Review

Autoimmune disease: A role for new anti-viral therapies?

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A B S T R A C T

Many chronic human diseases may have an underlying autoimmune mechanism. In this review, the author presents a case of autoimmune CIU (chronic idiopathic urticaria) in stable remission after therapy with a retroviral integrase inhibitor, raltegravir (Isentress). Previous reports located using the search terms “autoimmunity” and “anti-viral” and related topics in the pubmed data-base are reviewed suggesting that novel anti-viral agents such as retroviral integrase inhibitors, gene silencing therapies and eventually vaccines may provide new options for anti-viral therapy of autoimmune diseases. Cited epidemiologic and experimental evidence suggests that increased replication of epigenomic viral pathogens such as Epstein–Barr Virus (EBV) in chronic human autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus Erythematosus (SLE), and multiple sclerosis (MS) may activate endogenous human retroviruses (HERV) as a pathologic mechanism. Memory B cells are the reservoir of infection of EBV and also express endogenous retroviruses, thus depletion of memory B-lymphocytes by monoclonal antibodies (Rituximab) may have therapeutic anti-viral effects in addition to effects on B-lymphocyte presentation of both EBV and HERV superantigens. Other novel anti-viral therapies of chronic autoimmune diseases, such as retroviral integrase inhibitors, could be effective, although not without risk.

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Contents

1. Rational for anti-viral therapy of autoimmune disease ........................................... 89
   1.1. Chronic idiopathic urticaria, a prototype for the role of anti-viral therapy in autoimmune syndromes .......................... 89
   1.2. A case of steroid, cyclosporine and xolair resistant chronic autoimmune urticaria with response to anti-viral therapy, raltegravir ....... 89
2. Overview of immunopathology associated with Epstein–Barr Virus (EBV) ................. 91
   2.1. Epstein–Barr Virus infection as a model of epigenomic chronic viral infection associated with human immunopathology and autoimmune disease .... 91
   2.2. X-linked lymphoproliferative syndrome as a model of viral induced immunopathology ................................................ 91
3. Abnormal immune responses to EBV infection in prototypical autoimmune diseases ....... 92
   3.1. Rheumatoid Arthritis (RA) ........................................................................ 92
   3.2. Systemic Lupus Erythematosus (SLE) ............................................................ 92
   3.3. Multiple Sclerosis (MS) ............................................................................. 92
   3.4. Epigenomic pathogens (such as EBV) and endogenous viral pathogens (HERV) as triggers to lymphocyte activation and epitope spreading ........................................ 92
4. Evidence based medicine supporting anti-viral therapy for autoimmune diseases ............. 93
   4.1. Memory B lymphocytes as targets for anti-viral therapy in autoimmune disease ........................................... 93
   4.2. Retroviral integrase inhibitors as a novel form of anti-viral therapy for autoimmune disease ................................. 93
   4.3. Other forms of novel anti-viral therapy of autoimmune disease ......................... 94

Abbreviations: CMV, Cytomegalovirus; EBV, Epstein–Barr Virus; HSV, Herpes Simplex; HIV-1, Human Immunodeficiency Virus 1; VZV, Varicella-Zoster Virus; HSV6, Human Herpes Virus 6; ANA, Antinuclear Antibody; MS, Multiple Sclerosis; EGS, RNAse P External Guide Sequence; RNAi, Interfering RNA; RAG, Recombination Activating Genes; RA, Rheumatoid Arthritis; SH2D1A, Sarc Homology 2 Domain protein 1A; SAP, SLAM-Associated Protein; SLE, Systemic Lupus Erythematosus; XLP, X-linked Lympho-Proliferative syndrome; ERV, Endogenous Retro Virus; HERV, Human Endogenous Retro Virus.

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1. Rational for anti-viral therapy of autoimmune disease

1.1. Chronic idiopathic urticaria, a prototype for the role of anti-viral therapy in autoimmune syndromes

Chronic idiopathic urticaria (CIU, also termed chronic spontaneous urticaria or chronic autoimmune urticaria) is in many cases an autoimmune syndrome [1,2]. In some cases CIU is associated with autoimmune thyroid diseases [3,4], and in many cases CIU is associated with autoantibodies binding to and activating the IgE receptor, suggesting that autoantibodies may be part of the pathogenic mechanism of the disease, although other inflammatory mechanisms may also play a role [5]. Skin biopsies in CIU show an infiltrate of lymphocytes and other inflammatory cells and cytokines in contrast to allergic, post viral or physical urticaria, also consistent with an autoimmune pathogenesis. Like many autoimmune diseases, CIU can require oral corticosteroids or other anti-inflammatory therapies. As in other autoimmune diseases such as Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE), and multiple sclerosis (MS), CIU may be associated with abnormal response to common infectious agents, although a causal role of infectious agents in CIU and other autoimmune diseases has been difficult to prove as will be reviewed in more detail in Sections 2 and 3 of this review [6,7]. Because of the daily skin lesions on the skin, it is possible to directly monitor clinical response to novel therapy [8,9]. In contrast, other autoimmune diseases primarily targeting organs other than the skin must be monitored indirectly or by surrogate markers of disease progression.

