The replicative activity of human endogenous retrovirus K102 (HERV-K102) with HIV viremia

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Objective: To address the activation and replicative activity of HERV-K102 *in vivo* associated with HIV viremia.

Design and Methods: Initially serology was performed on HERV-K102 specific envelope peptides to determine if HERV-K102 may become activated with HIV viremia. Before developing a quantitative PCR (qPCR) assay, we first determined whether plasma associated particles contained DNA or RNA genomes in a pilot study which surprisingly revealed predominantly DNA genomes. A relative, ddCt qPCR ratio method was then devised to detect excess levels of HERV-K102 *pol* DNA templates over genomic levels which served as a surrogate marker to reliably index the level of particles found in plasma.

Results: Both the peptide serology and ddCt qPCR excess ratio methods suggested the activation of HERV-K102 in about 70–80% of HIV viremic cases whereas only 2–3% of normal healthy adults had marginally activated HERV-K102 ($P < 0.0001$). Moreover, by digestion with dUTPase we were able to confirm that the vast majority of excess DNA template in plasma related to cDNA production rather than representing genomic copies.

Conclusions: Our work uniquely suggests the common activation of HERV-K102 with HIV viremia and may be first to directly demonstrate HERV-K102 cDNA production *in vivo*. The potential implications of the induction of HERV-K102 activation and replication for the prevention and control of HIV are discussed.

AIDS 2007, **21**:2417–2424

Keywords: HERV-K102, HIV-1 host factors, plasma, qPCR, replication, serology

Introduction

Of human endogenous retroviruses (HERV), the HERV-K family is the most recent and the most biologically active [1–5]. Some HERV-K (HML-2) family members, such as HERV-K102 and K108, are unique to humans and continue to evolve [4,5]. Here, selection appears to be mediated through the envelope protein (Env) implying an infective process [4,5]. However, a fully infectious HERV has not been identified.

The HERV-K (HML-2) family is broadly divided into two types based on the absence (type I) or presence (type II) of a 292-bp insert at the *pol–env* boundary encoding *c-orf/Rec* [6,7]. It is often supposed that type I proviruses would be replication defective due to the lack

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Received: 5 March 2007; revised: 30 June 2007; accepted: 7 August 2007.

of *c-orf/Rec* domains. Remarkably, although the human specific HERV-K102 (type I) and HERV-K108 (type II) proviral genomes have stop codons [7], in quasispecies produced in association with various cancers, stop codons are commonly eliminated [8–10]. This might imply that HERV-K102 and K108 may be replication competent *in vivo*.

Particles relating to type II HERV-K (HML-2) proviral sequences have been identified in placenta [11], cancer cell lines [8,12,13–15] and/or have been artificially created [16,17]. Serological evidence has suggested temporal activation of HERV-K with germ cell tumors [18–20], in melanomas [12] and with HIV infection [21]. However the latter finding has been contested by others [18,20,22].

Type II HERV-K (HML-2) mRNA has been detected in peripheral blood mononuclear cell (PBMC) samples, placenta and other normal tissues [23–26], and may be enhanced with HIV infection in brain tissue [27] and possibly in PBMC [28,29]. In contrast, the available evidence suggests that the expression of HERV-K102 does not appear to be constitutive, but may be specifically induced in association with tumorigenesis [9,10,23] or activated in some placenta samples [9]. As the HERV-K PCR primers used for investigations appear unable to amplify HERV-K102, we questioned whether the discrepant results for HERV-K serology with HIV infection, might pertain in part to the activation of HERV-K102. Our goal then was to address if HERV-K102 activation and replication occurs with HIV viremia.

Methods

Sample collection

The Ottawa Hospital Research Ethics Board and Health Canada approved the collection of blood samples. These samples were part of an Archived Sample Bank which was initially set up for the development of screening methods for emerging bloodborne zoonotics and included collections from laboratory workers, farmers, normal healthy adults, as well as from a private infectious disease clinic. In addition, a collection of 22 plasma samples from the HIV clinic at the Ottawa Hospital were purposely collected for testing of HERV-K102 ddCt ratios in which CD4 counts, HIV plasma levels, and therapy status were also provided (reported in Table 3 and included in the results of Table 2 for the 37 HIV samples). Separate informed consent from the University of Manitoba to study potential HIV resistance factors was obtained by K. Fowke for the five HIV samples collected from Kenya, Africa (used in Table 2). We also routinely collected cord blood samples from the Ottawa Hospital under separate informed consent, which were primarily used for inducing HERV-K102.

Plasma (sodium citrate, siliconized tubes) and serum samples were aliquotted and stored at −80°C. Cord blood samples were collected in heparinized tubes and PBMC were isolated using Ficoll-Paque (GE Healthcare Bioscience Inc., Baie d'Urfe, Quebec, Canada) standard protocols. Cord blood samples were cultured at 5×10^5/ml in Iscove's Modified Dulbecco's media (IMDM) with 10% fetal calf serum (FCS) at 37°C for 7 days to induce HERV-K102 and the associated particles. Electron microscopy used standard fixation methods and was performed at the Children's Hospital of Eastern Ontario.

