majority of studies explored the role of EBV in the pathogenesis of MS. The first observation suggesting this role was published by Fraser et al. at the end of the seventies of the last century [8]. These authors showed that lymphocytes isolated from the blood of patients with MS exhibited a more intense transformation upon exposure to EBV, than those from healthy controls. Then, the greater incidence of EBV seropositivity and higher EBV antibody titers in patients with MS were first reported several years later [9].

During the 3 decades, which have elapsed since that time, evidence suggesting a close relationship between EBV and MS has kept accumulating very slowly but progressively *(Table 3)*. It was only in recent years that these studies have become the focus of attention. For example, searching the PubMed database using the search term "EBV and MS" in 2010 yielded twice as many hits as in the previous year and the number of hits in the first 8 months of 2011 was equal to that in the whole year of 2010. Evidence for this relationship is as follows *(Table 4):*

1. *The relationship between the onset of EBV infection and the incidence of MS*. As mentioned earlier, MS is much more common in populations enjoying good living standards than in those with poor hygienic conditions. Besides, it has been unequivocally demonstrated that the majority of children living in poor hygiene circumstances are infected by EBV during early childhood. The most straightforward explanation for this is that the virus – primarily transmitted with the saliva – spreads among children with the food eaten or the toys used in the company of peers. EBV infection contracted at this age is almost always symptom-free. In adolescents and young adults, by contrast (and as I have mentioned earlier), EBV infection spreads predominantly through kissing and can remain subclinical or manifest as IM. Comparing the incidence of MS in three age groups of individuals not yet infected by EBV, people infected by EBV during early childhood, and those infected by EBV at a later age revealed that *MS is extremely uncommon among non-infected (EBV-seronegative) individuals* (see the detailed discussion later). On the other hand, MS was much more common among people with a symptomatic infection than in those who had been infected during early childhood and without experiencing symptoms [10, 11]. The incidence of MS is twice higher among patients who have had IM than in those of the same age and whom this disease has spared. According to a meta-analysis originally [10] conducted on data from 14 – but recently expanded to analyze 18 – published studies [12], this difference is extremely significant ($p < 10^{-54}$). Evidently, accepting the presumption that EBV infection has a role in the pathogenesis of MS requires modifying the hygiene hypothesis of EBV/MS. In particular, the protective effect of infection in early childhood is unlikely to result from an age-related difference in the impact on some generic inflammatory-autoimmune process. Rather, it results from the fact that children in the developed countries are protected from early EBV infection, which leads to a substantial risk of contracting MS at a later age.
2. *Similarities between the epidemiological properties of IM and MS*. There are many of these similarities. IM typically occurs between the age of 15 and 24 years, whereas MS is usually seen in individuals aged 25–34 years [13]. In both disorders, age at the onset is lower in females than in males, and both are common in industrialized countries. By contrast – and similar to MS – IM is extremely uncommon in tropical countries with poor hygienic conditions, as well as among the Inuit, and the Japanese. The observation regarding the latter is particularly important [14], as Japan is a developed, industrial country, and its population is living in civilized and hygienic circumstances. This paradox is attributable to the Japanese tradition of eating from the same bowl when having a meal in company and accordingly, infection can spread with the saliva at a very early age already. In developed countries, both disorders occur more frequently in those better off economically; and in the United States, both afflict the Europid population

Table 3. Observations suggesting a link between EBV and multiple sclerosis (MS)

- Almost every patient with MS is infected by EBV; MS is extremely rare in individuals seronegative for EBV
- A new (recurrent) EBV infection has never been observed in children or adults with MS
- Age at infection by EBV is closely related to the incidence of MS. MS is more common among individuals with a history of infectious mononucleosis (IM)
- IM and MS share similarities in their epidemiological properties (age, gender differences, geographical similarities, relationship with social status)
- Patients with a high EBNA-1 IgG titer face several-fold higher chance of contracting MS subsequently, compared to those with a low titer
- MS follows a more severe clinical course in patients with a high EBNA-1 IgG titer.

