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REVIEW ARTICLE

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Systemic lupus erythematosus and human endogenous retroviruses

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Abstract Human endogenous retroviruses (HERV) are known to be widely present in the human genome. Several investigations have suggested a possible etiological role of HERV in certain human disorders, including systemic lupus erythematosus (SLE). Here we review and discuss the possible role of HERV, especially HERV clone 4-1, in the onset of SLE, based on recent findings including our own data.

Key words DNA methylation · Human endogenous retrovirus (HERV) · Interleukin (IL)-16 · Systemic lupus erythematosus (SLE)

Introduction

It is well known that certain viruses, such as cytomegalovirus (CMV), Epstein–Barr virus (EBV), and parvovirus B19, can induce immune abnormalities, including autoimmunity, and such viral infections can occasionally trigger the onset or exacerbation of systemic lupus erythematosus (SLE).[1–3] Retroviruses, including endogenous retroviruses, have also been suggested as an etiological factor for human autoimmune diseases such as SLE, although this is still controversial and the precise role of these viruses remains unclear. Extensive studies performed during the last decade on exogenous retroviruses, such as human immunodeficiency virus (HIV) and human T-cell leukemia virus (HTLV), have also led to marked advances in our knowledge about human endogenous retroviruses (HERV).[4–10] It is now known that HERV exist widely in the genomic DNA of humans and have usually lost the ability to replicate viral proteins, although HERV are presumably all remnants of ancient infections by exogenous retroviruses.[11–13] It may be said that HERV are potentially the nonself in human genes. From this point of view, autoimmune disorders (especially SLE) seem to be the most suitable diseases to investigate to clarify the biological or pathogenic activity of HERV. Here we review and discuss the possible role of HERV in the onset of SLE, as a representative autoimmune disease, based on our own data as well as a survey of the literature.

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Background of HERV

Retroviruses are plus strand RNA viruses with a mode of replication in which the RNA genome is transcribed into DNA by reverse transcriptase (RT) and is integrated into the host genome as a provirus. Two identical elements (the long terminal repeats, LTRs) are reconstituted at the 5′- and 3′-ends of the provirus, which contain the promoter, enhancer, and repressor sequences for tissue-specific expression. All genera of retroviruses encode three genes, which are expressed as polyproteins: *gag* that encodes the core proteins, *pol* that encodes the RT, and *env* that encodes the envelope proteins. Although exogenous retroviruses such as HIV and HTLV can reproduce viral RNA and viral particles from their proviral DNA, the ability of HERV to replicate viral proteins is generally blocked by several mechanisms. Endogenous retroviruses are known to exist not only in animals, but also in the genomic DNA of humans.[11,13]

Recent investigations have indicated that HERV may account for approximately 8% of the DNA in humans (450000 copies)[10,14,15] and thus contribute substantially to the architecture of the human genome.[12,13] HERV are

divided into classes, based on their relations in the viral phylogenic tree, and are named according to the sequences of their transfer RNA primer-binding sites, such as HERV-W and HERV-K.[13] All HERV are inherited in a Mendelian fashion, and therefore may be associated with genetic susceptibility or an increased risk of certain diseases. The precise origin of HERV remains unclear, but certain HERV-related sequences can be traced back to the time of the divergence of Old and New World monkeys (25–30 million years ago).[12,16]

Recent studies have revealed that certain HERV can undergo transcriptional and translational activation that results in protein expression and HERV particle formation.[6,8,11] The potential capacity of HERV elements to move about inside the genome, modifying host genes or their expression, and the ability of some retroviral proteins to modulate immune responses, has suggested that these prototypical persistent viruses might also have a role in autoimmune diseases.[10] Based on such characteristics, HERV could also be involved in the evolution of human beings. Discussion of the potential role of HERV in human autoimmune diseases has generally focused on five diseases: multiple sclerosis (MS), insulin-dependent diabetes mellitus (IDDM), primary biliary cirrhosis (PBC), Sjögren's syndrome (SS), and SLE.[10,11]