Peptide ELISAS

Two HERV-K102 Env peptides were selected first based on their relative antigenicity using a proprietary algorithm developed by Washington Biotechnology Inc. (Baltimore, Maryland, USA), and then selected further for their specificity for HERV-K (HML-2) type I family members (ML-4) or HERV-K102 (ML-5) based on their respective amino acid sequences (GenBank). The ML4 peptide has the amino acid sequence KRASTEMVTPVTWMDN (GenBank accession # AF164610) and is common to a number of type I HERV-K (HML-2) family members but not type II. The ML5 peptide is specific to HERV-K102, and has the sequence LETRDCKPFYTIDLNSS. Peptides and rabbit antisera made to the peptides were manufactured by Washington Biotechnology Inc. The peptide ELISA protocol followed the manufacture's instructions (Washington Biotechnology Inc.) except we used a horseradish peroxidase conjugated goat antihuman (heavy and light chain, IgG from Southern Biotech, Birmingham, Alabama, USA) diluted 1 : 2000 in 1% bovine serum albumin–phosphate buffered saline for screening human sera. Human sera were tested at a 1 : 150 dilution and a negative reference human serum was used to standardize background levels (typically around an optical density of 0.080). Thresholds for interpreting positive samples were set at three times the background rate (typically around an optical density of 0.240). In some cases positive reactions were verified by inhibition of the reactions in the presence of an excess of the appropriate peptide (100 μg/ml, data not shown). Screening was performed in duplicate and repeated three times, or performed in triplicate and repeated twice. The co-efficient of variation was generally less than 10%.

Particle isolation for pilot study

Particles were isolated from plasma with the QIAamp UltraSens Virus (Particle) Isolation Kit for DNA and RNA viruses according to manufacturer's instructions (Qiagen Inc., Mississauga, Ontario, Canada).

PCR and RT–PCR to determine type of genomes in isolated particles

For analysis of RNA, samples for RT–PCR were first digested with DNase according to the manufacturer's instructions (Promega, Madison, Wisconsin, USA). Reverse transcription using random hexamers followed

manufacturer's instructions (Promega) and was performed with and without murine leukemia virus (MuLV) reverse transcriptase. For DNA analysis, the reverse transcription and DNase treatment steps were omitted. For the β-actin PCR, 50 μl of PCR amplification reaction mix was made by combining 0.2 mM dNTP mixture, 1.5 mM $MgCl_2$, Taq PCR buffer, 0.1 μM β-actin primers (forward primer, 5′–TGACGGGGTCACCCACACTGTGCC CATCTA–3′; reverse primer, 5′–CTAGAAGCATTTG CGGTGGACGATG GAGGG–3′), 5 μl template (adjusted for concentration) and nuclease-free water. The reaction mixture was heated to 94°C for 5 min then placed on ice wherein 2.5 μl of a 1/10 dilution of AmpliTaq polymerase were added per tube. The cycling parameters were: 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min, for 35 cycles. The PCR product was 661 bp. For the HERV-K102 *pol* regular PCR, new primers were devised which amplified HERV-K102 *pol* which was validated by sequencing mRNA PCR products (data not shown). The primers used were: forward 5′–TGGCAGAGCAGGATTGTGAA–3′ and reverse 5′–CAGATGCTATTGCCAGTCCA–3′. The PCR product was 293 bp in length. Fifty μl of PCR amplification reaction mix was made by combining 50 pmol of forward and reverse primers, 50 μM dNTPs, 1.4 U/50 μl total reaction buffer AmpliTaq Gold polymerase, 2 mM $MgCl_2$, PCR buffer II, 5% dimethylsulfoxide, 5 μl template (same concentration as for the β-actin) and nuclease-free water. The AmpliTaq Gold polymerase was activated 12 min at 95°C. The cycling parameters were denaturation for 30 s at 95°C, annealing for 30 s at 50°C, strand extension for 30 s at 72°C, and the number of cycles totalled 35.

HERV-K102 *pol* ddCt ratios

DNA was extracted from 200 μl of plasma with the Qiagen Mini DNA extraction kit according to manufacturer's instructions. Real time quantitative PCR (qPCR) reactions employed the Applied Biosystems 7300 Real Time PCR system using standardized conditions and buffers for all PCR reactions according to the manufacturer's instructions, except Universal Master Mix without Amperase-UNG^{TM} was typically used. Novel primer sets and probe were designed by us and custom manufactured by Applied Biosystems, Inc. (Streetsville, Ontario, Canada) (forward primer, 5′–TCTTCAACCAGTTAGAGAAA AGTTTTCA–3′; reverse primer, 5′–TGGCAACCTCT GCTTGCA–3′; TaqMan probe: 6Fam-5′–gcagcaca-taaaatatcatcaat–3′). We used the 18S RNA kit from Applied Biosystems Inc. to control for genomic equivalents. For an internal reference standard, 25 pg per reaction mixture of a normal (male) DNA was used (Applied Biosystems Inc.). Testing was performed in triplicate on the 96-well plates. The ddCt ratio real time PCR method (relative quantitation) takes the difference between HERV-K102 *pol* and 18S RNA Ct (delta Ct), and subtracts this from the same performed on the internal standard DNA (the delta delta Ct). Then we used the algorithm $2^{-(ddCt)}$ to calculate the relative ratio of HERV-K102 to genomic DNA. The co-efficient of variation was $< 1\%$ and often $< 0.1\%$. In order to confirm the sequences were cDNA and nongenomic, samples showing positive excess HERV-K102 ratios were then subject to re-amplification in master mix containing Amperase-UNG^{TM} an enzyme (dUTPase) which cleaves DNA containing dUTP. While genomic DNA contains little or no dUTP, retroviral particles can contain significant amounts of dUTP depending on the cell type in which the viral RNA is reversed transcribed into cDNA [30,31]. Thirty normal healthy control samples all which had been tested to be negative by serology (Table 1) and negative for particles (Fig. 2) were used to set the threshold for the ddCt ratio of HERV-K102 DNA (template copy number) per genome.

