Neuropathology of NFHgp160 Transgenic Mice Expressing HIV-1 Env Protein in Neurons

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Abstract. The neuropathology of HIV-1 dementia remains largely hypothetical. Although several sets of evidence point towards an indirect multicellular inflammatory pathway, gp120, one of the HIV-1 env products, was shown to be very cytotoxic for neurons in vitro. To explore a direct pathway in the physiopathology of dementia in AIDS, we developed transgenic mouse models carrying the HIV-1 env proteins gp 120 and gp 41 (gp 160) under the control of the human light neurofilament and murine heavy neurofilament promoters. To date, this is the first mouse model in which the HIV-1 env protein can be detected in neurons by immunohistochemistry. The expression is found in several brainstem and spinal cord gray structures and in the cerebellum in one of the mouse lines bearing the NFHgp160 transgene. The morphological findings at 3 months are subtle and are dominated by a watery, dendritic degeneration and a reactive gliosis. At 12 months, the evidence of neuronal degeneration and loss is present along with various degenerative phenomena involving synapses, dendrites and axons, including axonal swellings. Cytoskeletal abnormalities were found by immunohistochemistry. Chronic inflammation was also observed in the leptomeninges of the spinal cord and brainstem and in the cerebellar white matter. These models thus offer an exciting sequence of morphological findings initiated by the neuronal expression of the HIV-1 env proteins and offer a different tool to explore the neuronal dysfunction in AIDS.

Key Words: gp160; HIV-1; Transgenic mice.

INTRODUCTION

The acquired immunodeficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV), with HIV-1 predominating over HIV-2. This member of the Lentivirinae subfamily of retroviruses targets the immune and nervous systems (1). As, observed early in the AIDS epidemic (2), neurological diseases became a significant part of the clinical picture (3, 4). The most frequent neurological disease of the central nervous system (CNS) is HIV-1 associated cognitive/motor complex (5) also referred to as AIDS dementia complex (6) or HIV-1 associated dementia (HIVD) (7). According to various estimates, its incidence ranges from 15% to 20% of patients with AIDS (8, 9) and it is now the leading cause of dementia in the 20–59 age group (10). Morphologically, 2 major entities are consistently associated with HIVD: HIV-1 encephalitis (HIVE) and HIV-1 leukoencephalopathy (HIVL) (7, 9–13). Yet, the lack of correlation between the severity of the dementing illness and the inflammatory changes was recognized early (11). Morphological abnormalities in early stages of HIV-1 infection were defined (14–16). The neuronal population, remarkably normal with conventional studies, was found to be normal or decreased (17–22) by quantitative analysis. When neuronal loss was identified, it was not generalized and neurochemical (23) or structural subpopulations were involved (24, 25). The consensus is that there is no good correlation between the quantitatively determined loss of neurons and the level of dementia. Subcellular abnormalities, such as dendritic simplification and loss of pre-synaptic terminals, have a better potential for correlating with the neurobehavioral abnormalities (21, 26).

The search for the physiopathogenic mechanisms involved in the AIDS dementia complex was further complicated by the lack of evidence of the HIV-1 virus in the neuronal cells. Using different approaches, several laboratories demonstrated the replication and/or expression of HIV-1 mainly in macrophages, multinucleated giant cells, microglial cells (27–32), and vascular endothelial cells (31, 33) but not in neurons. Additionally, Teo et al (32) showed that the presence of unintegrated circular HIV-1 DNA and the active replication and expression of the virus correlated with the presence of multinucleated giant cells and dementia. Thus, the previous reports (34, 35) that showed by in situ RT-PCR hybridization the presence of DNA and RNA in neuronal cells remain to be confirmed.

