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HIV-1 protein-mediated amyloidogenesis in rat hippocampal cell cultures

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ABSTRACT

Since the beginning of the highly active antiretroviral therapy (HAART) era, epidemiological evidence indicates an increasing incidence of Alzheimer's (AD)-like brain pathology in aging HIV patients. Emerging evidence warns of potential convergent mechanisms underlying HIV- and Aβ-mediated neurodegeneration. We found that HIV-1 Tat B and gp120 promote the secretion of Aβ 1-42 in primary rat fetal hippocampal cell cultures. Our results demonstrate that the variant of Tat expressed by the neurotropic subtype of HIV-1 virus (HIV-1 clade B) specifically induces both the release of amyloidogenic Aβ 1-42 and the accumulation of cell-bound amyloid aggregates. The results of the research rationalize testing of the ability of β-amyloid aggregation inhibitors to attenuate HIV protein-mediated cognitive deficits in animal models of NeuroAIDS. The long-term goal of the study is to evaluate the potential benefits of anti-amyloidogenic therapies for management of cognitive dysfunction in aging HIV-1 patients.

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The development of persistent cognitive deficits in aging HIV patients with suppressed viral replication is now recognized as a serious and growing medical problem [26]. HAART medication has shifted neuropathology from a subacute encephalitic condition to a more subtle neurodegenerative process involving synaptic and dendritic degeneration, particularly of hippocampal neurons [30]. Epidemiologic evidence indicates a growing incidence of AD-like brain pathology in aging HIV patients. Clinical studies reveal similar changes in Aβ 1-42 levels in CSF from cognitively impaired HIV-1 patients and in patients with mild dementia of the AD-type [9]. These alarming facts suggest that HIV-associated pathology is evolving [7]. Neurotoxic regulatory and core viral proteins, such as Tat and gp120, are thought to contribute to the development of HIV-associated cognitive dysfunction. Although the role of viral proteins in HIV-associated memory impairment has been documented, the mechanism of this dysfunction is poorly defined. Recent reports warn that Aβ biogenesis and clearance venues may be influenced by HIV-1 proteins [19,30].

Adverse effects of HIV-1 Tat and gp120 on neuronal function [6,11,29] resemble alterations elicited by soluble Aβ oligomers [28]. Existing evidence of strikingly similar characteristics of beta-amyloid- and HIV protein-mediated neurodegeneration rationalizes the idea that viral proteins, which are known to induce deficits in learning and memory, may facilitate accumulation of misfolded Aβ in hippocampal cells. Nevertheless, the potential ability of Tat or gp120 to induce formation of amyloidal aggregates has not been systematically investigated.

In this study, we investigate the connection between Tat or gp120-mediated cell injury and amyloid formation in primary cultures of rat fetal hippocampal neurons.

Purified proteins: Recombinant original Tat 1-86 B and (Cys22 → Gly22)-substituted Tat 1-86 B (LAI/Bru strain of HIV-1 clade B, GenBank accession no. K02013) were purchased from Diatheva (Italy). Tat 1-101 clade C was purchased from Prospec (Israel). The recombinant gp120 was purchased from Protein Sciences (Meriden, CT). Synthetic Aβ 1-42 was purchased from Anaspec (Fremont, CA).

Primary hippocampal cell cultures were prepared from 18-day-old Sprague–Dawley rat fetuses as previously described [2]. Cultures were used for experiments after 14 days in culture and were >85–90% neuronal as determined by anti-MAP-2/anti-GFAP/Hoechst fluorescent staining.

The treatment of hippocampal cell cultures was carried out by the addition of freshly prepared stock solutions of the recombinant gp120, Tat polypeptides, or pre-aggregated stock solutions of Aβ 1-42 into the cell culture growth medium. An equal volume of the vehicle was added to control cell cultures. The preparation of pre-aggregated Aβ 1-42 was carried out as previously described [1].

Hippocampal cell viability was assessed using the microplate reader-formatted [2,3,4] variant of the Live/Dead assay (Molecular Probes, Inc., Eugene, OR). Fluorescence was measured using a Bio-Tek Synergy HT microplate reader (Bio-Tek Instruments, Inc., Winooski, VT).

Direct ELISA measurements of the Aβ 1-42 immunoreactivity in cell-conditioned medium (CM) samples were performed using the rabbit polyclonal anti-Aβ 1-42 antibody (Abcam Inc., Cambridge,

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MA, 1:500). Specificity of anti-Aβ antibodies was tested using samples of fresh cell culture medium with and without recombinant HIV-1 proteins (negative controls). Serial dilutions of freshly prepared stocks of the synthetic rat Aβ 1-42 (0–2500 nM) with cell culture medium were used for calibration.

The formation of cell-bound amyloid β-sheet aggregates in living hippocampal cells was analyzed by fluorescent/DIC microscopy following Congo Red staining (20 μM Congo Red for 30 min). The Congo Red specific binding (543 nM excitation/560–615 nm emission) was visualized using a 20× objective of the inverted fluorescent microscope (Nikon Eclipse TE2000-E). Congo Red staining and DIC images were captured using a CCD camera. Merged images of the specific Congo Red binding and DIC were produced and analyzed by the NIS Elements imaging software (Nikon). Parts of merged Congo Red/DIC images were magnified by placing a selection box over the area of interest and saving the selection as a new image with higher resolution.

Numbers of Congo Red-positive cells were counted using the Object counting option of the NIS Elements imaging software package in 20× images of 4 random fields of vision. For each field of vision, total numbers of hippocampal cells were determined using Hoechst fluorescent staining of cell nuclei.

