Altered Transcriptional Activity of Human Endogenous Retroviruses in Neuroepithelial Cells after Infection with *Toxoplasma gondii*

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Human endogenous retroviruses (HERVs) arose in antiquity from stable integration into the human genome. The mechanism for activation of HERVs has not been fully elucidated. Toxoplasmosis, caused by *Toxoplasma gondii,* is a medically important parasitic infection with worldwide distribution. To search for a tentative link between toxoplasmosis and HERV activation, HERV expression profiles of human neuroepithelial SK-N-MC cells infected with *T. gondii* were analyzed. Increased transcriptional activity of class I, II, and III HERV elements was observed in infected cells, suggesting that *T. gondii* can influence the transcription of HERVs in neuronal cells.

Human endogenous retroviruses (HERVs) are elements in the human genome that arose from the stable integration of exogenous retroviruses into germ cells with subsequent vertical passage in a Mendelian fashion [1]. Activation of HERV transcription has been postulated to be a mechanism of the pathogenesis of complex human disorders involving autoimmunity, neoplasia, and psychiatric abnormalities. The mechanisms involved in HERV activation have not been fully elucidated. Recent studies have indicated that viruses such as herpes simplex virus can activate HERV transcription [2]. However, to our knowledge, there have not been studies of HERV activation by other infectious agents of medical importance.

Toxoplasma gondii is an intracellular protozoan of worldwide medical importance, affecting 20%– 80% of the human population. The definitive host of *T. gondii* is the cat. Humans can be infected directly through the ingestion of oocysts shed in infected cat feces, indirectly through consumption of rare or raw meats containing parasite tissue cysts, or transplacentally from infected mother to fetus. Infections of immune-competent individuals result in mild symptoms, fever, and adenopathy or are apparently asymptomatic. In either case, the central nervous system can be infected, leading to a lifetime chronic/latent infection [3]. *Toxoplasma* infections occur commonly in HIV-infected individuals, and immunological studies indicate that *Toxoplasma* infection can result in an augmentation of HIV replication in dual-infected individuals [4, 5]. Furthermore, *Toxoplasma* infection has been shown to increase HIV transcription in animal models [6].

Materials and methods. The human neuroepithelioma cell line SK-N-MC (American Type Culture Collection [ATCC]) was propagated in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (D-MEM/F-12; Gibco) supplemented with 12.5% fetal bovine serum (FBS; Atlas Biologicals), 15 mmol/L HEPES, 4 mmol/L L-glutamine (L-gln), 100 U/mL penicillin G, and 100 μg/mL streptomycin sulfate. Normal human fibroblasts (HFF; ATCC) were used to grow stocks of the tachyzoites. These cells were maintained in D-MEM containing 10% FBS, 25 mmol/L HEPES, 2 mmol/L L-gln, 50 U/mL penicillin G, and 50 μg/mL streptomycin sulfate (human foreskin fibroblast cell growth medium [HFF CGM]). A culture of the tachyzoites of *T. gondii* strain 2F, which constitutively expresses cytoplasmic β-galactosidase and is derived from strain RH, was a gift from V. Carruthers, Johns Hopkins University Bloomberg School of Public Health.

The *T. gondii* tachyzoites used in the assay were prepared by passing the growth medium from an infected cell monolayer twice sequentially through 18-, 23-, and 27-gauge needles and then through a 3-μm-pore filter. The filter was rinsed with 7 mL of Hank's balanced salt solution (HBSS) without sodium bicarbonate (Gibco BRL) containing 10 mmol/L HEPES. The combined flow-through was subjected to centrifugation at 250 *g* for 10 min, the pellet was resuspended in 1–2 mL of toxo CGM (D-MEM without phenol red containing 10% FBS, 2

