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Review

Activation of the innate immune response by endogenous retroviruses

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The human genome comprises 8 % endogenous retroviruses (ERVs), the majority of which are defective due to deleterious mutations. Nonetheless, transcripts of ERVs are found in most tissues, and these transcripts could either be reverse transcribed to generate ssDNA or expressed to generate proteins. Thus, the expression of ERVs could produce nucleic acids or proteins with viral signatures, much like the pathogen-associated molecular patterns of exogenous viruses, which would enable them to be detected by the innate immune system. The activation of some pattern recognition receptors (PRRs) in response to ERVs has been described in mice and in the context of human autoimmune diseases. Here, we review the evidence for detection of ERVs by PRRs and the resultant activation of innate immune signalling. This is an emerging area of research within the field of innate antiviral immunity, showing how ERVs could initiate immune signalling pathways and might have implications for numerous inflammatory diseases.

Introduction

It is estimated that 8 % of the human genome comprises endogenous retroviruses (HERVs) (Lander *et al.*, 2001), which can be classified into at least 31 distinct families (Bannert & Kurth, 2006; Katzourakis & Tristem, 2005). Of these, the HERV-K (HML-2) family shows evidence of recent activity within the human genome (Marchi *et al.*, 2014). Amplification of endogenous retroviruses (ERVs) within mammalian genomes occurred either by extracellular reinfection of germline cells (Dewannieux *et al.*, 2006) or by retrotransposition, with the latter producing the largest ERV families (Magiorkinis *et al.*, 2012). No single HERV locus has been found that can produce infectious virions (Kassiotis, 2014), but the reconstitution of an ancestral HERV-K (HML-2) genome resulted in the production of functional infectious viral particles (Dewannieux *et al.*, 2006; Lee & Bieniasz, 2007). Here, we review the biological role of HERV expression and how it could result in innate immune activation. We will first provide an outline of the mechanisms of innate immunity and then discuss how these may be used to detect and limit HERV expression.

Expression and biological roles of HERVs

The existence of endogenous retroviruses in mammalian genomes has been known since the 1970s (Coffin, 1979). Early studies showed the presence of retrovirus-like particles in placentae of humans and non-human primates (Kalter *et al.*, 1973). Further studies identified virus-like particles in cancers including human teratocarcinomas (Harzmann *et al.*, 1982) and cloned full-length sequences of several HERVs (Bonner *et al.*, 1982; Repaske *et al.*, 1985). Subsequently, the multicopy nature of these elements was described, with HERV-K initially thought to be present at 30–50 copies per haploid genome (Ono, 1986). HERV mRNA was detected in normal and malignant human tissues (Cohen *et al.*, 1988; Kato *et al.*, 1987; Medstrand *et al.*, 1992), with variation among tissues in the length, quantity and content of the HERVs (Rabson *et al.*, 1983). More recently, a survey was made of the genome for complete proviral elements and to quantify the transcription of these elements in healthy human tissues (de Parseval *et al.*, 2003). Of the more than 10 000 retroviral elements, there were 476 potential complete *env* genes and, of these, 16 were found to encode potentially full-length envelope proteins (de Parseval *et al.*, 2003). Ultimately, these full-length *env* genes were used to design primers for quantitative reverse transcription-PCR, which showed variation in tissue-specific expression of the HERV *env* genes (de Parseval *et al.*, 2003). Importantly, it also showed that the promoters are active, allowing transcription of these genes (de Parseval *et al.*, 2003).

The expression of HERVs is thought to be analogous to the replication of exogenous retroviruses, with transcription of the provirus followed by export of the mRNA and translation of viral proteins. Furthermore, the RNA transcript can be reverse transcribed to produce cDNA in the cytosol that could theoretically reintegrate into the host genome. Early studies showed the nascent viral particles budding from the plasma membrane into the extracellular space, but these were shown to be morphologically immature (Bieda *et al.*, 2001). These particles are more likely to be non-infectious retroviral-like particles (RLPs), possibly lacking the full complement of genes and resulting from

the transcription of HERV proviruses with intact *gag* genes, as the Gag protein is implicated in particle formation and budding (Akers *et al.*, 2013).

The expression of at least some HERVs is thought to be beneficial to the host. One mechanism by which this has occurred is through the domestication of a HERV gene for use by the organism (Patel *et al.*, 2011). A well-studied example of this is the case of the HERV-W and HERV-FRD *env* genes, which encode the proteins syncytin-1 and syncytin-2, respectively (Dupressoir *et al.*, 2012). These proteins are required for placenta formation, allowing fusion of cells to form the syncytiotrophoblast and contributing to immune tolerance of the fetus (Dupressoir *et al.*, 2012). In addition, genes and regulatory sequences from ERVs have been co-opted in the fight against viral infections, providing endogenous viral-element-derived immunity (Aswad & Katzourakis, 2012). For example, in mice, an ERV LTR-derived regulatory element in APOBEC3 allows the upregulation of expression of this intrinsic restriction factor (Sanville *et al.*, 2010). Also in mice, the capsid-binding restriction factor Fv1 was found to be derived from the *gag* gene of an ERV (Best *et al.*, 1996). Fv1 protects against infection by a number of exogenous retroviruses by interacting with the capsid and blocking viral DNA entry into the nucleus (Yap *et al.*, 2014). Thus, these restriction factors provide endogenous viral-element-derived immunity by protecting the cell from exogenous retrovirus infection. Recently, HERV-H was found to be expressed as a long non-coding RNA (Lu *et al.*, 2014), a type of regulatory RNA molecule that associates with other factors linked to the stem cell phenotype, such as Octamer-4 (OCT4) and Mediator subunits, and is expressed in embryonic stem cells (Lu *et al.*, 2014).

