Cell Cycle Proteins Exhibit Altered Expression Patterns in Lentiviral-Associated Encephalitis

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Cell cycle proteins regulate processes as diverse as cell division and cell death. Recently their role in neuronal death has been reported in several models of neurodegeneration. We have reported previously that two key regulators of the cell cycle, the retinoblastoma susceptibility gene product (pRb) and transcription factor E2F1, exhibit altered immunostaining patterns in simian immunodeficiency virus encephalitis (SIVE). Here we show that E2F1 and the inactivated, hyperphosphorylated form of pRb (ppRb) also exhibit altered immunostaining patterns in human immunodeficiency virus encephalitis (HIVE). Quantification of E2F1 and ppRb staining by immunofluorescent confocal microscopy confirms a significant increase in E2F1 and ppRb in both HIVE and the simian model. This increase in E2F1 and ppRb staining correlates with an increase in the presence of activated macrophages, suggesting a link between changes in cell cycle proteins and the presence of activated macrophages. Changes in ppRb and E2F1 staining in SIVE also correlate with alterations in E2F/DNA binding complexes present in the nuclear and cytoplasmic fractions from both midfrontal cortex and basal ganglia. These findings suggest that changes in cell cycle proteins occur in both HIVE and the simian model and that these changes have functional implications for gene expression in neural cells under encephalitic conditions mediated by macrophage activation or infiltration.

Key words: HIV; SIV; transcription; cell cycle; neurodegeneration; brain; encephalitis; neuron; phosphorylation; retinoblastoma; E2F

Received July 5, 2001; revised Dec. 17, 2001; accepted Dec. 21, 2001.

This work was supported by National Institutes of Health Grants MH46790, MH182273, NS35731, MH01717, and NS41202, and by the Pathology Post-Doctoral Fellow Research Training Grant from the University of Pittsburgh School of Medicine. We extend our gratitude to the California NeuroAIDS Tissue Network (Grant P50 MH45294) for providing us with paraffin-embedded tissue sections and frozen tissues from HIV patients for these studies. We also thank Dr. Kelly Stefano Cole for the kind gift of the SIV gp110 polyclonal antibody and Jonette Welley and John Caltagargone for technical support.

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Figure 1. In HIVE, increased E2F1 staining was observed predominantly in neuronal cytoplasm and not astrocytes. A–F, Triple-label immunofluorescent confocal microscopy for E2F1 (green), nuclear DNA (propidium iodide; red), and astrocytes labeled with GFAP (blue). In HIV autopsies without encephalitis (A–C), minimal E2F1 staining is observed. In HIV autopsies with encephalitis (D–F), abundant E2F1 staining is observed but does not localize to astrocytes. G–L, Triple-label immunofluorescent confocal microscopy for E2F1 (green), nuclear DNA (propidium iodide; red), and MAP2 (blue). In HIV autopsies without encephalitis (G–I), minimal E2F1 staining is observed. In HIV autopsies with encephalitis (J–L), increased E2F1 staining is observed in neuronal cytoplasm [colocalization of blue (MAP2) and green (E2F1) staining appears aqua]. The columns contain images from the following brain regions: Basal Ganglia, Hippocampus, and midfrontal cortex (Frontal Cortex). DNA staining may not be seen in all nuclei because of the staining protocol used. In these cases, MAP2 and GFAP, as cytoskeletal components, are used to delineate the cytoplasm. All micrographs are the same magnification. Scale bar, 20 μm (for all images).