This review begins with a description of a particularly severe case of CIU, resistant to anti-histamines as well as anti-inflammatory therapies including cyclosporine and oral corticosteroids, as well as the humanized monoclonal antibody xolair (omalizumab) [1,9]. A complete clinical response of this patient to therapy with Raltegravir, a drug developed for therapy of Human Immunodeficiency Virus (HIV-1) is described, and provides the introduction for a discussion of the possible mechanisms of raltegravir in this condition. In the model presented in Fig. 1 of this report, Raltegravir, a retroviral integrase inhibitor could have effects in CIU through direct suppression of epigenomic herpes virus replication, but also through suppression of an endogenous retroviral pathogen or human endogenous retrovirus (HERV) activated by herpes replication. The mechanism of Raltegravir is described in more detail in Fig. 2 and in Section 4 of this report, including evidence that Raltegravir could also cause suppression of inflammation through other indirect anti-inflammatory effects, for example by blocking generation of T and B lymphocyte receptors by the RAG (Recombination Activating Genes) which may also be inhibited by retroviral integrase inhibitors. The authors suggest that these observations may provide a basis for further studies of the risk and benefits of novel anti-viral therapies in autoimmune diseases.

1.2. A case of steroid, cyclosporine and xolair resistant chronic autoimmune urticaria with response to anti-viral therapy, raltegravir

A 36 year old woman was referred for evaluation of daily urticaria (hives) at times associated with respiratory symptoms and wheezing. A serum basophil cd203c activation assay demonstrated that she had autoantibodies capable of activating donor basophils (7.5% activation versus less than 5.0 control, testing by NJC, 1400 Jackson St., Denver, CO) while taking 40–60 mg daily prednisone for control of urticaria and associated wheezing. Laboratory testing was otherwise unremarkable including normal immunoglobulin, cellular immunity, serum protein electrophoresis and tryptase, with the exception of elevated total IgE (312 kU/L nl<114) and elevated IgG to EBV antigens (negative IgM to VCA, but elevated IgG to VCA->5 Units with positive >1.1, EBNA->5 with positive >1.1, EA 1.78 with positive >1.1). She also had evidence of past infection with CMV, HSV type 1, VZV, and HHV6, also with elevated IgG titers but negative IgM. ANA and thyroid function were normal, and there was no evidence of hepatitis.

Her symptoms were not affected by high doses of antihistamines, immunosuppressive agents such as cyclosporine, and responded only to high dose oral corticosteroids with significant steroid associated pathology. Because of the evidence of abnormally elevated IgG to several different human herpes viruses she was offered a trial of Acyclovir, an anti-viral agent that is approved for therapy of several different human herpes viruses [10–13]. Anecdotally and in the authors’ personal experience, Acyclovir can lead to decreased oral corticosteroid usage and in some cases remission of steroid dependent CIU, but this has not been studied in controlled series. However, Acyclovir was not associated in any change in her symptoms. After failure of 3 months of acyclovir therapy she began therapy with omalizumab (Xolair), a monoclonal antibody targeting the human IgE receptor by decreasing IgE binding to the receptor. Omalizumab was indicated because of her elevated IgE serum level and wheezing associated with the CIU [1,9]. She had a partial response omalizumab with oral corticosteroid dosing decreased from 40–60 mg daily to 10–20 mg daily. After more than 2 years on omalizumab she remained unable to discontinue oral corticosteroids and remained a partial responder with continued daily breakthrough symptoms.

Several recent reports suggest a role of retroviral integrase inhibitors as therapy for herpes virus infections either by effects on a herpes recombinase related to the retroviral integrase [14,15], or by effects on another conserved family of herpes virus proteins termed terminases required for genome cleavage and viral packaging [16]. Because of the patient’s documented elevated titers of IgG to both EBV and other human herpes viruses, she was offered a trial of Raltegravir (Isentris), a retroviral integrase inhibitor with FDA approval for use in HIV-1 infection [17–19]. Remarkably, in contrast to her complete lack of response to acyclovir 2 years previously she experienced a complete remission of all of her symptoms after less than one week of Raltegravir. Prednisone was slowly tapered followed by discontinuation of Raltegravir over a 3-month period with any recurrence of urticaria. As will be reported in more detail elsewhere, she remained completely free of any urticaria for approximately 3 additional months with no oral corticosteroid requirements, although she continued on omalizumab because of her past history of asthma. Many of her steroid related side effects as well as quality of life markedly improved.

The rapid and sustained response of CIU in this patient to therapy with an anti-viral drug, raltegravir may be of general interest, and is in sharp contrast to evidence of an autoimmune disease promoting effect of raltegravir in an autoimmune disease mouse model [20]. The remainder of this review will propose new directions for anti-viral therapy of other common autoimmune diseases such as RA, SLE, and MS that are associated with abnormal elevated serological responses to EBV and other chronic
Viral infections. To provide a framework for further discussion the author provides a model (Fig. 1). As shown in diagram form in Fig. 1, EBV infection in memory B-lymphocytes activates an endogenous interferon sensitive HERV encoding a T-lymphocyte activating superantigen. Because this HERV encoded superantigen stimulates T-lymphocyte activation and inflammatory cytokines in human autoimmune diseases, both EBV and HERV are also targets of antiviral therapies due to B-lymphocyte depletion (Fig. 1). This model may be helpful in developing future anti-viral therapies for autoimmune diseases, as will be reviewed in detail in the remainder of this work.
2. Overview of immunopathology associated with Epstein-Barr Virus (EBV)

2.1. Epstein–Barr Virus infection as a model of epigenomic chronic viral infection associated with human immunopathology and autoimmune disease