HERV clone 4-1 is one of the best understood with respect to its structure and other characteristics. It is a member of the HERV-E family that is widely distributed in human genomic DNA (being found in approximately 100% of Japanese individuals). Clone 4-1 shows sequence homology with Moloney murine leukemia virus (MuLV) and is approximately 8.8kb in size, with open reading frames in the *gag*, *pol*, and *env* regions (Fig. 1). This HERV sequence was originally detected in human brain fragments and a human DNA library using African green monkey DNA as a probe that hybridized to MuLV and endogenous baboon virus (BaEV).[17] Recent studies on human sequence data banks have shown that there are approximately 85 copies of clone 4-1 at 7 to 11 integration sites in the human chromosomes.[13,18,19] Clone 4-1 transcripts and gene products have been detected in syncytiotrophoblasts and in the vascular endothelium of some human placentas.[20] We have recently been investigating HERV clone 4-1 as a possible causative agent of SLE, as described later.

Retrovirus-related autoimmunity

The potential mechanisms by which retroviruses may cause the development of autoimmunity by retroviruses are summarized in Fig. 2. The direct effects of general viral infections, such as CMV and EBV, may also contribute to retrovirus-mediated autoimmunity. In addition, based on the characteristics of retroviruses, indirect effects may be important in the induction of autoimmune abnormalities (Fig. 2). A role for retroviruses as an etiological factor in SLE is supported by the following evidence: (1) the importance of endogenous retroviruses in mouse models of SLE;[29–31] (2) the detection of antibodies, antigens, and sequences for animal and human exogenous/endogenous retroviral components in the organs and serum of patients with autoimmune diseases such as SLE;[21,23,32,33] (3) electron microscopic detection of unknown retroviral particles in the organs of SLE patients;[34] (4) the finding that viral components derived from endogenous or exogenous retroviruses

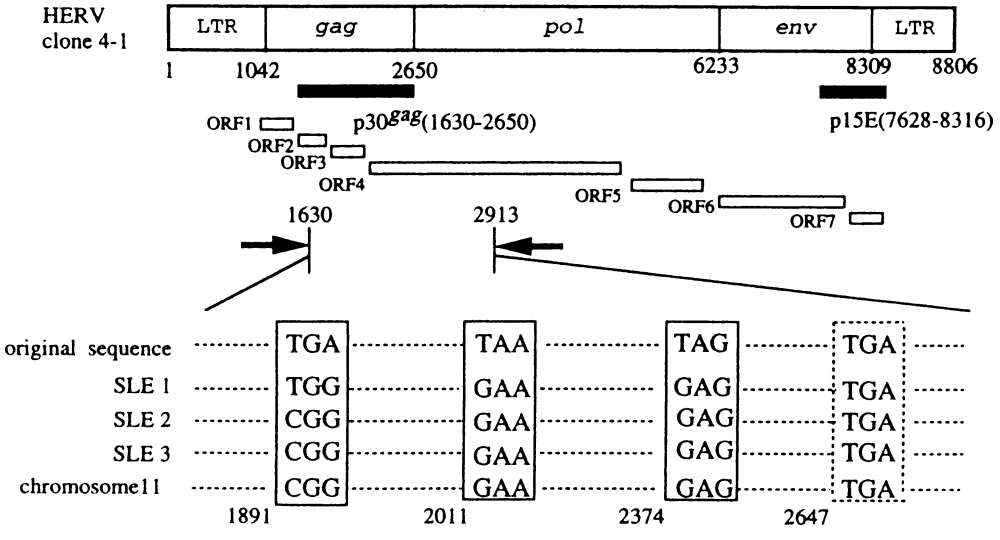

Fig. 1. General structure of human endogenous retrovirus (HERV) clone 4-1 and various genomic DNA sequences. The sequences between nt 1630 and nt 2913 were analyzed. Alterations to the original stop codon sequences that were found in the systemic lupus erythemotosus (SLE) patients are shown by *solid boxes*; the conserved sequence is shown by a *dotted box*. The prototype (original) sequence of clone 4-1 is based on data from Repaske et al.[17] and the consensus sequence of clone 4-1 in chromosome 11 is modeled from the GenBank database. The location of clone 4-1 on chromosome 11 (11p15) was indicated by data from Taruscio et al.[18,19] *Numbers* indicate the nucleotide numbers; *ORF*, open reading frame. With the approval of our ethics committee, written informed consent was obtained from all participants represented in this figure as well as Figs. 3 and 4