Table 1. Serology for antibodies to HERV-K102 envelope peptides by ELISA.

Serum sample cohort	ML4 (% positive)	ML5 (% positive)
Normal controls	1/51 (2%)[a]	1/51 (2%)[b]
HIV viremia	8/10 (80%)*	7/10 (70%)*
Herpes viremia	3/17 (18%)	3/17 (18%)

[a]Marginal reactivity to ML4 in an apparently healthy farm worker.
[b]Marginal reactivity to ML5 in an apparently healthy laboratory worker.
*$P < 0.0001$ Fisher exact test when compared to normal controls.

Statistical analysis

The Fisher exact test, a nonparametric method of proportions was used for statistical analysis using the SAS software (version 8).

Results

Initial serological evidence for HERV-K102 activation with HIV

In our analysis which was purposefully limited to type I (ML4 peptide) or HERV-K102 specific epitopes (ML5 peptide), only 2% of normal healthy controls were judged to be marginally positive for antibodies to HERV-K102 Env peptides ($n = 51$) by ELISA for either peptide (Table 1). However, eight of 10 HIV-1 viremic patients scored positive on the ML4 peptide and seven of 10 on the ML5 peptide ($P < 0.0001$). For comparison, only three of 17 herpes viremic samples (17.6%) showed positive reactions with either peptide. This initial result suggested that there may be activation involving envelope expression of HERV-K102 in 70 to 80% of patients with HIV viremia.

Pilot study to explore genome types in plasma associated particles

In order to develop a qPCR method to detect HERV-K102 particle associated genomes, it was imperative to determine whether these were predominately DNA or RNA. This is because nonpathogenic retroviruses, like

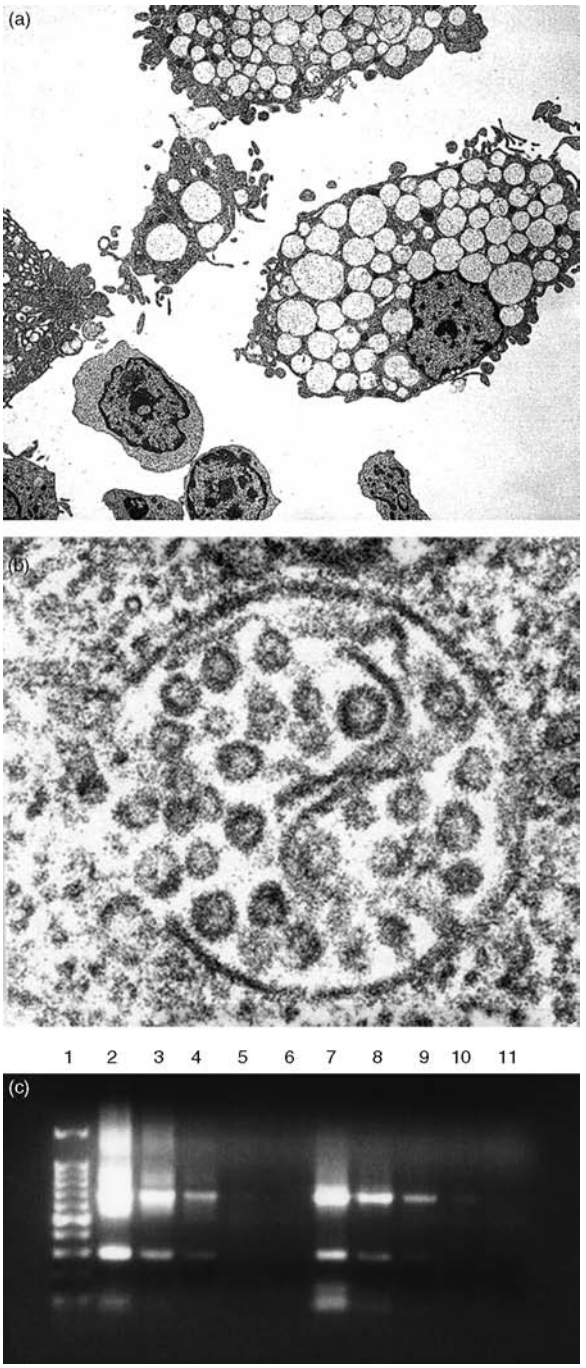

Fig. 1. Relative sensitivities of HERV-K102 versus β-actin PCR on cultured CB. HERV-K102 mRNA, cDNA and envelope protein are induced in CB cells by culture in IMDM media (data not shown, see below for mRNA) and correlates with the formation of immature particles as established in a time course assay and with blocking experiments (Laderoute *et al.* unpublished work). HERV-K102 activation in CB cells was induced in order to allow a comparison of the relative sensitivities of the PCR and RT–PCR reactions for β-actin verses HERV-K102 *pol* for the pilot study on genomes associated with putative plasma particles. This then allowed for the interpretation of the results in Fig. 2 on putative particles isolated from plasma.