As noted above, the CIU patient with described in this report showed evidence of abnormally high levels EBV (Human Herpes Virus 4), and past infection with other herpes viruses (HSV, Varicella, HHV6) possibly related to her response to raltegravir. EBV has been extensively characterized as a putative co-factor in human autoimmune disease since its identification as the cause of infectious mononucleosis, and this will be reviewed in more detail following a brief review of the biology of the virus and the role of the immune system in its control. Nearly all of the world's adult population is infected with EBV [28]. Soon after the discovery of the virus it was noted that the age of viral seroconversion was markedly different in different populations. Almost complete seroconversion noted by 1 year of age in Third World societies, while 50% or more of adolescents remain seronegative in First World societies such as the United States with some notable exceptions, for example Japan where relatively early seroconversion occurs despite First World economic conditions [28]. These epidemiologic observations may be important because infection in the first year of life may be partially neutralized by maternal antibodies transferred in utero and also in breast milk leading to a milder infection, and is consistent with a so-called hygiene hypothesis of infectious disease pathology in Western society [29,30]. In Western societies, infection with chronic viral pathogens such as EBV later in life may overlap developmental stages such as adolescence with associated proliferation of the virus in immune and reproductive tissues.

B-lymphocytes are the site of viral persistence [31–35]. In lymphocytes, epithelial cells and possibly macrophages the virus replicates and also may cause malignant transformation. The virus also infects some human T-lymphocytes, particularly immature T-lymphocytes undergoing repertoire development in the thymus, and T-lymphocytes also play a role in viral-associated immunopathology [36–38]. Typically, altered T-cell morphology termed “atypical lymphocytes” and abnormal T-cell function are a diagnostic feature of infection with EBV and these abnormalities resolve with recovery from primary infection [37,38]. A normal serologic response to primary infection with the virus is characterized by first the appearance of IgM against the viral capsid antigen (VCA) with maximal IgM response approximately 1–3 months after infection, followed by a lifelong low level of IgG response to VCA and EBNA (Epstein–Barr Virus Nuclear Antigen). IgG against another viral antigen complex termed EA (Early Antigen) is detected during the first years after infection but EA IgG titers decrease with time. The virus is associated with lymphocyte proliferation and cancer and production of viral specific IgA has also been identified as a marker for some viral associated malignancy such as nasopharyngeal carcinoma, possibly reflecting high levels of viral replication in tissues such as epithelial cells [39].

A combination of these serological markers can be useful in estimating the time from primary infection with EBV and identifying normal and abnormal response to viral infection. An abnormal serological response to EBV, often identified in autoimmune diseases can be defined by abnormalities such as chronically elevated titers to latency antigens such as EBNA or failure to produce IgG to EBNA, increasing rather than decreasing titers of IgG to VCA and EA with time, or IgA against viral proteins. The so called “monospot” assay used to determine acute EBV infection through the presence of an associated heterophile or cross species antibody is both less sensitive and specific in diagnosing EBV infection than viral protein specific immunoglobulin titers and is not a substitute for viral serology, although the monospot may be useful as a relatively inexpensive screening tool. Importantly, a lifelong IgG response to VCA and EBNA is normal and positive serology to these viral proteins should not be confused with immunopathology.

In B-lymphocytes the virus establishes a latent infection for the life of the host by immortalization of latently infected cells, related to its role as a co-factor in malignancy [39]. Because the virus persists for the life of the host in a latent form in B-lymphocytes, an intact T-cell response including both CD4 and CD8 T-cell subsets is critical for control of viral replication, and infusions of T cells prepared in vitro can control viral-associated tumors in immunocompromised patients [31]. Remarkably, serologic data confirms that acute mononucleosis is not evident in many individuals who seroconvert to EBV. Benign clonal proliferations of T-lymphocytes are also evident in patients without any evidence of active disease [31]. Factors which underlie the ability of some individuals to recover from primary infection with no clinical symptoms, while others have a prolonged typical mononucleosis characterized by lymphadenopathy, splenomegaly, fever, and fatigue, and still others develop viral associated malignancy are not well understood [40].

2.2. X-linked lympho-proliferative syndrome as a model of viral induced immunopathology

EBV triggers a fatal lympho-proliferative syndrome termed X-linked lympho-proliferative syndrome (XLP) in genetically predisposed males, which has provided recent insights into viral associated immunopathology and the immune responses required for control of viral replication [41–43]. XLP syndrome in most cases results from an inborn deficiency of an X-linked T-cell and NK-cell regulatory factor termed SH2D1A (Sarc Homology 2 Domain protein 1A), to indicate that the protein possesses a signaling domain similar to those of other Sarc proteins. Alternatively, this gene product has been named SAP (SLAM-Associated Protein) to indicate its association with the lymphocyte signaling molecule SLAM (Signaling Lymphocyte Activation Marker) [42]. Because of the usually fatal prognosis for XLP if untreated, bone marrow transplantation prior to EBV infection has been utilized to prevent fatal disease in both canonical and variant forms of XLP [41–43].

If EBV infection occurs in an individual with the XLP syndrome, a rapidly progressive and usually fatal T cell proliferation and dysfunction is evident with death resulting from hepatic necrosis often accompanied by lymphoma or other malignancy. In the absence of EBV infection, patients may appear clinically well, but absence of functional XLP gene product results in a progressive T cell dysfunction evident primarily as an increased risk of autoimmune syndromes and lymphoma. In general, hereditary immunodeficiency affects resistance to EBV directly in correlation to loss of T-cell function. Certain HLA types confer an increased risk immunopathology. Collectively, these observations suggest that a range of T and NK cell responses to EBV infection are possible, ranging from silent seroconversion to fulminant and fatal T cell dysfunction, depending upon the host response and other epidemiologic factors such as age of infection [41–43].