As the neuron is apparently not the primary target of the HIV-1 CNS infection, several indirect multicellular pathways were proposed and studied in a variety of in vitro and in vivo experimental models (9, 12, 36, 37). Activation of macrophage/microglial cells following infection by HIV-1 appeared to induce secretion of excitotoxins and/or neurotoxins such as glutamate, cytokines of
**Fig. 1.** Structure and restriction map of NFHgp160 plasmid. A: The plasmid Bluescript pSKNFH harboring the 2.5-kb NFH promoter was modified by introducing a Sal I site at the unique Not I restriction site. The 4.8-kb Sal I-Xba I gp160 fragment of HIV-1 HXBc2 strain was introduced in the pUC18 at Sal I and Xba I sites as previously described (56). The 2.5-kb Sal I segment containing the murine NFH promoter was inserted in Sal I site at the 5' end of the HIV-1 4.8-kb fragment. The NFHgp160 transgene was deleted from the vector pUC18 by EcoR I digestion before microinjection. B: The KS-Sty construct was derived to provide the 2.2-kb HindIII subfragment of the HIV-1 env gene as a probe for Southern hybridization analyses.

several types, arachidonic acid, quinolate, platelet-activated factor, nitric oxide synthetase and others (38), potentially targeting the neurons, astrocytes and oligodendrocytes (37, 39). Apoptosis (40–44) and necrosis (38) were also put forward as mechanisms of neuronal loss. Finally, axonal damage in close relationship with blood vessels (45, 46), axonal swellings (47), or focal accumulation of amyloid precursor protein (48) were observed in HIV-E, and even in HIV-1-positive patients without AIDS (49).

Direct pathways involving HIV-1 replication or gene products are still possible. In vitro, it has been demonstrated that the gp120 subunit of the HIV-1 env product was neurotoxic even at low concentration (50). This direct effect of gp120 was associated with an intracellular increase in free calcium (36), which seemed mediated by an excessive activation of N-methyl-D-aspartate (NMDA) receptor-operated channels (36, 51–54). In vitro also, HIV-1 and gp120 induced apoptosis of human central nervous system neurons through the activation of c-Jun N-terminal and p-42 extracellular-regulated kinase (55).

In patients, however, whether neuronal lesions are directly related to gp160 remains undetermined because of the HIV-1 infection process and its systemic effects. In order to better investigate the pathological effects of gp120 on the neuronal cells in vivo we developed transgenic mouse models carrying the HIV-l env gene under the control of 2 neuron-specific promoters: the human neurofilament light (NFL) and the murine neurofilament heavy (NFH) genes. We described the NFLgp160Xba transgenic mice, the expression pattern of the env protein in their CNS, and their pathological evaluation at 4...
months of age (56). In the present report, we describe the pathological changes in the NFHgp160 transgenic mice with emphasis on the neuropathological lesions in older animals, at approximately 1 yr of age.

**MATERIALS AND METHODS**

**Transgene Construct and Transgenic Mouse Development**

The plasmid harboring the NFHgp160 transgene was obtained as follows: the 4.8 kb Sall-Xbal HIV-1 fragment containing the sequence encoding the env protein of HXBc2 provirus was excised from the env expressor plasmid psvIlexE7 and inserted in the pUC18 vector, as previously described (56). The pSK-NFH plasmid (gift of Dr. J. P. Julien, McGill University), harboring the NFH gene promoter was modified by replacing the Not1 site with a Sal1 restriction site. This modified plasmid was used to excise the 2.5-kb Sal segment that contains the NFH promoter. The NFH gene promoter was then inserted in the Sal site at the 5’ end of the env sequence (Fig. 1A). The 7.2-kb NFHgp160 transgene was deleted from the plasmid construct by EcoRI digestion and then purified and microinjected, as previously reported (56, 57). The integration of the transgene into the mouse genome was assessed by Southern blot hybridization of mouse tail DNA using, as probe, the env 2.2-kb HindIII fragment of the KS-Sty construct (Fig. 1B) and conditions previously reported (56). All transgenic mice were developed and maintained in a pathogen-free facility.

**In Vivo Expression of the Env Protein**

Since the expression of the Env proteins has been targeted to the CNS tissue, its presence was primarily sought in the nervous tissue. This was assessed by immunohistochemistry, using vibratome serial sections and monoclonal antibodies against gp41 and gp120 (Dupont NEN Canada, Mississauga, Ontario, Canada) and HIV-1 patient serum. The anesthetic treatment and the fixative transcardiac perfusion of the control and transgenic animals as well as the immunoreactions were carried out as previously described (56). The stereotaxic atlas of Paxinos et al (58) for the rat and the atlas of the mouse brain and spinal cord of Sidman et al (59) were used as anatomical references; the nomenclature used in Paxinos et al (58) was adopted for the present description.