Table 4. Potential mechanisms behind the relationship between EBV and MS

- Molecular mimicry between certain EBV antigens and certain epitopes of myelin basic protein
- Bystander injury (hypothetical)
- Superantigen effect – αB crystalline is erroneously recognized as "self"
- Injury mediated by EBV-infected, autoreactive B lymphocytes

more often than the Afro-Americans or people of Asian descent [13].

3. *EBV seropositivity is more prevalent among patients with MS than in the control population of peers*. The investigation of this issue is made very difficult by the fact that infection by EBV is ubiquitous and thus, the virus and a proportion of the antibodies produced against it remain detectable lifelong. Notwithstanding this, a difference between the proportion EBV seropositive individuals could be demonstrated between patients with MS and the general population. The results of studies conducted on adult MS patients were first summarized by Ascherio and Munger in 2007 [5]. Having reviewed 13 publications reporting data from 1779 patients and 2526 healthy controls, the authors found that 94% of the controls and 99.5% of MS patients were EBV seropositive. A calculation using these data reveals that compared to EBV seropositives, the chance for contracting MS by EBV seronegatives is negligible (OR: 0.06; $p < 0.000000001$). This conclusion was confirmed also by studies conducted on pediatric patients. Infection by EBV is much less prevalent among children than in adults: Alotaibi et al. [15] found 83% of pediatric patients with MS and only 42% of age- and sex-matched controls EBV seropositive. Similar results have been reported also by others: in two recent studies, the proportions of EBV-seropositive subjects among pediatric MS patients and controls were 98.6% versus 72.1% [16], and 86% versus 64% [17], respectively. (Of note, a proportion of EBV-seronegative pediatric cases presumably do not have true MS, but suffer from some other CNS disease, which can be easily mistaken for MS.)
4. *The relationship between high EBV antibody titers and MS*. Ascherio et al. were the first [18], followed by Sundstrom et al. [19] to report that the titers of certain antibodies against EBV are higher in blood samples obtained from future patients before the onset of MS than in those from age- and sex-matched controls. Testing archived blood samples from patients, the former team found higher titers of EBNA-1, EBNA-2, and EA-D; the latter team ascertained the same for EBNA-1 antibodies. On the other hand, the titers of antibodies against the cytomegalovirus and the morbilli virus were not different between the samples from patients or controls. The longitudinal study conducted by Levin et al. in 2005 contributed invaluable information [20]. Using banked blood samples and archived data maintained by the US Army, the authors measured the titers of various antibodies against EBV in serial blood samples from 83 patients with MS and twice as many controls (matching the patients in age, sex, and ethnicity at the time of blood sampling). The levels of antibodies against EBV were not different in the samples obtained before the age of 20 years from future patients and controls. Thereafter, EBNA-1 titers were significantly higher in the samples from future patients, but remained unchanged in the samples from controls. The authors attributed the sudden elevation of anti-EBNA antibody titers occurring at the beginning of the second decade of life to superinfection, to the reactivation of EBV, or to some other, yet unknown mechanisms. The risk of contracting MS increased parallel with antibody titers and in subjects with a titer of 1:1280, it was almost ten times higher than in those with a titer of 1:80. These findings provided additional support for the existence of the close relationship between high levels of antibodies against EBV (nuclear antigens, primarily) and the subsequent onset of MS. In another study, DeLorenze et al. demonstrated the elevation (vs. matched controls) of anti-EBNA-1 antibody titers in blood samples obtained 15–20 years before the diagnosis of MS [21]. A fourfold elevation of antibody titers corresponded to an almost doubled risk of MS. Theoretically, the relationship between high anti-EBNA antibody level and MS could be interpreted that the susceptibility to MS, and the predisposition to the elevation of antibody titer might be attributed to the same genetic factors. However, this assumption has been largely refuted by the latest studies [22–24] that compared the presence of HLA-DRB1*1501 (the strongest hereditary risk factor for MS) and high titers of anti-EBNA-1 antibodies of the IgG type in patients with MS and in healthy controls. As demonstrated by these studies, both represent a high risk for MS, but are independent of each other: high levels of anti-EBNA antibodies were observed in both MS patients who were carriers or non-carriers of DR*1501. In 2010, Levin et al. contributed even more convincing evidence for the relationship between EBV seropositivity and MS [25]. The authors tested blood samples from 305 MS patients and 605 matched controls, all active servicemen of the US Army. The availability of serial banked samples made estimating the time of infection by EBV possible in the patients, and the date thus determined was then applied also to the controls. At baseline, 10 patients and 35 controls were seronegative. Each of the ten servicemen who subsequently contracted MS had turned seropositive before the onset of the disease, compared to 37.5% (10/35) only of the controls (who did not contract MS). This study showed that people who have not yet been infected by EBV face an extremely low chance of contracting MS; however, the risk increases dramatically after infection. Very recently, Munger et al. [26] confirmed these findings. In their study, MS risk increased with increasing titers of anti-EBNA complex ($p < 10^{-9}$) and anti-EBNA-1 ($p = 5.8 \times 10^{-9}$) titers. MS risk was 36-fold higher among individuals with anti-EBNA complex IgG titers 320 (95% CI: 9.6–136) than among those with titers <20 and eightfold (95% CI 2.6–23) higher among those with anti-EBNA-1 320 than among those with anti-EBNA-1 <20. These associations were consistent across gender and race/ethnicity groups. Authors concluded that serum titers of pre-onset anti-EBNA antibodies are strong, robust markers of MS risk and could be useful in an MS risk score. Such an evident relationship with MS cannot be detected in the case of any

