Epstein–Barr Virus and Rheumatoid Arthritis: Is There a Link?

Review

Epstein–Barr virus and rheumatoid arthritis: is there a link?

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Abstract

Rheumatoid arthritis is a systemic autoimmune disease characterized by chronic, destructive, debilitating arthritis. Its etiology is unknown; it is presumed that environmental factors trigger development in the genetically predisposed. Epstein–Barr virus, a nearly ubiquitous virus in the human population, has generated great interest as a potential trigger. This virus stimulates polyclonal lymphocyte expansion and persists within B lymphocytes for the host's life, inhibited from reactivating by the immune response. In latent and replicating forms, it has immunomodulating actions that could play a role in the development of this autoimmune disease. The evidence linking Epstein–Barr virus and rheumatoid arthritis is reviewed.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory polyarthritis that progressively destroys synovial joints and can cause systemic complications. RA affects about 1% of the world's population [1], and its prevalence in women is twofold to fourfold that in men [2,3]. RA has enormous personal, social, and economic impact [4,5]; women with RA have overall mortality rates 2.3-fold those in age-matched controls [6]. New biologic therapies, based on an increasing understanding of the molecular mechanisms involved in RA, afford a more normal life to many, but the burden of disease remains high. At present there is no known cure. Despite improved therapy, the long-term prognosis remains poor and average life expectancy is reduced by 3 to 18 years [7]. Both the direct costs of treatment of RA and the indirect costs of disability and loss from the workplace are high [8,9].

RA is marked by extensive synovial hyperplasia and infiltration by lymphocytes, monocytes, macrophages, and fibroblasts. RA is a predominantly $CD4^+$ T helper type 1 (Th1)-driven disease [10]. Aberrant T cell activation is one of the earliest events in the development of RA, with $CD4^+$ T cells stimulating monocytes and macrophages to produce inflammatory cytokines, including interleukin (IL)-1, IL-6, and tumor necrosis factor-α (TNF-α), as well as proteolytic enzymes, destroying synovium, cartilage, and underlying bone [11]. The T cells infiltrating the rheumatoid synovium are oligoclonal, implicating an antigen-driven process [12,13], but the inciting antigen or antigens remain unidentified. Activated T cells also signal B cells to produce increased levels of immunoglobulins, including rheumatoid factor (RF). Autoreactive B cells also have a central role in the development of RA, producing autoantibodies that might be involved in tissue damage in RA [14].

Genetic factors are important in disease susceptibility, but environmental exposures are probably crucial as well. Many exposures have been investigated as possible risk factors for the development of RA, including reproductive factors such as the use of oral contraceptives, hormone replacement therapy, and breast feeding [15-17], and dietary factors such as antioxidants [18,19], red meat protein [20,21], and fat intake [22,23]. However, most of these have shown only weak associations. Cigarette smoking is the only exposure that has repeatedly been found to increase the risk of RA, with a relative risk of about 1.8 [24-27].

Viruses and the development of RA

A viral trigger of RA in the genetically predisposed has been hypothesized for many years [28-36]. A virus could act as an adjuvant in the development of autoimmunity, non-specifically stimulating innate immune responses, including mast cells, dendritic cells, Toll-like receptors and complement receptors [37]. Polyarthritis resembling RA is seen clinically soon after exposure to multiple viruses including rubella, human T cell leukemia virus-1 (HTLV-1), parvovirus B19, and hepatitis B and C [36,38-40]. Exposure to a common virus would explain the ubiquity of RA worldwide. However, such a virus has

CCP = cyclic citrullinated peptide; EA-D = Epstein–Barr virus early antigen – diffuse; EA-R = Epstein–Barr virus early antigen – restricted; EBNA = Epstein–Barr virus nuclear antigen; EBV = Epstein–Barr virus; HLA = human leukocyte antigen; HTLV-1 = human T cell leukemia virus-1; IL = interleukin; RA = rheumatoid arthritis; RF = rheumatoid factor; SLE = systemic lupus erythematosus; Th1 = T helper type 1; TNF = tumor necrosis factor; VCA = Epstein–Barr virus viral capsid antigen.

eluded identification by modern techniques possibly because of a long latency period, with RA onset years after initial exposure. Viruses including Epstein–Barr virus (EBV), parvovirus B19, HTLV-1, human herpesvirus-6, human herpesvirus-8, and human endogenous retroviruses-5 have all been proposed to be involved in the pathogenesis of RA [31-35,41-44].

