



## Role of endogenous retroviruses in autoimmune diseases

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### **Molecular biology of endogenous retroviruses**

Considerable evidence indicates that pathogenesis of autoimmune diseases is mediated by an interplay of environmental and genetic factors. Concordance rates of approximately 25% in monozygotic twins for most common autoimmune disorders, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), insulin-dependent diabetes mellitus (IDDM), or multiple sclerosis (MS), show that genetic factors influence development of autoimmunity [1]. Alternatively, a 70% discordance rate emphasizes the importance of environmental factors. Based on the notion that molecular interactions between the host genome and environmental factors are critical for autoimmunity, endogenous retroviruses (ERVs) are of particular importance [2–9].

Endogenous retroviruses and other retroviral elements have been found in all vertebrates investigated. They belong to the larger family of retrotransposable elements (Table 1) that make up as much as 40% of the human genome [10]. These elements include short interspersed nucleotide elements (SINEs), such as ~300–base pair (bp) Alu repeats and ~-kilobase (kb) transaldolase-associated repetitive elements (TAREs), and long interspersed nucleotide elements (LINEs), such as L1. Alus and TAREs can be transcribed into nonpolyadenylated RNA by RNA polymerase III [11]. Long interspersed nucleotide elements are polyadenylated and transcribed by RNA polymerase II. Their reintegration is dependent on reverse transcription. Most retroelements, Alus, and truncated ERVs lack reverse transcriptase which can be provided in *trans* by other retroelements, such as L1

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Table 1  
Retroelements in the human genome

Designation	Example	Transcription	Length	Copy number/ prevalence <sup>a</sup>	Reference
ERV	HERV-E	RNA pol II	3–9 kb	1–100	[3]
LTR retrotransposon	THE-1	RNA pol II	2.3 kb	10,000	[123]
SINE	Alu	RNA pol III	300 bp	300,000	[124]
LINE	L1	RNA pol II	6 kb	10,000	[126]
Retropseudogenes	TALDOP1	RNA pol II	variable	?	[13]

*Abbreviations:* ERV, endogenous retrovirus; HERV-E, human endogenous retrovirus E; LTR, long terminal repeat; SINE, short interspersed nucleotide elements; LINE, long interspersed nucleotide elements; THE, transposon-like human element; TALDO, human transaldolase gene.

<sup>a</sup> Approximation based on hybridizations and frequency in the human genome sequence.

[12]. Occasionally, mRNA transcripts of functional genes can be reverse transcribed and reintegrated into the genome thus giving rise to retropseudogenes. These sequences lack introns and contain a polyadenylated tail at their 3' end. As an example, the human genome contains an intronless and polyadenylated transaldolase pseudogene on human chromosome 1 [13].

Human ERVs (HERVs) have the basic structures of the integrated proviral form of infectious retroviruses with long terminal repeats (LTRs) of several hundred nucleotides flanking sequences homologous to *gag*, *pol*, and *env* genes [3]. The *gag* gene codes for inner structural core proteins, such as matrix and capsid. The *pol* gene encodes reverse transcriptase (RT) which copies viral RNA into DNA as well as protease and integrase allowing for integration of proviral DNA into the host genome. The *env* gene codes for transmembrane and outer envelop proteins, the latter playing key roles in binding to cell surface receptors. Sequence homologies between the *pol* genes have been used to divide ERVs into two classes: class I with homologies to mammalian type C retroviruses and class II with homologies to mammalian type A, B, and D retroviruses and avian type C retroviruses (Table 2). Human ERVs are commonly designated as HERV followed by a single-letter amino acid code corresponding to a tRNA. The 3' terminus of tRNA is predicted to initiate reverse transcription by annealing to an 18-nucleotide long primer binding site (PBS) at the 5' LTR. Human ERVs have generally been found to be defective proviruses with accumulated deletions or stop codons in *gag*, *pol*, or *env* open reading frames (ORFs) [9].

Endogenous retroviruses may represent a key molecular link between the host genome and infectious viral particles. Endogenous retroviruses may have originated from exogenous retroviruses that integrated into the genome and became trapped because of mutations of essential genes [3]. They constitute a large reservoir of viral genes that may be activated by mutations caused by radiation or chemicals or by recombination with exogenous retroviruses. Although exogenous retroviruses are infectious, with a replication cycle that requires integration of proviral DNA into host cell DNA, ERVs are transmitted genetically in a classic mendelian fashion through the germline as proviral DNA.