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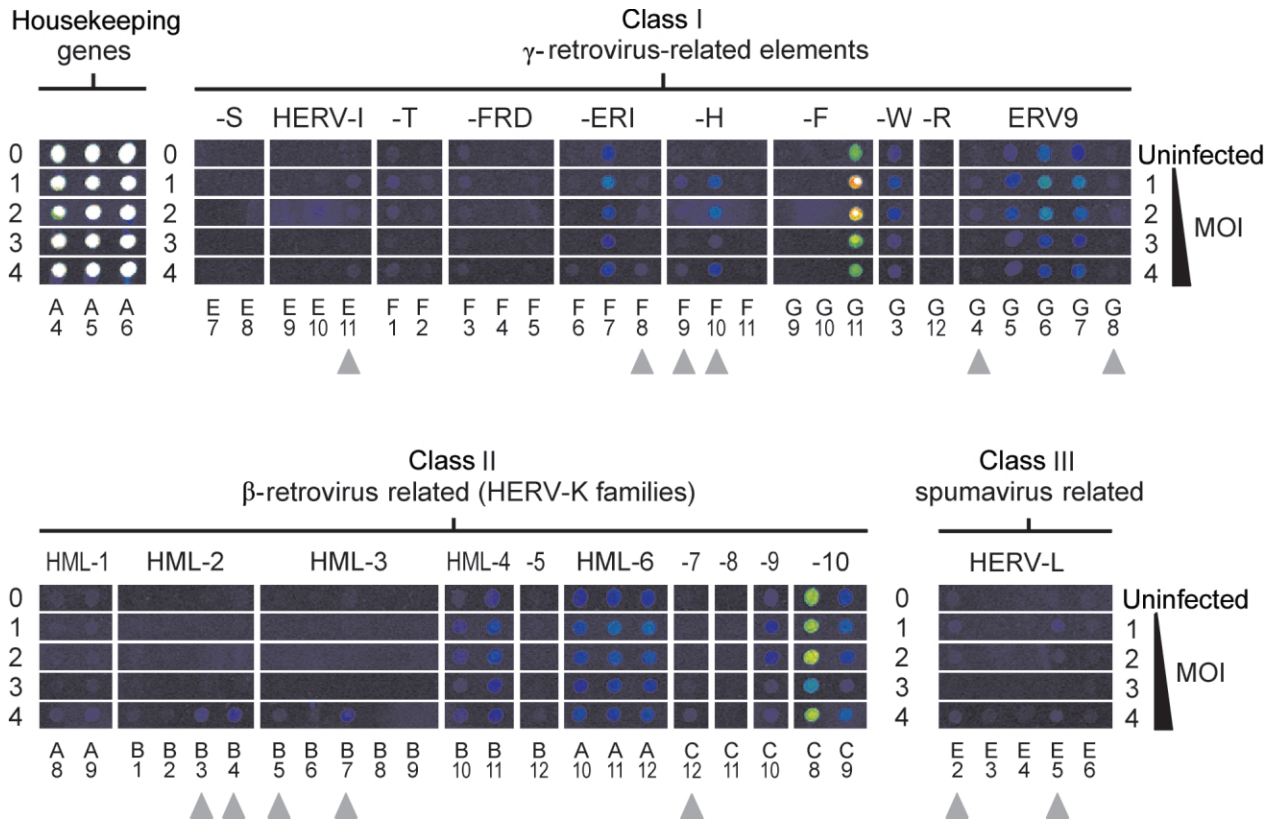

Figure 1. Alignment of false color chip data sets corresponding to human endogenous retrovirus (HERV) classes I, II, and III transcriptional patterns observed in SK-N-MC cells after infection with *Toxoplasma gondii* by HERV microarray hybridization. A housekeeping gene panel (RPL19 [A4], β-actin [A5], and HPRT [A6]) served as an internal control. For origin and identity of dot codes, see Frank et al. [8]. Changes in transcriptional pattern irrespective to MOI are depicted by arrowheads. 0 indicates uninfected cells; 1, 2, 3, and 4 MOI correspond to 10^3, 10^4, 10^5, and 10^6 *T. gondii* tachyzoites/well, respectively.

mmol/L L-gln, and 20 μg gentamicin/mL), and then the tachyzoites were counted in a hemacytometer chamber.

