Human Endogenous Retroviruses as Contributors of Exosome-mediated Autoimmunity in Systemic Lupus Erythematosus

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SUMMARY At least 8% of the human genome was formed by integration of retroviral DNA sequences. These sequences are remnants of ancient infections that have accumulated mutations over millions of years. Although human endogenous retroviruses (HERVs) were previously thought to be inactive, they are now emerging as potential contributors of autoimmune diseases. Environmental and epigenetic factors have been shown to activate the expression of HERVs in pathological conditions such as Systemic Lupus Erythematosus (SLE). SLE is a poorly understood non-organ specific autoimmune disease lacking diagnostic criteria and curative treatments. It has been suggested that an unfortunate interplay of genetic susceptibility and environmental factors play an important role in generating an abnormal autoimmune response in SLE patients. HERVs have been proposed to be the bridge linking environmental factors to the pathogenesis of SLE. However, no clear mechanisms of causation have been identified in this association. This review outlines the key HERVs implicated in SLE, the factors that influence their activation and the proposed mechanisms utilized by HERVs in the etiopathogenesis of SLE. In addition, I investigate the potential of exosomes in playing a role in HERV-mediated stimulation of the immune system that in turn may contribute to activation and progression of SLE. The characterization of exosomal cargo will allow the identification of HERV products or sequences that may be transmitting intercellular signals involved in the immune dysregulation of SLE. Elucidating this association between HERVs and SLE may facilitate the development of novel diagnostic and therapeutic tools to combat this debilitating multi-system disease.

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a chronic multisystem autoimmune rheumatic disease that is highly heterogeneous in its manifestation [1]. It is characterized by periods of active disease, known as flares, and periods of remission during which there are few symptoms [2]. A broad spectrum of clinical presentations appear along with interpatient
variability. This includes dermatological, musculoskeletal, haematological, cardiopulmonary, renal and neuropsychiatric manifestations [2].

Prevalence of SLE is variable and ranges from 40 to 200 per 100,000 [3]. This depends on the ethnic and geographic differences in the population being studied, the definition of SLE applied and the methods of case identification [3, 4]. SLE is more common in those of African and Asian ancestry and is nine times more prevalent in females than males [4]. The usual onset of the disease is between 15-45 years old, although it can appear at any age [4].

Being a non-organ specific disease makes it highly unpredictable and poses challenges to physicians responsible for the diagnosis and management of the disease. It takes an average of 6 years to get a definite SLE diagnosis, from the time symptoms appear [5]. By that time, many will develop serious complications such as kidney disease, cardiovascular problems and chronic arthritis [4]. Lupus nephritis (LN) is a major cause of the morbidity and mortality of SLE, where up to 30% of patients progress to end-stage renal disease [6]. The majority (63%) of SLE patients report being incorrectly diagnosed [5]. Of those, more than half (55%) report seeing four or more different healthcare providers for their symptoms before being accurately diagnosed [5]. Diagnostic criteria have not been developed yet, although classification criteria made for research purposes have been adopted for aid in clinical diagnoses [7]. The 1997 American College of Rheumatology (ACR) criteria has been revised in 2012 by the Systemic Lupus International Collaborating Clinics (SLICC), requiring patients to meet at least four out of 17 criteria to get a definite diagnosis [7, 8]. However, none of these classification criteria have 100% sensitivity and 100% specificity and may still fail to classify some patients [8]. Therefore, there is an urgent need for diagnostic tools to help in early detection and management of the disease.

Although the cause of SLE is still unknown, evidence suggests a complex interplay between genetic, epigenetic and environmental factors [9]. The disease is primarily driven by loss of immune tolerance and abnormal innate and adaptive responses [1]. One of the main candidates currently implicated in the etiopathogenesis of SLE are human endogenous retroviruses (HERVs) [10]. The observation of a long preclinical duration preceding the clinical onset of the disease, analogous to clinical symptoms of retrovirus infections, led to the proposal of endogenous retrovirus involvement in autoimmune diseases [10]. In light of relevant literature emerging in the field, this relationship is further examined in this article.