Table 2  
Human endogenous retrovirus families

Designation	PBS	Organization	Length	Copy number <sup>a</sup>	Reference
Class I					
HERV-E	Glu	LTR- <i>gag-pol-env</i> -LTR	8.8 kb	35–85	[3,84]
HERV-F	Phe	LTR- <i>gag-pol-env</i>	7.1 kb	1	[127]
HERV-H	His	LTR- <i>gag-pol-env</i> -LTR	8.7 kb	660	[83,128]
HERV-I	Ile	LTR- <i>gag-pol-env</i> -LTR	9.0 kb	25–85	[3,83]
HERV-P	Pro	LTR- <i>gag-pol-env</i> -LTR	8.2 kb	20–100	[3,129]
HERV-R	Arg	LTR- <i>gag-pol-env</i> -LTR	9.9 kb	10–15	[83,130]
HERV-W	Trp	LTR- <i>gag-pol-env</i> -LTR	7.6 kb	15–115	[34,83,125]
ERV-1	?	<i>gag-pol-env</i> -LTR	3–4 kb	1–15	[3,131]
ERV-9	Arg	LTR- <i>gag-pol-env</i> -LTR	9.6 kb	40–70	[3,83,132]
HRES-1	His?	LTR- <i>gag-Δpol</i>	6 kb	1	[3]
RRHERV-I	Ile	LTR- <i>gag-Δpol</i> -LTR	3.3 kb	15–20	[3,44]
S71	?	<i>gag-Δpol-env</i> -LTR	5.4 kb	1–20	[3,133,134]
Class II					
HERV-K	Lys	LTR- <i>gag-pol-env</i> -LTR	9.2 kb	170	[3,83,135]
HERV-L	Leu	LTR- <i>gag-pol-ΔLTR</i>	6.5 kb	200–575	[83,136]
HERV.HML6	Lys	LTR- <i>gag-Δpol-env</i> -LTR	7.5 kb	30–45	[83,137]

*Abbreviations:* PBS, primer binding site; HERV, human endogenous retrovirus; HRES, human T-cell lymphotropic virus–related sequence; ERV, endogenous retrovirus; RRHERV, retinoic acid-responsive HERV; LTR, long terminal repeat.

<sup>a</sup> Approximation based on hybridizations and frequency in the human genome sequence.

Expression of ERVs can influence the outcome of infections in different ways that can be either beneficial or detrimental to the host [3]. Endogenous retroviruses can provide genes for recombination with exogenous viruses, interfere with virion assembly, block cellular receptors for viral entry, and modulate immune responses to exogenous viruses (Fig. 1). Recombination with murine ERV can expand cellular tropism of HIV-1 [14].

Although expression of murine ERVs can lead to production of infectious virus and cause viremia, no production of infectious virion has been documented by human ERV. The high copy number of most ERV families makes it difficult to distinguish which members of a group are expressed. Although no single provirus with intact LTRs and uninterrupted *gag*, *pol*, and *env* ORFs has been identified, the HERV-K ERV, as a family, has been shown to encode *gag* [15], reverse transcriptase [16], integrase [17], and *rev* proteins [18,19]. The HERV-K *rev* protein, encoded by the LTR region, is functionally analogous to the HIV-1 *rev* and human T-cell lymphotropic virus type I and II human T-cell leukemia virus (HTLV-I/II) *rex* proteins. Human ERV-K *rev* binds to both the nuclear export factor Crm1 and to a cis-acting viral RNA to activate nuclear export of unspliced RNAs [19]. Alternatively, the HERV-K LTR is also recognized by HIV-1 *rev*, suggesting a potential interaction between these exogenous and endogenous retroviruses.

The HERV-K class of HERV may be most potent in terms of its ability to form virions [18,20]. Human ERVs show a characteristic pattern of tissue-specific expression (Table 3). Most ERVs are expressed in the placenta, teratocarcinomas, and