Figure 2. ppRb localized predominantly to nuclei of neurons in HIVE. A–F, Triple-label immunofluorescent confocal microscopy for ppRb (green), nuclear DNA (propidium iodide; red), and astrocytes labeled with GFAP (blue). In HIV autopsies without encephalitis (A–C), minimal ppRb staining was observed. In HIV autopsies with encephalitis (D–F), abundant ppRb staining was observed but only occasionally localized to GFAP-positive astrocytes. G–L, Triple-label immunofluorescent confocal microscopy for ppRb (green), nuclear DNA (propidium iodide; red), and MAP2 (blue). In HIV autopsies without encephalitis (G–I), minimal ppRb staining was observed. In HIV autopsies with encephalitis (J–L), increased ppRb staining was observed in neuronal cytoplasm and nuclei [colocalization of blue (MAP2) and green (E2F1) staining appears aqua], and colocalization between green (E2F1) and red (DNA) appears yellow]. Columns contain images from the following brain regions: Basal Ganglia, Hippocampus, and midfrontal cortex (Frontal Cortex). DNA staining may not be seen in all nuclei because of the staining protocol used. In these cases, MAP2 and GFAP, as cytoskeletal components, are used to delineate the cytoplasm. All micrographs are the same magnification. Scale bar, 20 μm (for all images).
with high salt buffer (0.42 M NaCl, 20 mM HEPES, pH 7.9, 1.5 mM MgCl2, 0.2 mM EDTA, 0.2 mM PMSF, 0.5 mM DTT) until there were no large chunks. Separated cells were collected by centrifugation at 3000 rpm for 5 min at 4°C. Supernatants were removed to a separate tube, and pellets were resuspended in 4 vol of hypotonic buffer (20 mM HEPES, pH 7.9, 1.5 mM MgCl2, 0.5 mM KCl, 0.5 mM DTT, 0.2 mM EDTA, 0.5 mM PMSF, 2 µg/ml pepstatin A, and 1 µg/ml leupeptin). The suspension was homogenized for 10 sec and incubated on ice for 15 min. The cells were collected by centrifugation at 13,000 × g for 30 min. The supernatant was labeled “S1,” and the pellet was further extracted with high salt buffer (0.42 M NaCl, 20 mM HEPES, pH 7.9, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM DTT, 25% glycerol, 0.5 mM PMSF, 2 µg/ml pepstatin A, and 1 µg/ml leupeptin) on ice for 20 min. Residual insoluble material was removed by centrifugation at 14,000 × g for 30 min. The supernatant fraction was collected and termed “S2.” Protein concentrations for each fraction were determined by Bio-Rad protein assay.

Electrophoretic mobility shift assay. Oligonucleotides containing the E2F DNA consensus site were end labeled with 32P. Protein extracted from fresh macaque brain tissue (24.5 µg) was incubated with 0.5 ng of 32P-labeled E2F probe in 20 µl of electrophoretic mobility shift assay (EMSA) buffer (20 mM HEPES, pH 7.9, 20% glycerol, 200 mM KCl, 0.2 mM EDTA, 0.5 mM PMSF, 0.5 mM DTT) for 20 min at room temperature (Ausubel et al., 1994; Jordan et al., 1994). Included in the reaction was 0.5 µg of salmon sperm DNA as a nonspecific competitor before addition of labeled probe to reduce nonspecific DNA/protein interactions. For competition reactions, unlabeled competitive molecules were preincubated with the protein for 5 min on ice before addition of labeled probe. The reaction mixture was loaded onto a pre-run 4% nondenaturing polyacrylamide gel (Ausubel et al., 1994; Jordan et al., 1994) and electrophoresed at 100 V in a low ionic-strength buffer (6.8 mM Tris, pH 8.0, 1 mM EDTA, pH 8.0, and 3.3 mM NaOAc). The buffer was recirculated every 45 min. After 1.5–2 hr, the polyacrylamide gel was removed from the apparatus, dried, and exposed to autoradiography film. Exposure time varied depending on the abundance of E2F protein complexes present in the extract. The double-stranded oligonucleotides containing an E2F site were taken from the c-myc promoter (Hall, 1990). These sites were as follows: 5′-AAATTCGTTTGCGGAAAAC-3′ and 3′-GCGGACC-GCCCCTTTTGTTAA-5′.

RESULTS

E2F1 and ppRb exhibited altered staining patterns in HIV encephalitis

To determine whether our observations in SIVE reflected similar processes occurring in HIV, we compared staining patterns for E2F1 and ppRb in six patients diagnosed with HIV and six HIV-infected patients with no encephalitic pathology as controls. In both sets of patients, we surveyed immunohistochemical staining patterns in three brain regions: the basal ganglia, the hippocampus, and the midfrontal cortex. Of the six HIV-positive control patients, only one exhibited any positive staining for E2F1 or ppRb (Table 2). Most of the HIVE cases exhibited abundant staining for E2F1 and ppRb in all three brain regions examined (Table 2). These results suggest that there is increased staining for both E2F1 and ppRb in HIVE consistent with our observations in the simian model. The HIV-infected, non-encephalitic autopsy case that stained for E2F1 and ppRb also exhibited abundant type II Alzheimer’s astrocytes, a condition associated with elevated serum ammonia levels. For this study, such a case may not be appropriate for use as a control.