**Neuropathologic Assessment**

*Histopathology:* After transcardiac perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, CNS tissues of 3- and 12-month-old transgenic mice were immersed in 2% paraformaldehyde in the same buffer for 24 h, then embedded in paraffin blocks. Serial 5-μm sections were used for the following stains: hematoxylin-phloxin-safran (HPS), Luxol-cresyl violet, modified Bielschowsky and Holzer.

*Immunohistochemistry:* The indirect immunoperoxidase technique of Sternberger (PAP) was applied on 5-μm sections using the following antisera: glial fibrillary acidic protein (GFAP)
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Fig. 3. Immunoreactions with anti-synaptophysin antibody. Synaptophysin immunostaining of the lumbar anterior gray horns of 3-month-old NFH 1932 transgenic mice (A) and normal control mice (B). Synaptophysin immunostaining of a megasynapse in the anterior gray horn of the spinal cord (C). Magnifications: A, B, ×250; C, ×400.

RESULTS

Development of the NFHgp160 Transgenic Mice

Prior to microinjection, the NFLgp160Xba and NFHgp160 transgenes were tested in transient expression assays. As previously reported (56), the data showed that both constructs expressed and correctly processed the Env precursor and the resulting gp120 and gp41 subunits maintained their biological properties (56). The transgenes were then used for microinjection and their integration in the mouse genome was determined by Southern blot hybridization as described (56). The mouse lines carrying NFLgp160Xba have been phenotypically and genetically characterized (56). With the NFHgp160 transgene (Fig. 1A), 5 out of 33 animals tested (NFH 354, NFH 1932, NFH 1243, NFH 2016, and NFH 2917) were found to harbor 2 to 10 copies of full-length copies of

(Dako, Glostrup, Denmark, 1: 1,000), synaptophysin (Boehringer Mannheim Canada, Laval, Quebec, Canada, 1: 20), neuronal specific enolase (NSE) (Dako, 1: 500). The neurofilament proteins were immunolabeled with antibodies directed against the phosphorylated heavy subunit present in axons (NF-a) and the nonphosphorylated heavy subunit mostly present in the neuronal perikaryon (NF-n) using, respectively, the monoclonals SMI 31 and SMI 32 (Sternberger Monoclonals, Baltimore, MD, 1: 500) and the indirect PAP method. All sections were deparaffinized and treated for 20 min in 3% hydrogen peroxide before incubation for either 1 h (NF-a, NF-n, synaptophysin) or 20 min (all other antibodies). HRP (Zymed Laboratories, San Francisco, CA) was used to label NF-a and NF-n antibodies, while the streptavidin-peroxidase method (Lipshaw Immunon, Detroit, MI) was used for all the other markers.

Finally, in order to screen for co-localization of neuronal loss and reactive gliosis, immunoreactions with monoclonal antibodies against GFAP (Boehringer Mannheim, Canada, 1: 500) and the microtubule-associated protein-2 (MAP-2) (Amersham Pharmacia Biotech, Baie d’Urfé, Quebec, Canada, 1: 400) and FITC-labeled goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, 1: 200) were performed on 5-µm step-sections from the same blocks as for immunoperoxidase studies. Because MAP-2 is mostly expressed in the dendrites it was chosen as a marker to quantitatively evaluate the changes in the dendritic tree. Field selection and image capture and storage were done with a confocal microscope (LSM 410, Zeiss, Germany). Quantitative analyses were carried out with an image analysis system (IBAS, Kontron Elektronik, Germany). Briefly, for each animal the area percentage occupied by the labeling was averaged from 8 microscopic fields of fronto-central cortex, 4 fields from trigeminal (5), facial (7), and hypoglossal (12) brainstem nuclei, and 4 fields from cervical and lumbar spinal anterior horns. These quantitative analyses were performed blindly by 3 different examiners on 5 transgenic mice and 5 age-matched normal control mice.

