

## THE ROLE OF EPSTEIN-BARR VIRUS IN SYSTEMIC LUPUS ERYTHEMATOSUS

### ULOGA EPŠTAJN-BAR VIRUSA U SISTEMSKOM ERITEMSKOM LUPUSU

Rada Mišković<sup>1,2</sup>, Sanvila Rašković<sup>1,2</sup>, Ana Banko<sup>3</sup>

<sup>1</sup> Univerzitet u Beogradu, Medicinski fakultet, Beograd, Srbija

<sup>2</sup> Univerzitetski klinički centar Srbije, Klinika za alergologiju i imunologiju, Beograd, Srbija

<sup>3</sup> Univerzitet u Beogradu, Medicinski fakultet, Institut za mikrobiologiju i imunologiju, Beograd, Srbija

**Correspondence:** rada\_delic@hotmail.com

#### Abstract

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Epstein-Barr virus (EBV),  
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Systemic lupus erythematosus (SLE) is a systemic autoimmune inflammatory disease that develops in a complex interaction of genetic and environmental factors. Viruses have long been recognized as important factors in the pathogenesis of lupus, especially the Epstein-Barr virus (EBV). A link between EBV and SLE has been suggested since the 1970s, and since then a growing body of evidence supports this link. In this mini-review, the current knowledge on the role of EBV in SLE has been summarized, focusing on the alterations in the immune response to EBV and the mechanisms of EBV-mediated autoimmunity induction in patients with SLE.

#### Sažetak

**Ključne reči:**

Epštajn-Bar virus (EBV),  
sistemski eritemski  
lupus (SEL),  
autoimunost,  
imunska disregulacija

Sistemski eritemski lupus (SEL) je sistemsko autoimunska zapaljensko oboljenje koje nastaje u kompleksnoj interakciji genetskih i faktora sredine. Virus su odavno prepoznati kao važni faktori u patogenezi lupusa, posebno Epštajn-Bar virus (EBV). Veza između EBV i SEL je predložena još sedamdesetih godina 20. veka i od tada ovu vezu potkrepljuje sve veći broj dokaza. U ovom mini preglednom članku sumiramo trenutna saznanja o ulozi EBV u SEL, fokusirajući se na disregulaciju imunskog odgovora na EBV i mehanizme indukcije autoimunosti posredovane EBV kod pacijenata sa SEL.

## Introduction

Systemic lupus erythematosus (SLE) is a multifactorial systemic autoimmune disease that occurs in a specific interplay of genetic factors, immune dysregulation, and environmental factors such as UV radiation, drugs, hormones, toxins, and diet. In most patients, SLE has a relapsing-remitting course, with a minority of patients having persistently active disease or prolonged remission (1). Although not completely revealed, SLE pathogenesis includes enhanced type I interferon (INF) production, resulting from prolonged plasmacytoid dendritic cells (pDC) stimulation, impaired clearance of apoptotic material, production of autoantibodies and immune complex formation and deposition. This leads to the development of diverse clinical manifestations, like musculoskeletal, mucocutaneous, hematological, polyserositis, lupus nephritis, neuropsychiatric lupus (2).

Epstein-Barr Virus (EBV) is an ubiquitous virus with a world-wide prevalence above 90%, which is mainly transmitted through saliva. It consists of double-stranded DNA, surrounded by a protein nucleocapsid and envelope containing glycoproteins which are important for the host cell infection. Primary infection occurs usually in early childhood and is mostly asymptomatic, while in adolescents it commonly presents as infectious mononucleosis (IM). Interestingly, there are many overlapping features between clinical presentation of IM and SLE (3).

The role of EBV in driving autoimmunity in genetically susceptible persons has been suspected for a long time. Evidence supports the role of EBV in induction, progression, and exacerbation of SLE. Given the complexity and diversity of the data, in-depth understanding of the link between EBV and SLE is still lacking. The aim of this review is to summarize current knowledge of the role of EBV in SLE, with the focus on dysregulated immune response to infection and EBV induced autoimmune humoral response.