other ubiquitous viruses, such as the herpesviruses (HSV1, varicella-zoster virus, cytomegalovirus, morbilli virus, mumps virus, and rubeola virus) [5].

5. *The relationship between high anti-EBV antibody levels and the course of MS*. As a multitude of studies has confirmed the elevation of the titers of antibodies against EBV – to EBNA-1, primarily – in patients with MS, it seems reasonable to seek a potential relationship between antibody titers and the course of MS. Although it has been suggested earlier that high anti-EBV titers in the cerebrospinal fluid result from the intrathecal synthesis of antibodies within the central nervous system, this has been clearly disproved by the latest studies [27]. Data on the clinical significance of high anti-EBNA-1 antibody titers are also controversial. Farrell et al. studied the relationship of EBNA-1 IgG levels with disease subtypes, and with the appearance of new, gadolinium-enhancing MRI lesions, characteristic of acute inflammation in 100 patients with MS [28]. Antibody titers were the highest in the relapsing-remitting form, whereas EBNA-1 IgG antibody levels were significantly lower in the clinically isolated syndrome (a single clinical event without relapse), and in the primary progressive disease type. Antibody titers exhibited a very significant, positive correlation with enhancing MRI lesions, with their size (T2 lesion circumference), and with the Expanded Disability Status Scale (EDSS – a quantitative index of disability in MS). According to the authors, their findings demonstrate a relationship between EBNA-1 IgG level and disease activity. Lünemann et al. [29] found that high anti-EBNA-1 antibody levels predict progression of the clinically isolated syndrome to clinically definite MS – that is, an impending second relapse. Additionally, high titers correlate with the number of T2 lesions and of Barkhof criteria (required to establish a diagnosis of MS by MRI), and with the EDDS score. Ingram et al., by contrast, did not find any significant relationship between the clinical activity of MS and EBNA-1 IgG levels [30].