It has been suggested that certain factors activate the expression of HERV elements [9]. This activation can influence the function of critical immune responses, leading to immune dysregulation or dysfunction characteristic of SLE [9]. Therefore, understanding the role HERVs play in the pathogenesis of SLE will facilitate the move towards developing targeted therapies that prevent their activation. This article will describe the potential of HERVs as the bridge linking the multifaceted factors predicted to cause SLE.

**FIG. 1 Structure of intact HERV DNA.** HERVs are similar to exogenous retroviruses in that they are composed of gag, pol and env genes, flanked by LTRs. gag stands for group specific antigens which encodes viral capsid proteins and nucleocapsids. pol encodes for the viral polymerase, composed of the reverse transcriptase, integrase and protease. env encodes for the viral envelope proteins.
RESEARCH QUESTIONS

SLE is a multifactorial disease that can be debilitating to affected individuals. There is no cure for SLE and current treatments are only aimed at easing symptoms [11]. In addition to SLE affecting the immune system, treatments will further reduce cellular and humoral responses due to their inhibition of T and B lymphocytes [11]. This weakened immune system can lead to secondary outcomes by predisposing patients to a range of infections and disorders that can be even more impairing than the disease itself. Since the aetiology of SLE is unknown, we have a limited ability to define the disease and design therapeutics that are able to target the disease directly.

HERVs have been emerging as candidates of autoimmune manifestations and not just “junk” DNA, as previously considered. Increasing evidence in the literature suggests that HERVs play a role in SLE, although there are no clear mechanisms of causation that have been identified. Therefore, I will present three research questions that will be address the link between HERVs and SLE. Firstly, the key HERV classes associated with the cause and/or subsequent development of SLE manifestations need to be identified, along with the factors that influence their expression or activation. Secondly, the proposed mechanisms utilized by HERVs in the etiopathogenesis of SLE need to be further explored. Finally, utilizing this information, this article aims to investigate the potential of endogenous retrovirus elements attributing to exosome-mediated autoimmunity in SLE patients.

PROJECT NARRATIVE

What are the key HERVs implicated in SLE and the factors influencing their activation?

Over the past few years, interest in HERVs as potential contributors to autoimmune diseases has increased [12-15]. The identification of the key HERVs implicated in SLE will allow us to study their structure and mechanisms of activation in order to subsequently elucidate their role in causing disease. HERVs are remnants of ancient infections which integrated into the germline 30 to 40 millions of years ago [16]. They are passed down through generations in a Mendelian fashion and constitute around 8% of human DNA [16]. Through phylogenetic studies, they have been classified into Class I, II or III and further categorized into families (e.g. HERV-K, HERV-R) [17]. Their structure is similar to exogenous retroviruses in that they are composed of gag, pol and env genes, which encode viral capsid proteins, reverse transcriptase and envelope proteins, respectively (Fig. 1) [17]. Additionally, they are flanked by long terminal repeats (LTRs), which contain the promoter and enhancer

<table>
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<tr>
<th>HERV</th>
<th>Class</th>
<th>Region</th>
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<td>Class I</td>
<td>Chromosome 1</td>
<td>LTR-gag-Δpol</td>
<td>• Elevated antibodies to Gag-generated proteins in patients with active SLE</td>
<td>23, 25, 26</td>
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<td></td>
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<td>• Integration into SLE susceptibility locus</td>
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<td>HERV-R (ERV-3)</td>
<td>Class I</td>
<td>Chromosome 7</td>
<td>LTR-gag-pol-env-LTR</td>
<td>• Elevated antibodies to env products in SLE patients</td>
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<td>• Homology between SLE autoantigens and env</td>
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<td>HERV-E 4-1</td>
<td>Class I</td>
<td>Mapped to several chromosomes</td>
<td>LTR-gag-pol-env-LTR</td>
<td>• PBMCs from SLE patients generate mRNA gag transcripts</td>
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<td>• Autoantibodies to recombinant Gag products together with manifestations of SLE in an animal model</td>
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<tr>
<td>HERV-K10/HERV-K18</td>
<td>Class II</td>
<td>Widely distributed, including chromosome 1</td>
<td>LTR-gag-pol-env-LTR</td>
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<td>31, 37</td>
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<td>• Superantigens on HERV-K18</td>
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TABLE 1 Summary of key HERVs implicated in the etiopathogenesis of SLE. This table outlines the class, region, structure and significance of HERV sequences that have been studied in relation to SLE. PBMCs refer to peripheral blood mononuclear cells. Δ refers to gene deletion.
regions important for viral transcription [17]. Although they are mostly inactive due to mutations and insertional events, increased expression and antibody reactivity to HERV products have been detected in patients with autoimmune diseases, including SLE, multiple sclerosis, rheumatoid arthritis and type 1 diabetes (T1D) [17-19].