## EBV life cycle and immune system evasion

One of the specific characteristics of EBV life cycle is its ability to maintain life-long latency in memory B-cells after primary infection, with occasional reactivations and switch to the lytic phase. This process is highly influenced by the host immune system. During the lytic phase, which occurs in the course of primary infection and further reactivations, most viral genes are expressed enabling viral replication. The virus has several mechanisms which enable immune system evasion, but two viral homologues are crucial. Viral IL-10 acts as a homologue of human IL-10 and is encoded by BCRF-1 gene, while restricted early antigen (EA/R), a Bcl-2 homologue is encoded by BHRF-1 gene. Viral IL-10 inhibits synthesis INF- $\gamma$ , MHC I expression and response of cytotoxic CD8<sup>+</sup> T-cell, while EA/R makes infected B-cells and epithelial cells resistant to apoptosis (4). Host immune response in most cases is capable of controlling EBV infection, which

enters latent phase. The EBV-infected cells differentiate into immortalized resting memory B-cells which persist throughout life. During the latent phase, most EBV genes are silenced to avoid T-cell recognition. Only 9 genes, encoding proteins crucial for viral survival, are encoded during the latent phase: 3 latent membrane proteins (LMP-1, LMP-2A, LMP-2B) and 6 EBV nuclear antigens (EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3 and leader protein). Both LMP-1 and LMP-2A provide signals needed to rescue infected B-cells from apoptosis. While the LMP-1 acts as a functional analogue of CD40, the LMP-2A acts as B cell receptor. Additionally, EBNA-1 is a crucial viral protein which represents a replication factor during latency. While containing Gly-Ala repeats, it cannot be degraded by proteasome and presented on the surface of B-cells, making the infected cell invisible for the immune system (3). During occasional reactivations virus switches to the lytic phase, infecting new B-cells and epithelial cells. However, triggers for the reactivation are not completely understood.

## Specific alterations of immune response to EBV in SLE

Cellular immunity is critical for the control of EBV infection and subsequent viral reactivations. The number of virus specific CD8<sup>+</sup> cytotoxic cells during primary infection significantly rises, comprising up to 50% of the CD8<sup>+</sup> T cells in patients with infectious mononucleosis (5). This CD8<sup>+</sup> T cell expansion and accompanying IFN- $\gamma$  secretion are responsible for the control of infection which eventually enters latent state. Humoral immune response is also triggered leading to the production of antibodies to several viral antigens. A stage specific anti-EBV antibody profile is present. Primary infection triggers development of anti-VCA IgM and shortly after that, anti-VCA IgG appears which persist for life. The titer of anti-EA(D) IgG rises during first 3 - 4 weeks after infection or reactivation and persists for up to 3 months in most individuals. Anti-EBNA1 antibodies appear later during primary infection and remain positive for life. During EBV reactivation, there is a rise in anti-VCA IgG titer and reappearance of anti-EA(D) IgG (6, 7).

However, in SLE, immune response to EBV is impaired in several ways leading to frequent viral replications. The patients with SLE have increased frequency of EBV infected peripheral B-cells compared to controls and significantly increased viral load (15 to 40-fold) in the peripheral blood mononuclear cells regardless from immunosuppressive therapy (8-10). Recent meta-analysis found that SLE patients are 3.86 times more likely to be positive for EBV DNA compared to healthy controls (11). This finding is a consequence of reduced number of EBV-specific CD8<sup>+</sup> T cells, which are functionally impaired, exhibit reduced cytotoxic potential, facilitating viral replication, and eventually with repeated reactivations lead to T-cells exhaustion (5, 12, 13). Increased frequency of EBV-specific CD4<sup>+</sup>

cells producing INF- $\gamma$  is found, representing a compensatory mechanism (10). Decreased Th17 and Treg responses additionally contribute to inadequate control of EBV infection (14). Since CMV-specific T-cell response is preserved, it is assumed that there is an intrinsic immune defect related to regulation of EBV infection, while general immune surveillance mechanisms are preserved (3). Draborg and collaborators showed significantly impaired cytokine responses to latent and lytic EBV antigens in SLE patients without lymphopenia, as well as general dysfunction of leukocytes, further corroborating defective immune regulation of immune response to EBV(15).

