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Everyone Comes from Somewhere: Systemic lupus erythematosus (SLE) and Epstein-Barr Virus, induction of host interferon (INF) and humoral anti-EBNA1 immunity

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As surely as we can infer that all progeny have had parents, we can be confident that the diseases at present thought to be “idiopathic” have origins in the differential effects of environment and genetics that await discovery.

Epstein-Barr virus (EBV), or human herpes virus 4 (HHV-4), is an obvious and recurrent suspect for the origin of inflammatory diseases. EBV transforms some infected B cells and has the potential to commandeer the immune system and inflammatory response, as is well known from EBV-induced infectious mononucleosis. Once infected, EBV persists in the host for life as a latent infection with a viral reservoir in resting memory B lymphocytes. Gene products expressed in latency and the low level of lytic virus continuously emerging from latency provoke sustained immune activation of focused anti-EBV immune responses. In the billions of seropositive, chronically EBV infected, healthy mass of humankind, the ~1% of all CD8 T cells specifically directed against EBV antigens belies the astonishing level of immune attention we humans ordinarily dedicate to this single virus (1). Every higher primate studied has its own unique specific EBV-like variant with little to no cross-species adaptation. Also, and unfortunately, there are no close homologues to EBV in the mouse and EBV does not infect the mouse, greatly retarding its experimental study.

The impressive magnitude of the human host immune response to EBV and the many now known pleiotropic interactions that this virus has with host inflammatory responses are clear opportunities to generate the dysregulation of immunologically mediated diseases. EBV has become, for example, a strong candidate etiologic agent for multiple sclerosis (reviewed in (2)). Indeed, for autoimmune diseases, the virus infection by itself is not the apparent culprit. Rather, the prime suspect is the host inflammatory response, as influenced by or in response to EBV gene products.

Interferon-alpha (INFα) induces dendritic cell differentiation with associated antigen presentation. SLE patients with active disease have been shown to have elevated levels of INFα in their serum (3). Multiple groups have found increased expression of interferon inducible or interferon stimulated genes in lupus patient peripheral blood mononuclear cells. Also, interferon activity, as measured by a gene expression reporter assay, correlates with disease activity, select autoantibody subsets, and genetic polymorphisms in some lupus-associated genes (reviewed in 4). How and when in the course of SLE pathogenesis these dysregulated interferon responses begin; and specifically, whether they precede the specific

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abnormalities of lupus autoimmunity or are a consequence of the particular immune responses characteristic of lupus, is not now known. Curious differences in the IFNα production of peripheral blood mononuclear cells of SLE patients compared to healthy seropositive controls in response to TLR9 ligands have already been described (5). Endogenous TLR and non-TLR pathways stimulate interferon production through various immune complexes and autoangenic nucleic acid/protein complexes and are reviewed in (6).

In this issue of the Journal Quan et al show that plasmacytoid dendritic cells (pDC) from healthy individuals produce high levels of IFNα in response to EBV (7). Induction of IFNα by pDC is stimulated by EBV binding, not necessarily requiring viral replication, and requires endosomal acidification and TLR9 engagement. Small RNAs, or EBERs, which are found in latent and lytic EBV infection also induce IFNα production through TLR7 engagement in pDCs. In latently infected B cells EBERs induce type I interferons via RIG-I, a pathway that is independent of toll-like receptors (TLRs) (8,9). These findings set the stage to merge two now extensive and previously largely independent literatures, one focused on the role of IFNα in SLE and the other on EBV in SLE, to develop a more complete conceptual construct of lupus pathogenesis.

The possibility that SLE is associated with EBV infection has been building sporadically since the first association was first accidentally found in 1969 (10). Since then the hypothesis that EBV somehow causes lupus has been entertained, discarded, and finally convincingly resuscitated (11,12). The association of EBV infection with SLE is most convincing in children where the lower background level of infection in the controls provides much improved statistical power (12) relative to adults. At this point, not only has the serologic association has been replicated multiple times, even in adults (13), but the association has also been found by recovering viral DNA sequence (12,14).

