Induction of Cell-Cycle Regulators in Simian Immunodeficiency Virus Encephalitis

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Neuronal degeneration associated with human immunodeficiency virus encephalitis has been attributed to neurotoxicity of signaling molecules secreted by activated, infected macrophages. We hypothesized that the barrage of signals present in the extracellular milieu of human immunodeficiency virus-infiltrated brain causes inappropriate activation of neuronal cell-cycle machinery. We examined the presence of three members of the cell-cycle control machinery: pRb, E2F1, and p53 in the simian immunodeficiency virus encephalitis (SIVE) model. Compared to noninfected and simian immunodeficiency virus-infected, nonencephalitic controls, we observed increased protein expression of E2F1 and p53 and aberrant cellular localization of E2F1 and pRb. In SIVE, E2F1 was abundant in the cytoplasm of neurons in both neurons and astrocytes proximal to SIVE pathology in the basal ganglia. pRb staining was nuclear and cytoplasmic in cortical neurons of SIVE cases. Antibodies to phosphorylated pRb also labeled the cytoplasm of cortical neurons. These data suggest that in SIVE, cell signaling results in phosphorylation of pRb which may result in subsequent alteration in E2F1 activity. As increased E2F1 and p53 activities have been linked to cell death, these data suggest that the neurodegeneration in SIVE could in part be because of changes in expression and activity of cell-cycle machinery. (*Am J Pathol* 2000, 157:497–507)

Human immunodeficiency virus encephalitis (HIVE) is observed in ~25% of AIDS autopsies^{1–4} and a more variable percentage of simian immunodeficiency virus (SIV)-infected macaques.^{5–12} Lentiviral encephalitis is a peculiar chronic encephalitis in that severe neuronal damage occurs despite an absence of significant neuronal infection.^{5,6,9,13,14} The pathogenesis of neurodegeneration in HIVE and simian immunodeficiency virus encephalitis (SIVE) is unknown; however, it appears linked to abundant activated and lentiviral-infected brain macrophages.^{5,8,12,15–24} Numerous studies have suggested

that these activated macrophages may secrete direct or indirect toxins that act on neuroglial elements and lead to synaptic damage and neuronal death.^{25–35}

Several studies have shown a tight association between the presence of activated and lentiviral-infected central nervous system (CNS) macrophages and aberrant cell-signaling molecules (eg, cytokines, chemokines, neurotrophic factors).^{25–35} As some of these molecules are capable of signaling the initiation of cell cycle in nonneuronal cell types, we hypothesized that the aberrant CNS milieu may lead to inappropriate expression of cell-cycle proteins within terminally differentiated neuronal elements leading to their chronic damage and eventual death.

The signaling molecules secreted by HIV-infected macrophages may stimulate a cell to divide, differentiate, or die. These processes are regulated by the activities of p53, pRb, and E2F1 proteins.^{36–41} The functions of pRb and E2F1 are linked through direct interaction between these two proteins.^{42,43} E2F1 is an activator of transcription that binds DNA as a heterodimer with the DP1 protein.^{44,45} This DNA:protein complex acts as a transcriptional repressor when interacting with pRb.^{46–49} Phosphorylation of pRb disrupts this interaction allowing the E2F complex to activate expression of genes needed for entry into the cell cycle and completion of S phase.^{36,40,50,51} In addition to regulation of S-phase genes, E2F1 has additional functions separate from other members of the E2F family. Both *in vivo* and *in vitro* evidence support a role for E2F1 in regulation of cell death.^{52,53} Key evidence supporting a role for E2F1 in cell death was the rescue of the severe neuronal death in the CNS of pRb deficient mice by concomitant deletion of E2F1.^{54,55} These data are supported by many *in vitro* studies showing that increased E2F1 expression mediates apoptosis.⁵³

Given that cell-cycle proteins have been found to participate in neuronal death and the environment in the HIV-infected brain is conducive to cell-cycle protein induction, we proposed to test if cell-cycle proteins were being activated. We elected to use the SIVE model to study the pathogenesis of neurodegeneration seen in AIDS. Depending on the specific simian host and SIV

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strain, immunocompromised monkeys develop neuropathological changes that share many similarities with the human disease.^{5,8,9,11,12,56} Although the time course and anatomical distribution of SIVE is model-dependent, the end-stage histopathology is remarkably similar to that observed in HIV. We used this model to study the expression patterns of cell-cycle proteins E2F1, pRb, and p53 in response to lentiviral infection by immunohistochemistry and immunoblot analysis.

