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Retrograde and anterograde transport of HIV protein gp120 in the nervous system

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ABSTRACT

Neurodegeneration and gliosis are prominent pathological features of subjects with human immunodeficiency virus (HIV) dementia complex (HAD). In these patients, neurodegeneration occurs in uninfected neurons. In addition, these patients develop sensory neuropathy despite the antiretroviral therapy. The HIV protein gp120, which mimics some of the pathological alterations seen in HAD, is retrogradely transported in rodent neurons. However, it is still unclear whether gp120 can also be transported anterogradely and whether axonal transport can occur in the peripheral nervous system (PNS). To determine whether gp120 is transported retrogradely and/or anterogradely, we injected gp120IIIB together with the retrograde tracer fluoro-ruby (FR) or the anterograde tracer 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyamine perchlorate (DiI) into the rat superior colliculi. We discovered that gp120 is retrogradely transported with FR along a direct pathway from the superior colliculus to the retina and anterogradely transported with DiI to several areas of the occipital cortex. To determine whether gp120 is also axonally transported in the peripheral nerves, gp120 and FR were injected into the sciatic nerve. No gp120 immunoreactivity was found in the sciatic nerve or dorsal root ganglia, suggesting that gp120 axonal transport does not occur in the PNS. Gp120 axonal transport may play a role in neuronal injury. Therefore, we examined apoptosis at various time points after gp120 injection. Activated caspase-3 was evident within neurons transporting gp120. These results indicate that axonal transport of gp120 might exacerbate the pathogenesis of HIV-1.

1. Introduction

Neurological abnormalities occur in a significant number of human immunodeficiency virus (HIV)-infected individuals during the late phase of infection (Janssen et al., 1989). Post mortem brains of these individuals have revealed neuronal loss, simplification of synaptic contacts, astrocytosis, and microglial nodules with multinucleated giant cells (Everall et al., 1993; Masliah et al., 1997). These pathological alterations occur even though HIV-1 productively infects only macrophages and microglia (Budka et al., 1987; Eilbott et al., 1989) and astrocytes can support only limited viral replication (Tornatore et al., 1994). Additionally, markers of neurodegeneration can frequently be detected in brain areas that are distant from sites of viral replication. Given the fact that HIV infection in the brain is relatively anatomically restricted, an unresolved issue is how low viral load in the brain can cause such widespread neuronal dysfunction.

Experimental evidence has shown that viral proteins such as gp120, the activator of HIV transcription Tat, and accessory proteins Nef and Vpr, can cause neuronal cell death *in vitro* as well as *in vivo* [reviewed in (Matarrese and Malorni, 2005)]. With regards to gp120, chemokine receptors, especially CXCR4, have been proposed to mediate its neurotoxic effect (Hesselgesser et al., 1998; Kaul and Lipton, 1999; Meucci et al., 1998; Zheng et al., 1999). Consequently, much effort has been directed toward characterizing CXCR4 signal transduction mechanisms responsible for gp120 toxicity. However, far less is known about the role of gp120 trafficking in light of recent studies showing that Tat and gp120 can migrate to different brain areas by axonal transport (Bachis et al., 2006; Bruce-Keller et al., 2003). Intriguingly, Tat internalization and transport occur independently from a receptor-mediated mechanism, whereas gp120, at least the T-tropic strain, requires the chemokine receptor CXCR4 for internalization in neurons (Bachis et al., 2003). Interestingly, compounds that prevent gp120 axonal transport also block gp120 neurotoxicity (Bachis et al., 2006).

Axonal transport is a universal mechanism in the nervous system that allows neurons to communicate between distant sites. Molecules that are transported include endogenous proteins, such as neurotrophic factors. These proteins travel along axons to be transported both anterogradely from cell bodies to the terminals or retrogradely from terminals to the cell bodies. Neurodegeneration may occur if the axonal transport is impaired. Moreover, in infectious diseases, exogenous proteins also utilize axonal transport. These include the prion protein (Borchelt et al., 1994) and the tegument proteins of the herpes virus (Bearer et al., 2000) which can be

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neurotoxic. Therefore, it is crucial to establish whether a "suicide transport" is one of the mechanisms that account for the widespread neurotoxic activity of gp120. In addition, it is important to establish whether HIV proteins can be both retrogradely and anterogradely transported and whether axonal transport is also operational in the peripheral nervous system (PNS).

The rat visual system is well characterized in terms of anatomical connections. The superior colliculus receives inputs from retinal ganglionic cells (RGC) (Beckstead and Frankfurter, 1983), primary visual cortex, and prefrontal cortex (Sefton and Dreher, 1995). Fibers from the superior colliculus innervate various nuclei of the thalamus and brain stem (Donnelly et al., 1983; Redgrave et al., 1993; Westby et al., 1990). By virtue of these connections, the visual pathway can be used as an experimental model to examine retrograde and anterograde transport of gp120 in the central nervous system (CNS). Conversely, the sciatic nerve, which contains motor and sensory axons of the dorsal root ganglia (DRG), represents a suitable model to study axonal transport in the PNS. The present study was undertaken to demonstrate axonal transport and neurotoxicity of gp120 in the CNS and PNS.