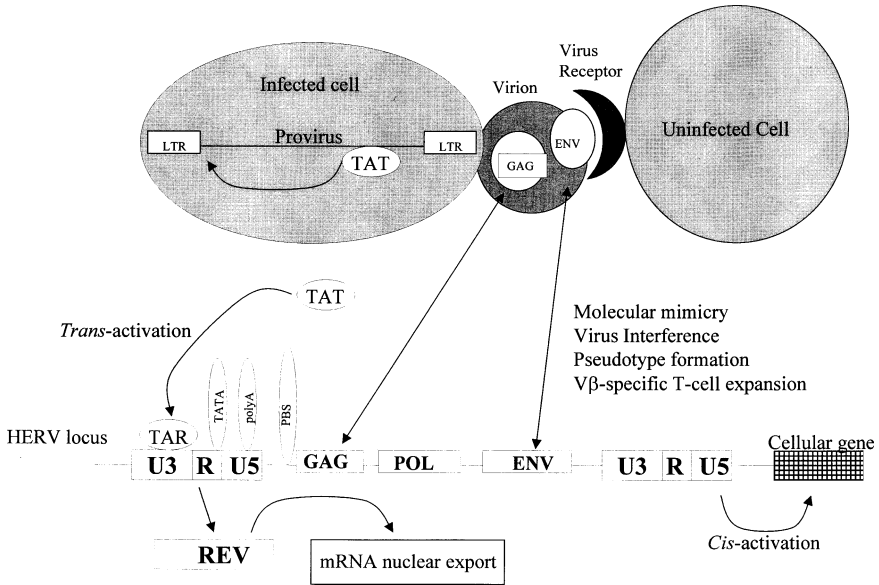


Fig. 1. Schematic diagram on pathogenicity of endogenous retrovirus (ERV) in autoimmunity. As an example, the human T-cell lymphotropic virus-related endogenous sequence (HRES-1) long terminal repeat (LTR) contains TATA box, polyadenylation site, tRNA primer binding site (PBS), an HIV-1 trans-activation region (TAR), and inverted repeats (IR) at typical locations [67]. Transcription from the ERV LTR may be stimulated by trans-acting factors (eg, *tat* of HIV-1). Endogenous retrovirus proteins may interfere with assembly or binding of virions to cell surface receptors, thus affecting replication, infectivity, and pathogenicity of exogenous viruses. Immune responses triggered by viral antigens may recognize similar ERV-encoded proteins, thus leading to autoimmunity.

other malignant tissues. Several ERVs are expressed in normal peripheral blood lymphocytes [21–23], salivary gland [24,25], breast [26], and keratinocytes [27].

### Interactions between endogenous retrovirus gene products and the immune system

Two possible mechanisms of endogenous retroviral pathogenesis are discussed here. The first scenario involves molecular mimicry causing abnormal self-reactivity [28]. Naturally, viral infections elicit potent antiviral immunity that may lead to cross-reactivity against self-antigens. Analysis of molecular mimics, that is, delineation of autoantigenic epitopes of self-antigens, may provide clues to the identity of viral antigens responsible for triggering the cross-reactive immune responses. Because of homologies between exogenous and endogenous viral antigens, ERV-encoded proteins are likely targets of cross-reactive antiviral immune responses.

In addition to serving as cross-reactive targets of antiviral immunity, ERVs may also have a direct role in regulating immune responses. Viral proteins mimic

Table 3  
Expression of human endogenous retrovirus genes

ERV	Tissue	mRNA <sup>a</sup>	Protein <sup>a</sup>
Class I			
HERV-E	Placenta, colon, spleen, liver	<i>env</i> , LTR [23,138,139]	38 kd <i>env</i> [142]
HERV-F	Placenta, liver, spleen, brain	LTR- <i>gag-pol-env</i> [127]	?
HERV-H	Teratocarcinoma, placenta	LTR- <i>gag-pol-env-LTR</i> [140]	62 kd <i>env</i> [33]
HERV-I	Teratocarcinoma	LTR, <i>gag</i> , <i>pol</i> , <i>env</i> env 2 [23]	?
HERV-R	Placenta, endothelium, adrenal, skin	LTR, <i>env</i> [45,130]	65 kd <i>env</i> [45]
HERV-W	Placenta, testis	LTR- <i>gag-pol-env-LTR</i>	65 kd <i>env</i> [34–36]
ERV-1	Placenta	<i>gag</i>	75 kd <i>gag</i> [143]
ERV-9	Embryonal carcinoma	LTR- <i>gag-pol-env-LTR</i> [132]	?
HRES-1	Brain, salivary ducts, T cells	LTR, <i>gag</i> [67]	28 kd <i>gag</i> [25,66,68]
RRHERV-I	Teratocarcinoma	LTR- <i>gag-Apol-LTR</i> [44]	?
S71	Placenta, K562 cells	<i>gag-Apol-LTR</i> [141]	?
Class II			
HERV-K	Teratocarcinoma	LTR, <i>gag</i> [20,23], <i>pol</i> , <i>env</i> , <i>rev</i>	<i>rev</i> [19,144], <i>env</i> [145,167], protease [15,146], integrase
HERV-L	RA synovial fluid cells placenta, breast cancer	<i>pol</i> [100] LTR- <i>gag-Apol</i> [26]	? ?

*Abbreviations:* ERV, endogenous retrovirus; HERV, human endogenous retrovirus; HRES, human T-cell lymphotropic virus–related sequence; RRHERV, retinoic acid-responsive HERV; LTR, long terminal repeat.

<sup>a</sup> References are in brackets.

immunoregulatory abnormalities of rheumatic disease (Table 4). Changes in production of cytokines similar to those in patients with SLE, a shift from a Th1- to a Th2-type cytokine profile, have been described as a result of HIV-1 infection [29]. CD4 T-cell decline is mediated by an increased rate of apoptosis or programmed cell death (PCD) [30]. Th1 cytokines protect against apoptosis, whereas Th2 cytokines increase PCD [29]. A synthetic heptadecapeptide (CKS-17) corresponding to the transmembrane domain of the *env* protein conserved among many exogenous and endogenous retroviruses has potent immunosuppressive properties [31], possibly through suppression of Th1 cytokine production [32]. Recently, a full-length *env* protein of HERV-H was found to suppress antitumor immunity in the mouse [33]. The *env* protein of HERV-W, also called syncytin [34], stimulates expression of the type D mammalian retrovirus receptor in placenta [35]. Human ERV-W *env* can function as an envelope protein, form pseudotypes with HIV-1 virions, and confer tropism for CD4-negative cells [36]. Human ERV-W *env* may also act as a superantigen, causing V $\beta$ 16-specific T-cell expansions [37].