Significantly, it has been shown that treatment of XLP with rituximab, a monoclonal antibody used for depletion of B lymphocytes was of therapeutic benefit in two patients with XLP and fulminant EBV infection [41]. Rituximab completely cured the immunopathology that is normally fatal in this syndrome. The effectiveness of B-lymphocyte depletion seems to involve an anti-viral mechanism although this has not been studied in detail. However, it seems plausible that depletion of memory B lymphocytes which are the viral reservoir of infection contributes to reduction of immunopathology due to EBV associated B lymphocyte dysfunction in XLP (Fig. 1). The remainder of this review will consider the role of EBV in other specific autoimmune syndromes as a prototype of a viral co-factor in autoimmune disease, and then critically review evidence that anti-viral therapy may have a role in therapy of these autoimmune syndromes.
3. Abnormal immune responses to EBV infection in prototypical autoimmune diseases

3.1. Rheumatoid Arthritis (RA)

An abnormal response to EBV infection in a specific autoimmune disease was apparently first identified and reported more than three decades ago in RA [44,45]. T-lymphocytes from patients with RA could not suppress transformation of B-lymphocytes in vitro in contrast to T-cell from control patients with evidence of past EBV infection but without RA. The inability of cells from patients with RA to control EBV replication in vitro was found to result from a specific defect related to control of EBV immunity rather than a general defect in T-cell function, because the T-cells proliferated normally when stimulated with lymphocytes expressing heterologous HLA markers. Evidence for viral replication in synovial tissue was presented [46].

Other evidence suggested an increased level of EBV viral replication in RA as reflected by immunoglobulin levels, and viral shedding [47]. More recently, specific T cells cross reactive with host proteins and EBV viral proteins have been described in patients with RA, and T lymphocytes isolated from inflamed joints of RA patients have been found to react specifically with EBV specific T-cell epitopes and EBV antigens are detected in germinal center like proliferations in joints with active RA [48–50]. However, infiltration of joints by T-cells in RA could be a general property of joint inflammation rather than a specific response to EBV. Thus, it has been difficult to distinguish whether the T-cell regulatory abnormalities and increased EBV replication noted in RA are a consequence or a cause of the disease. Not unexpectedly, given the role of memory B-lymphocytes as the reservoir and source of EBV (Fig. 1), rheumatoid arthritis responds to depletion of memory B-lymphocytes by rituximab [51]. However, it is not established to what extent the beneficial effects of rituximab in RA are due to depletion of EBV directly versus effects on B-lymphocyte function such as B-lymphocyte antigen presentation. It could be argued that depletion of EBV accounts for the beneficial effects of rituximab in RA, or alternatively that the depletion of EBV is an unavoidable but unrelated consequence of rituximab therapy and that the therapeutic effects of rituximab are entirely due to the effects of depletion of B-lymphocytes on B lymphocyte presentation to T lymphocytes.

3.2. Systemic Lupus Erythematosus (SLE)

An abnormal antibody response to EBV antigens was first evident through epidemiologic evidence and later confirmed by several different approaches in SLE [52–59]. Patients with recent onset SLE had evidence of recent infection with EBV but not with other ubiquitous herpes virus pathogens [54]. Elevated titers to EBV antigens and elevated EBV genome levels as determined by PCR (polymerase chain reaction) were also found in association with SLE in both adolescents and adults. Some authors suggest that a specific interaction between an EBV capsid antigen and a host antigen resulted in SLE through a combination of molecular mimicry and possibly T-cell regulatory defects peculiar to individuals with a predisposition to SLE [52,57,60]. Subsequently other independent investigators have confirmed that individuals with SLE invariably have increased evidence of viral replication and an abnormal immune response to the virus [56,59]. PCR titers of viral replication in these studies suggest that more EBV infected cells are present, rather than a higher level of EBV genomes per cell, but studies were not performed on tissues such as lymph nodes that might arguably be more relevant to immunopathological mechanisms [61]. As in the case of RA, SLE responds to B-lymphocyte depletion with rituximab, although the relative contributions of anti-viral versus effects on non-viral B lymphocytes functions has not been established [62].

3.3. Multiple Sclerosis (MS)

Perhaps the most compelling evidence supporting a causal or direct role of EBV in a human autoimmune disease has emerged recently with respect to Multiple Sclerosis (MS). A widely cited series of studies reported on a large database of nurses followed prospectively to identify independent risk factors for MS. Abnormal responses to EBV, but not cytomegalovirus antigens were noted in patients with MS after the onset of illness [63,64]. As with SLE, evidence of an abnormal response to EBV preceded the onset of clinical MS and appeared to refute the argument that abnormal response to EBV was secondary to, rather than causally related to the illness. Others have confirmed these results for both adult and pediatric forms of MS, and convincing evidence of abnormal cross reactivity is demonstrated between EBV antigens and human antibodies in MS [65–70]. Abnormal cellular immunity in response to EBV antigens has suggested the proposal that EBV specific vaccines may eventually be used in therapy of MS as will be discussed in more detail later in this review [71–75].