However, most of the evidence implicating viruses in the pathogenesis of RA is circumstantial and inconclusive. Tantalizing observations have often been based on *in vitro* or animal studies, case reports, or studies with small sample sizes, cross-sectional designs, or no control groups.

Epstein–Barr virus

EBV, the causative agent of infectious mononucleosis, is a DNA-containing herpesvirus that is extremely prevalent worldwide, infecting more than 98% of the human population by the age of 40 years [45]. It is highly associated with several malignancies including nasopharyngeal carcinoma, Burkitt's lymphoma, T/NK cell lymphomas, lymphoproliferative disease in immunocompromised hosts, and Hodgkin's disease, in most of which EBV genomes are detectable within tumor cells [45].

To initiate infection, EBV uses its major envelope glycoprotein, gp350, to bind to its receptor, complement receptor-2, on epithelial cells and B lymphocytes [46,47]. Major histocompatibility complex (MHC) class II molecules are cofactors for the infection of B cells by EBV [48]. During initial infection there is massive polyclonal expansion of B lymphocytes, followed by that of $CD8^+$ T lymphocytes in particular [49]. EBV then becomes latent within memory B lymphocytes and persists for the lifetime of its human host. While it is latent within the B cell, its viral genome is intact as an episome, but most viral genes are not active [50]. The proteins it produces are responsible for inhibiting apoptosis and blocking the antiviral effects of interferon-γ on EBV-transformed B cells [50,51]. EBV has multiple immunomodulating actions. Binding of its major envelope glycoprotein gp350 to complement receptor-2 leads to the upregulation of the important inflammatory cytokines IL-1β, TNF-α, and IL-6 [52-54]. EBV encodes an immunosuppressive viral IL-10 cytokine and a viral colony-stimulating factor-1 cytokine receptor, involved in its ability to escape immune detection [55-57]. B cell transformation by EBV also induces the expression of EBV-induced gene 3 (*EBI3*), which encodes a form of IL-12, responsible for the initiation of Th1-type immunity [58-60].

The host's cellular immune response has primary responsibility for the control of latent EBV infection within the B cells [49,50]. $CD4^+$ T cells activate the innate immune response to EBV and are required for the generation of robust memory responses by $CD8^+$ cells, which is important in suppressing EBV [61-63]. EBV reactivation and EBV-related lymphoproliferative diseases occur in immunosuppressed renal and bone marrow transplant patients [64] and in association with HIV [65].

During late incubation and the early infectious phase of mononucleosis, antibodies against EBV viral capsid antigen (VCA) and early antigen complex – diffuse (EA-D) appear [45,66]. Later, weeks to months after disease onset, antibodies against EBV nuclear antigen (EBNA) and early antigen complex – restricted (EA-R) emerge (Table 1) [45,67]. Antibodies against EBNA-2 are detected first and decline within a few weeks, followed by the rise of EBNA-1 antibodies, which normally persist at a stable level for life [45,67]. Thus, in a normal adult, latent EBV infection is associated with moderate, stable and highly correlated levels of IgG antibodies against VCA, EBNA-1, and EA-R, with very low or undetectable levels of antibodies against EBNA-2 and EA-D [67-69].

In situations of decreased cellular immunity, however, EBV reactivation, or the transition from latent to lytic infection, can occur. Anti-VCA IgG antibodies, anti-EBNA-2 antibodies, and anti-EA antibodies are often elevated in these situations, which is consistent with EBV reactivation. The relationship between EBV serologic responses and levels of viral replication, as detected by polymerase chain reaction, is variable [45,69]. Latent EBV can replicate and spread despite the presence of antibodies, and antibody titers correlate with viral activity rather than with the degree of protection afforded [68]. In many diseases strongly associated with EBV, such as nasopharyngeal carcinoma and Burkitt's lymphoma, anti-EBV serologies are abnormal many years before the onset of disease. In nasopharyngeal carcinoma, for example, levels of IgA anti-VCA antibody 10-fold those in normal subjects are found years in advance of the onset of disease [70], indicative of high levels of viral replication. IgA anti-VCA antibody titers are used for screening in Asia, where nasopharyngeal cancer is endemic [71,72].