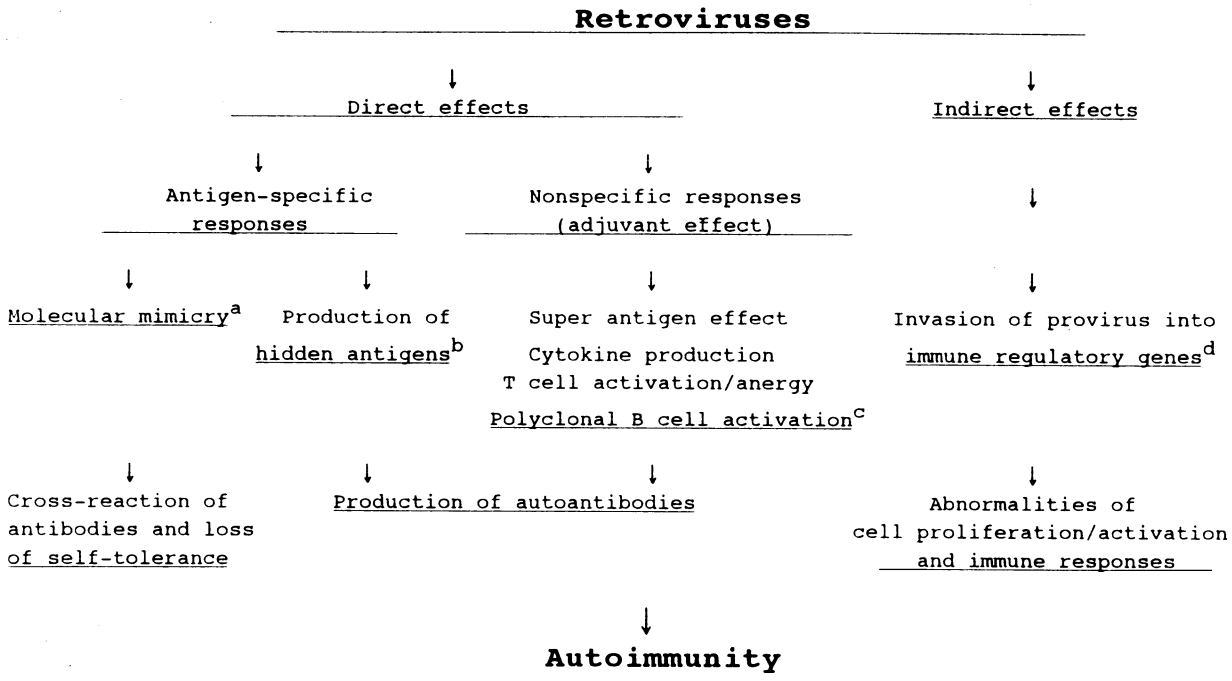

Fig. 2. Possible mechanisms of retrovirus-mediated autoimmunity. The main references associated with this schema of events are as follows: (**a**) 21–24; (**b**) 25,26; (**c**) 10,27,28; (**d**) 5

can induce the immune abnormalities observed in SLE;[27,28] (5) molecular mimicry of self-determinants by retroviral antigens;[21–24] and (6) the similarity of autoimmune manifestations and immune dysregulation between patients with SLE and patients infected with known human retroviruses. In particular, there are various similarities of immune abnormalities between HIV infection and SLE, including T- and B-cell dysfunction as well as similar changes of cytokines and chemokines along with variations in disease activity.[8,35,36]

Next, the nature of HERV clone 4-1 is discussed with reference to its possible pathogenic role in SLE based on the reported data, including our own studies.

HERV clone 4-1 and SLE

Transcription and translation of HERV clone 4-1 does not seem to occur in peripheral blood lymphocytes (PBL) from normal individuals, but messenger RNA (mRNA) for the clone 4-1 *gag* region can be detected in PBL from SLE patients by both the standard polymerase chain reaction (PCR) and the real-time quantitative PCR (RQ-PCR; TaqMan method), which can analyze mRNA quantitatively and is more sensitive than RT-PCR[37,38] (Fig. 3). This RQ-PCR also revealed significant differences in the amount of clone 4-1 *gag* mRNA between SLE and rheumatoid arthritis (RA) patients as well as SLE and normal individuals (average amount ± SD in PBL from 7 SLE patients, 7 RA patients, and 7 controls was 2458.3 ± 1165, 419.2 ± 274, and 185.8 ± 158.6 copies/μg, and statistical differences between SLE and RA patients or controls are