Materials and Methods

Animal Model

Rhesus macaques were housed and maintained according to strict American Association of Laboratory Animal Care standards. Macaques were derived from vaccine trials, infected, and sacrificed. SIVE was empirically defined as the presence of abundant perivascular mononuclear infiltrate and microglial nodules. Multinucleated giant cells were present in some lesions of all cases.

Protein Extracts and Immunoblotting

Protein extracts were prepared from basal ganglia and frontal cortex of three uninfected control monkeys, two SIV-infected monkeys, and three SIV-infected, encephalitic monkeys as previously described.⁵⁷ Tissues were homogenized on ice in phosphate-buffered saline with protease inhibitors (5 mmol/L phenylmethyl sulfonyl fluoride, 2 μ g/ml pepstatin A, and 1 μ g/ml leupeptin) until there were no large chunks. Separated cells were collected by centrifugation at 3,000 rpm for 5 minutes at 4°C. Supernatants were removed to a separate tube and pellets were resuspended in 4 volumes hypotonic buffer (20 mmol/L Hepes, pH 7.9, 1.5 mmol/L MgCl₂, 10 mmol/L KCl, 0.5 mmol/L dithiothreitol, 0.5 mmol/L phenylmethyl sulfonyl fluoride, 2 μ g/ml pepstatin A, and 1 μ g/ml leupeptin). The suspension was homogenized for 10 seconds and incubated on ice for 15 minutes. The cells were

collected by centrifugation at 13,000 $\times$ *g* for 30 minutes. The supernatant was labeled "S1" and the pellet was further extracted with high-salt buffer (0.42 mol/L NaCl, 20 mmol/L Hepes, pH 7.9, 1.5 mmol/L MgCl₂, 0.2 mmol/L ethylenediaminetetraacetic acid, 0.5 mmol/L dithiothreitol, 25% glycerol, 0.5 mmol/L phenylmethyl sulfonyl fluoride, 2 μ g/ml pepstatin A, and 1 μ g/ml leupeptin) on ice for 20 minutes. Residual insoluble material was removed by centrifugation at 14,000 $\times$ *g* for 30 minutes. The supernatant fraction was collected and termed "S2". Protein concentrations for each fraction were determined by the Biorad protein assay. Two hundred μ g of S1 and S2 extract from each sample was loaded onto a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for E2F1 and p53 and a 7.5% SDS-PAGE for pRb. These proteins were only detected in the S2 fraction.

The proteins were transferred from the SDS-PAGE to Immun-Blot polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA) by electrophoresis and blocked in 5% normal goat serum in Tris-buffered saline (TBS; 10 mmol/L Tris, pH 8.0, 150 mmol/L NaCl). All antibodies were used at 1:1,000 in 0.5% milk overnight at 4°C. Blots were washed three times in TBST (TBS + 0.1% Tween 20) for 15 minutes. Goat anti-mouse horseradish peroxidase (1:5,000; Jackson Laboratories, Bar Harbor, ME) and goat anti-rabbit horseradish peroxidase (1:5,000; Jackson Laboratories) was used to detect the appropriate primary antibodies. The secondary antibody was washed extensively in TBS, three times for 20 minutes. The antibody was then visualized using enhanced chemiluminescence (Renaissance; NEN Life Science Products, Inc.).