Potential mechanisms of the relationship between EBV and MS; observations incompatible with the EBV hypothesis

In view of the observations described above, EBV is very likely to have a role in the pathogenesis of MS and, moreover, it might influence also the clinical course of the disease. A number of hypotheses on the mechanism of this relationship have been suggested; however, each of these is supported by conflicting evidence *(Table 4)*. Several observations indicate that EBV itself cannot be regarded as the underlying cause of MS, or else the existence of cofactors must be presumed between the virus and the disease. These include, for example, the unexplained observation that the incidence of MS is lower among individuals relocating from a high-risk region to a lower risk domicile later than childhood. Another example is the MS epidemic that afflicted the Faroe Islands between 1943 and 1960 – four years after the occupation by the British troops in 1940. Considering that EBV was present already in the population of the island before the arrival of the soldiers, some activating infectious agent, or the introduction of a different strain of EBV could be held responsible for the epidemic. In recent studies of Simon et al. [31], however, findings do indicate that variation in EBNA1 N-terminus, EBNA1 C-terminus or LMP1 contributes to MS risk.

The following mechanisms have been proposed to explain the relationship between EBV and MS (summarized in: [32, 33]):

1. *Molecular mimicry among certain EBV antigens and certain epitopes of MBP*. According to this hypothesis, the primary target of the cellular immune response – that is, some antigens of the EBV – are homologous with certain epitopes of MBP. Owing to this "molecular mimicry," the cellular immune response against EBV would also involve MBP and therefore, it would be manifested as the autoimmune inflammation of myelin. Although it has been a favorite for long, this hypothesis is now considered less convincing because similar cross-reactions exist among the MBP and other viruses – however, a strong epidemiological relationship can be demonstrated only with EBV. Therefore, although its role cannot be ruled out completely, a cross-reaction itself can hardly be responsible for the relationship between EBV and MS. Most recently, however, Gabibov et al. [34] reported on studies which used a novel approach. They constructed a phage display library of single-chain variable fragments (scFvs) from blood lymphocytes of patients with MS as a potential source of representative MS autoantibodies. Structural alignment of 13 clones selected toward MBP showed high homology within variable regions with cerebrospinal fluid MS-associated antibodies as well as with antibodies toward Epstein–Barr latent membrane protein 1 (LMP1). Accoding to the author, conclusion antibodies induced against LMP1 during EBV infection might act as inflammatory trigger by reacting with MBP, suggesting molecular mimicry in the mechanism of MS pathogenesis.
2. *Bystander injury hypothesis.* The general idea of this hypothesis is that B cells infected by EBV are present also in the CNS and the attack by EBV-specific T cells on the former involves the destruction of surrounding

Table 5. Evidence for the relationship between EBV and SLE

• The increased prevalence of EBV infection among young patients with SLE
• High EBV viral load in patients with SLE
• Aberrant immune response to the nuclear antigens of EBV in childhood SLE
• Cross-reaction between certain EBV and SLE antigens (evidence from animal experiments)
• Defective control of subclinical EBV infection in SLE

tissue, as "collateral damage." This hypothesis is supported by a publication from Serafini et al. [35], which has aroused great interest. According to these authors, the brain of MS patients contains abundant, EBV-infected B cells in ectopic meningeal follicles, and in perivascular infiltrates present in the MS lesions. This could have been a straightforward explanation for the relationship between EBV and MS: tissue destruction would result from the specific immune response by cytotoxic T lymphocytes directed against the infected B cells. This attractive theory, which generated a great deal of comments [36], has in the meantime been challenged by subsequent publications. Specifically, three different studies failed to identify – or detected only small numbers of – EBV-infected B lymphocytes in the brains of patients with MS [37–39]. These discrepancies are usually attributed to differences in the sensitivity of the histological techniques used [40]. This conclusion was supported by a workshop which was organized recently under the umbrella of the European Union FP6 NeuroproMiSe project [41]

