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Envelope Gene of the Human Endogenous Retrovirus HERV-W Encodes a Functional Retrovirus Envelope

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A member of the human endogenous retrovirus (HERV) family termed HERV-W encodes a highly fusogenic membrane glycoprotein that appears to be expressed specifically in the placenta. It is unclear whether the glycoproteins of the HERVs can serve as functional retrovirus envelope proteins to confer infectivity on retrovirus particles. We found that the HERV-W envelope glycoprotein can form pseudotypes with human immunodeficiency virus type 1 virions and confers tropism for CD4-negative cells. Thus, the HERV-W *env* gene represents the first HERV *env* gene demonstrated to encode the functional properties of a retrovirus envelope glycoprotein.

Human endogenous retroviruses (HERVs) have been estimated to comprise about 0.5 to 1.0% of the human genome (4, 7). All known HERVs are replication incompetent; however, some proviruses have open reading frames capable of encoding functional proteins (7, 11). One member of the newly described HERV family termed HERV-W (2) encodes a highly fusogenic membrane glycoprotein that has been proposed to play a role in normal placental development (2, 8). The HERV-W envelope has been shown to induce syncytium formation in human, simian, and pig cells but not in avian, rodent, or feline cells (2). However, it is unclear whether this glycoprotein can serve as an envelope protein to confer infectivity on retrovirus particles.

We determined whether the HERV-W envelope can confer infectivity on an envelope-defective human immunodeficiency virus type 1 (HIV-1) strain. We used the HIV-1 vector NLEGFPΔBgIVprX, a derivative of NLthyΔBgIVprX (9) with a deletion within the HIV *env* gene and bearing an enhanced green fluorescent protein (EGFP)-encoding reporter gene. This *env* deletion-containing vector is dependent upon pseudotyping with an envelope for infectivity (data not shown). Virus was recovered by calcium phosphate-mediated cotransfection of 293T cells with a vector expressing the HERV-W envelope (phCMV-ENVpH74) (2). In addition to the entire HERV-W envelope open reading frame, phCMV-ENVpH74 contains 66 bp of the DNA sequence upstream of the HERV-W envelope start codon and 138 bp of the DNA sequence downstream of the HERV-W stop codon derived from the original HERV-W envelope cDNA. Virions were tested for infectivity on human embryonal kidney 293T cells (3) by measuring the EGFP fluorescence of infected cells by flow cytometry. Infection with virions derived by cotransfection of the HERV-W envelope (NLEGFPΔBgIVprX [HERV-W]) resulted in EGFP expression following infection of 293T cells (Fig. 1). Inclusion of the retrovirus reverse transcriptase inhibitors (RTIs) zidovudine and nevirapine as a control during infection led to loss of EGFP expression. Thus, pseudotyping of HIV-1 virions with the HERV-W envelope results in infectious virus. Consistent with the lack of fusion on mouse cells (2), NLEGFPΔBglVprX (HERV-W) did not infect mouse B16 cells (data not shown). Similar results were observed when the HERV-W envelope was utilized to pseudotype an extensive-deletion-containing, self-inactivating HIV-1 vector bearing an internal promoter expressing EGFP (SIN18RhMLVE) (5) rescued by complementation with a packaging plasmid to provide virion *gag* and *pol* products (data not shown). Compared to vesicular stomatitis virus G envelope pseudotypes, virions with the HERV-W envelope were approximately two- to fivefold lower in titer for comparable p24 Gag antigen levels. Supernatant titers ranged from 5×10^4 to 1×10^5/ml in different experiments. Freezing, thawing, and concentration by ultracentrifugation reduced titers considerably (data not shown). Consistent with previous reports (2), infectious pseudotypes were not observed with a murine leukemia virus (MLV)-based vector (Fig. 1). These results provide the first direct evidence that an HERV envelope glycoprotein can serve as a functional retrovirus envelope.

The HERV-W family of endogenous retroviruses consist of an estimated 30 to 100 provirus copies per haploid human genome (10). The HERV-Ws first entered the genome of primates following the divergence of New World and Old World monkeys (approximately 25 million years ago) (10). Several other HERV families have also been reported (7, 11). In all cases, the endogenous retroviruses are replication defective because of mutations within functional retrovirus genes (7, 11). However, individual open reading frames corresponding to *gag, pol*, and *env* have been observed and in some cases have been shown to encode proteins (7, 11). Our results raise the possibility that HERVs could potentially be assembled into infectious virions through transcomplementation with virion proteins encoded by different HERVs. A functional envelope glycoprotein would confer upon the retroviruses the ability to be transmitted vertically and/or horizontally and potentially

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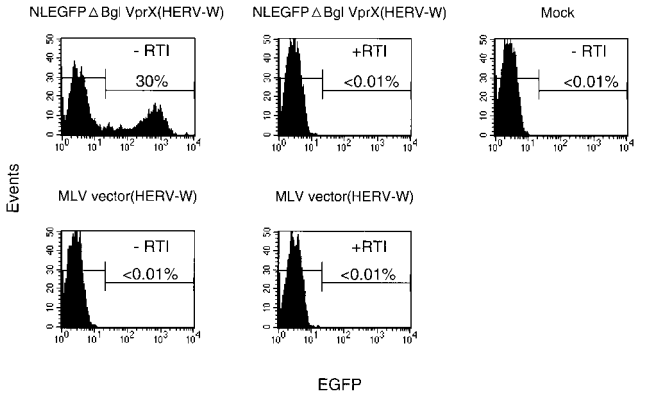

FIG. 1. HIV-1 can be pseudotyped with the HERV-W envelope. 293T cells were cotransfected with an HERV-W envelope expression construct and an HIV-1 vector (NLEGFPΔBgIVprX) or a MLV vector construct (SRαEGFP) (1) and packaging plasmid (SVφ$^-$env$^-$MLV) (6). 293T cells (5×10^4) were infected with 1 ml of the supernatant from the cotransfected 293T cells in the presence (+RTI) or absence (−RTI) of zidovudine (5 μM) and nevirapine (5 μM). The concentration of p24 in the HERV-W envelope- and HIV vector-cotransfected supernatant was 1,159 ng/ml. Two days postinfection, cells were analyzed for EGFP expression by flow cytometry. Mock-transfected cells (no infection) were analyzed in parallel. The *x* axis represents the logarithmic fluorescence intensity of EGFP; the *y* axis represents the number of events. The percentage of EGFP-positive populations was as indicated in each panel. Ten thousand events were acquired for flow cytometric analysis. The data shown here are representative of three independent experiments.

provide new roles for HERVs in normal physiology and/or pathology.

The pseudotyping of HIV-1 with the HERV-W envelope also raises the possibility that the expression of other HERV envelopes in other tissues of the body may alter the cell tropism of HIV-1. With the complete nucleotide sequence of the human genome near completion, the identification of novel HERVs with the potential to encode functional envelopes will allow testing of the possibility that other envelopes can pseudotype with HIV-1 and potentially contribute to HIV-1 pathogenesis.

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