The role of EBV specific antibodies either in the CSF or in the serum is controversial. Some groups have found evidence that serological immunoglobulin levels of EBV specific immunoglobulin predict relapse of MS, while other groups have not replicated this finding. Similarly, monoclonal bands in the CSF of MS patients do not appear to be specific for EBV proteins, although this cannot exclude EBV related epitopes generated by epitope spreading from EBV specific proteins. Similarly, CD4 B lymphocytes isolated from MS patients recognize different epitopes on EBV proteins than those from healthy EBV positive controls, but the role of these epitopes in disease progress is not established. Thus while an abnormal response to EBV predicts subsequent MS, once MS is clinically evident the role of EBV replication and specific immunoglobulins in disease progression is less clear [65,76,77].

The most direct evidence supporting a causal role of EBV in both MS initiation as well as disease progression is the finding that germinal center like B-lymphocyte proliferative centers in the brains of MS patients are EBV positive [78,79]. Possibly, monitoring EBV replication status in the brains of living MS patients could further confirm a causal role of the virus in MS, although this does not seem likely at present due to the lack of non-invasive technology for this purpose. As with RA and SLE, depletion of B lymphocytes with rituximab is highly effective in MS [80–87]. However, as noted in Fig. 1 of this work, and previously suggested by other authors [71,72], the extent to which rituximab is acting on b-lymphocytes as a viral reservoir versus non-viral effects on b-lymphocyte antigen presentation and T cell activation is not resolved.

3.4. Epigenomic pathogens (such as EBV) and endogenous viral pathogens (HERV) as triggers to lymphocyte activation and epitope spreading

The evidence reviewed above suggests that EBV is implicated as a co-factor in numerous autoimmune syndromes, raising the obvious question, why EBV rather than other infectious agents [7]? Autoimmune diseases are proposed to occur through activation of previously silent auto-reactive immune cells due to disturbances of natural regulatory mechanisms [88]. Unexpectedly, even healthy organisms contain both T and B lymphocytes that can recognize host antigens when activated through specific inflammatory signals or other “danger” signals. This discovery suggests a dynamic view in which the context of antigen presentation plays a critical role, rather than solely a deterministic interaction between a particular self-antigen and certain host antibodies or immune cells. T-cell regulatory defects in many common human autoimmune diseases have been demonstrated.

Both T and B cell epitopes to infectious agents can be cross reactive with cellular antigens (Fig. 1). In the prototypical mechanism of autoimmunity termed “molecular mimicry”, shared antigenic determinants between infectious agents and host tissues
trigger autoimmune responses in the presence of inflammation and tissue damage. The “molecular mimicry” hypothesis provides a link between common infectious agents such as Epstein–Barr Virus (EBV) and autoimmunity since EBV contains viral encoded proteins cross reactive with cellular antigens, and acute viral infection occurs in the presence of host cell inflammatory reaction. While dendritic cells, macrophages and other antigen presenting cells continually process self proteins and present them on the cell surface, in the absence of viral inflammation and cross reacting viral proteins these antigens are presumably not sufficient to trigger an immune response.

Subsequent “epitope spreading” could account for the progression of autoimmunity disease (Fig. 1). The “epitope spreading” hypothesis predicts that infectious agents triggering a vigorous or prolonged inflammatory response would be particularly associated with subsequent alterations in the T-cell repertoire favoring autoimmune disease. Related to this hypothesis, individuals with an inborn lack of resistance to specific infectious agents might be at higher risk for subsequent autoimmune disease. Similarly, cytokines such as interferon, important in coordinating antiviral host responses, may be more highly expressed in autoimmune disease target organs, and thus blocking inflammatory cytokines such as interferon is effective in a variety of autoimmune diseases.

In addition to horizontally transmitted infectious agents such as human herpes viruses that are present for life in the nucleus of host cells and thus termed by the author “epigenetic pathogens”, the genome encodes vertically transmitted human endogenous retroviral elements (HERV). Human B-lymphotrophic herpes viruses such as EBV as well as other viral and infectious pathogen activate a specific human endogenous retrovirus (HERV) inserted in an interferon responsive gene that encode a “superantigen.” Expression of this retroviral superantigen in the retroviral capsid protein is associated with activation of certain subsets of T-lymphocytes in human autoimmune diseases (Fig. 1). Similarly, in murine and other animal models of autoimmune disease, ERV are linked to autoimmune disease and encode a superantigen. The benefits of B-lymphocyte depletion in autoimmune disease may thus reflect a combination of decreased B-lymphocyte presentation of cellular antigens related to molecular mimicry and epitope spreading, as well as anti-viral effects on EBV replication, because EBV is resident in latent form in memory B-lymphocytes (Fig. 1).

4. Evidence based medicine supporting anti-viral therapy for autoimmune diseases

4.1. Memory B lymphocytes as targets for anti-viral therapy in autoimmune disease

Evidence based medicine is based on the principle that efficacy of medical therapies must be based on data from controlled human clinical studies. Although animal models are useful in developing theoretical models [89,90], these models are not identical to the human diseases that they seek to reproduce, nor are they a substitute for evidence based medicine. A body of evidence based medicine summarized previously in this report supports the efficacy of depletion of B-lymphocytes by rituximab in a variety of autoimmune diseases. As noted in Fig. 1, the memory B lymphocyte may play a variety of roles at the center of autoimmune disease pathogenesis. Depletion of memory B-lymphocytes with rituximab has therapeutic effects in autoimmune diseases such as RA, SLE, and MS in which the relationship between EBV as well as endogenous retroviral replication and disease is implicated (Fig. 1). However, rituximab illustrates a paradigm in which anti-viral effects on viral pathogens present in B lymphocytes cannot be clearly separated from the immunologic role of the B lymphocyte [71,72]. As noted above, depletion of memory B-lymphocytes has therapeutic effects both upon immunopathology and EBV viral replication in syndromes such as XLP.