Immunohistochemistry and Immunofluorescence

Paraffin-embedded sections were heated to 50°C for 20 minutes and deparaffinized in HistoClear (3 $\times$ 15 minutes) (National Diagnostics, Atlanta, GA). Sections were

Table 1. Antibody Dilutions Used for Immunohistochemistry and Immunoblot

Antigen	Antibody Type	Immunoblot dilution	IHC dilution	Source
E2F1	Mouse monoclonal	1:1,000	1:100	Santa Cruz Biotechnology, Inc. (Santa Cruz, CA)
pRb	Mouse monoclonal	1:1,000	1:100	Santa Cruz Biotechnology, Inc. (Santa Cruz, CA)
p53	Mouse monoclonal	1:1,000	1:100	Santa Cruz Biotechnology, Inc. (Santa Cruz, CA)
Phospho-Rb	Rabbit polyclonal	1:1,000	1:100	New England Biolabs, Inc. (Beverly, MA)
GFAP	Rabbit polyclonal	N/A	1:500	DAKO (Carpinteria, CA)
HAM-56	Mouse monoclonal	N/A	1:100	DAKO (Carpinteria, CA)
MAP-2	Mouse monoclonal	N/A	1:500	Sternberger Monoclonals, Inc. (Lutherville, MD)

Information on the antibodies used for immunoblot or immunohistochemistry (IHC) is listed. Also indicated are the type and source of each antibody used. NA, not applicable.

Figure 1. SIV-infected macaques with encephalitis show increased immunostaining for E2F1 and pRb. **A:** Macaques infected with SIV but without SIVE showed little to no staining for E2F1 in the basal ganglia. **B:** Macaques with SIVE showed robust staining for E2F1 in both nuclei and cytoplasm of basal ganglia cells. **C:** E2F1 staining was particularly intense in the cytoplasm of cells surrounding multinucleated giant cells. **D:** In the basal ganglia of macaques with SIVE Rb staining was restricted to select nuclei. **E and F:** In the cortex of macaques with SIVE Rb staining was observed in both cytoplasm and nuclei of cells with neuronal morphology particularly in regions with microglial nodules (arrows). Scale bar, 20 μ m.

rehydrated as follows: 100% alcohol for 10 minutes, two times; 95% alcohol for 10 minutes; 90% alcohol for 10 minutes; 70% alcohol for 10 minutes; and H₂O for 5 minutes. Endogenous peroxidase activity was inactivated by immersing in 3% H₂O₂ for 30 minutes. Antigen unmasking was performed by bringing sections to a boil in 10 mmol/L sodium citrate, cooling 5 minutes, and bringing to a boil again in the microwave. After gradual cooling to room temperature, tissue sections were blocked with 10% normal goat serum. Antibodies to E2F1, p53, and pRb previously characterized for immunohistochemistry^{58–60} were used at commercially recommended and

empirically defined dilutions (Table 1) and detected by the tyramide amplification system (New England Biolabs, Beverly, MA). For immunofluorescent studies, antibodies to MAP2, GFAP, and Ham-56 (Table 1) were used at stated dilutions without amplification. The fluorogen used is listed in the Figure legends. Immunocytochemical staining used an amino ethyl carabazole detection system (Biogenex, San Ramon, CA) and slides were mounted in crystal mount. Immunofluorescent slides were mounted in gelvetol⁶¹ and analyzed by laser confocal microscopy (Molecular Dynamics, Sunnyvale, CA), as previously described.⁶²

Figure 2. In the frontal cortex of macaques with SIVE nuclei of astrocytes and neurons immunostain for Rb as does the cytoplasm of some neurons. Frontal cortex from macaque with SIVE immunostained for pRb (red) and MAP2 (green) in (**top**) and GFAP (green) in (**bottom**) visualized by double-label immunofluorescent laser confocal microscopy. Single label for the cell type marker (red), pRb (green), and an overlay of the two images. Yellow-orange shows co-localization. Increased Rb staining was predominantly localized to nuclei of neurons and astrocytes, however, some MAP2-positive neurons also show cytoplasmic staining (**arrows**). Scale bar, 20 μ m.