Ultrastructure: It was carried out as previously described (60). Briefly, for transgenic as well as normal mice, 4 animals (2 at 3 months old and 2 at 12 months) were perfused with 20 ml of 3.5% glutaraldehyde, 1% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). Tissues were immersed in the same fixative solution for 4 h at 4°C. Tissues were then washed in 0.1 M sodium phosphate buffer (pH 7.4) overnight at 4°C, postfixed in 2% OsO4 for 1 h, dehydrated in graded ethanol solutions, immersed in propylene oxide, and embedded in epon resin (J.B. EM.Services, St-Laurent, Quebec, Canada). Sections of 60–90 nm thickness from representative regions of the CNS were mounted onto grids, counterstained with uranyl acetate and lead citrate, and blindly examined by 2 observers with a Philips EM201 microscope.
the transgene. All these transgenic founders except for NFH 354, which was infertile, reproduced normally and transmitted the transgene to their progeny. The mouse lines NFH 1932, NFH 1243, NFH 2016, and NFH 2917 derived from these founders, as well as the normal control animals, were maintained in a pathogen-free facility and have remained healthy and free of infections.

Expression and Distribution of Env Proteins in the CNS of NFH 1932 Mice

Expression of the transgene was assessed in CNS tissues by immunostaining of serial sections from brain and spinal cord of 3- and 12-month-old transgenic and normal control mice using monoclonal antibodies against gp41, gp120, or HIV-1 patient serum, as described (56). As detailed in the Table and illustrated (Fig. 2A–D), all the CNS structures that were immunoreactive in NFLgp160Xba mice (56) were similarly found expressing the transgene in NFH 1932 mice. In addition, immunopositive perikarya were also observed in the following structures: Mesencephalon: ventral pallidum (VP), medial longitudinal fasciculus (mlf). Pons: spinal vestibular nuclei (SpVe), medial vestibular nuclei (MVe), reticulo-tegmental nuclei pons (RtTg), dorsal paragigantocellular nuclei (DPGi), nucleus Y (Y), ventral cochlear nuclei anterior (VCA). Medulla: solitary tract (sol), rostroventrolateral reticular nuclei (RLV), cuneate fascicles (cu), prepositus hypoglossal nuclei (PrH), trapezoid body (tz), linear nuclei of medulla (Li). Finally, the expression of the transgenic proteins was detected in the cerebellum, in the interposed cerebellar nuclei anterior (Int A), medial cerebellar nuclei (Med DL) and lateral cerebellar nuclei (Lat:PC) (Fig. 2A, B) that always tested negative in NFLgp160Xba mice (56). Thus, with the exception of the cerebral and the cerebellum cortices, all the structures normally positive for the neurofilament proteins were found expressing the transgene in NFH 1932 mice.

Neuropathologic Phenotype

Because of the extended expression pattern observed in the NFH 1932 mice, the neuropathological changes and their eventual progression were investigated at 3 and 12 months of age in transgenic and normal control mice. In the 3-month-old transgenic animals, the results and findings obtained with the histochemical and immunohistological studies with the neurofilaments (NF-a, NF-n) and GFAP antibodies were similar to those previously reported for the NFLgp160Xba transgenic mice (56). Further investigations on the brain tissue from these animals using the anti-NSE and synaptophysin antibodies suggested a slight enhancement of the density of dendritic projections in the neuropil of the anterior horns of the spinal cord (Fig. 3A). In these structures, synaptophysin immunostaining showed grain irregularities when compared to controls; occasional aggregates of larger grains were also present (Fig. 3C), which signals a disturbance in distribution and/or size of the synapses. The GFAP and MAP-2 (Fig. 4) analyses of the fronto-central cortex, trigeminal, facial, and hypoglossal nuclei as well as anterior gray horns of the spinal cord by confocal microscopy showed a statistically significant reactive gliosis in the anterior horns of the spinal cord (Fig. 4A). The GFAP reaction was also increased in the cortex, the cranial and brainstem motor nuclei, but this increase was not statistically significant. The MAP-2 was also found slightly augmented for the transgenic animals in each sampled CNS area, but it reached statistical significance only in the cortex (Fig. 4B, C).

