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How compelling is the data for EBV being a trigger for systemic lupus and other autoimmune diseases?

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Abstract

Purpose of review: Systemic lupus erythematosus (SLE) is caused by a combination of genetic and acquired immuno-deficiencies and environmental factors including infections. An association to Epstein-Barr virus (EBV) has been established by numerous studies over the past decades. Here, we review recent experimental studies on this, and present our integrated theory of SLE development.

Recent findings: SLE patients have dysfunctional control of EBV infection resulting in frequent reactivations and disease progression. These comprise impaired functions of EBV-specific T-cells with an inverse correlation to disease activity and elevated serum levels of antibodies against lytic cycle EBV antigens. The presence of EBV proteins in renal tissue from SLE patients with nephritis indicates a direct involvement of EBV in SLE development. As expected for patients with immuno-deficiencies, studies reveal that SLE patients show dysfunctional responses to other viruses as well. An association to EBV infection has also been demonstrated for other autoimmune diseases including Sjögren's syndrome, rheumatoid arthritis, and multiple sclerosis.

Summary: Collectively, the interplay between an impaired immune system and the cumulative effects of EBV and other viruses results in frequent reactivations of EBV and enhanced cell death, causing development of SLE and concomitant autoreactivities.

Keywords: EBV, infections, immuno-deficiencies, SLE

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune rheumatic disease with female predominance [1]. Symptoms include rash, oral ulcers, arthritis, serositis, renal complications, hematologic and immunological disorders including production of autoantibodies against nuclear components (anti-dsDNA, anti-Sm, anti-histone, anti-ribonucleoprotein) (Table 1) [2].

The disease activity typically varies with periods of disease flares alternating with remissions. Known predisposing congenital and/or acquired immuno-deficiencies comprise complement deficiencies (especially, C1q and C4), impaired cytokine regulation, T-cell proliferation and B-cell functions including altered immunoglobulin production (Table 1) [1,4,5]. Together with external factors, predominantly infections, development of SLE is elicited.

Epstein-Barr virus (EBV) latently infects the majority of the world's population [6] (Figure 1, Table 2). Primary infection commonly takes place during childhood, causing a mild or asymptomatic infection. However, 30-70% of individuals acquiring a primary EBV infection during adolescence develop infectious mononucleosis (IM) [10] with symptoms resembling those of SLE [11,12,13]. Following primary infection, EBV persists in a latent form in memory B-cells, evading the host's immune system and preventing apoptosis of the infected cells [8]. Occasionally, EBV reactivates leading to expression of lytic genes and replication of the viral genome resulting in production and release of new infectious virus, which can infect epithelial-, B-, T- and NK-cells (reviewed in [8,9]). Also in the lytic cycle, EBV has effective immune evasion strategies including expression of proteins with anti-apoptotic, immuno-modulating and concealing functions (reviewed in [9]).

Various mechanisms have been proposed for activation of autoreactive lymphocytes caused by infections including molecular mimicry, bystander activation and superantigen activation (reviewed in [14]). However, none of these fully explain all aspects of SLE etiology.

EBV in SLE

EBV has for decades been suspected to be an environmental agent in the development of SLE and an association is by now widely accepted. An altered infection pattern with poor control of the latent EBV infection and exacerbations of an active lytic cycle has been established. Previous findings comprise high viral loads, increased EBV mRNA expression, and elevated humoral responses against EBV (reviewed in [11,15,16]). Already in 1971 an association was demonstrated by increased EBV antibody titres in SLE patients [15,17]. Numerous studies confirmed higher frequencies and elevated titres of EBV-directed antibodies, against lytic cycle antigens, in SLE patients compared to healthy controls (HCs), indicating frequent EBV reactivations (reviewed in [11,18]). These findings were again confirmed recently in two studies. Rasmussen *et al.* examined antibodies to EBV early antigen diffuse (EA/D) in plasma samples from 77 SLE patients and 29 HCs showing significantly higher titres of both IgM, IgA and IgG anti-EA/D in SLE patients compared to HCs with no association with immunosuppressant intake [19*]. Presumably, these results can be explained as the host's attempt to control widespread lytic infection in both B- and epithelial cells giving rise to the various antibody isotypes. Results of Fattal *et al.* confirm the findings on elevated anti-EA/D IgG together with increased titres of anti-EBV-p23 IgG in 49 SLE patients compared to 23 HCs [20*].