2. Methods

2.1. Animal treatment

All surgical procedures were performed in strict accordance with the Laboratory Animal Welfare Act, the National Institutes of Health Guide for the care and use of laboratory animals, and after approval from the Georgetown University Animal Care and Use Committee. Adult Sprague-Dawley male rats (250 g) were deeply anesthetized with ketamine/xylazine (80 and 10 mg/kg, i.p., respectively). Gp120 (strain IIIB) and heat-inactivated gp120IIIB (Immunodiagnostics, Woburn, MA) were used at 400 ng in 0.1% bovine serum albumin (BSA). Gp120 and the retrograde tracer fluoro-ruby (FR, 10%, Invitrogen Corp., Carlsbad, CA) or anterograde tracer 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyamine perchlorate (DiI, 10%, Molecular Probes) were delivered into both superior colliculi by a microperfusion pump (0.25 μl/min/10 min) using a 33 GA cannula. Injections into the superior colliculi (both left and right) were done according to the following coordinates: anteroposterior +6.0 mm; mediolateral ± 1.2 mm; dorsoventral −4.0 mm, from bregma (Paxinos and Watson, 1986). After the injection, the needles were left in place for an additional 5 min to accomplish quantitative diffusion of the volume delivered. Animals were then returned to their cages and allowed to survive for 3 or 18 days. At the appropriate survival times, animals were deeply anesthetized with ketamine/xylazine (80 and 10 mg/kg, i.p., respectively), followed by intracardiac perfusion of 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (PPB).

2.2. Preparation of retinae

Retinae were prepared as previously described (Ahmed et al., 2001). In brief, prior to excision of the eyeball, the center of the insertion of superior rectus muscle into the eye ball was marked with ink. Eyes were bisected at the equator, the lenses were removed, and the posterior segments were fixed with 4% PPB for an additional 30 min. Each retina was then isolated and post-fixed in 4% PPB, for 1 h. To prepare flat mounts, retinae were dissected from the underlying sclera/choroid, and flattened by four radial cuts.

2.3. Sciatic nerve injection

Rats were anesthetized as described above. The left sciatic nerve was exposed and firm pressure was applied to the nerve for 30 s to deliver a crush. FR alone or in combination with gp120 (400 ng in 0.1% BSA in 5 μl) or heat-inactivated gp120 (400 ng in 0.1% BSA in 5 μl) was directly injected into the nerve at the crush site. In a group of rats, the uncrushed sciatic nerve was wrapped with gelfoam (Pharmacia & Upjohn Co, Division of Pfizer, New York, NY), prepared in bands of approximately 4 × 8 mm, and saturated with gp120 (400 ng in 0.1% BSA) or heat-inactivated gp120 (400 ng in 0.1% BSA). Gelfoam was applied to the nerve 2–3 mm proximally to the trifurcation using care not to cause nerve injury or constriction. A group of animals received gp120 2 mm distal to DRG. Animals were euthanized by intracardiac perfusion with 4% PPB 3 days after the injection.

2.4. Immunohistochemistry

The brains and sciatic nerves were removed and post-fixed in 4% PPB, then transferred into buffered graded sucrose (10%, 20%, and 30%) and serial cross sections (16 μm) were prepared. Sections were incubated with 2.5% BSA and 0.01% Tritonx100 prior to immunohistochemistry. Retinae were incubated for 48 h and brain sections for 24 h at 4 °C with the following antibodies: Anti-gp120 (5 μg/ml; mouse monoclonal, Immunodiagnostics), anti-caspase-3 (1 μg/ml; rabbit polyclonal, Cell Signaling Technology Inc., Danvers, MA), anti-neurofilament (rabbit polyclonal 1:400, Millipore, Temecula, CA), anti-glial fibrillary acidic protein (GFAP, 4 μg/ml; guinea pig polyclonal, Advanced ImmunoChemical Inc., Long Beach, CA), and anti-CD11b/OX42 (10 μg/ml; mouse monoclonal, Serotech, Raleigh, NC). The latter visualizes active microglia. Sections were then incubated for 1 h at room temperature with the corresponding secondary antibodies: anti mouse IgG FITC conjugate (1:100, Sigma, Saint Louis MO), anti mouse IgG AlexaFluor© 594 (1:500, Invitrogen Corp), anti guinea pig Cy3 (1:100, Jackson ImmunoResearch Laboratories Inc., West Grove, PA). After a brief wash with PBS, slides were then mounted using Vectashield mounting medium with 4′,6′-diamidino-2-phenylindole (DAPI, Vector Lab, Burlingame, CA). Control sections were incubated either with primary or secondary antibody only.

Sciatic nerve sections (both coronal and horizontal) were incubated with anti-gp120, anti-neurofilament, or anti GFAP as described above. In addition, sections were incubated with anti-CXCR4 (5 μg/ml; mouse monoclonal 12G5, NIH AIDS Research and Reference Reagent Program, Rockville MD). Secondary antibodies are described above.

2.5. Fluoro-Jade

Degenerating neuronal somata and their processes were detected with Fluoro-Jade (Histochem, Jefferson, AK) as originally described (Schmued et al., 1997). Brain sections, prepared as described above, were incubated in each of the following solutions for the time indicated: 100% alcohol, 3 min; 70% alcohol, 1 min; dH_2O, 1 min; 0.06% potassium permanganate, 15 min; dH_2O, 1 min; 0.001% Fluoro-Jade in 0.09% acetic acid, 30 min; dH_2O, 2 × 1 min. Stained sections were allowed to dry at room temperature protected from light, dehydrated with xylene and coverslipped with DPX (Aldrich Chemical Company Inc., Milwaukee, WI).

2.6. Histological analysis

Immunofluorescence was analyzed with a Zeiss fluorescence microscope Axioplan2 (Carl Zeiss MicroImaging, Inc., Thornwood, NY) as previously described (Bachis et al., 2006). RGC labeled with FR or gp120 were counted in 72 microscopic fields of retina using a 20× objective and MetaMorph® Imaging software (Universal Imaging Corporation™, Downingtown, PA). Each microscopic field corresponded to an area of 120 × 160 μm. The selected fields were