Table 1 summarizes the class, region, structure and significance of the key HERVs implicated in SLE. Among those, HTLV-1-related endogenous sequence (HRES) is the most studied in SLE [18-21]. HRES-1 has been mapped to chromosome 1 which has a number of susceptibility genes that predispose to SLE [22, 23]. Its LTR region has been found to contain particular alleles correlated to clinical manifestations of SLE [24]. Studies have also shown SLE patients with clinically active disease producing high titer antibodies to HRES-1 Gag generated proteins [25, 26]. HERV-R is located on chromosome 7 and encodes a truncated Env polypeptide [27]. A key study highlighted a potential role of HERV-R in newborns with congenital heart block (CHB), an example of neonatal lupus syndrome [28]. The mothers of these babies were found to have elevated levels of antibodies to the HERV-R Env products [28]. HERV-E clone 4-1 is another key sequence found to generate mRNA gag transcripts in SLE patients [29]. Autoantibodies have been detected in SLE patients to recombinant HERV-E Gag proteins [30]. Moreover, the highly homologous HERV-K10 and HERV-K18 have been demonstrated to trigger antibody production through their gag matrix-derived peptide in renal lupus patients [31]. This association between different HERV elements and phenotypic manifestations of SLE should be further investigated.

These key HERVs have been shown to be influenced by estrogen, DNA hypomethylation and ultraviolet light (UVB) exposure [32, 33]. The 5’ LTR of HERV-K10 contains a hormone responsive element shown to enhance transcription levels when stimulated with estrogen, hence the bias of SLE towards females [34]. Certain drugs such as procainamide have also been shown to enhance HERV expression due to inhibition of DNA methylation [32]. UVB irradiation in epidermal keratinocytes also causes transcriptional activation of HERVs through poorly understood mechanisms [35]. This however could be a possible explanation of lupus flares associated with sunlight exposure [35].

Viral infections could also play a synergistic relationship (e.g. notion of ‘helper’ viruses). Epstein Barr virus (EBV) has been highlighted as a potential agent that plays a co-operative role in the upregulation of HERV-K10 activity in SLE [36]. In addition to HERV activation, insertional and sequence polymorphisms within the genome may cause disease in some individuals but not others, as mentioned with HRES-1 polymorphic genotypes [24]. All of these factors may be a single cause or together play a role in modulating the involvement of HERVs in SLE pathogenesis.

![HERV PRODUCTS](image1.png)

**HERV PRODUCTS**
- Molecular mimicry
- Superantigens

![HERV LOCI](image2.png)

**HERV LOCI**
- Insertional mutagenesis
- Transactivation

**FIG. 2** A brief outline of proposed mechanisms utilized by HERV loci and gene products in the etiopathogenesis of SLE. HERV products might be involved in molecular mimicry, where homology between HERV amino acid sequences and SLE autoantigens can lead to cross reactive antibodies to epitopes on human tissues. Superantigens can activate a polyclonal expansion of autoreactive T cells. Alternatively, HERV sequences may be involved in insertional mutagenesis within or near genes necessary for proper regulation of the immune system. Transactivation of host genes may also have a role in overexpression of cytokines leading to abnormal systemic inflammation. The affected innate responses impact the adaptive immune system and vice versa.
What are the proposed mechanisms utilized by HERVs in the etiopathogenesis of SLE?