Westergaard *et al.* found normal levels of both IgM, IgA and IgG anti-EBNA1 by investigating 28 SLE patients. [21*]. Similarly, Fattal *et al.* recorded a decrease in EBNA1 antibody titres in some SLE patients [20*]. These findings suggests that the altered humoral immune response to EBV in SLE patients primarily entails the lytic cycle.

An association between lupus nephritis (LN) and the EBV antigen latent membrane protein (LMP)1 has been shown in two studies. LMP1 on EBV-infected B-cells mimics the signal that otherwise is generated by the CD40 pathway in CD4+ T-cell help. Yu *et al.* detected a significantly higher positive rate of both LMP1 and EBV-encoded RNA (EBER)1 by investigating renal tissue from 58 LN patients compared to controls,

which was not associated with immunosuppressant medication, age or concurrent other infection [22**]. Ding *et al.* confirmed these findings in renal tissue from 51 young (6-16 years) SLE patients with LN, and furthermore showed a positive correlation between LMP1 and the classification of LN suggesting that renal EBV-infection may be directly involved in the development of LN [23*].

The latent state EBV-antigen LMP2A (that serves as a survival signal that normally is generated by antigen binding to the B-cell receptor) was recently demonstrated to be an important mediator of SLE in a mouse model [24**]. To mimic LMP2A expression in EBV-infected human B-cells, they used a system with conditional expression of LMP2A in B-cells. This resulted in B-cell hyperactivity and differentiation into plasma cells producing low-affinity antibodies and most interesting, it led to SLE-like symptoms including production of anti-dsDNA, anti-cardiolipin and development of glomerulonephritis-resembling lesions [24**].

Previous studies have demonstrated that SLE patients have impaired EBV-specific T-cell responses (reviewed in [9]). We have recently further evaluated these responses by *ex vivo* stimulation of whole blood samples from 28 SLE patients compared to 28 sex- and age-matched HCs. Results showed significantly fewer CD8+ and CD4+ T-cells responding to EBNA1 and EA/D by quantification of the surface activation marker CD69 and the production of interferon (IFN)γ by flow cytometry [25**]. Interestingly, an inverse association between EBV-specific T-cells and production of EBV-directed antibodies was observed, and disease activity was inversely correlated with the number of EA/D-specific T-cells. As cell-mediated immunity is crucial in order to control latent EBV infection, these results suggest impaired control of EBV in SLE patients with a shift towards more frequent reactivations and a humoral immune response attempting to control EBV activity. This impaired EBV control seems to contribute to the exacerbation of SLE disease symptoms [25**].

The decreased control of EBV in SLE patients was also lately demonstrated by the presence of EBV DNA detected by PCR in serum from SLE patients [26], and by several case reports. A 50-year-old woman

diagnosed with SLE (and concurrent autoimmune hepatitis) developed chronic active EBV infection with EBER-positive lymphocytes found in both liver and gastric mucosal biopsies and in high concentrations in the CD4+ T-cells in the blood [27]. Similarly, an 86-year-old woman with SLE developed a rare form of EBV-positive plasmablastic lymphoma in the colon [28]. Additionally, two case reports described how EBV-positive lymphoproliferative disorders can be initially misdiagnosed as a type of lupus since symptoms overlap and can entail the presence of antinuclear antibodies [29,30].