EBV and the pathogenesis of RA

In the quest to uncover an infectious trigger of RA, much research has concentrated on the potential for molecular mimicry presented by EBV. EBV was first implicated in the pathogenesis of RA by Alspaugh and Tan [30,73], who reported that sera from patients with RA were reactive against a nuclear antigen in EBV-transformed lymphocytes. This 'RA nuclear antigen' was determined as a glycine/alanine-rich repeat in EBNA-1 [74,75]. Antibodies against this repeat are cross-reactive with a 62 kDa protein present in the synovium of patients with RA, but not in that of controls [76-78]. Antigenic sequence similarities exist between other EBV proteins and RA-specific proteins as well. These include the EBV-encoded protein gp110, which has sequence homology with the QKRAA amino acid motif (the 'shared epitope') of the β-chain of human leukocyte antigen (HLA)-DR4 [79,80]. Humans with EBV infection have antibodies against the gp110 protein, as well as T cells with receptors that recognize the QKRAA motif in both gp110 and HLA-DR4 molecules. In addition, antibodies against EBV peptide

Table 1

Patterns of anti-Epstein–Barr virus (EBV) serologies observed in different disease states

Disease state	VCA	EBNA-1	EBNA-2	EA	References
Early, acute primary EBV infection	IgA, IgM	Undetectable	↑↑	EA-D	[45,66]
Primary infection (weeks to months)	IgG	↑	↓	EA-R	[45,67]
Latent EBV infection in healthy host	Stable IgG	Stable	↓	Stable EA-R	[67]
Reactivation/EBV replication	↑↑ IgG	↑↑	↑	↑	[67-69,117]
Nasopharyngeal carcinoma[a]	↑↑ IgG, IgA			↑ IgG, IgA	[70,118-120]
Burkitt's lymphoma[a]	↑↑ IgG	↑	↑↑	↑	[70,121]
Multiple sclerosis[a]	↑	↑	↑↑	↑	[111,112]
Systemic lupus erythematosus[a]	↑	↑			[113,114]
Rheumatoid arthritis	↑	↑	↑	↑	[82-86,122]

EA, EBV early antigen; EA-D, EBV early antigen – diffuse; EA-R, EBV early antigen – restricted; EBNA, EBV nuclear antigen; VCA, EBV viral capsid antigen. [a]Abnormalities observed before disease onset.

p107, the major epitope of the EBV-encoded EBNA-1 antigen, recognize and bind to denatured collagen and keratin [81]. These findings support the hypothesis that molecular mimicry, either by influencing T cell receptor recognition of the HLA 'shared epitope' or through the production of autoantibodies against joint proteins, is involved in RA disease pathogenesis.

Patients with existing RA have higher levels of antibodies against several EBV-encoded proteins, including VCA [82], early antigen (EA) [82], EBNA-1 [82-85], and EBNA-2 [86], than do healthy controls, and the presence of RF does not seem to be related to these elevations (Table 1). Patients suffering from RA have a 10-fold increase in EBV DNA load in peripheral blood mononuclear cells compared with that in controls; this elevation is stable and not influenced by the presence or absence of RF, age, duration of RA, disease activity, or RA treatment [87]. Patients with RA have significantly higher numbers of circulating EBV-infected B cells [88] and EBV DNA loads in saliva [42]. Several studies have shown that levels of EBV DNA and mRNA are much higher in the synovium of patients with RA than in that of healthy controls [83,89-91]. Synovial EBV DNA loads are highest in patients with RA with at least one copy of the HLA-DRB1 'shared epitope', the strongest known genetic risk factor for RA [89]. However, these cross-sectional findings have never been tested in a prospective cohort with blood drawn before the diagnosis of RA. Nevertheless, given the ubiquity of the virus in the population, a binary assay for the presence of anti-EBV antibodies years preceding the onset of RA would be less informative than a sensitive titer quantification compared with controls.