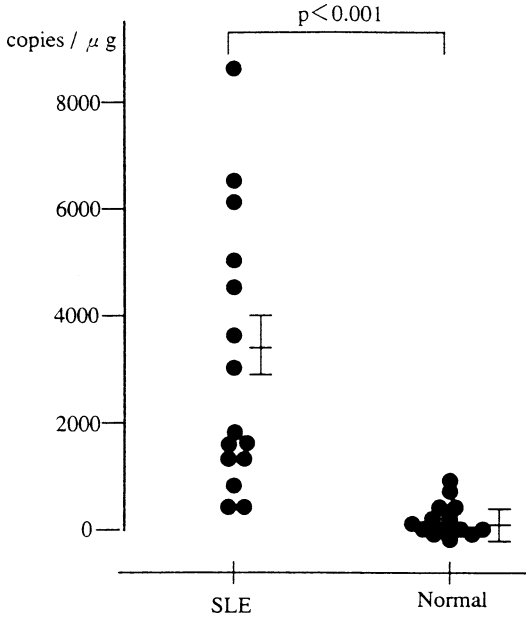

Fig. 3. Levels of HERV clone 4-1 mRNA in peripheral blood lymphocytes (PBL) from SLE patients and normal individuals. Analysis was performed with the TaqMan fluorogenic system (ABI Prime 7700 Sequence Detection System; Perkin-Elmer Applied Biosystems, Foster City, CA, USA) using two primers (*gag* primer 1, 1651–1668; *gag* primer 2, 2238–2250), as described previously.[38] *Bars* indicate the mean value ± SD in each population. Significant differences of this mRNA between SLE patients and controls were observed (Student's *t* test)

$P < 0.005$, respectively).[39] The *gag* region antigen (p30*gag*) of clone 4-1 has also been detected in PBL from certain SLE patients, but not in PBL from normal controls (Fig. 4).

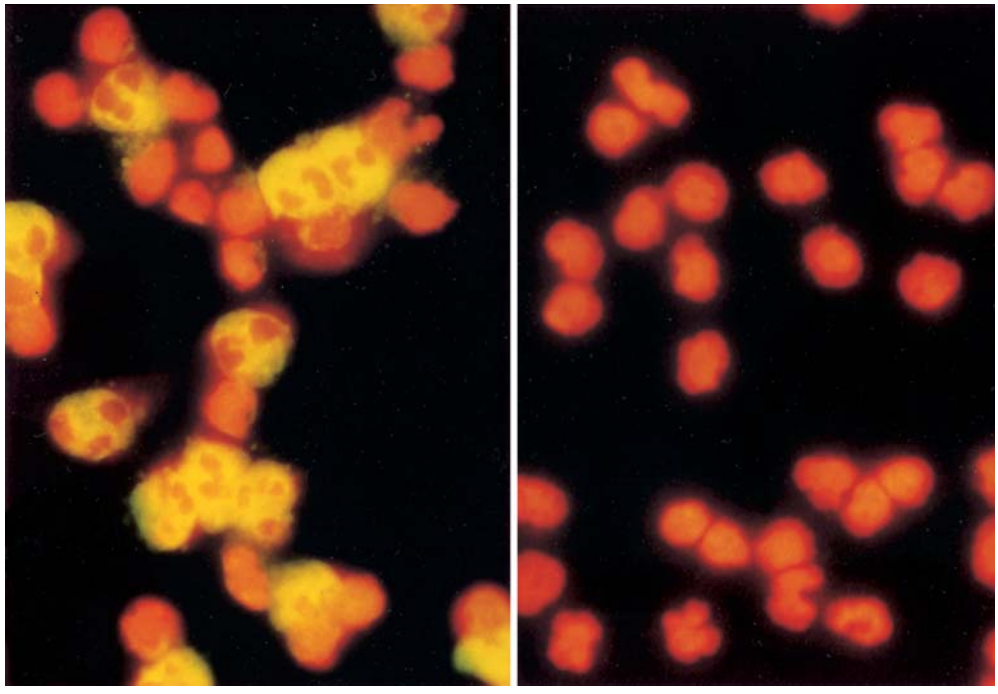

Fig. 4. Detection of HERV clone 4-1 (*gag*) antigen in PBL from an SLE patient (*left*) and a normal person (*right*). PBL were initially stained with goat-MulV p30*gag* antibody, which reacts with recombinant clone 4-1 p30*gag*, as reported previously,[40] and then were stained with fluorescein isothiocyanate (FITC)-labeled rabbit $F(ab')_2$ antigoat immunoglobulin (Cappel, West Chester, PA, USA) as the secondary antibody. A positive reaction was observed in SLE PBL (*left*) but not in normal PBL (*right*)