EBV has many properties that make it an ideal etiologic agent for SLE. EBV is ubiquitous in the human population which is required based upon the wide-spread occurrence of lupus. EBV makes its own version of IL-10, which would be expected to encourage TH2 responses, perhaps influencing the course of humoral autoimmunity in SLE. EBV produces BHRF1 (which has a protein structure similar to Bcl2) and inhibits apoptosis which could allow continued life of cells that should die in order to avoid autoimmunity (6). Clearly, the presence of increased levels of IFNα in sera from SLE patients and the induction of SLE by interferon treatment leads to the expectation that a virus causing lupus would be expected to induce interferon. (To complicate these issues CD21 the major receptor for EBV has been also shown to be a receptor binding IFNα (15).) EBV induces lupus-oriented humoral autoimmunity as part of its natural history of disease of infection, making the idea that it might be involved in generating the autoimmunity of lupus seem less far-fetched.

Although early control of EBV infection is determined at least in part by the innate immune system, adaptive T cell responses are primarily involved in long-term suppression of EBV in latency and in episodes of reactivation. Regulatory T cell numbers and activity is decreased in blood from infectious mononucleosis (IM) patients compared to EBV seropositive controls (16). Expansion of T cell responses after EBV infection leads to inflammatory cytokine production and B cell help, thereby inducing non-specific autoimmunity in infectious mononucleosis. Indeed, the autoimmunity has both specific and precursor characteristics of lupus autoimmunity (17,18). How these various interactions between EBV and the immune system may, or may not, be specifically dysregulated in SLE remain to be explored.

The host immune response to EBV is complex and complicated by the many strategies that the virus has to persist in the host and avoid immune recognition; latency is only one of

these. The major viral surface glycoprotein binds CD21 (complement receptor 2) and with the help of HLA class II molecules gains entry in to the B cell (19). Antibody responses against the surface protein are virtually universal among those infected, while antibodies binding other proteins, Epstein-Barr Nuclear Antigen 1 (EBNA1), EBNA2, Early Antigen, EBNA3A, EBNA3B, and latent membrane protein-2 (LMP-2) are progressively less frequent. The cellular response of CD8 T cells are most consistently directed against EBNA3 with other antigens, including EBNA1 being more of the focus of CD4 T cells. Class I HLA antigen presentation of EBNA1 is partially inhibited by a glycine-alanine rich central region of the protein (20).

Other autoimmune irregularities accompany EBNA1 immunity. The initiating events for autoimmunity of anti-60kd Ro and anti-Sm B appear to be consequent to the formation of separate cross-reactive antibodies. These are antibodies that bind EBNA1 structures and also bind structures in 60kD Ro or Sm B, respectively (Figure 1). Relative to healthy controls, lupus patients have not only a higher frequency of anti-EBNA1 antibody responses, thereby making this particular adaptive immune response a risk factor for disease beyond the association with EBV infection, but lupus patients also a tendency to produce anti-EBNA1 heteroimmune responses that predispose to forming cross-reactive self antibodies (21). Further, EBNA1 expression in mice induces anti-double stranded DNA and anti-Sm antibodies (22), showing that EBNA1 can generate autoimmunity in a naïve mammalian immune system. These and other data are consistent with the host response to EBNA1 being of critical importance in the pathogenesis of SLE. A hypothetical model for this process is presented in Figure 1.

SLE patients have a 10 to 100 fold more active EBV infection, as measured by viral load in peripheral blood, by the frequency of infected B cells, or by the amount of virus free in serum, seemingly independent of concurrent immunosuppressive SLE therapy (23–25). Extending the observations of Quan et al (7) by predicting that greater IFNα production by pDCs in those carrying a larger EBV viral load should increase the risk of developing or maintaining the pathologic changes of SLE. (The demonstration is so far lacking that an increased EBV viral load with greater IFNα production precedes the onset of SLE or that the increased load exacerbates the pathology accompanying lupus autoimmunity, once established.) The increased viral load is also consistent with the EBV specific T cell defect long appreciated in SLE (26) with higher CD4+ and lower CD8+ EBV-specific T cells (24).