Results

SIV-Infected Macaques with Encephalitis Show Increased Immunostaining for E2F1 and pRb

The soluble factors released by activated macrophages have the potential to initiate numerous intracellular signaling cascades. We hypothesized that cell-cycle machinery will be altered in neurons in response to a variety of signals introduced by infiltrating lentiviral-infected, activated macrophages. To examine this hypothesis, we assessed the expression patterns of three key cell-cycle regulators, E2F1, pRb, and p53, in the basal ganglia and frontal cortex of three control monkeys, three monkeys infected with SIV, and three monkeys with SIVE. Both control and SIV-infected monkeys without encephalitis exhibited no staining for E2F1 in the basal ganglia (Figure 1A) or frontal cortex (data not shown). In the basal ganglia of the SIVE cases there was robust E2F1 staining in the nuclei and cytoplasm of numerous cells (Figure 1B). This cytoplasmic staining was observed in cells surrounding multinucleated giant cells (Figure 1C), a hallmark pathological feature of SIVE. We did not observe any E2F1 staining in the frontal cortex of SIVE cases.

Staining for pRb was not observed in the basal ganglia and frontal cortex of control and SIV-infected monkeys without encephalitis. In basal ganglia of monkeys with

SIVE, pRb was found in select nuclei (Figure 1D). However, in the frontal cortex of monkeys with SIVE, pRb was cytoplasmic and nuclear in cells with the morphology of neurons (Figure 1, E and F). Nuclear staining was observed in cells with the morphology of astrocytes. As seen with E2F1, cytoplasmic pRb staining was observed in cells near microglial nodules, a pathological feature of the disease. Figure 1E shows cytoplasmic staining in the vicinity of a microglial nodule (arrows). These data indicate that cytoplasmic pRb increases during SIVE progression. Staining for p53 was predominantly nuclear and was the same in all three experimental conditions.

Rb Stains the Cytoplasm of Neurons in the Frontal Cortex

To confirm that pRb cytoplasmic staining in the frontal cortex occurs in neurons, we performed double-label laser confocal microscopy (DLCM) with pRb and CNS cell markers. Using DLCM we were now able to detect pRb in nonencephalitic controls showing that pRb staining was nuclear predominantly in cells staining positive for MAP2, a neuronal marker (data not shown). In SIVE cases pRb staining was also predominantly nuclear in the frontal cortex, but select cells staining for the neuronal marker, MAP2, showed cytoplasmic staining of pRb (Fig-

Figure 3. Neurons of the basal ganglia and frontal cortex of macaques with SIVE show cytoplasmic staining for E2F1. E2F1 (Cy3, red), and MAP2 (FITC, green) labeling in the basal ganglia (**top**) and frontal cortex (**bottom**) of a macaque with SIVE. Each marker is shown separately and as an overlay of the two images to show co-localization (yellow-orange). Scale bar, 20 μ m.

ure 2, top). pRb stains the nuclei of select astrocytes as indicated by the presence of pRb staining in GFAP-positive cells (Figure 2, bottom). pRb was also found in the nuclei of HAM-56-positive microglia (data not shown). In the basal ganglia, nuclear pRb was observed in neurons, astrocytes, and microglia. These data demonstrate that cytoplasmic staining of pRb occurs predominantly in neurons in the frontal cortex during SIVE.

Cytoplasmic Staining of E2F1 Occurs in Neurons of the Basal Ganglia and Frontal Cortex

Immunostaining for E2F1 in the basal ganglia of encephalitic monkeys shows a dramatic increase in cytoplasmic E2F1 staining. To determine whether neurons contained E2F1 cytoplasmic staining, we performed DLCM for E2F1 and the neuronal marker MAP2. Using DLCM we were able to determine that E2F1 was predominantly nuclear in MAP2-positive cells in the basal ganglia and frontal cortex of nonencephalitic controls (data not shown). In the basal ganglia of SIVE cases, cytoplasmic E2F1 was found to co-localize with MAP2 (Figure 3, top) showing that in the basal ganglia, E2F1 is found in the cytoplasm of neurons. Cytoplasmic E2F1 was also observed in MAP2-positive neurons in SIVE frontal cortex (Figure 3, bottom), although this staining was less robust than in the

basal ganglia. These data suggest that E2F1 is found in the cytoplasm of neurons in both the basal ganglia and the frontal cortex of SIVE monkeys.