the foamy retroviruses (FV) are instead, DNA [32–34]. As a source of mRNA for positive controls for our PCR, we cultured cord blood cells under specific conditions to induce HERV-K102 *pol* and envelope associated particles (Fig. 1 and data not shown). After determining that the β-actin method was twice as sensitive as the new HERV-K102 *pol* method for both PCR (DNA) and RT–PCR (mRNA) (see Fig. 1c), we then proceeded with purifying particles from plasma of individuals suspected of having HERV-K activation verses 30 normal adult healthy controls.

We chose cord blood (CB) and samples related to Epstein–Barr virus (EBV) activation as HERV-K102 induction had been shown to be associated with placenta [9] and HERV-K was known to be induced with diseases involving EBV [1,2,35,36]. With the regular PCR methods, no products were observed from plasma samples of 30 normal healthy adults (data not shown) suggesting that HERV-K102 associated particles do not circulate in normal healthy adults. Subsequently, as a method control, we spiked 1×10^5 peripheral blood mononuclear cells (PBMC) into 1 ml of plasma and then attempted to isolate particles (Fig. 2, lane 1). As can be seen in lane 1, while DNA products were identified for HERV-K102 *pol* and for β-actin, no cellular mRNA was isolated with the virus isolation kit, as expected. In contrast as shown in Fig. 2, lanes 2 to 7, selected samples suspected of being linked to EBV activity: acute EBV infection (lane 3), multiple sclerosis (MS, lanes 4 and 5), and a chronic fatigue syndrome (CFS) case (lane 2) all had HERV-K102 DNA. Also as expected, we did find HERV-K102 DNA in 2 CB plasma samples (lanes 6 and 7) but not in two other CB samples (data not shown) since HERV-K102 transcripts and/or associated particles may be produced in the placenta [9]. Interestingly RNA was also detectable in half of these samples which had HERV-K102 DNA (lanes 3–5). This indicated DNA would be a better

Fig. 1. (*Continued*).
(a) Electron micrograph showing extensive vacuolation in human CB cells cultured for 11 days (× 1500). (b) Higher magnification of electron micrograph, showing uniform, immature particles localize within the vacuoles of day 11 cultured CB where particles average about 100 nm (× 100 000). Cell surface budding was not found (data not shown). (c) Comparison of the relative sensitivities of the PCR for HERV-K102 *pol* versus β-actin on CB cells cultured for 6 days and extracted with Tri-reagent by titrating the amount of templates used. Amplified PCR products from both the β-actin (661 bp) and HERV-K (293 bp) PCR were loaded into each lane to facilitate comparison. Lane 1, 100 bp molecular weight standards. DNA templates were used neat (lane 2), or diluted 1/3 (lane 3), 1/10 (lane 4), 1/30 (lane 5), or 1/100 (lane 6). RNA templates were used neat (lane 7), or diluted 1/3 (lane 8), 1/10 (lane 9), 1/30 (lane 10), or 1/100 (lane 11). The results show the β-actin PCR was on average twice as sensitive when compared to the HERV-K102 *pol* PCR.

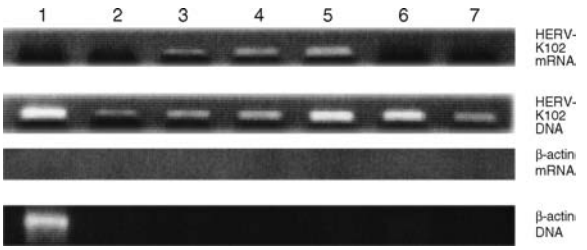

Fig. 2. Pilot study for the verification of type of proviral HERV-K102 *pol* containing genomes by PCR and RT–PCR in putative particles isolated from plasma samples. Particles were isolated from plasma with the virus isolation kit and then subject to PCR or to RT–PCR. HERV-K102 mRNA is shown in top panel, HERV-K102 DNA in the second from the top panel; β-actin mRNA in the third panel and β-actin DNA in the bottom panel. Lane 1 (positive control for methodology): 0.5×10^6 freshly isolated (uninduced) PBMC spiked into a negative control plasma. Lane 2: CFS patient off St. John's wort therapy for 84 h. Lane 3: severe, acute case of EBV infection. Lane 4: a MS patient sample at initial diagnosis. lane 4: the same patient as Lane 4 but at the time of progression. Lane 6: plasma from a cord blood. Lane 7: plasma from a cord blood. Two other cord blood samples and 30/30 normal healthy control plasma samples were found to be negative (data not shown). Samples from the same MS patient shown in lanes 4 and 5 while in remission on β-interferon therapy were negative as were various samples taken from the CFS patient while on St. John's wort therapy for insomnia.

substrate for the development of a qPCR method. Finally, prospective samples of the CFS or MS patient indicated the presence of particles when off therapy (lanes 2, 4, 5) but not when on therapy (data not shown), implying a correlation of particle production with disease symptoms (CFS) or activity (MS).