Despite the potential for co-option of HERVs or other beneficial effects, there is a long association of HERVs with diseases such as autoimmune diseases and cancer. One way the cells could deal with potential detrimental effects is by restricting the activity of endogenous retroviruses. The expression of HERVs in adult tissues can be silenced by epigenetic mechanisms (Lavie *et al.*, 2005), although the CpG methylation of HERVs is variable and is possibly due to the spread of methylation from neighbouring gene loci (Reiss *et al.*, 2007). In addition, a number of intrinsic antiviral factors have been described that block virus replication by directly binding to and acting upon viral nucleic acids or proteins. The intrinsic restriction factor APOBEC3G is a cytosine deaminase that inhibits the retrotransposition of endogenous retroviruses (Esnault *et al.*, 2005, 2006). It is hypothesized that APOBEC3G is most active in germ cells and during early embryogenesis, when HERVs are also thought to be active due to hypomethylation (Esnault *et al.*, 2005). Furthermore, there is evidence of past activity by a cytosine deaminase, resulting in the hypermutation of HERVs (Lee *et al.*, 2008). Other restriction factors include the TRIM family members, such as TRIM5α, which can restrict the expression of certain primate ERVs not found in humans (Kaiser *et al.*, 2007), and exogenous retroviruses such as human immunodeficiency virus type 1 (HIV-1) (Pertel *et al.*, 2011). Thus, there are cellular mechanisms that could repress HERV expression or cause deleterious mutations within the proviruses.

Furthermore, given that HERVs are part of the genome, they should theoretically be recognized as 'self', and immune tolerance of such self-antigens should be established during development (Tugnet *et al.*, 2013). Tolerance is not perfect and loss of self-tolerance is characteristic of autoimmune diseases. One mechanism by which HERVs could trigger an immune response is by producing nucleic acids or proteins that resemble the pathogen-associated molecular patterns (PAMPs) of exogenous viruses. Molecules generated by host tissues that are stressed or damaged are referred to as danger-associated molecular patterns (DAMPs) and activate the same classes of pattern recognition receptors (PRRs) as PAMPs (Tang *et al.*, 2012). In the case of HERVs, increased expression of RNA with retroviral-like structures or the presence of free cDNA in an unfamiliar compartment (i.e. the cytosol) could result in the activation of PRRs. The upregulation of HERV expression has been described in diseases such as cancer (Wang-Johanning *et al.*, 2014) and autoimmunity (Saresella *et al.*, 2009), but it is unclear whether HERV expression contributes to the disease phenotype or is a bystander effect. Should HERV molecules act as DAMPs, the activation of PRRs could thereby provide a mechanism by which HERVs contribute to pathogenesis.

Innate immune pathways activated by retroviruses

PRRs comprise an array of membrane-bound and cytosolic receptors that detect PAMPs and DAMPs (Tang *et al.*, 2012). The activation of PRRs invokes complex signalling pathways that ultimately alter gene expression. In particular, these pathways lead to the production of pro-inflammatory cytokines, chemokines and type I IFNs (IFN-α and IFN-β). These are the effector molecules that initiate an antiviral state and activate adaptive immune cells. There are a number of recent reviews on PRRs and signalling therefrom (Gürtler & Bowie, 2013; Lester & Li, 2014; O'Neill *et al.*, 2013); here, we provide an overview of the pathways that could be relevant to the detection of HERVs.

Toll-like receptors (TLRs)

One class of PRR for the detection of viruses is the TLRs, transmembrane proteins found on either the plasma or the endosomal membrane. There are 10 human TLRs (TLR1–TLR10), each with a distinct subcellular localization and ligand specificity (Lester & Li, 2014). TLRs have an extracellular ligand-binding domain comprising leucine-rich repeats and a conserved cytosolic domain, the Toll-IL-1 resistance (TIR) domain; the TIR domain is also found in the IL-1 receptor superfamily and is essential for signalling from both the TLRs and IL-1R1 (O'Neill *et al.*, 2013). Signalling from the TLRs requires receptor dimerization and the recruitment of TIR domain-containing adaptor

molecules, including myeloid differentiation factor 88 (MyD88), to the TIR domain of the receptor (Lester & Li, 2014). This is followed by activation of a cascade of intracellular kinases and ubiquitin ligases, resulting in the nuclear translocation of transcription factors including (NF-κB) and IFN regulatory factors (IRFs) 3 and 7 (O'Neill *et al.*, 2013). The transcription factors bind to response elements in genes, resulting in the expression of pro-inflammatory cytokines and type I IFNs (Fig. 1).

The plasma membrane-localized TLR4 was first described as the sensor for the bacterial product lipopolysaccharide (LPS) when it is bound to the protein myeloid differentiation factor 2 (MD2) (O'Neill *et al.*, 2013). Importantly, TLR4 can also detect viral glycoproteins, such as the Ebola virus glycoprotein and the envelope proteins of retroviruses including HIV-1 (Nazli *et al.*, 2013; Okumura *et al.*, 2010). TLR3, -7, -8 and -9 sense microbial nucleic acids in the endosomal lumen: TLR3 detects dsRNA, both TLR7 and -8 sense ssRNA, and TLR9 recognizes unmethylated CpG DNA (Gürtler & Bowie, 2013). More recently, TLR9 has been shown to recognize RNA : DNA hybrids (Rigby *et al.*, 2014). Given that the ligand-binding domain of these nucleic acid-sensing TLRs (NA-TLRs) is in the endosomal lumen, cytosolic PAMPs are unlikely to be sensed and this pathway is thought to be more important for the detection of exogenous viruses. However, the process of autophagy allows sampling of the cytosol and fusion of the autophagosome with the endosome (Tang *et al.*, 2012), possibly permitting the detection of cytosolic PAMPs by the endosomal TLRs.

Cytosolic PRRs

In addition to the TLRs, a number of PRRs have been described that reside in the cytosol or migrate to the cytosol following the detection of microbial nucleic acids (Fig. 2). The retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) are a class of RNA helicases that recognize viral RNA in the cytoplasm and induce type I IFNs (Gürtler & Bowie, 2013). The RLRs include RIG-I and melanoma differentiation-associated gene 5 (MDA5), both of which have two N-terminal caspase recruitment domains (CARDs) that are essential for signalling (Gürtler & Bowie, 2013). RIG-I detects short dsRNA and ssRNA with a 5′-triphosphate moiety, whereas MDA5 senses long dsRNA molecules (Kato *et al.*, 2008). A third RLR that lacks a CARD, laboratory of physiology and genetics 2 (LGP2), enhances the detection of RNA by MDA5 but not RIG-I (Childs *et al.*, 2013). Following detection of RNA ligands, RIG-I and MDA5 signal via homotypic CARD interactions with the mitochondrial outer-membrane-localized adaptor molecule mitochondrial antiviral signalling (MAVS), leading to the phosphorylation and activation of the transcription factors IRF3 and -7 (Loo & Gale, 2011). The phosphorylated IRFs can translocate to the nucleus, bind to the IFN-stimulated regulatory element and induce IFN expression (Hiscott, 2007).