In HIV encephalitis, increased E2F1 staining was observed predominantly in neuronal cytoplasm and not in astrocytes

We wanted to define the cell types exhibiting increased E2F1 staining in HIVE cases. Using triple-label immunofluorescent confocal microscopy, we assessed E2F1 localization in astrocytes (GFAP) and neurons (MAP2). Distribution of the nucleus or cytoplasm was determined by colocalization with nuclei stained with propidium iodide, a DNA intercalating agent. In HIV control cases, the neurons, astrocytes, and oligodendrocytes did not stain appreciably for E2F1 (Fig. 1A–C,G–I). In the basal ganglia of HIVE autopsy tissues, some E2F1 (green) localized to the nuclei (red) of cells that did not colabel with GFAP (blue) (Fig.
In all three brain regions of HIV-positive control cases that were using triple-label immunofluorescent laser confocal microscopy. Tissue sections from SIV and SIVE necropsies were stained for E2F1 (green), pRb (red), and ppRb (green) localized predominantly to nuclei of neurons in gray matter, midfrontal cortex were labeled by immunofluorescence for E2F1, pRb, or ppRb. The total fluorescence was obtained by multiplying the area by the average fluorescence intensity. Quantification of E2F1, pRb, and ppRb staining confirmed a statistically significant increase in the staining area and volume for each protein in midfrontal cortex (CTX), caudate (CAD), and putamen (PUT) of SIVE cases over non-encephalitic, SIV-infected controls (Fig. 3) (p < 0.001; data not shown).

In human autopsy tissue, we quantified the area of positive E2F1 and ppRb staining present in basal ganglia, midfrontal cortex, and hippocampus from six HIVE and six HIV cases. Because our staining protocol for pRb did not differentiate between HIV, HIVE, and non-infected individuals, these data were not subjected to statistical analysis at this time. Neuro-anatomic regions of the brain were marked on the slide (midfrontal cortical gray matter, midfrontal cortical white matter, CA1 region of the hippocampus, and putamen), five random sites within the marked regions were imaged, and the average positive staining area and total fluorescence were calculated. As in the simian model, both E2F1 and ppRb exhibited statistically significant increases in staining in HIVE as compared with non-encephalitic controls (Fig. 4) (p < 0.001). These data suggest that the number or availability of E2F1 and ppRb epitopes increased in both SIV and HIV encephalitis.
Table 3. E2F1, pRb, and ppRb immunostainings correlated with macrophages, whereas only pRb and ppRb correlated with virus in the simian model of HIV

<table>
<thead>
<tr>
<th>SIVE</th>
<th>A or T</th>
<th>Midfrontal cortex</th>
<th>Caudate</th>
<th>Putamen</th>
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<td></td>
<td></td>
<td>gp110</td>
<td>HAM56</td>
<td>gp110</td>
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<td>0.8101**</td>
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<tr>
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<td>0.6253**</td>
<td>0.7329**</td>
</tr>
<tr>
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<td>0.8052**</td>
<td>0.6769**</td>
<td>0.6374**</td>
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</table>

Fluorescent immunostainings for E2F1, pRb, and ppRb were quantified in midfrontal cortex, caudate, and putamen of SIVE macaques. For each cell cycle protein, double-label immunofluorescent staining for the SIV viral envelope protein gp110 (columns 1, 3, and 5) and HAM56 for activated macrophages (columns 2, 4, and 6) were also quantified in the same fields as the cell cycle proteins. Pearson’s correlation coefficients (r) were determined for each cell cycle protein compared with either SIV virus (gp110) or macrophages (HAM56) staining area (A) or total fluorescence (T). The r values are indicated in the table. *p ≤ 0.01; **p ≤ 0.001.

E2F1 staining correlated with macrophage activation in SIV encephalitis, whereas Rb and ppRb staining correlated with the presence of both SIV and activated macrophages

Because we observed alterations in the staining patterns and levels of E2F1 and ppRb in neurons of both SIV and HIV encephalitic individuals, we wanted to determine whether changes in these two proteins were spatially related to the presence of infected or activated macrophages. To decipher this relationship, we correlated the expression of our cell cycle protein of interest in the vicinity of macrophages or lentiviral infection.