3. *Superantigen effect; aB crystalline is erroneously recognized as "self"*. According to this tentative mechanism, certain antigens of EBV would be acting as superantigens and could thus activate a great proportion of MHC II-positive cytotoxic cells. Indeed, such polyreactive cells responsive to some epitopes of EBNA have been identified among peripheral T lymphocytes from patients with MS [42]. However, this hypothesis cannot explain why the CNS is attacked by these activated T cells. Alpha B-crystallin is a small molecular weight heat shock protein, expressed by lymphoid cells upon infection by various microorganisms. Assuming that the same occurs in a CNS infection, then the latter could lead to enhanced expression of alpha B-crystallin by the oligodendrocytes. As alpha B-crystallin is an immunodominant antigen of myelin, the T cell response against the former might also involve myelin and induce local inflammation. The EBV-specific nature of this process is supported by the fact that B cells indeed express alpha B-crystallin protein upon infection by EBV [43]. The primary limitation of this theory is that it cannot explain the initial steps of the immunological process occurring within the brain. Lately, Canadian authors reported that the (primary, human, cerebral microvascular) endothelial cells of the blood–brain barrier can be infected by EBV. This infection leads to activation of endothelial cells, to the production of proinflammatory cytokines and chemokines, and to the enhanced expression of adhesion molecules – along with an increased adherence of peripheral-blood mononuclear cells [44]. Damage to the blood–brain barrier, which is invariably present in MS, is a prerequisite to the penetration of myelin-specific lymphocytes into the central nervous system. Therefore, these findings cast light on a novel aspect of the relationship between EBV and MS. It is believed that the whole process is initiated by the reactivation of EBV.
4. *Injury mediated by EBV-infected, autoreactive B lymphocytes*. This theory presumes that in susceptible individuals, autoreactive, EBV-infected B cells penetrate the CNS [40, 45]. Here, they produce pathological antibodies, and generate co-stimulatory signals, which prolong the survival of autoreactive T cells by reducing their apoptosis. According to Pender, hereditary predisposition would result from a reduced CD8+ mediated immune response against EBV-infected B cells, which could thus proliferate indefinitely and enter the central nervous system. The autoreactive B cells, directed against myelin in genetically susceptible individuals (HLA-DRB1*1501 carriers), activate CD4+ T cells of matching specificity. The latter – cross-reacting with myelinic antigens – can trigger an inflammatory process leading to demyelinization within the CNS. Recently the same group [46] demonstrated that decreased CD8+ T cell response to EBV-infected B cells is not due to decreased HLA class I expression on B cells or monocytes. Although this theory can explain many observations, it is based on premises that have not yet been proven (the presence of EBV-infected B cells within the CNS and the defective function of CD8+ cells).

Findings supporting the relationship between EBV infection and SLE

Similar to MS, there is a variety of often diverse evidence (see later) for a relationship between EBV infection and SLE *(Table 5)*.

1. Several case studies reported the onset of SLE shortly after recovery from IM [47, 48].
2. Serological evidence has been found both in adults and in children. In 2001, James et al. [49] conducted a serology study on 192 patients with SLE and on 392 age- and sex-matched controls. They found that 99.5% of the patients, whereas "only" 95% of the controls were seropositive. This was a statistically significant difference, which could not be observed with any other herpesvirus studied concomitantly. An earlier comparative study by the same authors [50] revealed an extremely large difference in seropositivity between pediatric SLE patients (99%) and controls (70%) the odds ratio for EBV seropositivity was almost 50 times higher ($p < 10^{-12}$) in SLE patients.
3. In addition to seropositivity, also EBV antibody titers have been found different in SLE patients and in controls [51, 52]. However, this elevation of titers does not concern or concerns not only to anti-EBNA-1 antibodies. Thus, the new study by Esen et al. [53] did not find any difference between the anti-EBNA-1 antibody levels of SLE patients and controls; however – in agreement with earlier findings from other authors [54] – they found significantly higher levels of antibodies reacting with the EA/D antigen in the patients. In our unpublished study, however, we found highly significantly elevated anti-EBNA-1 IgG antibody levels in SLE patients as compared to healthy controls.