Table 4

## Viral proteins mimicking immunoregulatory abnormalities of rheumatic disease

Virus/viral product	Mechanism/effect	Reference
<b>Change in lymphokine milieu</b>		
All viral dsRNA or DNA	Induction of IFNs	[147]
HTLV-I p40/tax	Increases IL-2R expression	[148]
HIV-1 gp41 (aa581–597)	Inhibits PKC activity and IL-2 production	[149]
HIV-1 nef	Binds lck, inhibits Th1 cytokine production	[150]
HIV-1 tat	Inhibits Th1 cytokine IL-12 production	[151]
CMV US28	Binds C-C chemokines	[152]
HERV-H env	Inhibits anti-tumor immunity	[33]
CKS-17 env domain	Inhibits Th1 cytokine production	[31]
<b>Altered antigen presentation</b>		
EBV/BZLF2	Inhibits MHC class II binding	[153]
Adenovirus/E1A	Inhibits MHC class I expression	[154]
Adenovirus/E3 gp19	Inhibits MHC class I binding to antigen	[155]
HERV-K18 env	V $\beta$ 7-specific T-cell expansion	[40,41]
HSV/ICP47	Inhibits TAP (MHC) function	[156]
<b>Apoptosis pathways</b>		
HTLV-I p40/tax	Stimulation through blocking bcl-2	[157]
HTLV-I p40/tax	Inhibition of Fas and oxidative stress pathways	[158,159]
HIV-1 tat	Stimulation through Fas and oxidative stress	[160,161]
HIV-1 protease	Stimulation by cleavage of bcl-2	[162]
HIV-1 vpr	Stimulation through mitotic arrest	[163,164]
HIV-1 vpr	Disruption of mitochondrial transmembrane potential	[165]
Parvovirus B19	Stimulation by unknown mechanism	[166]
Influenza/NS1	Stimulation through Fas and bcl-2 pathways	[167]
Adenovirus E1B 19K	Bcl-2 homologue, inhibits apoptosis	[168]
EBV BHRF1	Bcl-2 homologue, inhibits apoptosis	[169]
HSV $\gamma_1$ 34.5	Inhibition by unknown mechanism	[170]
HBV pX	Inhibition, p53 antagonist	[171]
EHV-2 23 K E8-vFLIP	Inhibition of Fas pathway	[172,173]
MCV ORF159L-vFLIP	Inhibition of Fas pathway	[172,173]
HVS ORF71-vFLIP	Inhibition of Fas pathway	[172,173]
HHV-8 ORF189-vFLIP	Inhibition of Fas pathway	[172,173]

*Abbreviations:* HIV, human immuno deficiency virus; CMV, cytomegalovirus; HERV, human endogenous retrovirus; EBV, Epstein-Barr virus; HSV, herpes simplex virus; MCV, Molluscum contagiosum virus; HVS, Herpes virus saimiri; IFN, interferon; IL, interleukin; PKC, protein kinase C; MHC, major histocompatibility complex; ORF, open reading frame; HHV, human herpes virus; HTLV, human T-cell lymphotropic virus.

In turn, ERV expression may be induced by environmental signals and activation of the immune system. Interleukin 1 (IL-1) induces expression of xenotropic ERV in pancreatic  $\beta$  cells of NOD mice susceptible to IDDM [38]. Interleukin 1 and tumor necrosis factor alpha (TNF $\alpha$ ) stimulate transcription, whereas interferon gamma (IFN- $\gamma$ ) inhibits transcription of HERV-R in human vascular endothelial cells [39]. Interferon alpha (IFN- $\alpha$ ) induces expression of HERV-K18.1 env, which, acting as a superantigen, causes V $\beta$ 7- [40] or V $\beta$ 13-specific T-cell expansions [41]. Steroids have long been known to induce

expression and virion formation from ERV in the mouse [42]. Promoter of HERV-K can be induced by treatment with estradiol and progesterone [43]. Unlike related mouse mammary tumor viruses, HERV-K is not sensitive to stimulation by dexamethasone. Expression of retinoic acid-responsive HERV (RRHERV-I) [44] and HERV-R is enhanced by retinoic acid [45]. Human ERV-R can also be induced by vitamin D<sub>3</sub>, IFN- $\gamma$ , and phorbol esters [45]. Transcription of the ERV family members HERV-K, HERV-L, and ERV-9 was increased in UVB-irradiated skin and skin biopsies of lupus patients [27].