In the SIV model, paraffin-embedded brain sections containing the basal ganglia and midfrontal cortex were labeled by immunofluorescence for E2F1, pRb or ppRb, and HAM56, a marker expressed abundantly in macrophages, or gp110, a viral coat protein from SIV. The basal ganglia and gray matter of midfrontal cortex were identified grossly and marked on the slides. Within the marked regions, five random 67,600 μm² fields were imaged using double-label confocal microscopy by an individual blinded to the experimental design and to the cell cycle protein staining. As above, 10 planes at 0.34 μm intervals were captured, and the central image was subjected to analysis because we determined that there was no difference between using the value for this single image or the average of the 10 fields. In this field, the total number of pixels containing staining for the cell cycle protein and the cell phenotype marker were enumerated to give us a staining area. The staining area for the cell cycle protein being tested within each frame was then correlated with the staining area for macrophages or virus. There was little or no detectable staining for macrophages [HAM56(red)] or infected [gp110(red)] macrophages in either midfrontal cortex or the basal ganglia of SIV-infected macaques without encephalitis (Fig. 5A–C,G–I) (data not shown). Brains from macaques infected with SIV without encephalitis also demonstrated minimal staining for E2F1, pRb, and ppRb (green) (Fig. 5, A and G, B and H, C and I, respectively), whereas staining with the cell cycle proteins was abundant in macaques with encephalitis (green) (Fig. 5D–F,J–L). A strong correlation was observed between the area of E2F1 staining and the area of HAM56 staining present in the 67,000 μm² fields in all three brain regions examined for SIVE (Table 3) (r > 0.78). The E2F1 staining area did not correlate with the area of viral staining except in putamen (Table 3) (r = 0.7601). Similar results were obtained when total E2F1 fluorescence was compared with total fluorescence of macrophages or virus (Table 3, E2F1, T). E2F1 staining did not colocalize with the markers for either macrophages or SIV, and only minimal E2F1 staining was observed in HAM56- or gp110-positive cells, indicating that the observed correlations were not caused by E2F1-positive infiltrating cells but by increased E2F1 staining in adjacent and nearby cells (Fig. 5D,J). These results show that overall, the change in E2F1 staining observed in SIVE correlated with the presence of macrophages.

In SIVE, the pRb staining area correlated moderately with both gp110 and HAM56 in midfrontal cortex and caudate, but not in putamen (Table 3) (r > 0.65). The ppRb staining area correlated with both gp110 and HAM56 in midfrontal cortex, but correlated more strongly with gp110 than HAM56 in midfrontal cortex and caudate (Table 3). Neither pRb nor ppRb colocalizes with virus or macrophages and rarely exhibits positive staining in gp110- or HAM56-positive cells, indicating that any observed correlation was not caused by pRb-positive infiltrating macrophages but by increased staining of cells in the vicinity of the infiltrated and infected macrophages (Fig. 5E,F,K,L). These results show that changes in pRb staining correlate with the presence of both virus and macrophages in SIVE, but the inactive form of pRb, ppRb, correlates more strongly with virus.

In the human autopsy tissue, the spatial relationships between E2F1 and ppRb staining were compared with the presence of activated (HLA-DR) or HIV-infected (p24) macrophages. Because HIV infection of human brain tissue was most pronounced in deep gray matter, the correlation between ppRb or E2F1 and p24 was limited to the basal ganglia. The correlation between cell cycle proteins and HLA-DR was assessed in both gray and white matter from midfrontal cortex, hippocampus, and basal ganglia from each of six cases. By double-label immunofluorescent laser confocal microscopy, E2F1, ppRb, HLA-DR, and p24 staining was minimal in HIV cases without encephalitis in all brain regions that were assessed (Fig. 6A,D) (data not shown). Consistent with our previous observations, we observed increased staining for E2F1 and ppRb in basal ganglia from HIV+ cases in which there was also abundant staining for activated macrophages and HIV-infected macrophages (Fig. 6B,C,E,F). Although there was abundant virus and activated macrophage staining, neither correlated with the abundant E2F1 or ppRb staining in any brain region assessed (Table 4). These results suggest that although there was abundant E2F1 and ppRb in the vicinity of virus and macrophages, the amount of staining for each marker did not change proportionally with the marker for virus or macrophages.
E2F/DNA complexes were altered in SIVE