Since different autoimmune syndromes such as RA, SLE and MS all respond to rituximab, although with varying response profiles, it might be argued that all of these diseases might also respond to other anti-viral therapy if anti-viral as well as immunologic effects of B lymphocyte depletion are present (Fig. 1).

Alternatively, the effects of rituximab in these syndromes might reflect only non-viral or immunologic effects of B-lymphocyte depletion, rather than anti-viral effects, or some variable combination of the two possibilities in different autoimmune diseases. One means of distinguishing between these possibilities is to consider evidence based medicine studies of anti-viral therapy with anti-viral drugs that have no known effects on B-lymphocyte antigen presentation to T-lymphocytes. Anti-viral therapies reviewed in this work may be significantly less toxic than rituximab, and thus appropriate either as a prophylactic therapy before onset of clinical disease in patients “at risk” based on immunologic markers. These agents could also be used in conjunction with more toxic therapies such as rituximab in more advanced disease. Despite extensive theoretical evidence, summarized in this report, that defects affecting viral resistance predispose towards SLE, evidence based trials of antiviral therapy with acyclovir have apparently not been reported in lupus patients, and only anecdotal evidence favors use in RA [13]. In contrast to RA and SLE, a controlled study of acyclovir antiviral therapy has been conducted in MS and showed a trend towards improvement, however this study was not sufficiently powered to permit definite conclusions and unfortunately has not been repeated in larger studies to the present time [11].

Since Acyclovir and similar nucleoside analogs are relatively weak anti-viral agents particularly regarding effects on long term levels of viral replication and prone to select for viral resistance, it could be argued that the lack of greater effectiveness of these agents in late stage autoimmune diseases such as MS is not due to a failure of anti-viral therapy of autoimmune disease in principle but rather in practice due to the lack of highly effective anti-viral agents directed at b lymphotrophic viruses such as EBV [10,91]. Because of the possible anti-proliferative effects of nucleoside analogs such as acyclovir on b-lymphocytes, conversely it could be argued that any effects of these anti-viral agents are due to effects on b-lymphocyte activation and antigen presentation by these cells. Another problem associated with all mono-therapy of infectious diseases the development of viral resistance to a single agent. Response to nucleoside analog such as acyclovir may be limited in vitro due to the rapid origins of resistant viral strains that have not been evaluated in current studies.

In summary, very limited evidence-based medicine in humans suggests that anti-viral therapy such as the nucleoside analog acyclovir may be of benefit in autoimmune diseases. Interpretation of results is complicated by the limited potency of these medications, and potentially anti-proliferative effects of these medications on the immune system. While more potent nucleoside analog therapies for herpes viruses are available these agents are associated with more toxic and immunosuppressive effects, possibly an unavoidable consequence of the mechanisms of action of nucleoside analogs on nucleic acid replication. As with combination therapy of HIV, multiple different nucleoside analogs in combination could in principle address the problem of weak anti-viral activity and resistance to acyclovir, but not without cost in terms of increased toxicity and immunosuppression. Notably, the CIU patient described in this report had no response to acyclovir, but did respond to raltegravir.

4.2. Retroviral integrase inhibitors as a novel form of anti-viral therapy for autoimmune disease

Evidently, to demonstrate unequivocal effectiveness of anti-viral therapy of autoimmune disease what is required is anti-viral therapy that is both highly viral specific (unlike rituximab) and also highly
effective in both blocking both viral replication as well as long term viral persistence (unlike acyclovir). The remainder of this review will consider retroviral integrase inhibitors such as Raltegravir as potential anti-viral agents meeting these criteria as well as potential risks and benefits that might be associated with these novel anti-viral therapies in autoimmune disease. As shown in Fig. 2, the DDE site of the retroviral integrase binds a magnesium ion required for DNA cleavage and joining for integration of the HIV provirus and thus therapy with agents that block this pathway is highly effective as anti-HIV therapy [92,93].

Herpes viruses recombine using a DDE type recombinase, and thus the author has suggested previously that retroviral integrase inhibitors would be expected to be also highly effective as anti-herpes therapy [14,15]. Unpublished observations (work in progress by the author) suggest that different retroviral integrase inhibitors have variable effects upon herpes virus replication, and thus in principle could be optimized to maximize effects on herpes viruses in vitro and in vivo. Another viral enzyme required for terminal repeat processing of cytomegalovirus (human herpes virus 3) and highly conserved in other herpes viruses including EBV has recently been shown to both contain a DDE site similar to the retroviral integrase and this report demonstrated dose dependent inhibition of genome processing in vitro, although not in vivo [16].

The fact that retroviral integrase inhibitors are already approved by the US Food and Drug Administration (FDA) as safe and effective for therapy of HIV viral disease is reassuring since these trials were conducted on adult populations already infected with B lymphotropic virus with an infectious B lymphotrophic virus such as EBV as well as other herpes viruses [19,94–96]. The clinical safety profile of retroviral integrase inhibitors vastly simplifies the ethical and practical problems associated with a trial of these agents in autoimmune diseases, since the possibility of any catastrophic or unexpected side effects of these agents in large populations is effectively excluded. The author's personal experience in CIU summarized in this report also suggests that this class of medication may be rapidly effective in chronic autoimmune urticaria, an autoimmune syndrome possibly related to an underlying infectious process.