4. In addition to quantitative diversity, a substantial difference has been found between pediatric SLE patients and healthy controls as regards the epitope specificity of EBV antibodies. The antibodies of patients exhibited a preference for the carboxy- and aminoterminal regions, whereas those of healthy controls reacted with the middle region of the EBNA-1 protein [55]. Hypotheses supported by experimental findings assert that the antibody response after an EBV infection is different in individuals who will/will not contract SLE subsequently [56]. A relevant observation of probably great importance is that some of the antibodies with an essential role in the pathogenesis of SLE (anti-phospholipid, anti-Ro, anti-La, and antinuclear antibodies) appear long years before the onset of initial disease symptoms [57]. Lupus autoimmunity may be regarded as a progressive process, during which originally benign autoimmunity – prompted by some unknown triggering factor – first progresses into the stage of pathological autoimmunity, and the disease manifested by clinical symptoms follows only thereafter [58]. There is another important observation pertaining to blood samples obtained before the onset of the disease, and the knowledge of which is necessary to understanding the relationship with EBV. In SLE patients, both the anti-Sm, and the anti-Ro antibody responses originally occur as an antibody response against a single and characteristic epitope, which is typically PPPGMRPP or TKYKQRNGWSHK – from a very abundant pool of Sm or Ro antigenic determinants [59]. The tests conducted on serial blood samples from EBV-infected patients during the episode of IM identified an antibody against an EBNA-1 epitope (PPPGRRPFFHPVGEA), which cross-reacts with the Sm B1 epitope (PPPGMRPP) that produces anti-Sm antibody to appear first in the process [51]. This EBNA-1 sequence is recognized by the serum of SLE patients, but not by sera from healthy individuals. Following the immunization of rabbits or mice with the EBNA-1 cross-reactive epitope (PPPGRRP), autoantibodies against the Sm B' PPPGMRPP epitope appeared in the majority of animals – in association with lupus-like manifestations [60]. Similar experimental evidence has been obtained also for the anti-Ro system. In this case, the production of anti-Ro antibodies, and lupus-like symptoms (leucopenia, thrombocytopenia, renal impairment) were observed in rabbits, immunized with the EBNA-1 epitope cross-reacting with the first-occurring autoantibody against the Ro-epitope [59]. Based on all these findings, Harley and James [58] proposed a hypothesis asserting that the specific immune response occurring after infection by EBV follows a different course in individuals who will subsequently become ill with SLE than in those who will not. In the latter group, the antibodies against cross-reactive EBNA-1 epitopes disappear after the infection has resolved, but persist in SLE patients, and anti-Sm B' and anti-Ro antibodies appear as a result. Initially, these are directed to the disease-inducing epitope, but subsequently, the phenomenon known as "epitope spreading" results in the appearance of autoantibodies against other antigen determinants of these, and other proteins, as well as nucleic acids. Moreover, the latest studies have shown that the properties of autoantibodies against EBNA-1 also include anti-DNA specificity [61].
5. According to a number of studies, the EBV viral load – that is, the quantity of EBV DNA detected in the blood – is substantially higher in SLE patients than in controls. In patients, the number of B cells infected by EBV is approximately ten times higher, whereas viral load can be as high as 40 times higher [62, 63]. A correlation could be shown between viral load and disease activity [64]. These findings suggest that in SLE patients, chronic EBV infection follows a different course than in those without SLE – specifically, the patients experience a chronic, persisting infection or reactivation of the virus. Primarily, this might result from the defective control of EBV infection in patients with SLE. As shown by Kang et al. [63], the number of cytotoxic anti-CD69+ CD8+ EBV T cells, producing gamma-interferon is lower in SLE patients, although the difference was not statistically significant. Gross et al. attributed this difference to the dysfunctional regulation of B cell homeostasis [64].
6. Additional, indirect mechanisms may be considered for the interpretation of the relationship between EBV and SLE. For example, it has been demonstrated lately that latent membrane protein 1 (LMP1), which is believed to promote the neoplastic transformation of B cells by replacing CD40 signaling, can induce autoimmunity in a mouse strain rendered susceptible to lupus [65].