### **Impact of endogenous retroviruses integration on the immune system**

Endogenous retroviruses and other retrotransposable elements possess a relatively high mobility and cause immune dysregulation by insertional mutagenesis or *cis*- or *trans*-regulation of cellular genes [7]. The ERV HERV-K10 was found to have an integration site in the human complement C2 gene [46]. Variable repeats of HERV-K10 may have a role in polymorphism and differential expression of C2 loci. Two solo LTRs of HERV-K are located in the HLA-DR/DQ locus [47] oriented opposite to the transcription of the HLA-DQB1 genes \*0302, \*0402, \*0601, \*0202, and \*0305. By contrast, HLA-DQB1 \*0301, \*0303, and \*0502 alleles lack LTRs and display a massive 5635-nucleotide deletion [48]. Endogenous retrovirus-9 LTRs are located in the HLA-DRB region of human chromosome 6 [49]. Presence of the retroviral LTR may mediate association of these HLA class II alleles with IDDM and RA, as discussed later. Integration of a 5.3-kb ETn retrotransposon in the *FasR* gene locus resulted in disruption of this apoptosis pathway in lupus-prone MRL/lpr mice [50,51]. Inherited mutations of the human Fas receptor lead to autoimmune lymphoproliferative disease (ALPS) characterized by expansion of CD4<sup>+</sup>/CD8<sup>-</sup> T cells, lymphadenopathy, splenomegaly, and autoantibody production [52,53].

### **Role for endogenous retroviruses in animal models of autoimmune disease**

Independent lines of evidence suggest a viral cause in autoimmune rheumatic diseases. The possibility of a viral cause was raised by findings of virionlike tubuloreticular structures in endothelial cells and lymphocytes as well as by demonstration of elevated serum levels of type I IFN in lupus patients [54]. Viruslike particles were also noted in RA synovium [55]. Many viral infections are accompanied by production of autoantibodies, and viral proteins have profound effects on both antigen presentation and effector functions of the immune system. Dysregulation of programmed cell death has been documented in HIV-infected [30] and lupus patients [56]. Similar to SLE, anemia, leukopenia, thrombocytopenia, polymyositis and vasculitis have been widely reported in patients with AIDS [57]. Direct virus isolation and transmission attempts from tissues of autoimmune patients have not been successful [58]. Nevertheless, it is

possible that a (retro)virus responsible for provoking an immune response cross-reactive with self-antigens has been cleared from the host, so the absence of viral particles is not conclusive. An alternative retroviral cause, that is, activation of endogenous retroviral sequences (ERS), was initially proposed by a study of the New Zealand mouse model of SLE [59]. Endogenous retroviral envelope glycoprotein, gp 70, was found in immune-complex deposits of autoimmune lupus-prone NZB/NZW mice. Selective expression of an 8.4-kb full-length mink cell focus-forming (MCF) ERV was noted in the thymus of lupus-prone mouse strains [4]. Development of nephritis is controlled by a set of lupus-susceptibility loci [60]. High-level expression of the 8.4-kb full-length MCF transcripts was also noted in pancreatic  $\beta$  cells of NOD mice spontaneously developing IDDM [61,62]. Autoimmune vitiligo has been associated with expression with avian ERV in the Smyth line of chicken [63].

### **Endogenous retrovirus structural mimicry mediates cross-reactivity between viral protein and autoantigens in patients with systemic lupus erythematosus**

Under normal conditions, the immune system develops a potent virus-specific immune response that rapidly eliminates the virus with only minimal tissue injury. Minimal amounts of self-antigens are released, which are insufficient to induce autoreactive B and T lymphocytes, and autoimmune disease does not ensue. If the host and the virus share antigenic determinants, however, virus infection may result in autoimmunity, because virus-specific T cells and antibodies are cross-reactive with self-antigens. This scenario does not preclude the possibility that the infecting virus is eliminated by the immune response. Alternatively, similarities between proteins of the major histocompatibility complex (MHC) and microbial antigens, especially viral antigens, may allow the host to regard an infectious agent as self and, thus, forego an immune response. The shared epitope QKRAA sequence from the third hypervariable region of HLA DRB1 \*0401, which has been found in numerous human pathogens, is associated with susceptibility to RA [64]. The 70K protein of U1snRNP was the first lupus autoantigen shown to contain a region of homology and immunologic cross-reactivity with a conserved p30 *gag* protein of most mammalian type C retroviruses (Table 5). Based on this observation, Query and Keene [65] proposed that autoimmunity to U1RNP may be triggered by expression by an ERV *gag* protein. Anti-*gag* antibodies elicited by the ERS could cross-react with the 70K protein, and subsequently recognition could expand to additional 70K epitopes.