Quan et al, unify two previously independently developed concepts of SLE pathogenesis: the role of type I interferon and dendritic cells (5) and the many lines of evidence now known to implicate EBV in SLE. The data from Quan et al show that EBV induces IFNα from plasmacytoid dendritic cells, consistent with both of these hypotheses, and thereby adding another finding consistent with SLE being caused in part by EBV (or, more specifically, by the host response to EBV) (11). In addition, the rapidly expanding list of DNA variants associated with SLE, now >30, includes a few genes that specifically relate the host response to EBV infection and interferon (e.g., IRF5, TYK2, & STAT4) and a larger sub-set of putative lupus disease risk genes that influence EBV infection in the host.

As the action of viral gene products on the immune system are explained, the progressively more detailed knowledge of the host immune response has identified potentially important relationships. Small EBV RNAs (especially EBER1) form complexes with the La antigen and bind to TLR3, inducing type I interferon and other inflammatory cytokines (27). EBV infection also induces an expansion of activated T cells with associated expression of cell surface markers and inflammatory cytokines (1), many of which contribute toward the symptoms found in primary EBV infection, some of which are also found in lupus (e.g., fever, myalgia, arthralgia, fatigue, malaise, and lymphadenopathy).

Although Quan et al. focus on generation of interferons by pDCs through EBV, other work has shown that EBV interacts with TLRs and upregulates type I interferon and interferon inducible genes (Figure 2). UV-inactivated and untreated EBV increases TLR7 expression in naïve B cells. EBV binding also increases interferon stimulated gene expression and EBV/TLR interactions enhance B cell proliferation (28). Work from the Zhang laboratory (29) demonstrates that the major latent protein of EBV, latent membrane protein-1 (LMP-1), is able to induce expression of several interferon stimulated genes in transfection experiments, surprisingly without a detectable increase in interferon production, thereby arguing for direct interactions of LMP-1 with interferon regulatory elements in specific interferon stimulated genes (29). LMP-1 also potentially primes latently EBV infected cells for type I interferon production by a secondary infection (30). Chronic IFNα treatment of B cells may also encourage the transformed outgrowth of cells with the higher LMP-1 levels (31).

Quan et al. (7) do not experimentally address SLE directly and so set the stage for future studies to explore the interactions between SLE-associated environmental factors and interferon inducible gene expression changes. What is the role of EBV in these and other differences? Is the physiologic increase of interferon levels after EBV infection/reactivation sufficient to drive, relative to controls, the interferon signature of gene expression that has been reported in SLE? Are increased levels of interferon in SLE present before clinical disease onset and, if so, do these increases correlate with EBV seroconversion or reactivation? What influence on the natural history of disease do the immune complexes formed in lupus sera, which also induce interferon, have in relation to the interferon-inducing activity of the virus that Quan et al (7) describe? Of all of the ways type I interferon is induced and its consequences expressed in vivo, which of one or more of these are important in SLE pathogenesis (Figure 1)? Beyond SLE, do other autoimmune diseases with epidemiologic associations with EBV, such as multiple sclerosis and Sjögren's syndrome, have a differentially increased interferon production from pDCs or other cell types?

Knowing how EBV and other environmental factors influence the path toward pathogenic autoimmunity and clinical disease and how lupus disease risk loci interact with EBV will require additional study. Nevertheless, at this point, some features of the sequence of events from normal immune control to clinical autoimmunity with SLE as a consequence of EBV infection have become apparent (Figure 2). We can only hope that future work will validate and extend this scenario and fill the many steps involved with useful detail that presents an accurate conceptual construct of pathogenesis, fostering the development of important new diagnostics and therapeutics for SLE.