Furthermore, serum antibodies to recombinant clone 4-1 *gag* products (p30*gag*) are observed in approximately 50% of SLE patients but are not found in normal controls.[40] Similar data to those for the clone 4-1 *gag* region have also been obtained for the *env* region, although the details are not described here. Thus, transcription and translation of clone 4-1 appear to be strongly promoted in SLE patients when compared with normal individuals. In SLE patients, steroid therapy inhibits the transcription of this HERV (probably due to suppression of the promoter region),[38] and this action of steroids may be important for controlling SLE, in addition to various immunosuppressive effects on lymphocytes. Other immunosuppressants (cyclosporin A and FK506) have been shown to inhibit the promoter activity of HIV provirus integrated into the human genome and suppress its transcription.[41]

Components derived from endogenous retroviruses, such as p15E [which is a specific sequence of *env*-encoded transmembrane (TM) proteins that is conserved among retroviruses from several species, including murine, feline, and human viruses] are known to induce several immune abnormalities in vitro, such as inhibition of IL-2 production and suppression of the lymphocyte proliferative response,[28] although the mechanism involved is still unclear. The TM envelope proteins of clone 4-1 share amino acid sequence homology with p15E belonging to retroviruses from several species.[28] Our recent studies have indicated that synthetic clone 4-1-derived p15E peptides can induce CD4+ T-cell activation and anergy in vitro, as well as production of several cytokines (such as IL-6 and IL-16) and cytokine-related polyclonal B-cell activation (PBA) (submitted), all of which are also observed in SLE patients.

IL-16 seems to have the closest relationship with the disease activity among several cytokines that show increased serum levels in SLE,[42,43] although it is still controversial whether a high serum level of IL-16 is a cause or result of immune abnormalities in this disease.[43] IL-16 is mainly produced by activated CD8+ T cells and uses the CD4 molecule as its receptor, inducing SLE-like CD4+ T-cell abnormalities such as activation and anergy.[44,45] Several lines of evidence suggest that activated CD8+ T cells or IL-16 produced by such CD8+ T cells in SLE patients may induce their CD4+ T-cell abnormalities as well as a decrease of CD4+ cells.[45–48] In fact, CD8+ T cells are generally more strongly activated than CD4+ T cells in SLE patients.[48] Because endogenous antigens such as HERV are mainly recognized by CD8+ T cells,[49] viral components derived from HERV clone 4-1 could facilitate the activation of CD8+ T cells and subsequent IL-16 production, which would promote the CD4+ T-cell dysfunction seen in SLE. These CD4+ T-cell abnormalities may contribute to the loss of self-tolerance and the induction of SLE-related autoimmune phenomena in cooperation with PBA,[50] in addition to the role of molecular mimicry between retroviral components (including HERV clone 4-1)[51] and autoantigens, which is also reported to be important for the creation of autoantibodies.[24]

Mechanisms of HERV transcription and translation in SLE

In general, several interrupters (including termination codons, deletions, frameshift mutations, or methylation sites) inhibit the transcription or translation of HERV in normal individuals.[11] Based on our results, at least two mechanisms seem to play an important role in the promo-

tion of HERV clone 4-1 transcription/translation in patients with SLE. First, a low level of DNA methylation in SLE patients may contribute to increased transcription of clone 4-1,[38,52] and second, inactivation of a stop codon in clone 4-1 may allow translation to occur.[37] Cytosine methylation of the regulatory sequences of some genes is associated with transcriptional inactivation, whereas hypomethylation of these sequences is associated with active transcription.[53]