At 12 months of age the NFH 1932 transgenic and normal control mice were similarly investigated. With the HPS staining, the transgenic mice demonstrated chronic perivascular inflammation in the leptomeninges of the spinal cord (Fig. 5A) and brainstem and also in the cerebellar white matter (Fig. 5B). In the cerebellum, the anti-synaptophysin antibody reacted positively only with the neurons of the internal granular layer, indirectly confirming the inflammatory nature of these perivascular white matter infiltrates dominated by lymphocytes (Fig. 5B). The anterior gray horns of the spinal cord and the cranial nerve nuclei 5, 7, and 12 showed mild neuronal loss, with occasional degenerated or retracted acidophilic neurons (Fig. 5C, D). These areas demonstrated acidophilic amorphous or fibrillary-like deposits that do not seem to react with any immunohistochemical stains that have been used. Many neuronal intracytoplasmic acidophilic inclusion bodies and intravascular calcifications were more frequently observed in the thalamus of transgenic than in control mice. Additionally, a variety of dendritic and axonal swellings were detected with HPS staining (Fig. 6A, arrows) and also by immunoreactions with the HIV-1 antiserum, synaptophysin, NF-a, as well as NF-n antibodies, with NF-n giving a much stronger signal (Fig. 6B). They were observed mostly in the motor cranial nerve nuclei 5, 7, and 12, in the gray matter of the spinal

Fig. 4. Confocal microscopic evaluation of the GFAP (A) and MAP-2 (B) in the cortex, the motor trigeminal (Mo5), facial (7) and hypoglossal (12) nuclei, and in the anterior gray horns of the spinal cord of control and 3-month-old NFH 1932 transgenic mice. C: Laser confocal images of MAP-2-stained cortex, hypoglossal nuclei (12), and spinal cord in normal (N) and transgenic mice (T).
Fig. 5. Pathological evaluation of the NFH 1932 transgenic mice at 12 months of age. A: Inflammation in the leptomeninges with HPS staining and (B) in the cerebellum white matter with synaptophysin immunostaining that positively reacted with the ectopic granular neurons in the neighboring structures but not with perivascular inflammatory lesion. C: HPS-stained, retracted acidophilic and (D) degenerated neurons. Magnifications: A, C, D, ×640; B, ×500.

cord, particularly in the gray commissures and in the thoracic nuclei and also in the posterior or anterior funiculi. The immunostainings with NSE, NF-n, NF-a, GFAP, and synaptophysin led to the following observations: 1) an increase of the reactive immunohistochemical densities in the neuropil of the anterior gray horns of the spinal cord comparatively to controls; 2) clusters of larger synaptic grains and even megasynapses (Fig. 3C), with some merging the size of axonal swellings; 3) a significant number of perikarya reacting positively with the NF-a antibody (Fig. 6D) in the transgenic mice while they remain negative in the controls (Fig. 6C); 4) a moderate but significant gliosis in the anterior gray horns of the spinal cord (Fig. 7A) when compared to controls (Fig. 7B). The GFAP was also moderately positive in the cortex of the cerebellum (Fig. 7C). In the molecular layer there was patchy Bergmann gliosis. Mild fibrillary gliosis was also present in the internal granular layer. In the deep white matter and nuclei of the cerebellum, the reactive gliosis was present but the reaction was closer to that seen in the control mice. The control mice showed only focal and mild areas of gliosis in the molecular layer (Fig. 7D). No gliosis was observed in the cerebral cortex or in the deep structures of the cerebral hemispheres.

Ultrastructural Analysis

Ultrastructural studies done essentially on the anterior gray horn of the cervical, thoracic and lumbar spinal cord and on the seventh cranial nerve nuclei revealed in transgenic animals several findings largely confined to their dendrites and axons. An early change regularly observed in 3- and 4-month-old transgenic animals was a watery swelling of dendrites with preservation of a few organelles like mitochondria or neurotubules. Complex membranes could be present but these were more frequently found at 12 months (Fig. 8A). At this late age, watery degeneration was still very much present with occasional aberrant mitochondria in the abnormal dendrites (Fig. 8B). The most frequent changes involved the cristae and their orientation, and the matrix, which was sometimes watery, was sometimes replaced by a fibrillar or granular substance. Axonal swellings were observed regularly with myelin sheath degeneration (Fig. 9A) of variable severity. These lesions could be associated with an abnormal accumulation of intermediate filaments. In addition, some myelinated axons were, without being swollen, focally replaced by granular material or dense granular bodies (Fig. 9B). Vacuolar degeneration was observed in certain neuronal projections and it was also...
present focally in neuronal nuclei and the adjacent cytoplasm. Moreover, the neuronal perikarya seemed to be spared although occasional neurons demonstrated a vesicular hyperplasia in the Golgi area. Finally, some dendrites harbored occasional microtubuloreticular inclusions (Fig. 10).