Differences in the relationship between EBV and the disease in MS and in SLE

Although the evidence for the relationship between EBV infection and the pathological process are strong in both disorders, it must be understood clearly that this relationship differs in MS and in SLE. Differences can be identified in several important aspects:

1. As mentioned earlier, meta-analyses have shown a substantially higher incidence of MS in individuals who have had IM previously – this does not apply to SLE. In addition earlier observations, evidence for this difference comes from a new Danish study of data accumulated in a cohort of SLE patients over 20 years: no correlation could be demonstrated between the positivity of the Paul-Bunnel test (proof of previous IM) and the subsequent occurrence of SLE [66]. As IM is an indicator that predicts EBV infection occurring at a later age, this difference suggests that – unlike the observation made in MS – in SLE, another risk factor may exist during adolescence or young adulthood in addition to EBV infection.

2. While the viral load is extremely high in SLE, in MS it is similar to that seen in the healthy population [67]. This suggests that chronic reactivation of the EBV infection, as well as a detectable immune response to early EBV antigens occurs only in SLE, but not in MS.
3. As it has been discussed in the foregoing, the epitope specificity of the anti-EBV immune response is different in SLE patients than in healthy individuals, and the immune reaction against certain cross-reactive epitopes of the virus might contribute to the pathogenesis of SLE. In SM, by contrast, the difference in the immune response to EBV antigens – and to EBNA-1 primarily – is only quantitative, and not qualitative [27]. Recent observation of Sundqvist et al. [68] on elevated antibody titers for each epitope tested of the EBNA-1 antigen in SM patients supports this conclusion.

EBV and other autoimmune disorders

Compared to MS and SLE, relatively few studies have investigated the relationship between EBV and other autoimmune disorders, primarily *rheumatoid arthritis* (RA). The following observations support the existence of such a relationship:

1. The titers of antibodies against EBNA, the nuclear antigens of EBV are higher in patients with RA than in healthy controls [69].
2. The higher incidence of lymphoblastoid transformation in cultured peripheral blood lymphocytes of RA patients indicated a defective cellular immune response. Similar to the (controversial) research mentioned in the discussion of SLE [63], a study observed reduced production of gamma interferon by EBV-specific cytotoxic T cells [70].
3. Studies pertaining to the existence of molecular mimicry contribute the most convincing evidence indicating the potential role of EBV in the pathogenesis of RA. Of these, the similarity between the QKRAA sequence of HLA-DR1*0401 – known as the "shared HLA genotype" and associated with a high risk of contracting RA – and the gp110 glycoprotein of EBV (BALF4) appears to be the most important. The gp110 antigen is expressed only during the lytic cycle. The antibodies and the cellular immune response exhibit a cross-reaction between HLA genotype and the EBV antigen, as well as these immune reactions are accompanied by an inflammatory response in RA patients [52]. Moreover, it seems important that CD8+ lymphocytes carrying two of the EBV proteins (BZLFI and BMLFI) are present in the joints of RA patients [71].
4. EBV can be identified in the synovial fluid in 8–62% of RA patients. Compared to controls (and similar to SLE), the viral load is much higher in the lymphocytes [72], as well as in the synovial membrane and fluid of RA patients [73]. The level of EBV DNA (viral load) in the synovial tissue and HLA-DR4 carrier state increased the risk for RA 40-fold, compared to DR4- and EBV-DNA-negative individuals [73].
5. EBV might have a role in the production of anti-citrullinated protein antibodies specific for RA. EBV antigens have been shown capable of undergoing post-translational citrullination, which enables them to react with antibodies against citrullinated proteins. Thus, citrullinated EBNA-1 may induce an immune response directed against citrullinated proteins [74].
6. Finally, the use of TNF-alpha inhibitors as biological therapy for RA has raised the suspicion that such treatment can reactivate EBV and this may contribute to the evolution of lymphomas, which develop occasionally in RA patients. According to recent studies, however, such a reactivation does not occur [75, 76]. Another, most remarkable study conducted in 2010 [77] showed that patients with detectable EBV in their bone marrow respond much better to rituximab therapy, than EBV negatives.

