SUPPLEMENTARY MATERIAL

Further Evidence that Human Endogenous Retrovirus K102 is a Replication Competent Foamy Virus that may Antagonize HIV-1 Replication

Marian P. Laderoute[#,1,4], Louise J. Larocque[1,$], Antonio Giulivi[2,4] and Francisco Diaz-Mitoma[3,4]

[1]*Bloodborne Pathogens Division, Blood Zoonotics Unit, Public Health Agency of Canada, Ottawa, Ontario Canada, and* [#]*Recently Retired from Immune System Management Clinic and Lab, Ottawa, Ontario Canada;* [$]*Presently at the Centre for Biologics Evaluation, Health Canada, Ottawa, Ontario Canada*

[2]*Division of Hematopathology and Transfusion Medicine, The Ottawa Hospital, Ottawa, Ontario Canada*

[3]*The Advanced Medical Research Institute of Canada, Sudbury, Ontario Canada*

[4]*Department of Pathology and Laboratory Medicine, The University of Ottawa, Ottawa, Ontario Canada*

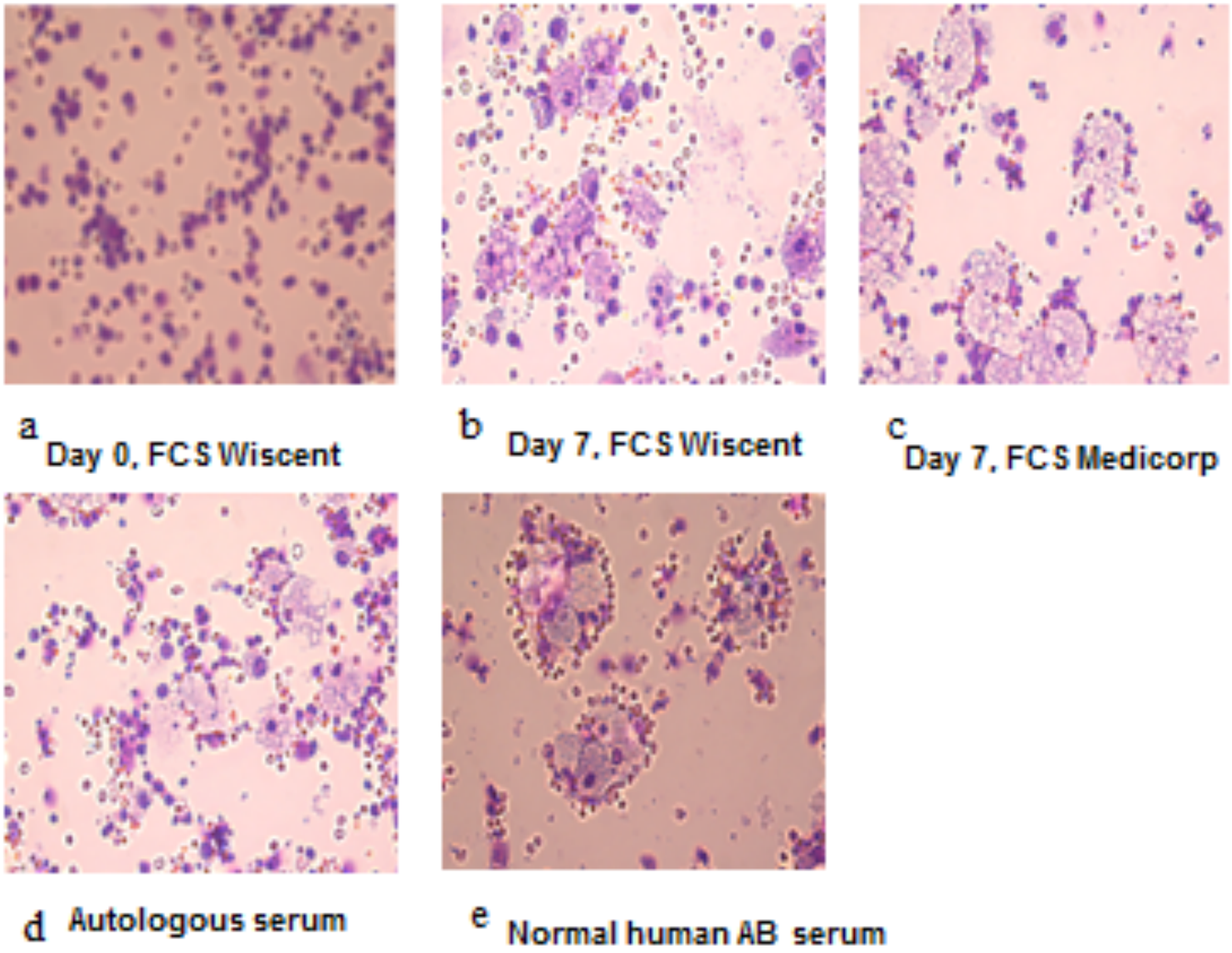

Fig. (S1). Vacuolation does not depend upon source of serum used for culture. Cytospins stained with H & E of uncultured CB day 0 (**a**) or cultured for 7 days in different sources of serum in IMDM as noted above **b**) Wiscent FCS **c**) Medicorp FCS, **d**) autologous serum **e**) normal human AB serum. The results imply the foamy retrovirus was not derived from the FCS used, but was endogenous as all supported vacuolation in cells.

Supplementary Table S1. Comparison of Features of Prototypic Foamy Virus[#] to Type 1 HERV-K (HML-2) HERV-K102[++].

	Property	Prototype Foamy Virus[#]	HERV-K102[++]
1	Causes vacuolation in cultured human mononuclear cells *in vitro*	YES **(hallmark)** [4]	YES *
2	Particles predominately bud into vacuoles rather than from cell surface membrane for cultured human PBMCs	YES (**hallmark**) [4]	YES * [5]
3	Immature particles (no electron dense core)	YES **(hallmark)** [1-3]	YES *
4	Abundant intracellular particles	YES **(hallmark)** [1-3]	YES *
5	Can cause lytic infection in some human fibroblast cell lines *in vitro*	YES **(hallmark)** [1, 2, 8, 9]	YES *(MRC-5 but not HFL-1 cells nor Vero cells, see* Laderoute MP *et al*, Patent CA2673395, 2006*)*