Several cytosolic receptors for DNA have also been described, including the DNA-dependent activator of IFN regulatory factors (DAI), which detects long, dsDNA (Takaoka *et al.*, 2007). In addition, the absent in melanoma 2 (AIM2)-like receptors include the proteins AIM2, which senses dsDNA (Hornung *et al.*, 2009), and IFN-γ-inducible protein 16 (IFI16), which detects ssDNA (Unterholzner *et al.*, 2010). Following detection of DNA in the cytosol, IFI16 recruits the adaptor stimulator of IFN genes (STING), leading to IFN-β production (Unterholzner *et al.*, 2010). Initially, this receptor was discovered using DNA from vaccinia virus (Unterholzner *et al.*, 2010), but it has since been found to detect DNA produced during lentivirus replication (Jakobsen *et al.*, 2013). The study showed that ssDNA with a stable stem region and dsDNA induced IFN-β production in an IFI16-dependent manner (Jakobsen *et al.*, 2013). Other cytosolic DNA sensors have been described, including DEAD box helicase 41 (DDX41) and cyclic GMP-AMP synthase (cGAS), the latter of which detects DNA from retroviruses and signals via STING and IRF3 for IFN induction (Gao *et al.*, 2013). More recently, cGAS has been shown to play a pivotal role in the antiviral response to both DNA and RNA viruses via the induction of IFN and IFN-stimulated gene (ISG) expression (Schoggins *et al.*, 2014). There are thus a number of cytosolic sensors that detect viral nucleic acids and initiate innate immune signalling. Whilst much of this work has been done with exogenous viruses, there is evidence that the same cellular mechanisms could also detect HERV expression.

Sensing of HERVs by innate immune receptors

Recently, a number of candidate receptors have been proposed that could sense retroviral DNA in the cytoplasm and RNA in the endosome. However, much of this work has been done with exogenous retroviruses, such as HIV-1, or murine ERVs (MuERVs), not with HERVs. Whilst the nucleic acids and proteins produced by HERV expression could generate endogenous ligands for the innate immune response (Fig. 3), there is limited evidence to date for a direct interaction.

TLRs and HERVs

Only a few studies have investigated the role of TLRs in detecting HERVs. One member of the HERV-W family that is potentially associated with multiple sclerosis has been shown to interact with TLRs and to produce a pro-inflammatory response (Rolland *et al.*, 2005, 2006). In this case, the surface subunit of the Env protein (ENV-SU) interacts with TLR4 and CD14 to stimulate the production of pro-inflammatory cytokines including IL-1β, IL-6 and TNF-α (Rolland *et al.*, 2006). Furthermore, this subunit can activate dendritic cells and induce a Th1 response (Rolland *et al.*, 2006), which is the activation of a subtype of helper T-cells that contribute to phagocyte-dependent inflammation and cell-mediated immunity (Murphy & Reiner, 2002). Single-nucleotide polymorphisms in a