Cytoplasmic Staining of E2F1 Occurs in Astrocytes of the Basal Ganglia but Not the Frontal Cortex

In the basal ganglia, cytoplasmic E2F1 staining co-localized with GFAP-positive cell bodies and processes (Figure 4, top). This is in contrast to what we observed in the frontal cortex where E2F1 was predominantly nuclear in GFAP-positive cells (Figure 4, bottom). Some E2F1-positive cell bodies were observed in the frontal cortex, but process staining was virtually absent. In both brain regions, some GFAP-negative cells exhibited E2F1 staining in the nucleus or cytoplasm. On the basis of their size and morphology these cells are likely neurons. In areas containing HAM-56-positive microglia, E2F1 was found in the nuclei of microglia, but in the cytoplasm of surrounding cells (data not shown). These data support a redistribution of E2F1 from the nucleus to the cytoplasm in neurons and astrocytes of the basal ganglia in areas containing activated microglia, whereas in the frontal cortex, it is the neuronal nuclei and cytoplasm that contain E2F1.

Figure 4. In SIVE, cytoplasmic staining of E2F1 occurs in astrocytes of the basal ganglia but not the frontal cortex. Double-label immunofluorescent confocal microscopy for E2F1 (Cy3, red) and GFAP (FITC, green) are shown for the basal ganglia (**top**) and the frontal cortex (**bottom**) of a SIVE case. Single labels are shown in the appropriate color followed by double-label on right. Co-localization will appear yellow-orange. In SIVE E2F1 staining extends into the cytoplasm of astrocytes in the basal ganglia, however in the frontal cortex staining is predominantly restricted to nuclei. Scale bar, 20 μ m.

The pRb Observed in the Neuronal Cytoplasm of SIVE Cases Is Phosphorylated

Because pRb is regulated by phosphorylation, an antibody recognizing the serine-795 phospho-isoform of pRb was used to assess the phosphorylation status of pRb in SIVE. In nonencephalitic controls, there was no staining for phosphorylated pRb in the frontal cortex (Figure 5, SIV-CTX). However, in the cortex of SIVE cases, staining for phospho-Rb was observed in the nucleus and cytoplasm of cells with the morphology of neurons (Figure 5, SIVE-CTX). The staining in the cortex is consistent with the previous observation that pRb is cytoplasmic in neurons suggesting that cytoplasmic pRb in cortical neurons is phosphorylated.

Expression of E2F1 and p53 Is Increased in Basal Ganglia of SIVE Cases

The robust staining for E2F1 in encephalitic monkey basal ganglia in comparison to the complete absence of staining in the nonencephalitic cases suggests an increase in expression of E2F1. To test this, a quantitative immunoblot was done using protein extracts from the basal ganglia and frontal cortex of three uninfected controls, two SIV-infected, nonencephalitic cases, and three

monkeys with SIVE. By immunoblot, E2F1 protein levels increased in the basal ganglia of SIVE cases (Figure 6). These data suggest that E2F1 expression is altered in SIVE. p53 also exhibited an increase in protein levels in SIVE cases as compared to nonencephalitic controls in the basal ganglia (Figure 6), whereas pRb remained constant in the basal ganglia and frontal cortex (data not shown, Table 2). These data suggest that p53 protein levels increase even though changes in subcellular localization were not detected.

Discussion

Neurodegeneration in lentiviral encephalitis has been linked to several soluble molecules released by activated macrophages including chemokines and neurotrophic factors. Yet the effects of these signals on the cells of the CNS are not understood. Potential targets for these factors include key regulators of the cell cycle: pRb, E2F1, and p53. Here we show changes in expression and subcellular localization of these proteins in SIVE (summarized in Table 2).