Inadequate T-cell response, and consequential frequent viral replication lead to an exacerbated humoral response to the virus and increased production of antibodies to different viral antigens (16-19). Zhao-Xia and collaborators report in their meta-analysis significantly higher rate of seropositivity for most anti-EBV antibodies except for anti-EBNA1 in SLE compared to controls (11). Interestingly, humoral immune response seems to be qualitatively different too (20, 21). Significantly higher proportion of SLE patients have IgA antibodies against different EBV antigens, with most evidence in support of anti-EA(D) IgA. Also, Draborg and his team reported 58% seropositivity rate of anti-EA(D) IgA in SLE compared to 0% in healthy controls (22). Since EA(D) is expressed during early lytic phase, this indicates increased EBV reactivation not only in lymphocytes but in epithelial cells too. In the same study, authors found that SLE patients are more frequently positive for 2 or more antibody isotypes (65% vs 10%) possibly reflecting disseminated infection. Neither disease activity nor immunosuppressive therapy affected these results. Overall, these findings indicate difficulties in the control of EBV infection with frequent viral replications in SLE patients.

## EBV related autoimmunity in SLE

Significant homology between common lupus antigens and EBV antigens exists, making structural molecular mimicry the most important mechanism of autoimmunity response in SLE. Several regions of EBNA 1 protein exhibit cross-reactivity with lupus autoantigens such as Ro, dsDNA, SmB, SmD and C1q (23-25). In an experimental study, immunization of animals with cross-reactive EBNA 1 epitope induced autoantibodies to several Ro epitopes, and subsequently development of lupus-like manifestations. Autoimmune response triggered by a single epitope, further expanded through the process of epitope spreading causing development of additional autoantibodies (26). Animal studies also showed cross-reactivity between anti-EBNA1 antibodies and dsDNA and Sm antigens (27). Peptides derived from other EBV antigens are also involved in the induction of autoimmune response. All the enlisted -EA-, LMP1- and LMP 2A-derived peptides - were found to increase ANA positivity, anti-SmB and anti-SmE in animal model (28).

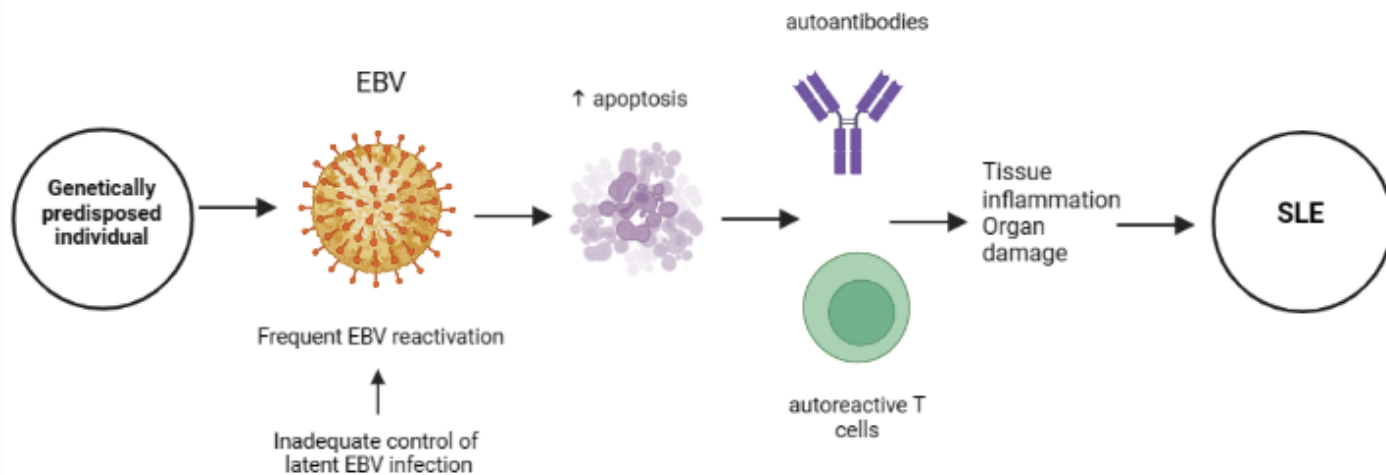
Recent study provided new perspective on the role