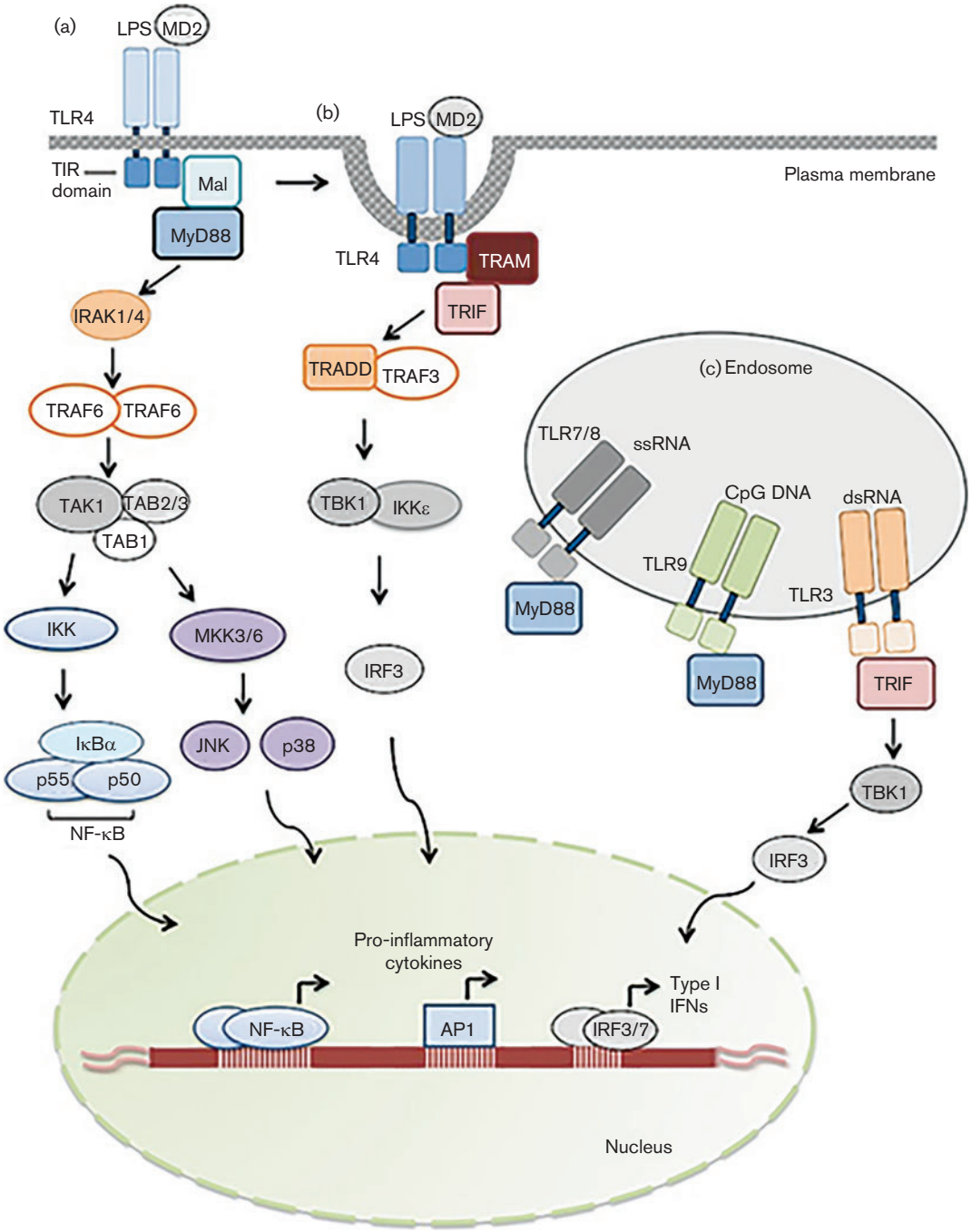

Fig. 1. Signalling from TLRs in response to ligand binding. TLRs are localised to either the cell surface or the endosome. TLRs that are localised to the cell surface (TLR1, -2, -4, -5, -6 and -11) typically respond to lipid or protein ligands. Of the cell surface TLRs, only TLR4 is shown in the figure for clarity. (a) MyD88-dependent signalling. Following the binding of the LPS–MD2 complex, the TIR adaptors MyD88 or Mal are recruited to the TIR domain of TLR4. Subsequently, recruitment of IRAKs leads to the activation of TRAF6, an E3 ubiquitin ligase. TRAF6 then ubiquitinates and activates the TAK1 in complex with TAB1 and TAB2. Activated TAK1 then phosphorylates the IκB kinase (IKK) and the MAPK kinase (MKK3/6) to activate NF-κB and MAPK, respectively. MKK3/6 activates the MAPKs, JNK and p38, which phosphorylate transcription factors including AP-1, CHOP and ATF2. (b) MyD88-independent signalling. TLR4 can be internalized following ligand binding, forming a second signalling complex. In this case, it uses the adaptors TRIF and TRAM and signals via the recruitment of TRADD and TRAF3. TRAF3 activates TANK-binding kinase-1 (TBK1) and IKKε, which phosphorylate IRF3, allowing it to translocate to the nucleus and induce type I IFN. (c) Endosomal TLRs. The nucleic acid-sensing TLRs are localized to the endosome and respond to DNA or RNA ligands. TLR7/8 and TLR9 utilize the MyD88-dependent pathway outlined above, whereas TLR3 uses a TRIF-dependent

pathway that signals via TBK1 to activate IRF3. Activation of the signalling pathways leads to the production of pro-inflammatory cytokines and type I IFNs. AP-1, activated protein-1; ATF2, activating transcription factor 2; CHOP, CCAAT enhancer-binding protein homologous protein; IRAK, IL-1R associated kinase; JNK, Jun N-terminal kinase; LPS, lipopolysaccharide; Mal, MyD88 adaptor-like; MAPK, mitogen-activated protein kinase; MD2, myeloid differentiation factor 2; TAK1, TGFβ-activated kinase 1; TAB, TAK1-binding protein; TIR; Toll/IL-1R' resistance domain; TRADD, tumour necrosis factor receptor-associated death domain; TRAF, tumour necrosis factor receptor-associated factor; TRAM, TRIF-related adaptor molecule; TRIF, TIR domain-containing adaptor inducing IFN-β.

HERV-K dUTPase were found to be significantly associated with psoriasis (Ariza & Williams, 2011). The recombinant WT and mutant dUTPase were able to induce NF-κB in a TLR2-dependent manner, resulting in a T-helper 1 (Th1) and Th17 cytokine response (Ariza & Williams, 2011). Whilst TLR2 is thought normally to respond to bacterial lipopeptides, other protein ligands including the measles virus haemagglutinin protein are sensed by TLR2 (Kawai & Akira, 2009). It is thus possible that other proteins including the HERV-K dUTPase could be detected by TLR2, although whether this reaction occurs *in vivo* remains to be demonstrated, particularly as it is thought to be intracellular whereas TLR2 is expressed on the cell surface. These two studies indicate that TLRs can respond to HERV proteins to induce a pro-inflammatory response.

There are a few reports of a role for the NA-TLRs in detecting MuERVs. First, the MuERV envelope glycoprotein, gp70, is considered to be an antigen against which a TLR7-dependent autoimmune response is directed in lupus-prone mice (Yoshinobu *et al.*, 2009). The expression of gp70 is increased following activation of TLR7 and -9 in a mouse model of lupus (Baudino *et al.*, 2010), providing a direct link between TLR activation and the upregulation of MuERV expression. Interestingly, the TLR-mediated expression of gp70 was distinct from the mechanism controlling basal levels of gp70 expression (Baudino *et al.*, 2010). Thus, there is a mechanism by which inflammation could lead to elevated serum gp70 levels, consistent with gp70 acting as an acute-phase protein in mice (Baudino *et al.*, 2010). TLR7 and -9 have a role in the pathogenesis of murine lupus, both by inducing an adaptive immune response to autoantigens and by increasing the expression of at least one class of autoantigen, the MuERV gp70 (Baudino *et al.*, 2010).

Another study showed an important role for NA-TLRs in the control of MuERV viraemia and the development of ERV-induced tumours (Yu *et al.*, 2012). Mice deficient in NA-TLR or the requisite signalling molecules showed enhanced MuERV mRNA expression and viral particle formation (Yu *et al.*, 2012). The absence of TLR7, alone or in combination with deficiencies in the other TLRs, resulted in an increase in MuERV *gag* mRNA levels and gp70 Env protein expression (Yu *et al.*, 2012). Likewise, deficiency in IFN regulatory factor 5 (IRF5), a specific signalling molecule used by TLR7 and -9, leads to increased gp70 *env* or *gag* mRNA levels, suggesting that TLR7 restricts MuERV expression via an IRF5-dependent pathway (Yu *et al.*, 2012). Only TLR7 had a non-redundant role in this immune surveillance. The role of the NA-TLRs was examined further in the context of MuERV-induced tumours. Acute T-cell lymphoblastic leukaemia developed only in the NA-TLR-deficient mice, suggesting that the combined effect of these TLRs is to provide a protective response against ERV-induced tumours (Yu *et al.*, 2012).