6	Induces lysis in HIV-1 or HTLV-1 infected PBMCs	YES [4]	Unknown
7	Extracellular particles contain DNA and RNA genomes	YES **(hallmark)** [1-3]	YES [5]
8	Non-pathogenic	YES **(hallmark)** [1-3]	YES (HERV-K102 is not known to cause disease, particles are found in normal cord blood [5] and as shown here, appears to be induced in monocytes as part of innate immunity, also lacks CKS17 immunosuppressive motif in TM of Env, see below)
9	Lacks the REC/REV/REX Domain in *env*	Yes **(hallmark)** [3]	YES (HERV-K102 which is a **Type 1 HERV-K (HML-2) lacks this domain** but Type 2 have this domain called 'REC, cORF, or K-Rev, [10-12])
10	5'LTR proviral genome begins with "TGTG" (evidence of asymmetric integration process)	YES **(hallmark)** [13]	YES (**distinguishes** HERV-K (HML-2) from all other HERVs but is shared with HERV-L, the latter which has some homology to foamy viruses but no *env* and thus is non-functional, [14])
11	Lacks the CWLC (CXXC) motif in the surface unit of Env	YES (**distinguishes** from most retroviruses, [15])	YES (**distinguishes** HERV-K HML-2 from all other HERVs, [16, 34])
12	Capable of intracellular retrotransposition	Yes **(hallmark)** [see 2, 17]	Unknown
13	Infectious particles contain DNA genomes	YES **(hallmark)** for review, see [16, 18]	YES, DNA containing HERV-K HML-2 virions are infectious and replication competent [37]
14	Env is required for particle formation	YES (**distinguishes** from most retroviruses, [1])	Unknown but processed Env detected with particles*
15	Env must be processed/cleaved for infectivity	YES [19]	N/D a*ppears to be cleaved when isolated from induced CB cells**
16	Env can substitute for orthoretrovirus Env in *trans*	NO [20]	NO, HERV-K102 Env does not substitute HIV-1 VLPs [38]
17	Uses lysine as tRNA for priming reverse transcription	YES	YES
18	"Complex" retrovirus	YES	YES [7]
19	Temporal transcription regulation: first uses internal promoter 3 ' to *env*, then LTR for full length transcripts	YES (**distinguishes** from most retroviruses, [1])	N/D