As mentioned previously, immune dysregulation and lack of immune tolerance are evident in SLE. Several mechanisms have been proposed as possible explanations by which retroviral endogenous elements can participate SLE disease progression (Fig. 2) [37]. The potential of HERVs to initiate and substantiate an autoimmune response can be through the effects of both its genes and gene products [37]. HERVs retain their ability to transposition and are subsequently found to integrate within or near host genes necessary for proper functioning of the immune response, such as fas, MHC and complement genes [37, 38]. An example of endogenous retroviral integration is evident in MRL/1pr mouse model, where HRES-1 integrates into the murine fas apoptosis-promoting gene [38]. This results in failure of apoptosis in autoreactive lymphocytes through decreased expression of Fas, leading to a severe systemic autoimmune disease in these mice similar to human SLE [38]. HERVs may also encode elements like Tat in HIV-1 or Tax in HTLV-1 which enable transactivation of cellular genes [37, 39]. In HIV-1-infected cells, Tat has been shown to activate the expression of tumor necrosis factor (TNF), involved in systemic inflammation [37, 39]. Therefore, it is possible that HERV encoded transactivating elements play a similar role to those encoded by HIV-1 or HTLV-1.

Alternatively, HERV gene products can sustain the disease by acting as superantigens or as cross-reactive antigens through molecular mimicry [37]. Sutkowski et al. showed that EBV, acting as an exogenous environmental factor, activates a superantigen on HERV-K18 [40]. Superantigens bind to conserved regions of MHC class II outside of the peptide-binding groove leading to expansion of T cell subsets and excessive cytokine production harmful to the host [40]. In addition, HERVs encode products that mimic common nuclear protein sequences such as sn-RNP [41]. This stimulates the production of antiretroviral antibodies that are cross-reactive with common nuclear antigens [41]. Perl et al. illustrated a correlation between the presence of antibodies to HRES-1 (found in 52% of SLE patients) and anti-RNP antibodies that may serve as a priming mechanism in the ongoing autoimmune response to self-antigens [42].

Studies that support the association between HERVs and SLE still remain limited. However, as the key HERVs implicated in autoimmunity emerge, further experimental evidence is needed to support the above proposed mechanisms. For the final question, I bridge these proposed mechanisms by hypothesizing a model by which HERVs hijack the exosome secretion pathway for induction of SLE pathogenesis.

Can HERV elements be attributed to exosome-mediated autoimmunity in SLE patients?

Exosomes are secreted, nano-sized membrane vesicles, 50-100 nm in diameter that contain DNA, RNA, proteins, lipids, and metabolites of producing cells [43]. They are released under both physiological and pathological conditions into the extracellular space, and in recent years, have been increasingly studied in association to several diseases [43]. Recently, Lee et al. found serum exosome levels to be significantly higher in SLE patients than healthy controls (HCs) and their levels correlate with disease activity [44]. SLE-isolated exosomes were found to induce a higher production of IFN-α, TNF-α, IL-1β and IL-6 compared to HCs-isolated exosomes [44]. This evidence suggests that circulating exosomes are immunologically active and contain potent immunomodulatory material associated with SLE pathogenesis. Therefore, exosomes may also be circulating HERV elements and propagating their pathogenic potential through direct or indirect mechanisms (Fig. 3). This model could lead to exciting research advances in understanding SLE pathogenesis and developing diagnostic and therapeutic tools.

HERVs could utilize the exosomal pathway to directly spread signals which may regulate or disrupt genes critical in normal functioning of the immune system, for example insertional mutagenesis or transactivation as described previously. Recent evidence revealed a newly discovered ability of a retrovirus-like Gag protein, known as Arc1, in auto-assembling and forming a virus-like capsid [45, 46]. This capsid structure was found to bind copia mRNA, a common Drosophila retrotransposon, and traffic across synapses in the nervous system via exosomes [45, 46]. This process is thought to mediate intercellular RNA transfer and play a role in synaptic plasticity [45, 46]. Arc’s evolutionary history is not fully understood, however recent studies have suggested that Arc emerged from a vertebrate
lineage of Ty3/gypsy retrotransposons, which are also ancestors to retroviruses [46-48].
Therefore, the presence of other Arc-like proteins encoded by HERVs is possible.
Investigating the identity of the RNAs that bind into these capsids and undergo secondary
envelopment by exosomal membranes will be essential to unravel the role of HERVs in SLE.

