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Characterization of neuropathology in the HIV-1 transgenic rat at different ages

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ABSTRACT

The transgenic HIV-1 rat (Tg) is a commonly used neuroHIV model with documented neurologic/behavioral deficits. Using immunofluorescent staining of the Tg brain, we found astrocytic dysfunction/damage, as well as dopaminergic neuronal loss/dysfunction, both of which worsening significantly in the striatum with age. We saw mild microglial activation in young Tg brains, but this decreased with age. There were no differences in neurogenesis potential suggesting a neurodegenerative rather than a neurodevelopmental process. Gp120 CSF levels exceeded serum gp120 levels in some animals, suggesting local viral protein production in the brain. Further probing of the pathophysiology underlying astrocytic injury in this model is warranted.

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1. Introduction

Since the introduction of anti-retroviral therapy (ART), the prevalence of HIV-associated dementia (HAD) decreased, however less fulminant forms of HIV-related neurological dysfunction became more common and currently affect around 52% of the HIV-positive (HIV+) patient population (McArthur et al., 2003, 2010). The exact neuropathology leading to milder forms of HIV-associated neurocognitive disorders (HAND) is not fully understood but is probably multifactorial. Unlike microglia and astrocytes, neurons do not seem to get productively infected with HIV (Kovalevich and Langford, 2012). The neurologic damage is rather thought related to persistent low level neuroinflammation (Desplats et al., 2013), neurotoxic effects of viral proteins (Agrawal et al., 2012; Hu et al., 2009; Mocchetti et al., 2011), as well as the disruption of the supportive and neurotrophic role of astrocytes (Bezzi et al., 2001) and oligodendrocytes (Radja et al., 2003).

Short of using expensive and sentient SIV infected monkeys, or sophisticated humanized mice models, the HIV-1 transgenic (Tg) rat is used by many groups as an HIV model. This non-infectious rat model expresses 7 of the 9 HIV-1 viral proteins including gp120, nef and tat and is known to develop clinically relevant neuropathologies (Reid et al., 2001) and cognitive deficits (Lashomb et al., 2009; Moran et al., 2012, 2013a; Peng et al., 2010; Vigorito et al., 2007). It thus appears to be of particular importance and practicality for the evaluation of neurological HIV complications.

In this study, we wanted to better understand the pattern of neurotoxicity underlying the neurological and behavioral problems described in the commercially-available Tg rat. Towards this goal, we compared immunofluorescence staining of various neuronal and glial markers, dopaminergic function and neurogenesis potential between the Tg and WT rats, at different ages. We also measured the levels of gp120 (as a representative viral protein) in the cerebrospinal fluid (CSF) and compared to serum levels of.

Abbreviations: ART, antiretroviral therapy; CC, corpus callosum; CSF, cerebrospinal fluid; CX, cortex; HAART, highly active antiretroviral therapy; HAD, HIV associated dementia; HAND, HIV-1 associated neurocognitive disorders; HC, hippocampus; IACUC, Institutional Animal Care and Use Committee; PCNA, proliferating cell nuclear antigen; PET, positron emission tomography; ST, striatum; SVZ, subventricular zone; Tg, HIV-1 transgenic; TH, tyrosine hydroxylase; WT, wild type.

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2. Materials and methods

2.1. Animals

Male HIV-1 Tg rats (F344/Hsd) and male age-matched controls (F344, WT) were purchased from Harlan Inc. (Indianapolis, IN) and used in various experiments. We used a total number of 43 Tg and 34 WT rats in all the experiments, ranging in age from 1 to 20 months. Some of the animals used for non-terminal procedures (serum and CSF collection) were then re-used for the terminal procedures (ELISA of brain lysates, PCR and immunostaining). Animal care and all experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the National Institutes of Health/Clinical Center (NIH/CC).

2.2. Immunofluorescence staining and quantification

Rat brain sections were evaluated using immunofluorescence and compared in four groups of animals: 3 Tg and 3 WT one-month-old rats, 5 Tg and 3 WT three-month-old rats (total young: 8 Tg and 6 WT), 3 Tg and 2 WT seven-month-old and 3 Tg and 3 WT nine-month-old rats (total middle-aged: 6 Tg and 5 WT). The relevant slices were obtained from two areas of interest: striatum, bregma 0.48 to 0.12 millimeters (mm) and hippocampus, bregma −2.92 to −3.24 mm, as per the Paxinos and Watson atlas (The Rat Brain in Stereotaxic Coordinates, Sixth Edition, Academic Press, San Francisco, CA).

Multi-epitope immunolabeling protocols were applied to identify the cellular phenotypes in fresh frozen brain slices using different combinations of primary antibodies. The labeled targets included NeuN, GFAP, Iba1, tyrosine hydroxylase (TH) and proliferating cell nuclear antigen (PCNA). Each of the above primary immunoreactions was visualized using appropriate fluorophore-conjugated (Alexa Fluor dyes) secondary antibodies. The cell nuclei were counterstained using 1 μg/ml DAPI to facilitate cell counting.