20	3' polypurine tract (PPT) conservation of D element	YES, "agg aga ggg" (3' to pol gene, [21])	Partial, has almost identical sequence "gg aga ggg" in reverse orientation at 3' polypurine tract (8943-8936) and one copy at end of *pol* (4203-4196)
21	Sequence: clustering away from most retroviruses using *env* or *pol*	YES (**distinguishes** spuma viruses, infectious HIV/HTLV and HERV-K HML-2 from all other retroviruses; *env* and *pol* analyses, see [15, 22, 23])	YES (**distinguishes** HERV-K HML-2 from all the other HERVs, [15, 22-24])
22	Lacks the "CKS17" immunosuppressive peptide in the TM region of Env which is common to most pathogenic retroviruses except HIV	YES (**distinguishes** from most retroviruses, see [15])	YES [15]
23	Nuclear staining of Gag	YES (**hallmark and diagnostic for foamy retroviruses,** [1])	N/D
24	Lacks the major homology region (MHR) in the capsid (Gag) $QX_3EX_4(F/Y)X_2R$ motif used for particle assembly/egress [25]	YES (**distinguishes** from most retroviruses, [1])	Partial (Both HERV-K102 and K108 have QxxxE at aa 124 and 128 in Gag but **not the rest of the motif**)
25	Lacks the Cys-His boxes in the nucleocapsid of Gag: $CX_2CX_4HX_4C$ motif (for RNA genome binding)	YES (**distinguishes** from most retroviruses, [1])	**No** [both Type 1 and Type 2 HERV-K (HML-2) have the $CX_2CX_4HX_4C$ in Gag see GenBank for AF164610 (K102- Type 1 and AF164614 K108- Type 2)]
26	Nucleocapsid (nc) not made from Gag (*i.e.* no cleavage products for nc and capsid)	YES (**distinguishes** from most retroviruses)	Possibly (see [6] for classical HTDV particles without spikes)
27	Lacks gag-pol fusion protein which is then cleaved (*i.e. pol* separately transcribed)	YES (**distinguishes** from most retroviruses, [1])	Not clear
28	Naturally "oncolytic"	YES (**distinguishes** from most retroviruses, see [26])	N/D. However, the immune system may use HERV-K antigens for lysis and removal of tumor cells [7, 15, 27-30]. Recent report suggests antibodies to Type 1 HML-2 Env promotes apoptosis of tumors [33].
29	Intratumoral injection of replication-competent FV in skin leads to widespread integration *i.e.* spleen, bone marrow, brain, gonads (appears to easily cross blood-brain and other physical barriers and infects many cell types)	YES (**distinguishes** from most retroviruses, [26])	Unknown
30	Integration pattern unique, *i.e.* not like other retroviruses (*e.g.*, does not integrate into active genes like HIV, or into transcription-start regions like MLV)	YES (no preference nor for certain chromosomal regions, [31])	N/D
31	Up to 20 copies of integrated provirus per cell	YES [32]	Up to 12 proviral copies per genome detected in HESN *
32	Infects human T cells and monocytes/dendritic cells, but not B lymphocytes	YES [4, 32]	Unclear (expression and activation does not appear to involve B cells, unpublished observations)*
33	Uses heparin sulphate to gain access to cells	YES [35]	Unknown.
34	PFV infected cells may increase lentivirus binding and entry	YES [36]	Unknown
35	Immature capsids uniquely congregate into cytoplasm and bud through endoplasmic reticulum generating vacuoles	YES [39]	This hallmark property is known to be shared by B/D retroviruses like HERV-K HML-2 [39] and is shown here in Figure 1c for HERV-K102*