The ERVs capable of triggering antibodies cross-reactive with the 70K protein may correspond to HRES-1, a human T-cell lymphotropic virus-related endogenous sequence [66,67]. Human RES-1 encodes a 28-kd nuclear autoantigen, HRES-1/p28, which is expressed in a tissue-specific manner [24,25,68, ]. Antibodies to HRES-1/p28 were detected in 21% to 50% of patients with SLE and overlap syndromes in various laboratories [24,66,68–70]. By contrast, 3.6% (4/111) of normal donors and none of 42 patients with AIDS or 50 asymptomatic

Table 5  
Molecular mimicry between viral proteins and autoantigens

Autoantigen	Prevalence <sup>a</sup>	Viral protein	Virus	Reference
70k/U1 snRNP	30%	<i>gag</i>	MoMLV, HRES-1	[65,66]
HRES-1	21–52%	<i>gagp24</i>	HTLV-I	[24,66,68,69]
Sm B/B'	30%	<i>gagp24</i>	HIV-1	[174]
C/U1 snRNP	30%	ICP4	HHV-1	[78]
Sm D	36%	EBNA-1	EBV	[175]
Sm B/B'	25–40%	EBNA-1	EBV	[73]
La	15%	<i>gag</i>	FSV	[79]
p542	10–50%	EBNA-1	EBV	[176]
Topoisomerase I	20% <sup>b</sup>	<i>gag</i>	MoMLV	[177]
ERV-3	32%	<i>env</i>	MoMLV	[81]
HERV-E	48.3%	<i>gagp30</i>	MoMLV	[82]

*Abbreviations:* HRES, human T-cell lymphotropic virus–related sequence; ERV, endogenous retrovirus; HERV, human endogenous retrovirus; EBNA, Epstein-Barr virus nuclear antigen; MOMLV, Moloney murine leukemia virus; EBV, Epstein-Barr virus; FSV, feline sarcoma virus; HTLV, human T-cell lymphotropic virus.

<sup>a</sup> Prevalence of antibodies in patients with systemic lupus erythematosus.

<sup>b</sup> Prevalence of antibodies in patients with scleroderma.

HIV-infected patients had HRES-1 antibodies [66]. The retroviral gag-related region of the 70K protein shares three consecutive highly charged amino acids, Arg-Arg-Glu (RRE), an additional Arg, and functionally similar Arg/Lys residues with HRES-1/p28 which represent cross-reactive epitopes between the two proteins [65,66,68,]. Interestingly, the RRE triplet is repeated three times in the 70K protein at residues 248–250, 418–420, and 477–479, respectively (GenBank accession number X04654). This repetition suggests that recognition of the retroviral domain may lead to epitope spreading through binding to RRE triplets within the 70K protein. Because highly charged polypeptides can elicit high titer antibodies [71], the presence of charged amino acids in the mimicking epitopes may have important implications in triggering cross-reactive antibodies of high affinity.

Pathogenicity of Epstein-Barr virus (EBV) in lupus may also be mediated through ERV. Epstein-Barr virus is a ubiquitous human DNA virus that infects B cells and causes their polyclonal activation and thus polyclonal antibody production. Such polyclonal B-cell activation may be an early step in pathogenesis of SLE [72]. The prevalence of EBV infection was reported to be as high as 99% in young SLE patients, in comparison to a prevalence of 70% in controls [73]. Moreover, pathogenic interactions between EBV and retroviruses have been documented [74–76]. The authors therefore examined the influence of EBV on expression of HRES-1. Although HRES-1/p28 is not detectable in peripheral blood lymphocytes and the DG-75 EBV-negative lymphoblastoid B-cell line [68], it is expressed in human peripheral blood B cells transformed with EBV from the marmoset cell line B95-8 [77]. Human RES-1/p28 was found in EBV-transformed B cells from two HRES-1 genotype I donors but not in a genotype II or two genotype III donors (Fig. 2). Thus, EBV may contribute to autoantigenicity of HRES-1. Pathogenicity of EBV may also be related to increased expression of the