Importantly, however, in one mouse model of SLE associated with an endogenous retroviral agent a trial of an integrase inhibitor surprisingly was associated with a more rapid progression of lupus-like disease [20]. This surprising and counter-intuitive finding seems in direct contrast to the favorable response to therapy of a human autoimmune syndrome, and remains to be explained. One possibility might be related to the differences between a murine endogenous retrovirus constitutively expressed in a variety of tissues and a human endogenous retrovirus only expressed in the presence of co-infection with other viral pathogens such as EBV. In the human disease the beneficial effects of the retroviral integrase inhibitor might predominate due to decreased replication of the actively replicating herpes virus, while in the murine model as yet unknown mechanisms directly related to the retrovirus not blocked by therapy might predominate. Evidently, an endogenous retrovirus is not equivalent to a HERV in the presence of other viral pathogens, it is evident that human autoimmune diseases are complex and reflect interactions between infectious agents as well as endogenous HERV. However, it is evident that any trial of novel anti-viral therapies such as retroviral integrase inhibitors in autoimmune disease must be carefully designed and monitored to exclude the possibility of accelerated rather than delayed progression of disease.

Worsening of disease in the murine ERV model could arise primarily from a secondary mechanism of constitutive activation of an endogenous retrovirus rather than by a primary activation of a latent HERV with an infectious B lymphotrophic virus such as EBV (Fig. 1). No increased autoimmune disease has been evident in studies of retroviral integrase inhibitors in HIV/AIDS despite the fact that these patients are at increased risk of immune dysfunction. Whatever the mechanism, it is apparent that SLE might not be the best disease for initial study of this class of anti-viral therapies. Instead, either RA or MS might be more attractive targets for initial studies since evidence suggests that direct proliferation of EBV in the targeting tissues, synovial tissue ectopic germinal centers in RA and brain ectopic germinal centers in MS, is the primary mechanism of viral involvement in the disease, rather than primary activation of ERV as proposed for SLE.

4.3. Other forms of novel anti-viral therapy of autoimmune disease

A more futuristic approach to anti-viral therapy of autoimmune diseases would build upon the current explosion in genetic information and technology to directly target viral replication through vaccines [71,72]. Vaccines have a long duration of action and/or purified neutralizing antibodies specific for EBV have been developed and may in the future be available for chronic pathogens such as EBV [97–100]. A paradigm for a successful herpes virus vaccine already exists, since vaccination for Varicella (human herpes virus 3) has proven both safe and also effective in reducing complications of Varicella in both young and elderly populations.

Intravenous immunoglobulins (IVIG) pooled from multiple donors are of proven utility in a broad spectrum of autoimmune diseases [101]. While the efficacy of IVIG is at least in part due to non-specific anti-inflammatory properties of the immunoglobulin chain [102,103], some of the efficacy may be due to neutralizing anti-bodies from donors directed at either HERV or chronic viral pathogens. In support of this hypothesis, the so called hygiene hypothesis suggests that altered age of infection with common viral and bacterial agents may be more pathogenic due to the lack of protective maternal antibodies in the host after infancy as reviewed above with respect to the biology of EBV. Oral or peptide induced tolerance has also theoretical possibilities, although results in practice have been disappointing [104,105].

Since considerable epidemiologic evidence implicates a later age of infection with EBV and subsequent increased viral replication as an important factor in viral pathogenesis, it seems logical that vaccination in early childhood for EBV or EBV-activated HERV could prevent a variety of later autoimmune syndromes such as RA, SLE and MS [71,72]. Alternatively, rather than targeting an entire population for vaccination against EBV or HERV, since certain populations can be determined to be at immediate increased risk of EBV related autoimmune disease such as MS long before onset of clinical disease, initially at least vaccines could be targeted to groups “at risk” of disease later in life based on antibody titers. It might also be possible to generate monoclonal antibodies directed at HERV, EBV or other inflammatory pathogens activated in autoimmune syndromes for therapeutic purposes.

Of course, vaccines are not without their own risks. Vaccine associated risks are largely in the category of autoimmune syndromes related to vaccine induced inflammation (ASIA) [106]. Thus, if the immune defect in auto-immunity is related not only to increased or abnormal viral replication but also to a defect in suppression of post viral inflammation of “epitope spreading” with a genetic or inherited basis, vaccination against EBV in patients at increased risk of EBV-associated immune disease could be counter-productive due to vaccine related auto-immunity. Administration of high titer monoclonal antibodies against viral epitopes associated with autoimmune disease could in principle avoid the inflammatory effects of vaccine adjuvant, although this would have a drawback of frequent administration and risk of anaphylaxis.

Anti-sense DNA, a form of gene silencing in which a nucleic acid complementary to a viral sequence is introduced into cells in order to block viral replication, was described decades ago and yet remains largely in the future due to problems with in vivo targeting [107,108]. More recent advances in gene silencing such as SiRNA targeting RNA
for sequence specific destruction by RISC (RNA induced silencing complex) and EGS (External Guide Sequences) targeting RNA for sequence specific destruction by endogenous RNAsP may increase the specificity and effectiveness of this type of targeted anti-viral therapy [109]. However, these novel anti-viral therapies are not currently available for human use, in part because of a lack of interest expressed by peer review panels that consider chronic human viral infections unimportant (unpublished observations). Interestingly, EBV and probably other chronic viral pathogens secrete viral encoded microRNA capable of silencing host lymphocyte genes, an entirely new pathway that in principle could be blocked to limit viral pathogenesis [110]. Perhaps in the future if the importance of epigenomic viral infections is better appreciated in chronic human disease, as summarized in this review, more promising therapies based on gene silencing will be possible.