#Prototype Foamy Virus (PFV) ***(Y07725, NC_001795; GenBank)*** is formerly known as Human Foamy Virus (HFV) and had been referred to as SFVcpz (hu) because it was found to have originated in chimpanzees despite having been isolated from a human tumor line. For general reviews on the features of foamy viruses see references *1-3* below.

++ ***(AF164610; GenBank)***

* in this manuscript

N/D = Not determined.

SUPPLEMENTARY REFERENCES

[1] Linial ML. Foamy viruses are unconventional retroviruses. J Virol 1999; 73: 1747-55.
[2] Meiering CD, Linial ML. Historical perspective of foamy virus epidemiology and infection. Clin Microbiol Rev 2001; 14: 165-76.
[3] Delelis O, Lehmann-Che J, Saib A. Foamy viruses: a world apart. Curr Opin Microbiol 2004; 7: 400-6.
[4] Mikovits JA, Hoffman PM, Rethwilm A, Ruscetti FW. *In vitro* infection of primary and retrovirus-infected human leukocytes by human foamy virus. J Virol 1996; 70: 2774-80.
[5] Laderoute MP, Giulivi A, Larocque L, *et al*. The replicative activity of human endogenous retrovirus K102 (HERV-K102) with HIV viremia. AIDS 2007; 21: 2417-24.
[6] Bieda K, Hoffmann A, Boller K. Phenotypic heterogeneity of human endogenous retrovirus particles produced by teratocarcinoma cell lines. J Gen Virol 2001; 82: 591-6.
[7] Lower R, Boller K, Hasenmaier B, *et al*. Identification of human endogenous retroviruses with complex mRNA expression and particle formation. Proc Natl Acad Sci USA 1993; 90: 4480-4.
[8] Murray SM, Picker LJ, Axthem MK, Hudkins K, Alpers CE, Linial ML. Replication in a superficial epithelial cell niche explains the lack of pathogenicity of primate foamy virus infections. J Virol 2008; 82: 5981-5.
[9] Linial M. Why aren't foamy viruses pathogenic? Trends Microbiol 2000; 8: 284-9.
[10] 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: 861-8.
[11] 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: 937-44.
[12] Mayer J, Ehlhardt S, Seifert M, *et al*. Human endogenous retrovirus HERV-K (HML-2) proviruses with Rec protein coding capacity and transcriptional activity. Virology 2004; 322: 190-8.
[13] Enssle J, Moebes A, Heinkelein M, *et al*. An active foamy virus integrase is required for virus replication. J Gen Virol 1999; 80: 1445-52.
[14] Cordonnier A, Casella JF, Heidmann T. Isolation of a novel human endogenous retrovirus-like elements with foamy virus-related pol sequence. J Virol 1995; 69: 5890-7.
[15] Benit L, Dessen P, Heidmann T. Identification, phylogeny and evolution of retroviral elements based on their envelope genes. J Virol 2001; 75: 11709-19.
[16] de Parseval N, Lazar V, Casella JF, Benit L, Heidmann T. Survey of human genes of retroviral origin: identification and transcriptome of the genes with coding capacity for complete envelope proteins. J Virol 2003; 77: 10414-22.
[17] Rethwilm A. The replication strategy of foamy viruses. Curr Top Microbiol Immunol 2003; 277: 1-26.
[18] Yu SF, Sullivan MD, Linial ML. Evidence that the human foamy virus genome is DNA. J Virol 1999; 73: 1565-72.
[19] Bansal A, Shaw KL, Edwards BH, Goepfert PA, Mulligan MJ. Characterization of the R572T point mutant of a putative cleavage site in human foamy virus Env. J Virol 2000; 74: 2949-54.