Establishment of the HERV-K102 ddCt ratio method and its validation

Since qPCR is significantly more sensitive than regular PCR, we exploited the fact that all plasma samples contain residual contaminating cellular debris. Accordingly we designed a novel ddCt relative qPCR method to provide a ratio of HERV-K102 *pol* DNA to the levels of genomic DNA present, the latter as indexed by 18S RNA. By analysing 30 samples from normal healthy controls (negative for serology and for demonstrable particles), we determined that on average the normal human genome contains 0.88 ± 0.37 (near 1 : 1) gene copies of HERV-K102 to 18S RNA ($n = 30$). From this a cutoff threshold ratio of 1.60 was arbitrarily set at two standard deviations above the mean. Under this setting, only one of the 30 samples from normal healthy controls was scored as being positive (3.3%) having a marginally increased ratio of 1.74 (Table 2). This proportion of positives was similar to that obtained through the specific HERV-K102 Env peptide serology at 2% (Table 1) which provided validation of the new qPCR method. Further validation was obtained by testing plasma samples which we knew from the pilot study had or did not have particles (data not shown) and by examining the increase in gene copy number associated with particle production *in vitro* (data not shown).

Table 2. Testing for particle-associated HERV-K102 *pol* cDNA templates in plasma samples by the ddCt ratio qPCR method.

Cohort	% Positive Ratios (positive/total)	Range
Normal[a]	3.3% (1/30)	0.41 to 1.74
Hepatitis B and C viremia	78.6% (22/28)*	0.81 to 4.32×10^9
Herpes viremia	61.9% (13/21)*	0.24 to 2.02×10^9
HIV viremia	75.7% (28/37)*	0.49 to 121.9

[a]The HERV-K102 *pol* to 18 sRNA ddCt qPCR ratio for the 30 samples from serologically negative, normal healthy controls was 0.88 ± 0.37.
*$P < 0.0001$ Fisher exact test when compared to normal by nonparametric proportions.

Testing for excess HERV-K102 DNA templates in plasma

We were curious as to whether there would be differences in the level or incidence of HERV-K102 activation with other bloodborne pathogens when compared to HIV. As shown in Table 2, HERV-K102 activation was found associated with other types of bloodborne pathogens as well as for HIV. In this study, 22 of 28 hepatitis samples were judged to be positive (78.6%) in which 14 of the 22 positives showed excess ratios at 10^8 or 10^9 over controls (data not shown). Of 14 positive hepatitis samples retested in the presence of UNG (dUTPase), all but two samples reverted to normal ratios (data not shown) indicating that most of the excess templates in plasma related to HERV-K102 *pol* encoding cDNA. Similarly for herpes viremic samples (which involved cytomegalovirus, EBV and human herpes-7 cases), 13 of 21 plasma samples were found to be positive by qPCR (61.9%), but here only four of the 13 had excess ratios in the 10^7 to 10^9 range (data not shown). On the other hand, 28/37 of HIV viremic samples were found to be positive (75.7%) for excess HERV-K102 *pol* DNA templates (above 1.60), but the range for the ratios was notably lower than that found for other bloodborne pathogens (ratio range for HIV samples, 0.49–121.9). The proportion of positive samples associated with HIV viremia or with other bloodborne pathogens (Table 2), was statistically significant when compared to normals ($P < 0.0001$). In addition, the incidence for activation of HERV-K102 in HIV viremic samples, corroborated what had been obtained earlier by serology (70–80%) further substantiating that HERV-K102 is commonly activated with HIV viremia.

Evidence for cDNA production with HIV infection

In a special cohort involving 22 HIV viremic cases, the CD4 cell counts, HIV viral loads and therapy status were known. As shown in Table 3, 16 of 22 samples in this cohort (72.7%) met the criteria of having ratios greater than 1.60, while six were judged to be negative. For the 16 samples scoring positive, UNG treatment reverted all

Table 3. HERV-K102 ddCt ratios with and without UNG treatment in a special cohort of 22 HIV viremic patients.