Other viruses in SLE

Other infectious agents especially other human herpes viruses (HHVs) have been associated with SLE including cytomegalovirus (CMV) and Varicella-zoster virus (VZV), and recent studies have confirmed this link. Rasmussen *et al*. showed elevated titres of IgG and IgA antibodies against the lytic cycle CMV antigen pp52 in SLE patients (n=77) compared to HCs (n=29), but not to the lytic cycle HHV6 antigen p41, indicating that not all anti-HHV responses are dysfunctional in SLE patients [19*]. Chen *et al.* demonstrated a significantly increased positivity of *UL55* expression (coding for CMV envelope glycoprotein gB) in peripheral blood mononuclear cells (PBMCs) from 60 SLE patients compared to 111 HCs and also increased titres of both IgM and IgG CMV antibodies [31*].

Moreover, both an increased humoral response (elevated levels of IgG anti-gpVZV) and a decreased cell-mediated response (lower frequency of IFNγ-producing PBMCs and decreased CD4+ T-cell proliferation upon stimulation) to VZV have recently been revealed in SLE patients compared to HCs [32**].

Finally, parvovirus B19, transfusion-transmitted virus (TTV) and human endogenous retroviruses (HERVs) [33,34] have also been associated with SLE [14,15,35], presumably as a result of general immuno-deficiencies.

EBV in other autoimmune diseases

EBV has been associated with other autoimmune diseases including Sjögren's syndrome (SS), rheumatoid arthritis (RA), and multiple sclerosis (MS), all of which (like SLE) are believed to be multifactorial with a genetic susceptibility combined with environmental triggers, and are characterized by partly overlapping symptoms and autoantibodies.

SS can present both as a primary and secondary disease (often associated with SLE or RA) and is mainly characterized by disorders of the exocrine glands [36]. RA is the most common of these diseases and symptoms especially comprise arthritis [37]. MS is an autoimmune disorder in the central nervous system (CNS) with chronic inflammatory demyelinating conditions [38].

Two recent papers have extended the knowledge on the association between EBV infection and SS. Croia *et al.* focused on specific lymphoid structures in the salivary glands of SS patients and observed both latent (LMP2A-positive) and lytic (BFRF1-positive) EBV-infected memory B-cells and plasma cells, respectively. Furthermore, it was revealed that especially Ro52-specific antibody-producing plasma cells were infected with EBV [39**]. Kivity *et al.* investigated 82 SS patients and 139 HCs and found increased positivity of EBV-EA IgG in SS patients which correlated with the presence of anti-Ro/SSA and anti-La/SSB, indicating a connection between EBV reactivation and development of SS [40*].

Interestingly, Westergaard *et al.* found that the immune response to the latent stage EBNA1 was altered in RA patients (n=77) with highly increased titres of EBNA1 antibodies of both IgM, IgG and IgA isotypes compared to both HCs (n=28) and SLE patients (n=28). The EBNA1 IgM and IgA were furthermore found to associate with presence of rheumatoid factors IgM and IgA, respectively, indicating a direct connection between development of these autoantibodies and the altered immune response to the latent EBV infection [21*]. In addition, Cornillet *et al.* demonstrated an association between antibodies to a citrullinated EBNA1 peptide and antibodies against other characteristically citrullinated peptides in RA patients [41]. The altered immune response to EBV in RA patients was also demonstrated by Erre *et al.*, by

showing increased EBV DNA positivity and load in PBMCs from 77 RA patients compared to 58 HCs and increased prevalence of both EBNA1 IgG and EBV-EA IgG [42].

The role of EBV in MS development has for long been investigated. A characteristic (although not specific) feature of MS is the intrathecal production of antibodies (oligoclonal bands) [38]. This presumably reflects the presence of EBV-infected B-cells in the CNS, in line with a theory of entry of EBV-specific T-cells into CNS causing attacks (reviewed in [43]). Actually, Nierop *et al.* recently demonstrated increased frequencies of intrathecal T-cell reactivity in MS patients using peripheral autologous EBV-infected B-cells as antigen-presenting cells [44*]. An uncontrolled latent EBV infection was also established by Nejati *et al.* with a significantly higher EBV DNA load in PBMCs from MS patients compared to HCs, which was inversely associated with vitamin D concentrations, suggesting a vitamin D contribution to the immune-modulation of EBV in MS patients [45*]. The altered immune reaction to EBV in MS patients also entails elevated EBV antibody titres [45*,46,47,48,49**].