With respect to pRb, we observed a change in subcellular localization in neurons proximal to microglial nodules. The change in subcellular localization was accompanied by alteration in the state of phosphorylation of

Figure 5. The pRb observed in the neuronal cytoplasm of SIVE cases is phosphorylated. SIV-infected macaques exhibit little staining for a phospho-isoform of pRb in the frontal cortex (SIV-CTX). In SIV-infected monkeys with encephalitis phosphorylated pRb is present in the cytoplasm of numerous cells in the frontal cortex (SIVE-CTX).

pRb. The presence of hyperphosphorylated pRb is consistent with activation of numerous signaling cascades.^{38,39,63,64} Although it has not been shown specifically for neurotrophic factor receptors, activation of other tyrosine kinase receptors akin to trkA, trkB, and trkC results in phosphorylation of pRb.^{65,66} Hyperphosphorylation of pRb renders it inactive, abrogating its ability to bind the transcriptional activator E2F1.^{38,39} When pRb is complexed with E2F1 in the nucleus, expression from promoters containing E2F1 binding sites is repressed. Cytoplasmic localization of hyperphosphorylated pRb would suggest an alteration in the activity of E2F1. The function of cytoplasmic pRb is not clear but its presence in the cytoplasm of neurons has not been previously reported (Figure 7).

Staining for the E2F1 protein was also distinct in SIVE. E2F1 protein levels increased in the basal ganglia where there was abundant cytoplasmic staining in both neurons and astrocytes. This cytoplasmic staining was observed in the vicinity of multinucleated giant cells and activated microglia, suggesting that SIVE alters E2F1 expression

and subcellular localization. This spatial association should be further evaluated when double-label protocols are available to co-localize the cell-cycle regulators and SIV.

As a transcription factor, E2F1 activities are believed to take place primarily in the nucleus where increased E2F1 expression normally initiates S phase of the cell cycle.⁶⁷ In differentiating neurons increased expression of E2F1 has been found to cooperate in p53 dependent apoptosis or initiate p53-independent apoptosis.^{54,55} Increased p53 protein levels may suggest that E2F1 is acting in a p53-mediated apoptotic mechanism. We did not observe altered p53 subcellular localization in SIVE compared to control and SIV nonencephalitic cases, however, by immunoblot analysis there was an overall increase in p53 protein levels in the basal ganglia of SIVE cases. These findings show that components of the cell cycle are altered in neuroglial elements during lentiviral encephalitis. Future studies will examine whether the alteration is associated with altered neuronal function or survival.

Figure 6. Expression of E2F1 and p53 is increased in basal ganglia of SIVE cases. The amount of E2F1 and p53 present in control (lanes 1–3), SIV (lanes 4 and 5), and SIVE (lanes 6–8) cases was determined by immunoblot. Two hundred μ g of protein were loaded into each lane of a 10% SDS-PAGE. The gel was immunoblotted for E2F1, p53, and actin as a control. Expression of these proteins was assessed in protein extracts from both the basal ganglia (top) and the frontal cortex (bottom).

A role for E2F1 in cell death independent of p53 has recently been linked to the ability of E2F1 to reduce protein stability of the TRAF2 protein⁶⁸ (Figure 7). TRAF2 prevents apoptosis initiated by death receptor activation.⁶⁹ Because changes in TRAF2 stability by E2F1 are dependent on the level of E2F1 protein expressed and not on its transcriptional activity, the observed increase in cytoplasmic E2F1 expression in SIVE may lead to degradation of TRAF2 (or a TRAF2 family member) by E2F1.⁶⁸

Cytoplasmic localization of E2F1 in SIVE would be consistent with its role in TRAF2 degradation leading to insufficient TRAF2 levels compromising cell survival. It is possible that either or both of these pathways are contributing to neuronal dysfunction in SIVE. Further investigation is required to elucidate the specific pathways involved.

Increased expression of E2F1 or deregulation of its activity in the nucleus would be expected to result in re-entry into the cell cycle.^{50,51} Because neurons are postmitotic, they may have a mechanism to prevent entry of E2F1 into the nucleus. However, if the amount of E2F1 were to exceed the amount of resident pRb, the cell may induce S-phase specific genes. At low levels this may result in production of enzymes needed for DNA and cellular repair. However, at high levels, increased E2F1 levels could turn on genes that would be inappropriate in a terminally differentiated cell. It will be interesting to study whether this leads to neuronal death or CNS dysfunction.