HERV-K102 ddCt ratios	UNG digested ddCt ratios	CD4 counts	HIV viral load (copies/ml)	Therapy status
121.9	1.04	613	24 843	None
60.5	0.60	NA	>500 000	NA
27.1	0.18	NA	17 613	None
23.1	1.27	474	22 142	None
13.6	0.36	210	30 898	C, R, At
12.8	0.47	341	14 211	None
9.90	0.89	180	>500 000	Ab, At, L
7.64	1.51	13	332 420	None
4.38	1.11	NA	2512	NA
4.26	2.36	NA	37 118	None
2.77	1.00	610	343 843	None
2.77	1.92	423	385 185	None
2.50	0.69	476	38 171	None
2.49	1.30	7	>500 000	None
2.08	0.84	34	304 581	R, C, At
1.96	1.53	NA	1020	NA
1.45	NA	120	217 125	Ab, L, K
1.34	NA	455	1240	Ab, E, C
0.83	NA	221	1410	C, At
0.81	NA	52	2664	Te, R, Tr
0.67	NA	1010	27 717	None
0.49	NA	378	919	C, K

NA, Not applicable or not available; C, combivir; R, ritonavir; At, atazanavir; Ab, abacavir; L, lamivudine; K, kaletra; E, efavirenz; Te, tenofovir; Tr, trepanovir.

but two samples (12.5%) to normal ratios, indicating that the majority of transcripts were cDNA. Of the six samples judged to be negative for HERV-K102 activation, five were found to be on antiviral therapy, of which four of five would be considered to have HIV viral loads under control. As it is known that protease inhibitors which have shown efficacy in clinical trials generally do not block HERV-K10 protease [37] and others have reported HERV-K activation despite HAART [28,29] it is unlikely that the negative ratios observed here for HERV-K102 relate to the use of antiviral HAART. The finding of positive HERV-K102 ddCt ratios in three resistant patients on anti-HIV therapy further corroborates this notion. The two of six samples negative for HERV-K102 cDNA but with high HIV viral loads (one on therapy and one not) are of unknown significance.

We found four of the five treatment naïve African HIV samples to have activated HERV-K102 (data not shown) which is similar in incidence to what we found on our North American samples.

Discussion

We are first to identify the specific activation of HERV-K102 commonly with HIV viremia. This was achieved initially by peptide serology and was confirmed by qPCR methods. Moreover we have provided evidence that activation involves the replication of cDNA genomes *in vivo*, suggesting that HERV-K102 quasispecies production as reported for breast cancers, probably relates to its replication *in vivo* [9,10]. Newer evidence now suggests the potential up-regulation of HERV-K transcripts associated with HIV infection both *in vivo* [28,29] and *in vitro* [38]. Although HERV-K102 was not specifically tested, these findings raise the possibility that HERV-K102 induction may also be in response to HIV infection.

Serological investigations have indicated that HERV-K antibody production is temporally regulated in that they disappear when tumors are excised, or are regained with tumor relapse [18,19]. This suggests that HERV-K antibody production may be an innate clearance mechanism by the host. Whether HERV-K102 antigens can be found at the cell surface of virally or tumor transformed cells, or whether HERV-K102 antibodies in fact contribute to CD4 loss or other HIV associated pathology [39], clearly needs further investigation.

An unanticipated finding of the present work was the discovery of predominately DNA genomes in purified putative particles from plasma. This was not totally unexpected as nonpathogenic retroviruses, the FV, have infectious genomes that are DNA rather than RNA [32–34,40]. The finding of HERV-K102 cDNA in plasma indicates that the lifecycle of HERV-K102 is most probably reversed when compared to HIV but is similar to that of FV. For the latter, reverse transcription occurs around the time of release from cells rather than soon after viral entry into cells. Interestingly, FV also lack the *c-orf*/Rec like domains in their envelopes, yet are fully infectious [32–34]. Thus, that HERV-K102 also lacks this domain, does not preclude replicative activity of the type I HERV-K (HML-2) family members, as is often supposed. The particles associated *in vitro* with HERV-K102 activation in cultured cord blood cells appear to be

distinct from those ascribed to type II HERV-K (HML-2) artificially created viruses [16,17] due to budding into the endoplasmic reticulum rather than through the cell surface membrane. Interestingly, the associated vacuolation and lack of cell surface budding found for HERV-K102 associated particles is reminiscent of the prototypic foamy virus (PFV) [32–34]. Thus, HERV-K102 may uniquely share some salient properties with PFV. The significance of this remains to be established, however as HERV-K102 is not genetically similar to PFV or to other known FV.

Of interest is the finding that HERV-K protease cleaves HIV Gag in the wrong places leading to reduced infectivity of released HIV particles [41]. Conversely, HIV protease may also cleave HERV-K Gag in the wrong places [42] suggesting that mutual antagonism exists at the molecular level. It is tempting to speculate that the relatively low plasma HERV-K102 ddCt ratios found with HIV viremia, when compared to other bloodborne pathogens, might reflect this mutual molecular antagonism. This antagonism along with our work raises the notion that humans may mount a defence strategy against HIV involving a viral antiviral attack. Clearly this new hypothesis needs experimental validation along with an evaluation of the role of HERV-K102 activation in HIV pathogenesis.

In summary, our work is first to suggest a provirus exclusive to humans, HERV-K102, may be induced and may replicate in association with HIV infection potentially as a novel host protective mechanism. It remains to be seen whether exploiting this provirus directly for the prevention and control of HIV infection or indirectly as a gene therapy vector for the newer 'intracellular immunization' approaches to HIV vaccines, will assist in extinguishing the HIV pandemic.

Sponsorship

Supported through operational funds for the Blood Zoonotics Unit through the blood safety program, and in part, by an Innovative Science Grant from the Office of the Chief Scientist at Health Canada.