Although a full discussion of gene silencing, vaccines, and related anti-viral therapy for chronic viral infections such as EBV is beyond the scope of this review, notably one FDA approved indication for anti-sense therapy is available currently as injection of therapy against cytomegalovirus (CMV) infection of the retina, and it is certainly plausible that in the future such targeted anti-viral agents could be developed for EBV, or HERV. Such a specific anti viral agent injected directly into the site or sites of abnormal viral replication, for example the brain in MS or the joint in RA might be safe and effective based upon the success of targeted therapy of CMV in the retina. CMV replication and retroviral gene expression are also readily targeted by EGS in vitro, although the effects of this new technology against EBV have not been studied either in vitro or in vivo.

5. Anti-viral therapy of autoimmune diseases: conclusions

A primary basis of medical ethics is to “do no harm.” This concept is highly relevant to the topic of therapy of autoimmune diseases in general and anti-viral therapy of autoimmune diseases in particular. Herpes viruses have been co-evolving with vertebrates for at least the past 400 to 600 million years, perhaps prior to the emergence of acquired immune system [115,111,112]. It has been suggested that interference with herpes virus replication for extended periods could have unknown consequences to the immune response to other types of pathogens [113]. Autoimmune syndromes are not only triggered by viral pathogens but also bacterial pathogens [114], and the effects of antiviral therapy on bacterial infections are unknown. The autoimmune response may also play a role in response of the immune system to other stress such as malignancy [115,116]. Another related theoretical concern is that the shared recombination mechanism between retroviral integrase and herpes virus recombinase blocked by retroviral integrase inhibitors (Fig. 2) is also present in the RAG-1 gene required for generation of the acquired immune system, and thus that prolonged use of retroviral integrase inhibitors could be immunosuppressive [14,15].

Limited evidence summarized above such as trials of the available anti-viral agent Acyclovir directly targeting b-lymphotrophic viral replication, and clinical observations tend to confirm the safety and therapeutic benefits of anti-viral therapy in human autoimmune diseases. In addition, autoimmune diseases are a growing problem in society, justifying consideration of new therapeutic approaches [6]. To facilitate evaluation of anti-viral therapy of autoimmune disease, the author presents a model of the effects of anti-viral therapy of autoimmune diseases (Fig. 1). The author also suggests that rituximab antibody depletion of memory B-lymphocytes is effective in a wide spectrum of autoimmune diseases in part because the depletion of memory B lymphocytes is anti-viral therapy directed against EBV, a lymphotrophic virus implicated in triggering autoimmune diseases. Rituximab is clearly at least in part effective as an anti-viral agent in the rare syndrome of XLP, and thus supports the concept that some of its effects in autoimmune diseases may also be related to antiviral effects of the medication.

The author also proposes that a novel class of anti-viral medications, termed retroviral integrase inhibitors (Fig. 2) may be class of therapy for chronic viral pathogens such as EBV and hence be useful in therapy of autoimmune diseases, perhaps in combination with b-lymocyte depleting therapies such as rituximab or existing anti-viral agents such as Acyclovir. Combination therapy, as in therapy of other infectious diseases combining agents with different mechanisms of action against infectious agents is in general preferable to therapy with a single agent, both because of decreased toxicity due to increased efficacy of combination therapy as well as decreased possibility of viral resistance. The retroviral integrase inhibitors may have benefits in autoimmune disease through a variety of mechanisms. Perhaps most importantly, retroviral integrase inhibitors are already available clinically and are known to be safe and effective in large populations of patients with HIV and associated HIV related immunopathology.

Other anti-viral therapies such as anti-sense and gene silencing as well as anti-viral vaccines or therapeutic anti-viral monoclonal antibodies may be on the horizon. For the present it seems that well controlled studies of various anti-viral therapies already available would be helpful to clarify new safe and effective therapies of common autoimmune diseases such as CIU, RA, SLE, and MS. In particular, the authors propose that in the near future and without large development expense, combination therapy with FDA approved anti-viral agents such as nucleoside analogs (Acyclovir) and retroviral integrase inhibitors (Raltegravir) could identify combinations of therapy with increased therapeutic index versus single agent therapy. Combination of these anti-viral agents with b-lymocyte depleting therapies such as rituximab and additional anti-viral therapies such as viral vaccines, targeted immunoglobulins and viral specific gene silencing may also be anticipated.

Take-home messages

- Endogenous viruses (HERV) and Epi-Genomic viral pathogens (Herpes Viruses) are implicated as co-factors in many or all human autoimmune diseases and could in theory be treated with new broad spectrum drugs such as Raltegravir, a retroviral integrase inhibitor effective against both retroviruses and herpes viruses.
- The role of anti-viral therapy in human autoimmune diseases is controversial with some mouse models suggesting no benefit or worsening of disease with Raltegravir, but in contrast the author provides a case of a patient with chronic autoimmune urticaria highly responsive to this medication and cites additional literature supporting a benefit of valacyclovir in other human autoimmune diseases.
- Further evidence-based medicine is required to clarify the role of anti-viral therapy such as Raltegravir as well as anti-viral gene silencing and vaccines, possibly in combination with other new therapies such as B-lymphocyte depletion with Rituximab that may have anti-viral effects.

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