SK-N-MC cells were plated 20 h before infection in 6-well tissue culture trays. At the time of infection, the cell growth medium was aspirated, and then the cells were rinsed once with HBSS without Ca^{2+} or Mg^{2+}. Duplicate wells were inoculated with HFF CGM without (negative control) or with known amounts of *T. gondii* (10^3–10^6 tachyzoites/well). The infections proceeded for 24 h at 37°C in 5% CO_2, after which time the cells were lysed in buffer and the total RNA was extracted in accordance with the manufacturer's instructions (RNeasy Mini Kit; Qiagen). To examine the retroviral transcriptome of *Toxoplasma*-infected cells, we employed a recently established retrovirus-specific microarray (HERV chip) that allows simultaneous detection and identification of 50 representative HERV reverse transcriptase–derived sequences from 20 major HERV families [7]. Microarray hybridizations were performed in triplicate as a blind study in accordance with the standardized protocol described elsewhere [8].

Results. Comparative qualitative analysis of HERV activity profiles revealed changes in HERV transcription on infection with *T. gondii* tachyzoites. Figure 1 shows a digitally processed alignment of a representative image data set comparing uninfected *(row 0)* and infected *(rows 1–4)* SK-N-MC cells. A panel of 3 human housekeeping genes *(upper left panel)* served as internal control, confirming equal RNA quality and comparability of all tested samples.

As expected from previous studies confirming tissue-specific HERV activity in humans [9], not all HERV taxa appear to be equally active in SK-N-MC cells. Preferentially, class I HERV elements of the taxa HERV-ERI (F7), HERV-F (G11), HERV-W (G3), and ERV9 (G5–G7) and class II elements of the HERV-K families HML-4 (B11), HML-6 (A10–A12), HML-9 (C10), and HML-10 (C8 and C9) are transcriptionally active irrespective of *Toxoplasma* infection status. Except for 2 HERV-K (HML-2) subgroups (B3 and B4) that are not expressed in uninfected SK-N-MC cells, the observed pattern is identical to the retroviral core activity signature in the human brain [8]. These differences may be due to the complex composition of the human brain, representing various cell types with differential HERV expression patterns.

Conspicuously, transcriptional activation was observed in 8 HERV families on *Toxoplasma* infection. Compared with uninfected control cells, members of HERV-I (E11), HERV-E (F8),

HERV-H (F9 and F10), ERV9 (G4 and G8), HML-2 (B3 and B4), HML-3 (B5 and B7), HML-7 (C12), and HERV-L (E2 and E5) families display positive signals after *T. gondii* infection (figure 1, *arrowheads*). Interestingly, the HML-2 elements (B3 and B4) activated by *T. gondii* belong to the same HML-2 subgroups as the HML-2 transcripts constitutively expressed in human brain tissue but missing in untreated SK-N-MC cells.

However, the observed signals do not always correlate with MOI. Whereas some signals (F9 and F10) become already evident at low MOI, others (B3, B4, and B7) are restricted to cells treated with the highest MOI (10^6 tachyzoites/well) (figure 1). The observed phenomena may be due to stress-related availability or levels of cellular transcription factors acting on HERV promoters [10]. In addition, the observed transcriptional activation might not require active replication of the parasite but rather a protein of the invading tachyzoite. Therefore, increasing abundance of this putative transactivating protein, rather than MOI per se, may contribute to the observed signal patterns. It is noteworthy that no deactivation of HERV activity on *Toxoplasma* infection was detectable.

Discussion. Our data demonstrate that SK-N-MC cells infected in vitro with the intracellular protozoan parasite *T. gondii* undergo up-regulation of certain HERV elements, which points to an association between *Toxoplasma* infection and retroviral activation in human neuronal cells. This phenomenon is in line with the observations of Gazzinelli et al. [6], who reported on activation of HIV-1 transcription in HIV-1 transgenic mice with *T. gondii* infection. Although mechanisms of HERV activation on *Toxoplasma* infection are still to be clarified, up-regulation of proinflammatory cytokines and modulation of numerous host cell processes associated with organelle distribution and apoptotic behavior could account for the observed findings [11]. Moreover, epigenetic mechanisms such as methylation may play a role in the observed phenomena [12]. Additional studies should be directed at defining the mechanisms involved in *Toxoplasma*-associated HERV activation as well as the role played by this activation in human disease.

Our comprehensive study establishes a link between *Toxoplasma* infection and activation of class I, II, and III HERVs in human neuronal cells in vitro and warrants future studies on a molecular level of HERV activation by infectious agents.

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