A general role for TLRs in the antibody response to exogenous retroviruses has been described recently. TLR7 was found to be involved in the germinal centre reaction, which facilitates antibody production and class switching, specifically in the acute response to murine exogenous retroviruses (Browne, 2011, 2013). Furthermore, TLR7 was critical for the production of autoantibodies to gp70 (Yoshinobu *et al.*, 2009), and mice lacking TLR7 were unable to produce antibodies to MuERVs (Yu *et al.*, 2012). It was proposed that MuERVs are activated sporadically and that this allows for a protective, TLR7-dependent antibody response to control MuERV viraemia (Yu *et al.*, 2012). Whether a similar system functions in the surveillance of HERVs in humans is not yet known. The activation of NA-TLRs in response to HERV expression could potentially contribute to the development of antibodies to HERVs, which has been observed in a number of studies of human cancers (Wang-Johanning *et al.*, 2008) and infection with HIV-1 (Michaud *et al.*, 2014). The NA-TLRs also promote autoimmune responses as they are fundamental to the activation of autoreactive B-cells (Koh *et al.*, 2013), which could include ERV antigens as well as other types of autoantigen (Baudino *et al.*, 2010). Thus, the NA-TLR-mediated control of ERVs could provide a link with the development of self antibodies in autoimmune diseases.

Detection of HERVs by cytosolic PRRs

As a large part of retrovirus replication takes place in the cytoplasm, the presence of cytosolic PRRs could allow early detection of the products of HERV expression. To date, no studies have identified a role for cytosolic RNA receptors in sensing HERV RNAs. There is evidence that HIV RNA can induce a RIG-I-dependent response (Berg *et al.*, 2012), so it is theoretically possible that other retroviral RNAs, including HERVs, could also be detected by this PRR. Importantly, enzymes have been described that maintain nucleic acid homeostasis in the cytosol, mainly by degrading excess nucleic acids to prevent immune activation. A recent study has identified a mechanism that would restrict cytosolic RNA and could thus prevent the accumulation of HERV RNAs. The 3′⟶5′ RNA exosome containing