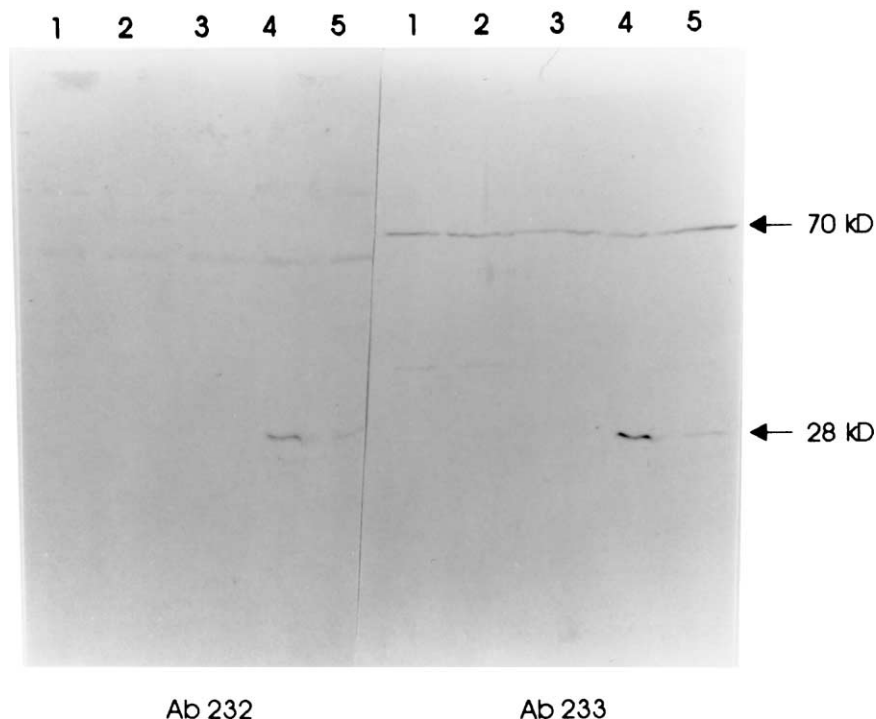


Fig. 2. Western blot analysis of expression of human T-cell lymphotropic virus–related endogenous sequence (HRES)-1/p28 in endogenous retrovirus (EBV)–transformed human peripheral blood B cells using antibodies 232 and 233 recognizing two nonoverlapping domains, pep14–24 and pep117–127 [11]. Donors 1 and 2 had HRES-1 genotype III/heterozygous alleles; donor 3 had genotype II/homozygous alleles; donors 4 and 5 had genotype I/homozygous alleles. Both Ab 232 and AB 233 detected expression of HRES-1/p28 in donors 4 and 5. The 70 kd component of U1snRNP detected by the cross-reactive Ab 233 [66] was uniformly expressed in all cells.

*env* gene of HERV-K18 [41]. This gene may have superantigen activity causing V $\beta$ 13-specific T-cell activation. The ICP4 protein of another ubiquitous human DNA virus, human herpesvirus type I (HHV-1) shows cross-reactivity with the C component of U1 snRNP [78]. A region with limited sequence homology to feline sarcoma virus (FSV) *gag* protein was noted in the La antigen [79].

Endogenous retroviruses, which are expressed on the protein level, are likely targets of cross-reactivity for virally induced immune responses. Such cross-reactivity (ie, molecular mimicry between self-antigens and viral proteins) has been proposed as a trigger of autoimmunity [25,28,80]. Along this line, antibodies to the *env* protein of ERV-3 were reported in patients with SLE, with the highest prevalence in mothers of babies with complete heart block [81]. Antibodies to a recombinant HERV-E 4-1 *gag* p30 protein were also reported in SLE patients [82]. There are at least 85 copies of HERV-E/4-1 per haploid genome scattered on 12 different chromosomes [83]. All isolates of HERV-E are

either truncated or contain multiple stop codons in their ORFs, thus rendering them incapable of encoding proteins [9]. The recombinant antigen was generated by correcting several stop codons in a genomic 4-1 sequence [82]. Existence of a native 4-1 *gag* p30 protein has not yet been reported. Therefore, the antigen responsible for triggering these autoantibodies remains to be determined.

Transcription of HERV-E, previously termed 4-1 [9,84], is also increased in patients with SLE [85]. Human ERV-E *gag*-specific RNA is detectable in peripheral blood mononuclear cells of healthy donors [23]. Thus, the amount, precise sequence, and chromosomal origin of lupus-specific HERV-E transcripts require further studies. Human ERV-E RNA was also noted in normal skin and keratinocyte cell lines [27]. Transcription of ERV family members HERV-K, HERV-L, and ERV-9 was increased in UVB-irradiated skin and skin biopsies of lupus patients [27]. Transcriptional inactivation of ERV is often associated by host-directed methylation of CpG islands in the ERV promoters [3]. 5-Azacytidine (5-AZA), a demethylating agent, was found to enhance expression of type C ERV in the mouse [86,87]. 5-Azacytidine was also described as enhancing expression of HERV-E (4-1) RNA in normal lymphocytes [85]. This mechanism is particularly interesting with regards to induction of T-cell autoreactivity by 5-AZA [88] and impaired DNA methylation in T cells of patients with SLE [89]. Demethylation of DNA plays an important role in toxicity of hydralazine and other lupus-inducing drugs [89].

Endogenous retroviruses are part of the human genome and may genetically predispose for autoimmunity. In 1991, the ERV HRES-1 was mapped to human chromosome 1 at q42 [90]. Polymorphic genotypes in the LTR/promoter region of the HRES-1 genomic locus have been associated with SLE [91]. Genotype I alleles seem to protect against SLE and autoreactivity to HRES-1 in white subjects [70]. Human RES-1 is centrally located at 1q42 with respect to microsatellite markers associated with susceptibility to SLE [92]. Thus, HRES-1 or a gene in linkage disequilibrium with this genomic locus may influence autoimmunity in SLE. A differential segregation of HRES-1 alleles was also reported in patients with multiple sclerosis, as discussed later.

The HRES-1 LTR contains polyadenylated runs varying from 8 to 14 bases. Increased rate of point mutations was observed in the vicinity of the polyadenylated tract [70]. These data can be related to recent observations that mismatch repair is inhibited and mutation rates are increased by  $10^3$  to  $10^4$ -fold at polyadenylated tracts ranging between 8 and 14 bases [93,94]. Variation of the polyadenylated tract (11 or 12 As) of the HRES-1 LTR was also noted by others [95]. Somatic mutations were more frequent in patients with SLE ( $\chi^2 = 16.88$ ;  $P < 0.001$ ). Hypermutability at the HRES-1 LTR may be influenced by a generally higher rate of somatic mutations in SLE [96].