Previous studies have detected hypomethylation (implied by low methyltransferase activity) in the T-cell nuclear proteins of SLE patients.[54] In addition, treatment with DNA methylation inhibitors such as 5-aza-deoxycytidine (5-aza C) increases the transcription of mRNA for HERV clone 4-1 and decreases transcription of mRNA for DNA methyltransferase 1 (DNMT-1, a member of the enzyme family responsible for methylation of DNA in mammalian cells)[55] in PBL from normal individuals.[52] Our recent data indicate that there is a significant negative correlation between the increase of clone 4-1 mRNA and the decrease of DNMT-1 mRNA in 5-aza C-treated normal PBL, and that transcription of DNMT-1 mRNA in PBL from SLE patients is lower than in PBL from normal controls (submitted). Thus, DNA hypomethylation may play a significant role in the transcription of HERV clone 4-1 and may also be involved in the pathogenesis of SLE. Certainly, treatment with 5-aza C has been shown to induce SLE-like autoreactivity of T cells and autoantibody production both in vitro and in vivo.[54]

The prototype clone 4-1 *gag* region reported by Repaske et al. has four stop codons between nt 1630 and nt 2650.[17] Our analysis using direct sequencing revealed that clone 4-1 DNA from several SLE patients showed changes of the same nucleotides when compared with the original sequence.[37] As shown in Fig. 1, in all three SLE patients studied, adenine (A) at nt 1893 was changed to guanine (G), thymine (T) at nt 2011 was altered to G, and T at nt 2374 was also altered to G. As a result of these variations, three of the four stop codons in the *gag* region of clone 4-1 were inactivated. Our computer search of current entries in sequence libraries (GenBank database) revealed greater than 90% sequence homology between genomic DNA from the SLE clone 4-1 *gag* region and the consensus sequence of clone 4-1 located on chromosome 11 (11p15) of normal individuals, including inactivation of these stop codons, unlike clone 4-1 from other chromosomes[37] (see Fig. 1).

We cannot completely exclude the possibility that the virus detected by our PCR primers was not actually clone 4-1, because many members of the HERV-E family are genetically similar to this clone.[11] Despite this limitation, our findings suggest that clone 4-1 transcribed in SLE patients may be derived from chromosome 11, and that inactivation of stop codons may contribute to the translation and production of *gag* proteins. The development of SLE is thought to be related to both the genetic background of the patients and environmental factors. Chromosome 11 is known to contain a breakpoint that is initiated by several activators, including X-rays.[18,19] This is also interesting in view of the potential role of environmental factors in the induction of SLE, because endogenous retroviruses can be activated by various stimuli, such as X-rays, ultraviolet light, chemical agents, bacteria (and other microorganisms), hormones, and cytokines.[11]

Thus, DNA hypomethylation and inactivation of stop codons appear to be important in the transcription and translation of HERV clone 4-1 in SLE. In this context, it is noteworthy that drugs such as procainamide and hydralazine, which have a demethylating effect, are known to occasionally trigger an SLE-like illness, especially in genetically predisposed individuals.[56] These drugs may promote the transcription of endogenous antigen-related genomic DNA, including HERV DNA, through demethylation and thus cause the onset of autoimmune phenomena. In contrast, stimulation of cultured normal PBL by mitogens, such as lipopolysaccharide (LPS), concanavalin A (Con A), and phytohemagglutinin (PHA), does not induce a quantitative increase of clone 4-1 mRNA.[52] This finding indicates that clone 4-1 expression is not a consequence of cellular activation, although lymphocytes are known to be activated in SLE patients.[50] Further investigations, such as a study of gene promoter activity in the clone 4-1 LTR region, are required to clarify the mechanisms of hypertranscription/translation of this clone in SLE.

Conclusion

Data revealed by the human genome project have suggested the possibility that the human genome contains many more endogenous retrovirus-like sequences than was previously thought. HERV seem to be one of the most plausible causative agents for various autoimmune diseases such as SLE. On the other hand, certain studies have indicated that an increase of HERV transcription or translation in several autoimmune disorders is a consequence (rather than a cause) of inflammatory changes.[7,10] Whether or not the data support an etiological role of HERV in SLE, further studies on these viruses should provide useful clues for solving several problems regarding this disease. Investigation of the role of HERV in SLE should be performed to clarify the etiology and to develop new therapeutic strategies, including the possible inhibition of HERV transcription/translation.

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