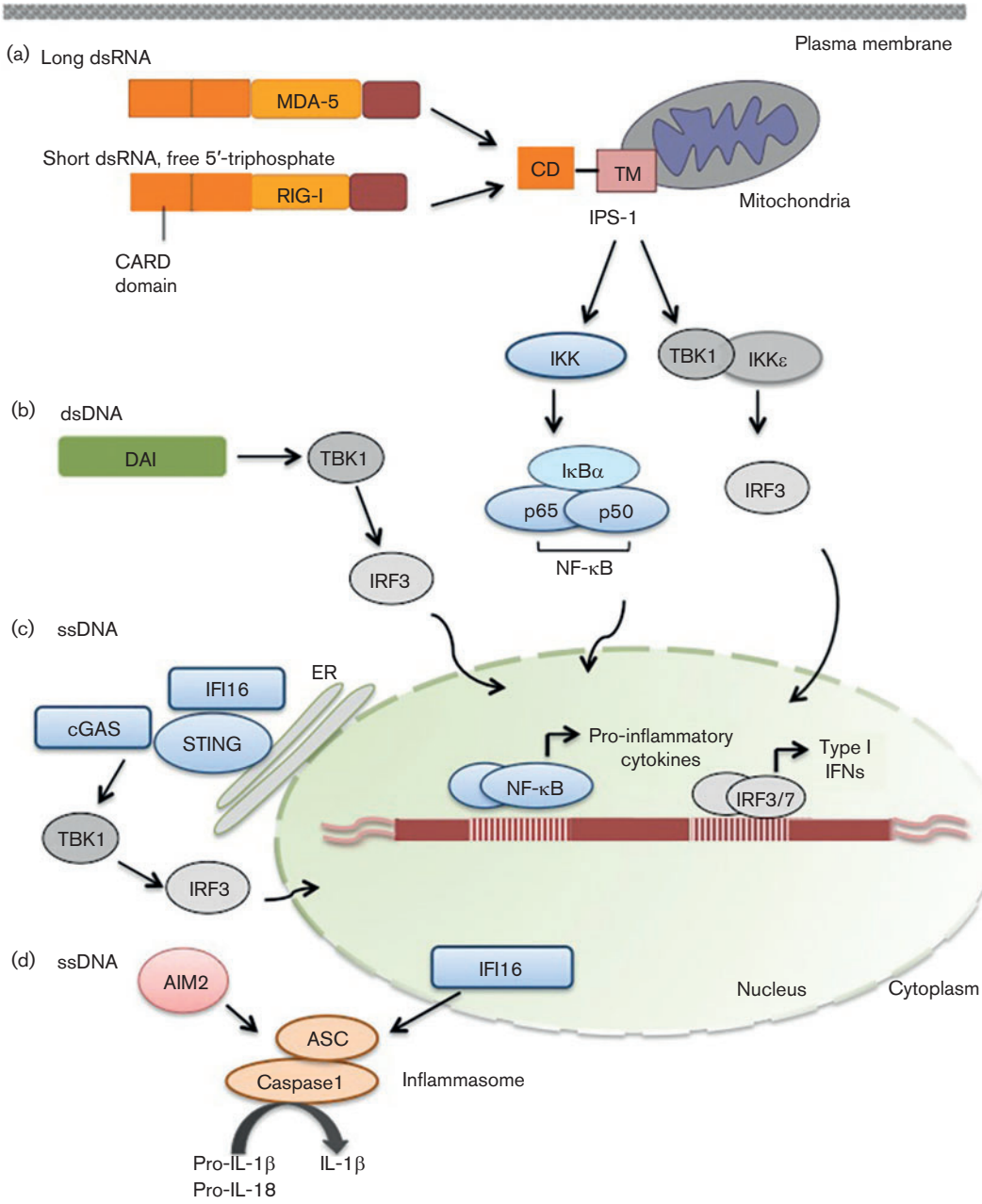

Fig. 2. Signalling from cytosolic receptors. (a) RIG-I and MDA5 detect 5′-triphosphate ssRNA or dsRNA, respectively. They mediate antiviral signalling via CARD–CARD interactions with the adaptor, IFN-β promoter stimulator 1 (IPS-1; also referred to as MAVS, Cardif and VISA). The transmembrane domain of IPS-1 is anchored in the mitochondrial outer membrane, and this localisation is required for it to transduce the signal to pro-inflammatory cytokine production and type I IFN induction via activation of NF-κB and IRF3. (b) The DNA-dependent activator of IRFs (DAI) detects dsDNA in the cytosol and signals via TBK1 and IRF3 activation to induce type I IFNs. DAI can also activate NF-κB in an RIP1-dependent manner. (c) IFI16 and cGAS are cytosolic sensors for ssDNA that signal via the ER-localized protein STING, to induce IFN gene expression. STING signalling is dependent upon TBK1 and IRF3. (d) IFI16 is one PRR that is found in both the nucleus and the cytosol. It can translocate from the nucleus to the cytosol to trigger the formation of the inflammasome, thereby activating caspase 1-mediated cleavage and maturation of IL-1β and IL-18. AIM2 can also direct formation of the inflammasome following detection of ssDNA in the cytosol. ASC is an adaptor molecule involved in inflammasome formation. AIM2, absent in melanoma 2; ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; CARD, caspase recruitment domain; CD, card domain; cGAS, cyclic GMP-AMP synthase; ER, endoplasmic reticulum; IFI16, IFN-γ-inducible protein 16; IKKε, IκB kinase ε; MDA5, melanoma differentiation-associated gene 5; RIG-I, retinoic acid-inducible gene I; STING, stimulator of IFN genes; TBK1, TANK-binding kinase-1; TM, transmembrane domain.

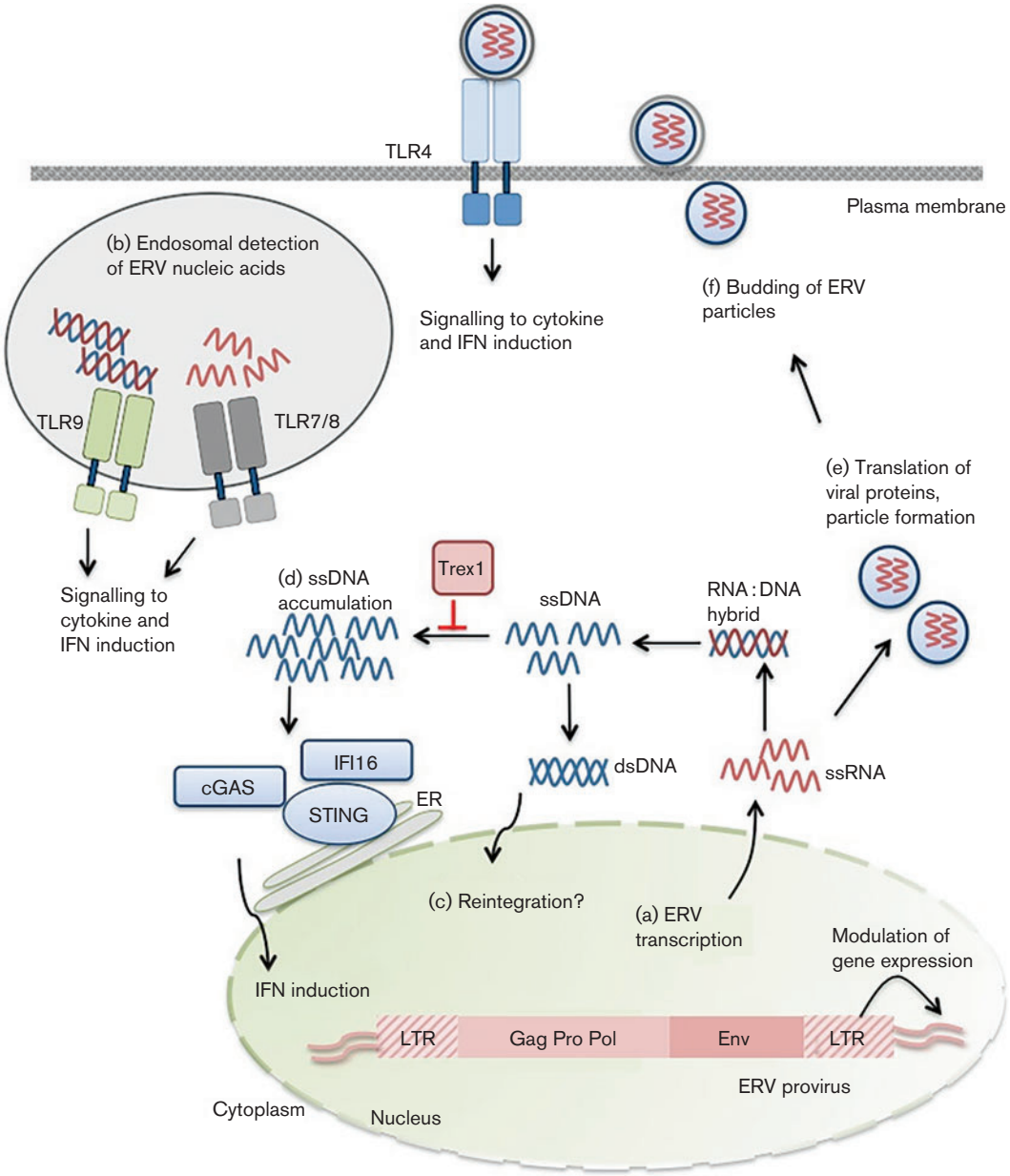

Fig. 3. Expression of ERVs and how they could activate components of the innate immune response. (a) RNA transcripts of ERV proviruses could be exported from the nucleus and used to produce cDNA. (b) The ssRNA and the RNA : DNA hybrid intermediate generated during reverse transcription would be sensed by TLR7 and TLR9, respectively, if they reached the endosome. There is no evidence yet that the ssRNA is sensed by cytosolic RNA PRRs. (c) The ssDNA produced following reverse transcription could be used to generate a dsDNA molecule for reintegration into the genome. (d) Alternatively, the cDNA could be degraded by the enzyme TREX1 or else detected by cytosolic PRRs such as IFI16 or cGAS, leading to type I IFN induction. (e) The RNA transcripts could also be read as mRNA for the production of viral proteins, possibly leading to particle formation. There is some evidence that even if only the Gag protein is synthesized, RLPs can be produced, although these may not package the viral genome. (f) The viral particles or RLPs could bud from the plasma membrane. TLR4 has been shown to sense the Env protein of at least one HERV, so it is feasible that the particles could activate a TLR4-mediated innate immune response.