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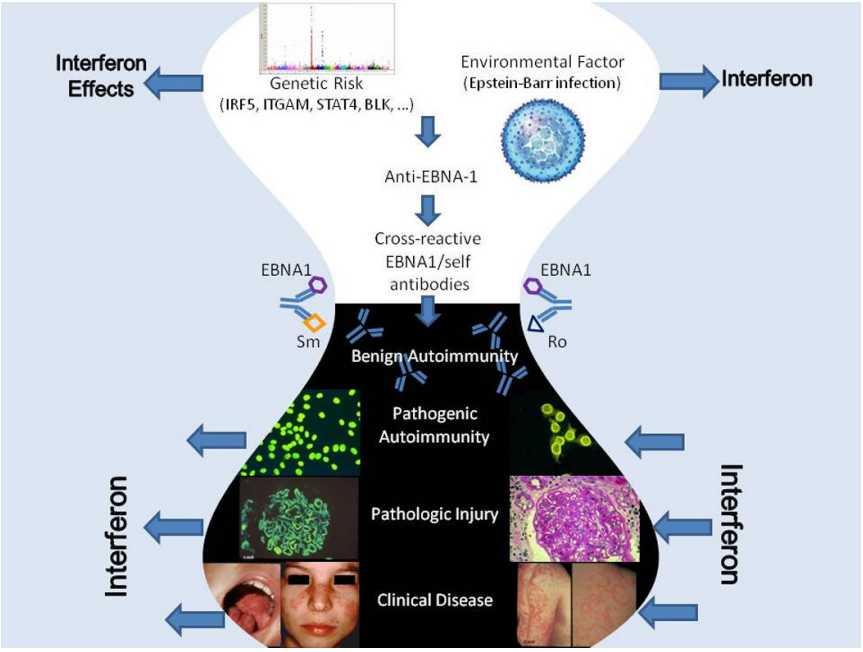

Figure 1.
Hypothetical pathways for SLE development through abnormal immune responses to Epstein-Barr virus.

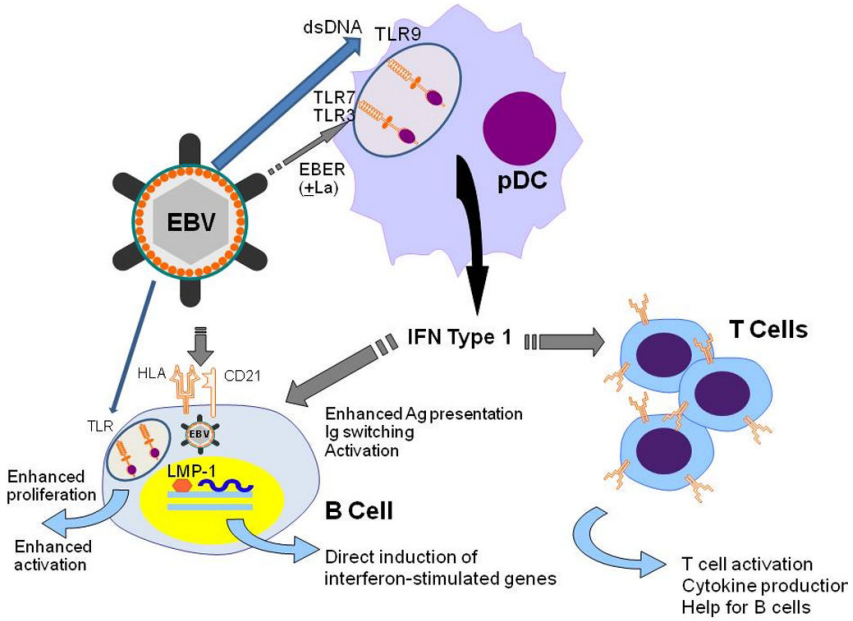

Figure 2.
Potential roles for Epstein-Barr virus in interferon mediated responses.