SUPPLEMENTARY MATERIAL

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

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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\(^{\text{a}}\) to Type 1 HERV-K (HML-2) HERV-K102\(^{\text{++}}\):**

<table>
<thead>
<tr>
<th>Property</th>
<th>Prototype Foamy Virus(^{\text{a}})</th>
<th>HERV-K102(^{\text{++}})</th>
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</thead>
<tbody>
<tr>
<td>1 Causes vacuolation in cultured human mononuclear cells <em>in vitro</em></td>
<td>YES (hallmark) [4]</td>
<td>YES *</td>
</tr>
<tr>
<td>2 Particles predominately bud into vacuoles rather than from cell surface membrane for cultured human PBMCs</td>
<td>YES (hallmark) [4]</td>
<td>YES * [5]</td>
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<tr>
<td>3 Immature particles (no electron dense core)</td>
<td>YES (hallmark) [1-3]</td>
<td>YES *</td>
</tr>
<tr>
<td>4 Abundant intracellular particles</td>
<td>YES (hallmark) [1-3]</td>
<td>YES *</td>
</tr>
<tr>
<td>5 Can cause lytic infection in some human fibroblast cell lines <em>in vitro</em></td>
<td>YES (hallmark) [1, 2, 8, 9]</td>
<td>YES (MRC-5 but not HFL-1 cells nor Vero cells, see Laderoute MP et al, Patent CA2673395, 2006)</td>
</tr>
<tr>
<td>6 Induces lysis in HIV-1 or HTLV-1 infected PBMCs</td>
<td>YES [4]</td>
<td>Unknown</td>
</tr>
<tr>
<td>7 Extracellular particles contain DNA and RNA genomes</td>
<td>YES (hallmark) [1-3]</td>
<td>YES [5]</td>
</tr>
<tr>
<td>8 Non-pathogenic</td>
<td>YES (hallmark) [1-3]</td>
<td>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)</td>
</tr>
<tr>
<td>9 Lacks the REC/REV/REX Domain in <em>env</em></td>
<td>Yes (hallmark) [3]</td>
<td>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])</td>
</tr>
<tr>
<td>10 5' LTR proviral genome begins with “TGTG” (evidence of asymmetric integration process)</td>
<td>YES (hallmark) [13]</td>
<td>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])</td>
</tr>
<tr>
<td>11 Lacks the CWLC (CXXC) motif in the surface unit of Env</td>
<td>YES (distinguishes most retroviruses, [15])</td>
<td>YES (distinguishes HERV-K HML-2 from all other HERVs, [16, 34])</td>
</tr>
<tr>
<td>12 Capable of intracellular retrotransposition</td>
<td>Yes (hallmark) [see 2, 17]</td>
<td>Unknown</td>
</tr>
<tr>
<td>13 Infectious particles contain DNA genomes</td>
<td>YES (hallmark) for review, see [16, 18]</td>
<td>YES, DNA containing HERV-K HML-2 virions are infectious and replication competent [37]</td>
</tr>
<tr>
<td>14 Env is required for particle formation</td>
<td>YES (distinguishes from most retroviruses, [1])</td>
<td>Unknown but processed Env detected with particles*</td>
</tr>
<tr>
<td>15 Env must be processed/cleaved for infectivity</td>
<td>YES [19]</td>
<td>N/D appears to be cleaved when isolated from induced CB cells*</td>
</tr>
<tr>
<td>16 Env can substitute for orthoretrovirus Env in trans</td>
<td>NO [20]</td>
<td>NO, HERV-K102 Env does not substitute HIV-1 VLPs [38]</td>
</tr>
<tr>
<td>17 Uses lysine as tRNA for priming reverse transcription</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>18 “Complex” retrovirus</td>
<td>YES</td>
<td>YES [7]</td>
</tr>
<tr>
<td>19 Temporal transcription regulation: first uses internal promoter 3' to <em>env</em>, then LTR for full length transcripts</td>
<td>YES (distinguishes from most retroviruses, [1])</td>
<td>N/D</td>
</tr>
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</table>
### Prototype Foamy Virus (PFV) (Y07725, NC_001795; GenBank)

PFV infected cells may increase lentivirus binding and entry to the cytoplasm and bud through endoplasmic reticulum generating vacuoles. The hallmarks of PFV that are known to be made by the BFV are listed below.

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| 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]) |
| 23 | Nuclear staining of Gag | YES (hallmark and diagnostic for foamy retroviruses, [1]) |
| 24 | Lacks the major homology region (MHR) in the capsid (Gag) QXEX(F/Y)X-R 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: CX2CX3HX2C motif (for RNA genome binding) | YES (distinguishes from most retroviruses, [1]) 
No [both Type 1 and Type 2 HERV-K (HML-2) have the CX2CX3HX2C 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]) |
| 28 | Naturally ‘oncolytic’ | YES (distinguishes from most retroviruses, see [26]) |
| 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] |
| 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