the SKIV2L helicase degrades endogenous RNA molecules that would otherwise be immunostimulatory, thereby acting as a negative regulator of the RLRs (Eckard *et al.*, 2014). The SKIV2L gene is associated with susceptibility to systemic lupus erythematosus (Eckard *et al.*, 2014), providing further support for the theory that cytosolic nucleic acids can be

linked to aberrant immune activation in autoimmune diseases. Whether HERV RNAs are also degraded by SKIV2L remains to be demonstrated.

TREX1 and cytosolic DNA accumulation

There is some evidence that HERV DNA could be detected by cytosolic PRRs and that the production of HERV DNA is restricted by the 3′ repair exonuclease 1 (TREX1), a 3′→5′ exonuclease that degrades endogenous DNA (Paludan & Bowie, 2013). TREX1 is found at the endoplasmic reticulum as part of the SET (suppressor of variegation, enhancer of zeste and trithorax) DNA repair complex (Yan *et al.*, 2009) and is thought to have a critical role in restricting the amount of endogenous DNA in the cytosol (Stetson *et al.*, 2008). Activation of TLRs in conventional dendritic cells results in the upregulation of TREX1 expression via type I IFN and NF-κB (Xu *et al.*, 2014). Thus, TREX1 is induced by pro-inflammatory conditions and is thought to play a role in the innate immune response to DNA PAMPs and DAMPs.

The critical role for TREX1 in degrading DNA in the cytosol was discovered in TREX1 knockout mice, which develop a type I IFN response and multi-organ inflammation (Gall *et al.*, 2012). In TREX1-deficient cells, short ssDNA sequences were found to accumulate at the endoplasmic reticulum (Yang *et al.*, 2007). These ssDNA sequences were thought to be produced during replication, probably as a by-product of recombination and the repair of double-stranded breaks (Yang *et al.*, 2007). A subsequent study identified retroelements, including MuERVs, among the cytosolic DNA that accumulates in TREX1 deficiency (Stetson *et al.*, 2008). Based on this study, endogenous retroviral DNA is a substrate for TREX1 and can accumulate in TREX1-deficient cells. However, further work is required to confirm these findings and to identify the types of endogenous DNA molecules that are present in TREX1 knockout cells.

Whilst the TREX1 knockout mice displayed an IRF3-dependent immune response to the cytosolic DNA (Stetson *et al.*, 2008), the PRR that detects the DNA and induces this response was not identified in this study. More recent work has implicated one or more of the cytosolic receptors in the detection of DNA in TREX1-deficient cells. A critical role was identified for the adaptor molecule STING, in the development of type I IFN-dependent inflammation and autoimmunity (Gall *et al.*, 2012). This adaptor is used by a number of cytosolic DNA PRRs, including IFI16 and cGAS. IFI16 has been found to recognize retroviral DNA: either retrovirus-derived ssDNA with a stable stem region or dsDNA induced IFN-β production in an IFI16-dependent manner (Jakobsen *et al.*, 2013). The same study found similar responses to lentiviral ssDNA and dsDNA with cGAS and DDX41, and the knockdown of these PRRs also impaired IFN production in response to these DNA ligands (Jakobsen *et al.*, 2013). This suggests that there could be overlapping or redundant functions of these sensors. Importantly, a recent study showed that TREX1-deficient cells in which cGAS was also knocked out were unable to induce ISG expression in response to cytosolic DNA (Ablasser *et al.*, 2014). This is strong evidence of a primary role for cGAS in the detection of immunostimulatory DNA in the cytosol. The source of the ssDNA could be HERV cDNA; cGAS can induce IFN expression in response to retroviral cDNA from exogenous retroviruses including HIV-1 (Gao *et al.*, 2013). Thus, it is possible that endogenous retroviral DNA could serve as a ligand for cGAS, but this remains to be demonstrated directly.

It has been proposed that the level of HERV cDNA in the cytosol is kept below a threshold level by the action of TREX1 (Volkman & Stetson, 2014); in the case of TREX1 deficiency, the normally immunologically silent cDNA accumulates such that it becomes a DAMP that could activate the innate immune response. At least one human autoimmune disease, Aicardi–Goutières syndrome (AGS), can be caused by mutations in the TREX1 gene that lead to disruption of the normal DNA damage response and the accumulation of DNA in the cytosol (Yang *et al.*, 2007). Indeed, the lack of TREX1 in patients with AGS is thought to lead to an accumulation of HERV DNA in the cytoplasm, ultimately inducing a type I IFN response (Stetson *et al.*, 2008). Thus, TREX1 deficiency could cause chronic activation of DNA-sensing PRRs, accounting for the inflammatory disease and elevated type I IFN observed in these patients (Yang *et al.*, 2007). Interestingly, the use of reverse transcriptase inhibitors in TREX1 knockout mice resulted in improved survival and reduced markers of inflammation in autoimmune myocarditis (Beck-Engeser *et al.*, 2011). Thus, it is likely that there is a role for ERV nucleic acids in triggering the autoimmune response in TREX1 knockout mice and in humans with AGS. TREX1 is one of six genes the mutation of which is associated with AGS; other genes include components of the endonuclease complex that target RNA : DNA hybrids (*RNASEH2A*, *-B* and *-C*), the adenosine deaminases acting on RNA (*ADAR*), the SAM domain- and HD domain-containing protein 1 and the cytosolic dsRNA receptor *MDA5* (Rice *et al.*, 2013, 2014). Along with TREX1, most of these genes encode enzymes that function in degrading or editing nucleic acids, minimizing the production of immunostimulatory nucleic acids including those produced following HERV expression. For example, SAMHD1 is a dNTPase that has been shown to restrict HIV-1 reverse transcription by depleting the pool of available dNTPs (St Gelais *et al.*, 2012; Sze *et al.*, 2013). A more recent paper suggests that SAMHD1 could specifically degrade ssRNA molecules, including HIV-1 transcripts (Ryoo *et al.*, 2014), although this finding remains to be confirmed by other studies. It is possible that this enzyme could limit the production of HERV transcripts, which might otherwise accumulate in cells. Much of the work on AGS has been done using gene-knockout mice. Further work to study this in primary human cells would be of interest, particularly for the purposes of identifying which HERVs, if any, are the source of immunostimulatory nucleic acids.