References

1. Bannert N, Kurth R. **Retroelements and the human genome: new perspectives on an old relation.** *Proc Natl Acad Med USA* 2004; **101 (Suppl 2)**:14572–14579.
2. Christensen T. **Association of human endogenous retroviruses with multiple sclerosis and possible interactions with herpes viruses.** *Rev Med Virol* 2005; **5**:179–211.
3. Benit L, Dessen P, Heidmann T. **Identification, phylogeny and evolution of retroviral elements based on their envelope genes.** *J Virol* 2001; **75**:11709–11719.
4. Belshaw R, Pereira V, Katzourakis A, Talbot G, Paces J, Burt A, *et al*. **Long term reinfection of the human genome by endogenous retroviruses.** *Proc Natl Acad Sci USA* 2004; **101**:4894–4899.
5. Belshaw R, Dawson ALA, Woolven-Allen J, Redding J, Burt A, Tristem M. **Genomewide screening reveals high levels of insertional polymorphism in the human endogenous retrovirus family HERV-K (HML2): implications for present day activity.** *J Virol* 2005; **79**:12507–12514.
6. Ono M. **Molecular cloning and long terminal repeat sequences of human endogenous retrovirus genes related to types A and B retrovirus genes.** *J Virol* 1986; **58**:937–944.
7. Barbulescu M, Turner G, Seaman MI, Dienard AS, Kidd KK, Lenz J. **Many human endogenous retrovirus K (HERV-K) proviruses are unique to humans.** *Curr Biol* 1999; **9**:861–868.
8. Muster T, Waltenberger A, Grassauer A, Hirschi S, Caucig P, Romirer I, *et al*. **An endogenous retrovirus derived from human melanoma cells.** *Cancer Res* 2003; **63**:8735–8741.
9. Wang-Johanning F, Frost AR, Johanning GL, Khazaeli MB, LoBuglio AF, Shaw DR, *et al*. **Expression of human endogenous retrovirus K envelope transcripts in human breast cancer.** *Clin Cancer Res* 2001; **7**:1553–1560.
10. Wang-Johanning F, Frost AR, Jian B, Epp L, Lu DW, Johanning GL. **Quantitation of HERV-K env gene expression and splicing in human breast cancer.** *Oncogene* 2003; **22**:528–535.
11. Simpson GR, Patience C, Lower R, Tonjes RR, Moore HD, Weiss RA, *et al*. **Endogenous D-Type (HERV-K) related sequences are packaged into retroviral particles in the placenta and possess open reading frames for reverse transcription.** *Virology* 1996; **222**:451–456.
12. Buscher K, Trefzer U, Hofmann M, Sterry W, Kurth R, Denner J. **Expression of human endogenous retrovirus K in melanomas and melanoma cell lines.** *Cancer Res* 2005; **65**:4172–4180.
13. Lower R, Boller K, Hasenmaier B, Korbmacher C, Muller-Lantzsch N, Lower J, *et al*. **Identification of human endogenous retroviruses with complex mRNA expression and particle formation.** *Proc Natl Acad Sci USA* 1993; **90**:4480–4484.
14. Bieda K, Hoffmann A, Boller K. **Phenotypic heterogeneity of human endogenous retrovirus particles produced by teratocarcinoma cell lines.** *J Gen Virol* 2001; **82**:591–596.
15. Patience C, Simpson GR, Colletta AA, Welch HM, Weiss RA, Boyd MT. **Human endogenous retrovirus expression and reverse transcriptase activity in the T47D mammary carcinoma cell line.** *J Virol* 1996; **70**:2654–2657.
16. Tonjes RR, Boller K, Limbach C, Lugert R, Kurth R. **Characterization of human endogenous retrovirus type K virus-like particles generated from recombinant baculoviruses.** *Virology* 1997; **222**:451–456.
17. Dewannieux M, Harper F, Richaud A. **Identification of an infectious progenitor for the multiple-copy HERV-K human endogenous retroelements.** *Genome Res* 2006; **16**:1548–1556.
18. Boller K, Janssen O, Schuldes H, Tonjes RR, Kurth R. **Characterization of the antibody response specific for the human endogenous retrovirus HTDV/HERV-K.** *J Virol* 1997; **71**:4581–4588.
19. Kleiman A, Senyuta N, Tryakin A, Sauter M, Karseladze A, Tjulandin S, *et al*. **HERV-K (HML-2) Gag/Env antibodies as indicator for therapy effect in patients with germ cell tumors.** *Int J Cancer* 2004; **110**:459–461.
20. Goedert JJ, Sauter ME, Jacobson LP, Vessella RL, Hilgartner MW, Leitman SF, *et al*. **High prevalence of antibodies against HERV-K10 in patients with testicular cancer but not with AIDS.** *Cancer Epidemiol Biomarkers Prevent* 1999; **8**:293–296.
21. Lower R, Lower J, Kurth R. **The viruses in all of us: characteristics and biological significance of human endogenous retroviral sequences.** *Proc Natl Acad Sci USA* 1996; **93**:5177–5184.
22. Vogetseder W, Dumtahrt A, Mayersbach P, Schonitzer D, Dierich MP. **Antibodies in human sera recognizing a recombinant outer membrane protein encoded by the envelope gene of the human endogenous retrovirus K.** *AIDS Res Hum Retroviruses* 1993; **9**:687–694.
23. Armbruester V, Sauter M, Krautkraemer E, Meese E, Kleiman A, Best B, *et al*. **A novel gene from the human endogenous retrovirus K expressed in transformed cells.** *Clinical Cancer Res* 2002; **8**:1800–1807.
24. Sugimoto J, Matsuura N, Kinjo Y, Takasu N, Oda T, Jinno Y. **Transcriptionally active HERV-K genes: identification, isolation and chromosomal mapping.** *Genomics* 2001; **72**:137–144.