The 1q42 chromosomal region has been associated with genetic instability [97] and has been identified as one of the three most common fragile sites in the human genome [98]. Genomic loci harboring ERV display increased chromosomal fragility [86]. Instability at 1q42 has shown an evolutionary conservation in man, gorilla, and chimpanzee [99], similar to the appearance of HRES-1

in Old World monkeys [67]. Moreover, fragility at 1q42 can be triggered with 5-AZA, a demethylating agent and a relative inducer of ERV genes in chicken cells [99]. Many ERVs are transcriptionally silent; this transcriptional inactivity is associated with host-directed methylation of CG sequences in the provirus [3]. Thus, activation of ERVs such as 4-1 and HRES-1 may mediate demethylation-induced autoreactivity.

### **Endogenous retroviruses in other autoimmune diseases**

Expression of ERV-9, HERV-K, and HERV-L transcripts are elevated in synovial cells from joints of patients with RA [100]. Human ERV-W is expressed in synovial fluid cells of RA patients [101]. Rheumatoid arthritis synovial fluid cells contain increased RNA amounts of the L1 retrotransposon which can be further stimulated by 5-AZA treatment [102]. Distinct HLA-DR/DQ alleles containing the two solo LTRs of HERV-K [47] correlate with RA [103] and IDDM [104]. Patients with IDDM, however, do not show humoral reactivities against endogenous retroviral envelope protein nor do they differ in retroviral mRNA expression from healthy relatives or normal individuals [105]. The mechanism by which the LTR-containing alleles confer susceptibility to RA and IDDM remains unknown. The HERV-K LTRs are oriented opposite to the transcription of the HLA-DQB1 gene. The LTRs are unlikely to influence transcription of the downstream DQB1 alleles, \*0302, \*0402, \*0601, \*0202, and \*0305, although they may serve as a recombination hotspot. Indeed, \*0301, \*0303, and \*0502 alleles lacking LTRs also display a massive 5635-nucleotide deletion [48]. Expression of an N-terminal superantigen encoded by the *env* region of IDDMK1,2, an ERV belonging to the HERV-K10 family, by pancreatic B cells and preferential expansion of corresponding V $\beta$ 7 T-cell receptor-positive T cells were reported in patients with IDDM [106]. Five subsequent studies failed to confirm these findings, however [105,107–110]. Thus, the involvement of HERV-K–encoded superantigen in IDDM remains controversial.

Autoantibodies to retroviral peptides, including HRES-1 p28, were found in sera of patients with Sjögren's syndrome [24,68]. In salivary glands of patients with Sjögren's syndrome, antibodies to HRES-1 detect antigens of aberrant size on Western blot assay [25]. These antigens may correspond to modified HRES-1 proteins or represent cross-reactivity with an exogenous virus. Human retrovirus-5 has been found only in 2 of 92 salivary gland biopsies, arguing against its involvement of pathogenesis of Sjögren's syndrome [111].

Multiple sclerosis is a chronic inflammatory disease of the central nervous system characterized by demyelination in the white matter [112]. Multiple sclerosis is thought to be mediated by an autoimmune process resulting in a selective loss of oligodendrocytes and subsequent demyelination. Although infection by HTLV-I has been associated with demyelinating myelopathy called tropic spastic paraparesis (TSP) [113,114], HTLV-I is not detectable in patients with MS [115]. Immunologic cross-reactivity between viral proteins and the

oligodendroglial autoantigens transaldolase (TAL) [116] and myelin basic protein has been documented in MS patients [117]. Transaldolase contains a high-copy-number repetitive element within its coding sequence [11,118]. Transaldolase is expressed at selectively high levels in oligodendrocytes in the brain [116] and is targeted by antibodies and T cells in MS patients [116,119]. T-cell responses to the CKS-17 peptide and a similar octadecapeptide from HERV-E were found in patients with acute MS [120]. The expression of HERV-K and HERV-W, but not HERV-H or HERV-E, was greater in the brain tissue of patients with MS or AIDS than in brain tissue of patients with other neurologic diseases [121]. An association of HRES-1 alleles with MS was noted in studies of 110 patients and 100 healthy controls from the United Kingdom. The influence of HRES-1 was independent of disease type or HLA-DR2 positivity [122]. Up to 29% of patients with MS may contain autoantibodies to HRES-1 p28 [68]. Thus, HRES-1, in combination with other susceptibility loci, may influence development of MS, SLE [2], and perhaps other autoimmune diseases. Continued research on structure, expression, and autoreactivity of ERVs is required for understanding the evolution of the genome. Such research can be expected to provide future breakthroughs in the diagnosis and treatment of autoimmune diseases.

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