Consequences of innate immune activation by HERVs

One of the consequences of activation of the innate immune response is the production of inflammatory mediators, including type I IFNs, cytokines and chemokines. Ordinarily, the immune response is curtailed once the threat (e.g. virus infection) has been eliminated; if it is not resolved, chronic inflammatory diseases can arise (Turner *et al.*, 2014). Thus, activation of the innate immune response by HERVs could be an initiating event that could lead to chronic inflammation, and this is one way in which HERVs could contribute to autoinflammatory and autoimmune diseases. The chronic induction of type I IFNs in particular could have a pathological effect, given the potent biological activity of these molecules. For example, type I IFNs released from one cell could bind to the IFN receptor (IFNAR1/2) on the same and neighbouring cells, inducing signalling cascades leading to the induction of ISG expression. This could put the cells into an antiviral state, in which many cellular metabolic processes are shut down and which can ultimately lead to apoptosis (Zuniga *et al.*, 2007). Apoptotic cells are normally cleared by phagocytes to prevent an immune reaction to cellular contents; improper clearance of apoptotic cells and chronic disease states are two factors contributing to the onset of autoimmunity (Szondy *et al.*, 2014). Furthermore, type I IFNs have immunomodulatory roles that include activating natural killer cells and stimulating the clonal expansion of cytotoxic T-cells (Zuniga *et al.*, 2007), thereby linking an initial innate immune response to the HERVs to a cellular immune response. In addition, the detection of HERVs by PRRs could ultimately potentiate the production of autoantibodies. This has been shown for self nucleic acids that activate TLRs and promote the production of autoantibodies in systemic lupus erythematosus (Barrat *et al.*, 2005). Whether these self nucleic acids comprise or even include HERVs remains to be demonstrated, although work with MuERVs (Yoshinobu *et al.*, 2009) suggests it is possible.

Importantly, there is potential for the development of a positive-feedback loop by which the inflammation induced by HERVs could lead to increased HERV expression. Cellular transcription factors can bind to the LTRs of HERVs in order to regulate expression of the provirus. This is important given the alteration in transcription factor expression and activation in different disease states. A recent *in silico* study identified numerous transcription factor binding sites within the HERV-K 5′ LTR, including those for the pro-inflammatory transcription factor NF-κB and the IFN-stimulated regulatory element (Manghera & Douville, 2013). NF-κB is an important transcription factor in the inflammatory response; it is activated following signalling from the TLRs and it induces the expression of pro-inflammatory cytokines and type I IFNs. The presence of NF-κB-binding sites in HERV LTRs could thus result in increased expression during inflammation. Experimentally, the treatment of cells with LPS, the ligand for TLR4, or TNF-α increased the expression of HERV-K, HERV-H and HERV-W RNA (Johnston *et al.*, 2001). Both TLR4 and TNF-α receptor signalling can induce NF-κB, which could then bind to the response elements found in the HERV LTRs. This was demonstrated for HERV-W, wherein TNFα signalling resulted in NF-κB binding to the promoter and the induction of HERV-W (specifically, the Env protein syncytin-1) expression (Mameli *et al.*, 2007). This could lead to chronic activation of the innate immune response.

Alternatively, the expression of certain HERVs could result in immune suppression. This has been demonstrated for a couple of HERV Env proteins, HERV-FRD and HERV-H. The HERV-FRD Env protein (syncytin-2) is immunosuppressive, preventing a maternal immune response to the alloantigens of the fetus (Mangeney *et al.*, 2007). In addition, the HERV-H Env protein was also found to be immunosuppressive, preventing immune rejection of tumour cells implanted in mice (Mangeney *et al.*, 2001). The transmembrane domain of the Env protein of HIV-1 and of some endogenous retroviruses has an immunosuppressive domain (isu) that modulates cytokine production by PBMCs (Denner, 2014). Further to this, the HERV-K Env protein has been found to induce the production of IL-10 (Morozov *et al.*, 2013), which is an immunosuppressive cytokine. However, compared with other retroviruses, the HERV-K isu was relatively variable (Denner, 2014), and therefore it might not be as potent at immunosuppression. It has been proposed that immunosuppression by HERVs could contribute to immune evasion by cancer cells, permitting tumour growth (Kassiotis, 2014). Thus, the consequences of HERV expression could include promotion of tumorigenesis by immune suppression.

Epilogue

The products of HERV expression have the potential to interact with components of the innate immune response and to activate pro-inflammatory signalling pathways. There is direct evidence of an interaction between certain HERV proteins and TLRs, as well as indirect evidence that HERV nucleic acids could activate cytosolic PRRs. With respect to the latter, further studies into which PRRs are involved and which structures of HERV nucleic acids form the ligands for the PRRs would help to define the mechanism of HERV-induced immune activation. This activation could contribute to the inflammation observed in autoimmune and autoinflammatory diseases, as well as increasing HERV expression further by the binding of pro-inflammatory transcription factors (such as NF-κB) to the HERV LTRs. In addition, HERV expression could increase autoantibody formation via TLR activation. Alternatively, there is evidence that certain HERV Env proteins could be immunosuppressive, potentially impairing the immune response to exogenous pathogens and tumours. Many questions remain about the expression of HERVs in cells and whether they contribute to pathogenesis. An interaction with the immune response is one way in which HERVs could have pathogenic potential.

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