25. Stauffer Y, Theiler G, Sperisen P, Lebedev Y, Jongeneel CV. **Digital expression profiles of human endogenous retroviral families in normal and cancerous tissues.** *Cancer Immunol* 2004; **4**:2–19.
26. Seifarth W, Frank O, Zeilfelder U, Spiess B, Greenwood AD, Hehlmann R, *et al*. **Comprehensive analysis of human endogenous retrovirus transcriptional activity in human tissues with a retrovirus-specific microarray.** *J Virol* 2005; **79**:341–352.
27. Johnston JB, Siva C, Holden J, Warren KG, Clark AW, Power C. **Monocyte activation and differentiation augment human endogenous retrovirus expression: implications for inflammatory brain diseases.** *Ann Neurol* 2001; **50**:434–442.
28. Contreras-Galindo R, Gonzalez M, Almodovar-Camacho S, Gonzalez-Ramirez S, Lorenzo E, Yamamura Y. **A new Real-Time-RT-PCR for quantitation of human endogenous retroviruses type K (HERV-K) RNA load in plasma samples: increased HERV-K RNA titers in HIV-1 patients with HAART nonsuppressive regimens.** *J Virol Meth* 2006; **136**:51–57.
29. Contreras-Galindo R, Kaplan MH, Markovitz DM, Lorenzo E, Yamamura Y. **Detection of HERV-K (HML-2) viral RNA in plasma of HIV Type 1-infected individuals.** *AIDS Res Hum Retroviruses* 2006; **22**:979–984.
30. Chen R, Wang H, Mansky LM. **Roles of uracil-DNA glycosylase and dUTPase in virus replication.** *J Gen Virol* 2002; **83**:2339–2345.
31. Priet S, Sire J, Querat G. **Uracils as a cellular weapon against viruses and mechanisms of viral escape.** *Curr HIV Res* 2006; **4**:31–42.
32. Yu SF, Sullivan MD, Linial ML. **Evidence that the human foamy virus genome is DNA.** *J Virol* 1999; **73**:1565–1572.
33. Linial ML. **Foamy viruses are unconventional retroviruses.** *J Virol* 1999; **73**:1747–1755.
34. Delelis O, Lehmann-Che J, Saib A. **Foamy viruses – a world apart.** *Curr Opin Microbiol* 2004; **7**:400–406.
35. Sutkowski N, Chen G, Calderon G, Huber BT. **Epstein-Barr virus latent membrane protein LMP-2A is sufficient for transactivation of the human endogenous retrovirus HERV-K18 superantigen.** *J Virol* 2004; **78**:7852–7260.
36. Lerner AM, Beqaj SH, Deeter RG, Fitzgerald JT. **IgM serum antibodies to Epstein-Barr virus are uniquely present in a subset of patients with the chronic fatigue syndrome.** *In Vivo* 2004; **18**:101–106.
37. Towler EM, Gulnik SV, Bhat TN, Xie D, Gustschina E, Sumpter TR, *et al*. **Functional characterization of the protease of human endogenous retrovirus, K10: can it complement HIV-1 protease?** *Biochemistry* 1998; **37**:17137–17144.
38. Contreras-Galindo R, Lopez P, Velez R, Yamamura Y. **HIV-1 infection increases the expression of human endogenous retroviruses type K (HERV-K) *in vitro*.** *AIDS Res Hum Retroviruses* 2007; **23**:116–122.
39. Mehandru S, Poles MA, Tenner-Racz K, Manuelli V, Jean-Pierre P, Lopez P, *et al*. **Mechanisms of gastrointestinal CD4+ T-cell depletion during acute and early human immunodeficiency virus type I infection.** *J Virol* 2007; **81**:599–612.
40. Brooks JI, Merks HW, Fournier J, Boneva RS, Sandstrom PA. **Characterization of blood-borne transmission of simian foamy virus.** *Transfusion* 2007; **47**:162–170.
41. Padow M, Lai L, Fisher RJ, Zhou YC, Wu X, Kappes JC, *et al*. **Analysis of human immunodeficiency virus type I containing HERV-K protease.** *AIDS Res Human Retroviruses* 2000; **16**: 1973–1980.
42. Kuhelj R, Rizzo CJ, Chang CH, Jadhav PK, Towler EM, Korant BD. **Inhibition of human endogenous retrovirus-K10 protease in cell-free and cell-based assays.** *J Biol Chem* 2001; **276**: 16674–16682.