[20] Pietschmann T, Heinkelein M, Heldmann M, Zentgraf H, Rethwilm A, Lindemann D. Foamy virus capsids require the cognate envelope protein for particle export. J Virol 1999; 73: 2613-21.
[21] Peters K, Barg N, Gartner K, Rethwilm A. Complex effects of foamy virus central purine-rich regions on viral replication. Virology 2008; 373: 51-60.
[22] Belshaw R, Pereira V, Katzourakis A, *et al.* Long term reinfection of the human genome by endogenous retroviruses. Proc Natl Acad Sci USA 2004; 101: 4894-9.
[23] 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: 12507-14.
[24] Tristem M. Identification and characterization of novel human endogenous retrovirus families by phylogenetic screening of the human genome mapping project database. J Virol 2000; 74: 3715-30.
[25] Willems L, Kerkhofs P, Attenelle L, Burny A, Portetelle D, Kettmann R. The major homology region of bovine leukaemia virus p24gag is required for virus infectivity *in vivo.* J Gen Virol 1997; 78: 637-40.
[26] Heinkelein M, Hoffmann U, Lucke M, *et al*. Experimental therapy of allogeneic solid tumors induced in athymic mice with suicide gene-transducing replication-competent foamy virus vectors. Cancer Gene Ther 2005; 12: 947-53.
[27] Wang-Johanning F, Frost AR, Johanning GL, *et al.* Expression of human endogenous retrovirus K envelope transcripts in human breast cancer. Clin Cancer Res 2001; 7: 1553-60.
[28] 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: 1528-35.
[29] 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: 4581-8.
[30] Wang-Johanning F, Radvanyi L, Rycaj K, *et al*. Human endogenous retrovirus K triggers an antigen-specific response in breast cancer patients. Cancer Res 2008; 68: 5869-77.
[31] Nowrouzi A, Dittrich M, Klanke C, *et al*. Genome-wide mapping of foamy virus vector integrations into a human cell line. J Gen Virol 2006; 87:1339-47.
[32] Yu SF, Stone J, Linial ML. Productive persistent infection of hematopoietic cells by human foamy virus. J Virol 1996; 70:1250-4.
[33] Wang-Johanning F, Rycaj K, Plummer JB, *et al.* Immunotherapeutic potential of anti-human endogenous retrovirus-K envelope protein antibodies in targeting breast tumors. J Natl Cancer Inst 2012; 104: 189-210.
[34] Contreras-Galindo R, Kaplan MH, Contreras-Galindo AC, *et al.* Characterization of human endogenous retroviral elements in the blood of HIV-1-infected individuals. J Virol 2012; 86: 262-76.
[35] Plochmann K, Horn A, Gschmack E, *et al*. Heparan sulfate is an attachment factor for foamy virus entry. J Virol 2012; 86: 10028-35.
[36] Schiffer C, Lecellier CH, Manniou A, *et al*. Persistent infection with primate foamy virus type 1 increases human immunodeficiency virus type 1 cell binding *via* a Bet-independent mechanism. J Virol 2004; 78: 11405-10.
[37] Dube D, Contreras-Galindo R, He S, *et al*. Genomic flexibility of human endogenous retrovirus type K. J Virol 2014; 88: 9673-82.
[38] Brinzevich D, Young GR, Sebra R, *et al*. HIV-1 interacts with human endogenous retrovirus K (HML-2) envelopes derived from human primary lymphocytes. J Virol 2014; 88: 6213-23.
[39] Hütter S, Zurnic I, Lindemann D. Foamy virus budding and release. Viruses 2013; 5: 1075-98.