Statistical comparisons were made using ANOVA and planned comparisons were used to determine specific treatment effects. Significant differences were set at $P < 0.05$.

Results of the anti-Aβ 1-42 ELISA analysis of conditioned medium (CM) samples from hippocampal cultures exposed for 3 days to a toxic dose (100 nM) of Tat or gp120 revealed a significant increase in Aβ 1-42 production (Fig. 1). At the age of 14 days *in vitro* (DIV), approximately 20–25% of neurons in hippocampal cultures express amyloid protein precursor (APP) isoforms and 1.5–3.0 nM of extracellular Aβ 1-42 immunoreactivity can be detected in the cell culture medium. According to the anti-Aβ 1-42 ELISA measurements, CM samples from cultures treated with Tat; gp120; or vehicle contained subsequently 28.2 ± 1.6 nM; 18.4 ± 5.2 nM; or 2.4 ± 1.4 nM of the immunoreactive Aβ 1-42.

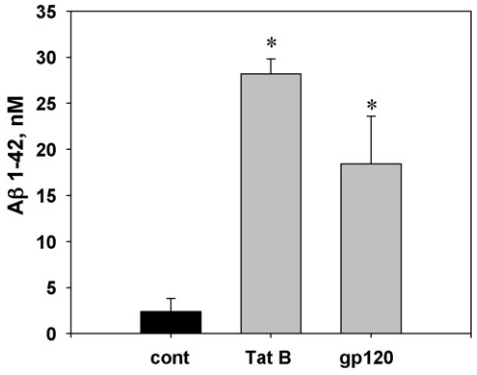

Fig. 1. The Aβ 1-42 immunoreactivity in the conditioned medium from hippocampal cell cultures exposed to HIV-1 Tat or gp120. The graph represents results of direct anti-Aβ 1-42 ELISA measurements in the CM samples collected from hippocampal cultures ($n = 8$ per group) treated with either 100 nM Tat 1-86 B, 100 nM gp120, or the equal volume of vehicle. The freshly prepared Aβ 1-42 stock was serially diluted with cell culture growth medium to obtain ELISA calibration curves. Data presented as mean values ± SEM. *Marks significant ($P < 0.05$) differences in extracellular Aβ 1-42 immunoreactivity.

The analysis of Aβ 1-42 immunoreactivity in hippocampal cell cultures treated with 50 nM dose of Tat 1-86 B for different periods of time (typical protocol for the Tat cytotoxicity analysis described in [3]) revealed that the increase of soluble Aβ 1-42 in CM from hippocampal cell cultures becomes evident early after the start of the exposure and precedes the cell viability decline (Fig. 2A and B). The maximum level of specific Aβ 1-42 immunoreactivity in the CM occurred after 24-h Tat exposure and was 46.1 ± 17.1 nM.

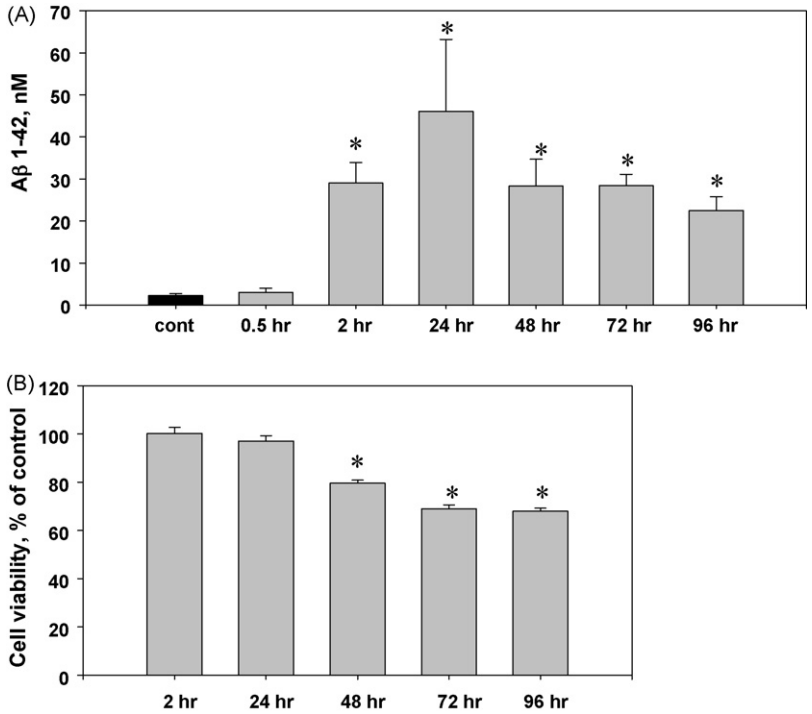

Fig. 2. The temporal relationship between the Aβ 1-42 release and cell viability changes in hippocampal cell cultures exposed to a toxic dose of Tat 1-86 B. (A) The Aβ 1-42 immunoreactivity was measured in CM samples collected from hippocampal cultures during the continuous exposure (0.5–96 h) to 50 nM Tat 1-86 B or vehicle ($n = 5$ per group). Data are presented as mean values ± SEM. *Marks significant ($P < 0.05$) differences in extracellular Aβ 1-42 immunoreactivity between Tat-treated and vehicle-treated cell cultures. (B) Cell viability in hippocampal cell cultures (treated side-by-side, $n = 8$ per group) was determined using Live/Dead assay. Data are presented as mean % of control values ± SEM. *Marks incubation time points for significant ($P < 0.05$) differences in Live/Dead ratios between cultures treated with 50 nM Tat 1-86 B and vehicle-treated controls have been observed.