**DISCUSSION**

Transgenic mice harboring and expressing the HIV-1 env gene under the control of neurofilament promoters were successfully generated (56). So far these models are unique in that they are the only ones in which both Env proteins, gpl20 and gp41, are produced in neurons at levels detectable by immunostaining technique. The topography of the protein expression in both NFLgp160Xba and NFH 1932 transgenic mice included several structures in the brainstem, cerebellum, and the spinal cord, but spared the cerebral cortex, the limbic system and essentially all the subcortical supratentorial structures. Explanation for this topography remains as yet unclear. We can reasonably exclude developmental intra-uterine death of Env producing neurons as the expression of the protein appears at day 8 of the postnatal period. In addition, these spared structures do not show any reactive gliosis that could signal a postnatal neuronal loss, even if enhanced intra-uterine or neonatal developmental apoptosis presumably would leave no trace. Nevertheless, these mice allowed us to demonstrate at the cellular and subcellular levels some of the pathological changes induced by the transgene products in absence of the effects related to systemic HIV-1 infection.

Among the possible viral factors suspected for the neuronal dysfunction or loss in HIVE or HIVL, gpl20 became a prime suspect following the in vitro demonstration that, at low concentration, this protein was highly neurotoxic to neurons. Several direct and indirect pathways were evoked to explain this gp120-induced toxicity (38, 50–52, 61). Our transgenic mouse models allow selection of 1 specific hypothesis (direct pathway) out of several others in which neurons are not directly affected by the virus but are secondarily involved through different cellular reactions (37).

The morphological evaluation of these 2 transgenic mouse lines strongly suggested an initial neuronal involvement. By ultrastructure, at 3 months of age the initial lesion was a segmental watery degeneration of the...
neuronal dendritic tree. Its enhancement with some cytoskeletal markers (NF-a, NF-n, for example) without ultrastructural abnormalities of the cytoskeleton at that stage, suggested a prefibrillogenesis derangement. At 3 months this watery change was found associated with axonal swellings that were best revealed with the HIV-1 antiserum, indicating an axonal transport of the transgenic proteins. These swellings were not very numerous when compared to our findings in the 12-month-old mice. This very early change probably reflects the effect(s) of the transgene (or part of it v.g. gp120) on the cellular membrane of the neurons. However, whether these effects could be related to an increase of intraneuronal free calcium by gp120 as reported for neuronal cell cultures (36, 51–54) remains to be determined. Further studies aimed at measurements of intracellular calcium as well as the state of the calcium dependent channels in the Env expressing neurons are needed to help determine the underlying mechanisms for these lesions. In addition, studies on 2 different calcium binding proteins, calbindin and parvalbumin, in the CNS of moderate and severe HIVE patients suggested that different pathogenetic mechanisms could be involved and these may vary in different regions of the brain (62).

Beyond the watery dendritic and axonal changes, the morphometric analysis with the MAP-2 protein and the synaptophysin immunostaining patterns suggested an increased density of the neuronal projections along with irregularities of synapses. Moreover, the neuronal loss became obvious at 12 months of age as degenerating neurons were observed by conventional histology. The gliosis already detected at 3 months by conventional histological and immunohistochemical techniques and confirmed by morphometric analysis is likely secondary to all these neuronal changes. However, the cellular reaction went beyond the reactive gliosis as chronic inflammation was found in the leptomeninges and white matter of the cerebellum in the NFH 1932 line at 12 months of age. The spleens of these animals also showed a reactive hyperplasia (data not shown). We cannot exclude that this

Fig. 7. Immunoreactions with anti-GFAP antibodies on 12-month-old NFH 1932 transgenic and normal control mice. This antibody showed a moderate but significant gliosis in the anterior gray horns of the spinal cord of transgenic mice (A), similar to that observed in younger animals of the same line. In the normal control (B), only rare astrocytes are observed in the same areas. In the cerebellum, the GFAP antibodies demonstrated moderate gliosis in the cerebellar cortex of the transgenic mice (C). The same antibodies show focal limited areas of gliosis in the molecular layer of the normal control (D). Magnifications: A, B, ×250; C, D, ×160.
inflammation could influence the morphological picture in the CNS and, more specifically, the neuronal damage by the introduction of indirect cytotoxic pathways.