Alternatively, exosomes can expose HERV elements such as Env glycoproteins on their
surface, mediating indirect autoimmune induction. Exosomes express both self-antigens and
peptide-MHC complexes and therefore, they could represent a source of HERV antigens that
might activate autoreactive T cells in the context of MHC [49]. A recent study found
endogenous retrovirus (ERV) proteins enriched in exosomes secreted in T1D mouse models
[50]. These ERV antigens were found to be targets of autoreactive T and B lymphocytes and
attributed to the autoimmune induction [50].

In summary, exosomes can potentially serve as “nano-shuttles” that spread HERV
information, leading to the systemic manifestation of SLE. Their cargo can range from viral
RNAs, proteins and surface antigens that potentially induce autoimmunity through direct
and/or indirect mechanisms. This might explain the role of HERVs in the initiation and
perpetuation of the autoimmune response in SLE.

SUMMARY AND CONCLUSION

SLE is the prototype multisystem autoimmune disease that presents as the great mimic to
many other diseases due to its wide spectrum of clinical manifestations [1, 2]. Although
relatively common throughout history, very limited advancements have been made in
facilitating the diagnosis and development of therapeutics that directly target SLE [6-8].
HERVs represent a key molecular link between the environmental and genetic factors that
govern SLE etiopathogenesis [18, 33]. Understanding this complex interplay and mechanisms
by which HERVs are associated with SLE can provide insight into the potential contribution of endogenous viruses in the development of other multifactorial autoimmune diseases.

The purpose of this review was to outline the associations of HERVs in SLE and to analyse possible means by which these associations could lead to the onset or exacerbation of this non-organ specific disease. This article also presents a model by which HERVs exploit exosomal pathways to induce abnormal immune responses characteristic of SLE. Studies that examine the role of HERVs in SLE are very limited due to the challenges that arise with this disease. However, as HERVs emerge in having a role in pathological conditions, further experimental evidence is needed to support the research questions presented in this article. Once the causative mechanisms of HERV involvement in SLE are unravelled, new frontiers open up for biomarker detection to track and monitor disease progression and therapeutic strategies for treatment of the disease. Additionally, it will enable a more refined definition of SLE and the development of diagnostic criteria with enhanced sensitivity and specificity.

Future studies should focus on experimentally proving the associations of the key HERVs presented in this article and other environmental and epigenetic factors that might influence their activation. The pathological mechanisms explored in this article should be further investigated and put into context with the proposed exosome-mediated autoimmunity model. This can be done through a multicentre study cohort consisting of HCs, SLE lab-confirmed cases (definite, probable and possible), those with currently active versus low level activity SLE, and other types of lupus (e.g. cutaneous, drug-induced and neonatal lupus). Isolation and purification of exosomes from these individuals’ biofluids will allow characterization of the cargo in healthy and diseased samples. Deep sequencing and tandem mass spectrometry should subsequently be performed to establish transcriptomic and proteomic profiles for each sample, which will allow thorough comparison of exosomal content.

ACKNOWLEDGEMENTS

I would like to thank Dr. Jean for his constant assistance and support during the development of my project. His guidance was extremely helpful when it came to writing this paper.

REFERENCES


ACRONYMS

Systemic Lupus Erythematosus (SLE), Human endogenous retrovirus (HERVs), Lupus nephritis (LN), American College of Rheumatology (ACR), Systemic lupus international collaborating clinics (SLICC), long terminal repeat (LTR), Human T-cell lymphotropic virus- related endogenous sequence type 1 (HRES-1), endogenous retrovirus type R (HERV-R), endogenous retrovirus type E (HERV-E), endogenous retrovirus type K (HERV-K), Type 1 diabetes (T1D), Congenital heart block (CHB), Ultraviolet radiation (UBV), Epstein Barr virus (EBV), endogenous retrovirus (ERV), Human immunodeficiency virus type 1 (HIV-1), Human T-cell lymphotrophic virus (HTLV-1), Tumor necrosis factor (TNF), small nuclear ribonucleoprotein (snRNP), healthy controls (HCs), interferon alpha (IFN-α), interleukin 1 beta (IL-1β), interleukin 6 (IL-6), Major histocompatibility complex (MHC).