Quantification of immunofluorescent staining was performed using FIJI image processing package, based on ImageJ (NIH, Bethesda, MD). The locations of the striatal and cortical ROIs were identical for all the animals. For NeuN, Iba1 and PCNA cell counts, the RGB bitmap images were converted to 8-bit grayscale and the threshold was adjusted to include only cells of interest and eliminate the background, and this was followed by counting, using the image based tool for counting nuclei plugin (ITCN). All images were processed using the same analysis parameters. The cell density (cells/mm^2) was calculated from the total number of positive cells divided by the total area. For the staining intensity measurements of GFAP and TH also calculated for the total area (total intensity/mm^2), the background fluorescent signal was removed by a thresholding process, visually selected by the user. The same cutoff value was then used to analyze all the slides stained concurrently.

Since our animal brains were stained in batches at different dates, we decided to use the ratios of staining instead of absolute measurement values to compare young and middle-aged animals. For each set of animals stained at the same time, we used the ratio of counts or the ratio of intensities of each Tg animal with respect to the corresponding co-stained WT rat(s). We then compared the individual animal ratios in the young (1–3 month-old) to those in the middle-aged (7–10 month-old) animals.

2.3. Viral protein analysis

2.3.1. Serum and CSF collection

Serum was collected from 28 Tg and 5 WT rats (age range: 3 to 20 month-old) by retro-orbital sampling, under anesthesia. We had CSF samples from 17 Tg rats (age range = 3 to 20 month-old), after excluding samples that were blood-tinged. Out of the latter, 13 rats had CSF and serum collected within a few days of each other.

For CSF collection, animals were first anesthetized and placed in a stereotaxic apparatus (Stoelting, Wood Dale, IL, USA), with their head positioned downward at around 45 degrees angle to their back and secured with ear bars. The back of the neck and base of skull were shaved and disinfected. The lower border of the skull was then palpated and a one inch, 30 gauge needle attached to silicone tubing which ends in a 1 cm^3 Syringe was inserted horizontally, in a central location, between the occipital protuberance and the first cervical vertebra. The needle was then advanced slowly, with gentle negative pressure maintained within the syringe. Sometimes a slight change in the resistance upon puncturing the dura could be felt. As soon as the CSF was seen to flow into the silicone tubing, the needle was manually stabilized in place. Collection of CSF was then achieved by another operator pulling back the syringe plunger very slowly. The CSF was immediately placed into 0.5 ml Eppendorf tubes and frozen at −80 °C. The volume of CSF collected varied from 50 to 100 μl. Some of the samples were minimally contaminated with blood and were included in the analysis. When the blood contamination was deemed to be significant, the sample was discarded.

2.3.2. Brain lysate collection

We collected brain lysates from 7 Tg and 2 WT rats at different ages ranging from 2 to 20 month-old.

2.3.3. ELISA

Serum, CSF and brain lysate gp120 levels were quantified by enzyme-linked immunosorbent assay (ELISA) using HIV-1 gp120 antigen capture assay (ABL, Rockville, MD) with slight modification to the manufacturer's instruction. In general, gp120 standards were diluted either in rat sera (AbD Serotec, Raleigh, NC; SPF Fisher 344), rat CSF (Bioreclamation LLC., Westbury, NY; SPF Fisher 344) or brain lysate extraction buffer (Novateinbio, Woburn, MA) for standard curve. Samples were run in duplicates. Samples where duplicate absorption values differed significantly from each other were excluded.

2.4. Quantitative real-time polymerase chain reaction (qRT-PCR) of brain tissues

For the assessment of viral protein expression in the Tg rat, brain tissue samples were obtained from 3 young Tg rats (2 month-old) and 4 old Tg rats (age > 1 year). Two control rat brains were used as negative controls to test the primers by regular PCR and running gel. Control brains did not show any viral protein bands on the running gel. For analysis of tat, gp120 and nef expression, 100 mg samples of frontal cortex, striatal and hippocampal tissues from each animal were used. Total cellular RNA was isolated from each tissue section using an RNeasy Lipid Tissue mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Synthesis of first-strand cDNA was performed using Quantitect reverse transcriptase kit (Qiagen, Hilden, Germany) and analyzed using IQ SYBR Green PCR kit (Bio-Rad, Hercules, CA). Relative levels of specific mRNA were quantified by using the CFX96 Real-time System (Bio-Rad, Hercules, CA). Primer Sequences for tat, gp120, nef and GAPDH as well as real-time PCR conditions have been previously described (Peng et al., 2010). Primers were synthesized by RealTimePrimers (Elkins Park, PA). Samples were run in triplicate and the yield of PCR product was normalized to GAPDH. Primer efficiencies were determined in triplicate using serial 10-fold dilutions of cDNA. Primer efficiencies (E) were calculated as $E = 10^{[-1/\text{slope}]} - 1$; all primers used in these studies had good efficiency (E = 90–110%). To control for DNA contamination, equal amounts of RNA were used without reverse transcriptase. The relative quantification of the template was