of EBNA2, another latent viral protein. Harley and collaborators wanted to investigate gene-environment interaction in relation to EBV using new computational method (bioinformatics algorithm - RELI). They found that almost half of SLE risk alleles are occupied by EBNA2 protein (which serves as a transcription factor), providing evidence for a new mechanism of SLE pathogenesis related to EBV (29). Similar association were found with several other autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, type 1 diabetes, inflammatory bowel disease and celiac disease, which authors termed EBNA2 disorders.

In addition to structural molecular mimicry, evidence suggests a role of functional molecular mimics in autoimmunity response and SLE pathogenesis. Most evidence for functional molecular mimics exists for LMP1, a functional homologue of C40. After LMP1 induces expression of BAFF (B-cell activating factor of TNF family) and APRIL (proliferation inducing ligand), it provides signals needed for survival of B-cells, T-cell independent antibody production, and class switch recombination in the absence of germinal center reaction (30). It also mediates activation signals cooperating with host predisposing genetic factors, leading to amplification of autoimmune response (31). Expression of LMP1 gene is shown to be increased in SLE, and associated with disease activity and type I INF pathway (32).

Recent study provided additional data linking frequent EBV reactivation assessed by serological measures with increased risk of transitioning to SLE in unaffected relatives of patients with SLE (33). The study reported that increased anti-VCA IgG and anti-EA(D) IgG at baseline were associated with significantly increased risk of transitioning to SLE, though all relatives had similar anti-VCA IgG seropositivity rate indicating similar previous EBV exposure. A genetic component was further explored. Analysis of genes implicated in viral-related pathways identified significant interaction between CD40 variant rs48100485 and anti-VCA IgG level, and IL10 variant rs3024493 and anti-VCA IgA level in transitioning to SLE. These data show that a genetic predisposition influences immune response to latent EBV infection, leading to frequent reactivation in susceptible individuals and increasing the risk of transitioning to classified SLE.

Based on the current data from experimental and clinical studies, numerous authors agree with the hypothesis that frequent EBV reactivations due to inadequate control of latent EBV infection in genetically susceptible individuals result in increased number of EBV infected cells, and apoptosis, which together with impaired removal of the waste load trigger production of autoantibodies and formation of autoreactive T-cells. Consequent immune response cause tissue inflammation and organ damage, leading eventually to clinically manifested SLE (**figure 1**) (4,5). However, precise mechanisms of this process need to be further explored.



**Figure 1.** Proposed model of the role of EBV reactivation in SLE. (Image created with the help of BioRender.com).

## Conclusion

Current knowledge supports significant role of EBV in etiology and pathogenesis of SLE. Patients with SLE manifest dysregulated immune response to EBV infection, probably due to genetically determined intrinsic immune defect leading to increased frequency of EBV reactivation. Recent study proposed novel mechanism by which EBV promotes autoimmunity which implies interaction of EBV gene products with SLE susceptibility loci. Subsequent research and better understanding of EBV-host interaction may provide clues to new diagnostic and therapeutic strategies.

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