Re-activation of cell-cycle proteins has been implicated in another neurodegenerative disease, Alzheimer's disease.^{70,71} Increased p53 and pRb staining have been reported^{72,73} as well as regulators of pRb activity the cyclin dependent kinases and their inhibitors.^{74–77} Cyclin dependent kinases, pRb, and E2F activity have been implicated in β -amyloid toxicity in an *in vitro* model of Alzheimer's disease.⁷⁸ These data suggest that further experiments defining the role of cell-cycle proteins in neuronal loss may have implications for other neurodegenerative diseases.

Deciphering the pathogenesis of lentiviral-associated neurodegeneration remains a daunting task. Primary neuronal damage must in some way be related to activated and infected macrophages,^{25–35} but the pathway may be very indirect. Simply deciphering involved cells is complicated by the time course of the disease, number of potentially involved cells and downstream effects of damage between interconnected regions of the brain. The clinical symptomatology and pathology of HIVE suggests that the disease begins in subcortical structures and spreads to cortical regions.^{35,79–84} It is not clear that this

Table 2. Summary of Protein Localization and Expression for pRb, E2F1, and p53

	pRb		E2F1		p53	
	Non-E	SIVE	Non-E	SIVE	Non-E	SIVE
Basal ganglia						
Neurons	Nuclear	Nuclear	Nuclear	Cyto	Nuclear	Nuclear
Astrocytes	Nuclear	Nuclear	Nuclear	Cyto	Nuclear	Nuclear
Microglia	Nuclear	Nuclear	Nuclear	Nuclear	NT	NT
Frontal cortex						
Neurons	Nuclear	Cyto	Nuclear	Cyto	Nuclear	Nuclear
Astrocytes	Nuclear	Nuclear	Nuclear	Nuclear	Nuclear	Nuclear
Microglia	Nuclear	Nuclear	Nuclear	Nuclear	NT	NT
Protein expression						
Basal Ganglia	Same-NS		Increased in SIVE		Increased in SIVE	
Frontal Cortex	Same-NS		Same		Same	

Subcellular localization of pRb, E2F1, and p53 in specific CNS cell types is indicated as nuclear or cytoplasmic (Cyto). Changes in protein expression are also listed with regard to specific brain regions tested. NT, not tested; Non-E, non-encephalitis; NS, not shown; SIVE, simian immunodeficiency virus encephalitis.

Figure 7. Model of the proposed role for cell-cycle regulators in the SIVE progression. In normal neurons, E2F1 and pRb are found in the nucleus in complex with DP1 bound to promoter elements of S-phase-specific genes maintaining low expression of these genes (**left**). At this time TRAF2 is bound to the death receptor (DR) such as p75^{NTFR} and initiates a survival signal (**left**). In response to extracellular signals released by infiltrating macrophages, pRb becomes phosphorylated and accumulates in the cytoplasm (**right**). E2F1 expression increases and accumulates in the cytoplasm where it causes degradation of TRAF2 (**right**). Degradation of TRAF2 allows the death receptor (DR) to interact with death domain containing proteins. Stimulation of the death receptor by neurotrophic factors (NTF) will lead to activation of the death domain proteins leading to cell death.

is the case with SIVE. Nevertheless, our quantitative immunoblots suggest the E2F1 expression in the basal ganglia of SIVE cases exceeds that observed in the frontal cortex. If E2F1 were related to neurodegeneration, this observation would be consistent with early involvement of the basal ganglia.

In the current study we have attempted to examine the hypothesis that induction of cell-cycle regulators may spatially correlate with presence or absence of histopathology. In addition to identifying quantitative increases in cell-cycle regulatory proteins, we localized these proteins to neuroglial elements in differentially involved regions of the brain. Inferences regarding the connection between presence of SIV-infected macrophages and neuroglial levels of E2F1 would be strengthened by quantitation of viral expression and comparison to E2F1 levels. Correlation of E2F1 expression with markers of neuronal dysfunction and cellular death will further delineate mechanisms of dementia in SIVE.

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