The immunohistochemical reactions on control and transgenic animals with NF-n and NF-a antibodies revealed abnormal enhancement in the neuropil and/or aberrant reactions in the perikarya, all indicating profound dysregulation in the processing of these cytoskeletal proteins. Recently, similar alterations of the neurofilaments and severe cortical neuronal changes were reported in cats infected with the feline immunodeficiency virus (FIV), a lentivirus sharing many properties with HIV-1 (63). In this animal model, the SMI 32 antibodies used for CNS investigations reacted with pyramidal neurons.
only in the frontal neocortex and showed a significant increase in their number in the infected animals. In the rat neonate model, dystrophic neurons were generated in the neocortex following systemic injection of purified gp120. This was associated with delayed developmental milestones and complex motor behavior (64). Thus, both these studies and our findings clearly indicate early alterations of the neuronal cytoskeleton by viral infection or gp120 alone. Following our findings on the transgenic mice with NF-a and NF-n antibodies, we used the same
Antibodies for preliminary studies on 5 HIV-1 patients (including one already reported [47]) and 3 controls. Striking changes were observed in some of these patients, with marked increase and/or aberrant localization of the immunoreaction in the neocortex. Although these changes were similar to those observed in the transgenic mice it is premature to draw definite conclusions and establish a firm parallel between these and HIV-1 patients because of the possible artifacts associated with postmortem material. Nevertheless, these results underline the frequent presence of neurofilament modifications in HIV-1-associated neurological diseases.

The watery vacuolization found in our transgenic mice is reminiscent of the observations made in humans. The vacuolization of the dendritic processes was observed in patients by electron microscopy (49, 65) but it was never illustrated, likely because autopsy material is not optimal for electron microscopic studies. Indeed, the vast literature on AIDS remains silent on ultrastructural subcellular neuronal changes. In vitro, by electron microscopy, Yeung et al (66) described large cytoplasmic vacuoles in neural cells with morphology consistent with neurons. They used primary cultures of human brain cell aggregates that contained all cerebral cell types. The vacuoles were obtained by addition of interleukin-6 to the culture medium, while addition of gp120 produced changes that were mostly nuclear. The neuronal dendrites and axons were not described (66). In vivo, pathologic distension of the smooth endoplasmic reticulum (SER) was found to correlate with the dendritic vacuolization of pyramidal neurons in the neocortex of gp120 transgenic mice (65). The authors suggested that calcium toxicity could play a role through aberrant activation of the inositol phospholipid pathway, which is linked to the glutamate receptors known to be highly concentrated in dendritic SER.

In conclusion, the NFLgpl60Xba and NFHgpl60 transgenic mice demonstrate an exciting sequence of events initiated with neuronal expression of HIV-1 Env proteins in neurons at 8–10 days after birth. The subsequent morphological changes, although nonspecific, can lead to a variety of cellular, biochemical, and molecular investigations. Prior to the development of chronic inflammation, it will be possible to explore a direct pathway linking gp120, the neuron, and its glial environment. With
the presence of inflammation, the exploration of indirect pathways becomes a possibility not only in the confinement of the CNS but also between the CNS and the extracranial compartment. This laboratory animal model could contribute to the understanding of neuronal dysfunction in AIDS and in other neurodegenerative disorders.

ACKNOWLEDGMENTS

Jean Michaud would like to thank Jean-Jacques Hauw and the medical staff of the Laboratoire de Neuropathologie R. Escourroulé, Hôpital La Salpêtrière, Paris, for stimulating discussions and academic support during his sabbatical year. We also thank M. E. Weinreb for reviewing the manuscript. We are grateful to F. Lacourse-Brière, K. Dupuis, F. Saker, D. Rodrigue and G. Lambert for technical and photographic support.

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