EBV-specific T cell function is also impaired in RA [92-98]. A large proportion of the $CD8^+$ T cells infiltrating rheumatoid synovium recognize the EBV transactivating factors, BZLF-1 and BMLF-1, important in the control of EBV reactivation [99]. The HLA-DR4 shared epitope, a strong genetic risk factor for RA, is associated with low frequencies of T cells specific for the EBV gp110 glycoprotein, also critical in the control of EBV infection [98]. Clonal expansion of peripheral $CD8^+$ $CD28^-$ EBV-specific T cells is observed in patients with RA but not in controls [100]. These cells are thought to be dysfunctional, senescent suppressor T cells, possibly caused by recurrent EBV stimulation and/or a primary defect of T cell differentiation and proliferation in RA.

Antibodies directed against cyclic citrullinated peptides (CCPs) are increasingly important in the early diagnosis of RA [101,102]. Citrullination is the process of deimination of peptidyl arginine to peptidyl citrulline, recognized specifically by anti-CCP antibodies. These autoantibodies are directed against citrullinated proteins in the rheumatoid synovium, including fibrin, filaggrin, perinuclear factor, and keratin [103]. They are highly specific for RA (sensitivity 68%, specificity 98%) [101] and in prospective cohort studies are present several years before the onset of RA [104-106]. Klareskog and colleagues in Sweden have found that cigarette smoking may trigger HLA-DR restricted immune reactions to autoantigens modified by citrullination, potentially explaining the interaction between HLA shared epitope and cigarette smoking that greatly increases the risk of anti-CCP-positive RA (L Klareskog, personal communication). Although it has not yet been studied in relation to the citrullination of autoantigens or the formation of autoantibodies, EBV could potentially have a similar role. Moreover, the regulation of B cell apoptosis might be important in the production of anti-CCP antibodies [107]; EBV persists indefinitely in host B cells and encodes at least two proteins that interfere with apoptosis, namely BHFR1 (a viral homologue of the anti-apoptotic protein Bcl-2) [108] and LMP-1 (latent membrane protein-1) [109].

Chicken or egg?

Although the observations noted above support an association between EBV, or the host's immune response to it, and RA, this association need not be causative. Elevated anti-EBV antibody titers have also been found in other autoimmune diseases, including Sjögren's syndrome [110], and years before the onset of both multiple sclerosis [111,112] and systemic lupus erythematosus (SLE) [113,114]. Anti-EBV antibody titers rise gradually from their first detectable levels years before the first symptoms of SLE until the time of SLE diagnosis, paralleling, and in some cases preceding, the development of SLE-specific antibodies [113,114].

Whether the observed abnormalities in EBV-directed immune responses and EBV viral loads are a cause or a consequence of RA remains a mystery. Through its potential for molecular mimicry, by polyclonal activation of B cells, or via some other mechanism, EBV or an EBV-specific immune response could be a trigger for the development of RA in the genetically predisposed. Alternatively, an innate or acquired immune defect in those with or at risk for RA could handicap the host's ability to suppress this chronic viral infection. There is mounting evidence that patients with lupus, for example, have impaired EBV-specific immune responses [115] and the frequency of EBV-infected cells in the blood of patients with SLE increases during SLE disease flares, independently of immunosuppressive therapy and in concert with aberrant expression of viral proteins [116]. This suggests that in those with SLE, and perhaps similarly in those with RA, T cell control of latent EBV infection is defective. Whether the virus actually has an etiologic role in these autoimmune diseases, or whether underlying immune abnormalities allow dysregulation of latent EBV as an epiphenomenon, is the crux of the matter.

Conclusion

The cause of RA, a highly disabling systemic autoimmune disease, remains unknown. Family studies and genome-wide scans have shown that there is an important genetic influence in the susceptibility to RA; evidence points to a common virus, such as EBV, that could act as a trigger in genetically susceptible hosts. So far, studies looking for an association between EBV infection and RA have been characterized by small numbers and retrospective or cross-sectional designs. Patients with established RA seem to have elevated levels of anti-EBV antibodies and viral loads. These study designs have not been able to address the timing of these abnormalities with regard to the development of RA, nor have they been able to exclude the possibility that RA itself, or its treatment, is responsible for abnormally elevated EBV serologic responses and viral loads. Understanding the timing and directionality of the EBV–RA relationship is crucial to distinguishing inciting from secondary events in RA pathogenesis and to advancing our understanding of the etiology of RA.

Competing interests

The author(s) declare that they have no competing interests.

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