Discussion: The role of EBV and other viruses in the etiology of SLE and other autoimmune diseases

EBV is an important, extremely successful HHV, which infects essentially all of mankind (Figure 1, Table 2). However, this fact is also a common objection to EBV having a causative role in SLE and other autoimmune diseases. A related question is how EBV can cause several seemingly different autoimmune diseases. These questions can simply be answered by the role of individual genetically determined immuno-profiles and by individual combinations of infections, environmentally induced immunosuppressions, viral reactivations and treatment regimes (Figure 2).

EBV infects epithelial cells and other cell types and it affects the immune system of the host directly by infection of lymphocytes with subsequent immortalization and indirectly by expression of immuno-modulating viral antigens. EBV infection may develop diverse autoreactivities in different individuals according to genetic susceptibility and immune condition (Table 1), the types of infected cells, the sites of

reactivation and immune responses against EBV and in this way be a contributive agent to diverse yet overlapping autoimmune diseases.

An apparent paradox is how current treatment, based on immunosuppression, can be successful against a virus-induced autoimmune disease like SLE. This paradox may be resolved by the biology of EBV, its tropism for B-cells and the ability of immunosuppression to limit the spreading of EBV and infected B-cells in addition to its dampening of inflammation. This is supported by the ability of anti-CD20 drugs to control disease activity [50].

Conclusion

In our opinion, the evidence for a causative role of EBV in SLE and other autoimmune diseases is so compelling, that future research should be focused on the additional role(s) of other (herpes)viruses and possible new therapies based on immuno-modulation and/or anti-viral drugs. Currently, there is an aggravating scarcity of vaccines and drugs with efficacy against EBV.

Key points:

- SLE patients have characteristic immuno-deficiencies
- EBV infects SLE patients, which cannot control the virus effectively
- EBV evades the immune system and can switch between latent and lytic life stages
- Repeated reactivations of EBV causes SLE disease flares and associated autoimmune manifestations

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Conflicts of interest

None.

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This study is the first to compare the humoral responses (IgM, IgG and IgA) to early antigens of the three HHVs: EBV, CMV and HHV6 in SLE patients and HCs. SLE patients had an increased response to EBV-EA/D and CMV-pp52 but not to HHV6-p41, suggesting that not all anti-HHV responses are dysfunctional in SLE patients.

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Figure legends

Table 1. SLE characteristics [2] and predisposing immuno-deficiencies [1,3,4]

Table 2. EBV characteristics and associated diseases [7,8,9]

Figure 1. Epstein Barr virus

EBV consists of a dsDNA genome within a capsid surrounded by tegument within a lipid envelope with surface glycoproteins. EBV is transmitted via saliva and enters epithelial cells and B-cells. EBV shifts between a resting latent state in memory B-cells with a limited expression of genes (EBNAs and LMPs) and reactivation to lytic cycle with expression of immediate early (IE) antigens (transcription factors), early (E) antigens (including EBV-EA/D) and replication of the viral genome and subsequent expression of late (L) antigens (structural proteins including EBV-p23) resulting in production and release of new virus. [7,8]

(**electronmicrograph:** http://www.sciencedaily.com/releases/2005/07/050725065240.htm)

Figure 2. Gene – environment interactions in the development of SLE with an emphasis on the role of EBV

Timeline showing the immuno-profile and environmental elements affecting the development of SLE in genetically predisposed individuals with certain immuno-deficiencies. The primary infection with EBV leads to production of EBV-directed antibodies and generation of EBV-specific T-cells. Over time (and with multiple reactivations) the quantity of EBV-specific T-cells decreases and the latent infection gets even harder to control resulting in increasing number of disseminated EBV-infected cells and eventually production of autoantibodies triggered by the cumulative waste load and further development of SLE symptoms. The EBV-specific antibodies stay at high titres reflecting the increased antigen load and attempts to control the numerous reactivations as compensation for lack of cell-mediated control.