At the end of this review, it seems important to mention another novel finding. Cavalcante et al. [78] reported in 2010 that they have compared the incidence of EBV-infected cells in thymus glands from 17 patients with *myasthenia gravis* (MG) and six patients without MG. Using a battery of different methods (*in situ* hybridization, immunohistochemistry, PCR), the signs of an active EBV infection could be detected in the germinal centers of the thymus in all patients with MG, but not even in a single control subject. CD8+ T lymphocytes NK cells and plasmacytoid dendritic cells were also identified in the presence of EBV-infected cells. These findings suggest that in MG, a dysregulated EBV infection is active in the thymus, but the latter is under attack by the immune system. These processes might contribute to the occurrence of thymus-dependent pathological events in MG. In the accompanying editorial Kaminski and Minarovits [79] speculated that an increased EBV load or altered presentation of certain EBV proteins that cross-react with, or mimic, the acetylcholine receptors may trigger the development of MG. In line with it, we (Csuka et al., accepted for publication) recently found elevated anti-EBNA-1 IgG serum concentration in early-onset MG patients.

Open issues and their possible solutions

Autoimmune disorders are characterized by a strong genetic determination, and class II MHC genes are essentially responsible for the susceptibility to these conditions. In particular, DRB1*1501 predisposes to MS and SLE, whereas the DR3-DQ2 haplotype (which is part of the 8.1 extended haplotype) to SLE, and DR*0401 to RA. Although the carriers of these genotypes face a greater risk, only a minority does contract the disease they are predisposed to. This might even suggest the role of other genes, but any effect by the latter is dwarfed by that of genes from the MHC II class. Thus, it is evident that the pathogenesis of autoimmune disorders is dependent also on environ-

mental factors, including infections. The relationship of EBV infection with MS, SLE, RA, and MG seems very likely. The mechanism and pathological significance of this relationship is, however, far to be definitely understood. In addition, as it was pointed out above, there are similarities and differences between the EBV connection to various autoimmune diseases. In a recent book chapter, Niller et al. [80] summarized eleven different potential mechanisms which may facilitate the development of EBV-associated pathogenic and pathoepigenetic changes in major autoimmune disease. Most of these mechanisms concern MS, SLE, and RA, but it seems that there are disease-specific mechanisms as well. This relationship is not proven to be causal, although this cannot be ruled out. Unfortunately, demonstrating a causal relationship appears extremely difficult, if not impossible. As we have seen, EBV infects almost everybody. The infection can occur at various ages – the later this happens, the more likely it will be symptomatic. Theoretically, it seems feasible to prevent infection by EBV through vaccination in early childhood. The EBV gp350 subunit vaccine has proven safe and immunogenic [81] and therefore, it has been tested for the prevention of lymphoma in pediatric patients undergoing immunosuppressive therapy before organ transplantation. The outcome of this experiment was that although vaccination did preclude the symptoms of IM after EBV infection, but it definitely failed to prevent the latter in all vaccinated children [82]. Thus, it appears doubtful whether the risk of autoimmune disorders could be eliminated or reduced by EBV-immunization during childhood.

The other evidence would be the therapeutic efficacy of antiviral agents in autoimmune disorders related to EBV. Several experiments have indeed been conducted in MS, but these proved unsuccessful or not unequivocally successful [13].

Even if we cannot expressly demonstrate a causal relationship between infection by EBV and the occurrence of autoimmune disorders, investigation into this relationship can yield extremely important information on the pathomechanism of autoimmune disease, which has been elucidated only partially so far. Thus, identifying the processes responsible for the high anti-EBNA-1 IgG level – which predisposes to MS and that might facilitate disease progression – will undoubtedly take us closer to elucidating the pathomechanism of MS. Our team is actively researching in this direction.

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