Our results in both the simian model and human autopsy material suggest that the staining patterns and levels for E2F1 were altered under encephalitic conditions. We also observed increased phosphorylation of E2F1 regulatory proteins, such as ppRb. Because phosphorylation of ppRb reduces its ability to bind and regulate E2F1 activity, the changes that we observed suggest a change in E2F1 activity. As a transcription factor, one way of assessing E2F1 activity is to determine its ability to bind DNA. Protein extracts were prepared from three macaques with SIVE, two macaques infected with SIV without encephalitis, and three macaques that were neither infected nor encephalitic. A detergent soluble “cytoplasmic” fraction was collected from each sample, leaving the nuclei to be extracted with 0.42 M sodium chloride. Cytoplasmic and nuclear fractions from both midfrontal cortex and basal ganglia were used in the EMSA. When nuclear extracts from midfrontal cortex were assayed for E2F1/DNA binding activity, a slow migrating complex was observed in the control and SIV cases (Fig. 7A, C–I–C 3, SIV 1, SIV 2). In SIVE cases 2 and 3, two complexes that migrate more quickly than the complex observed in the control lanes were observed in extracts from midfrontal cortex (Fig. 7A, SIVE). Similar results were observed when extracts from the basal ganglia were used (data not shown). When the cytoplasmic extracts were used, we observed a reversal in the pattern of complexes for both brain regions (Fig. 7B, midfrontal cortex) (data not shown for basal ganglia). Cytoplasmic extracts from the control or SIV cases produced a band that migrated more quickly. In the SIVE cases, the quicker migrating band was present, but a slower migrating band appears in all three cases (Fig. 7B). To demonstrate that the observed bands were specific for the E2F DNA binding site, we included increasing concentrations of unlabeled E2F oligonucleotides or an unrelated sequence (Fig. 7C). The upper complexes generated by both the nuclear and cytoplasmic extracts were competed away by the E2F sequence, but not by the unrelated sequence (Fig. 7C), suggesting that the complexes present in both nuclear and cytoplasmic extracts are specific for the E2F site. The same results were obtained when extracts from basal ganglia were used (data not shown). Taken together, these data suggest that the protein composition of the E2F/DNA complex is altered in SIV encephalitis. This alteration appears to involve a switch in the E2F DNA binding proteins present in the different subcellular fractions.

DISCUSSION

In the simian model for HIV encephalitis, we previously demonstrated alterations in expression levels and subcellular localization of the E2F1 transcription factor with concomitant alterations in ppRb subcellular localization and phosphorylation (Jordan-Sciuotto et al., 2000). Here we have shown similar results in human autopsy tissue. In HIVE, we observed increased staining for E2F1 in midfrontal cortex, hippocampus, and basal ganglia. This staining localized predominantly to neurons in both the cytoplasm and nucleus. Changes in E2F1 staining were similar to changes in ppRb staining. Staining for ppRb also increased in all three brain regions of HIVE and was predominantly nuclear in neurons. Phosphorylation of ppRb is believed to disrupt interaction with E2F family members, freeing them to increase transcription from E2F target promoters. Our observations suggest that such events may be occurring in response to encephalitic conditions.

The increased staining for E2F1 could result from changes in a number of cellular regulatory processes, from an increase in gene expression at the transcriptional or translational level to changes in post-translational modification leading to increased protein stability. We favor the interpretation that increased staining is

<table>
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<th>HIVE</th>
<th>Frontal cortex gray matter</th>
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<th>Basal ganglia putamen</th>
<th>Hippocampus CA1</th>
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<td>NA –0.4285*</td>
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<td>NA –0.3277</td>
<td>NA –0.1674</td>
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<tr>
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<tr>
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<td>T</td>
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<td>NA –0.1850</td>
<td>0.6138*</td>
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Table 4. E2F1, pRb, and ppRb immunostainings did not correlate with macrophages or HIV virus in the human disease

Fluorescent immunostaining for E2F1 and ppRb was quantified in HIVE midfrontal cortex gray matter, frontal cortex white matter, putamen, and the CA1 region of the hippocampus. For each cell cycle protein, double-label immunofluorescent staining for the HIV viral capsid protein p24 (columns 1, 3, and 5) and HLA-DR for activated macrophages (columns 2, 4, and 6) were also quantified in the same field as the cell cycle proteins. Pearson’s correlation coefficients (r) were determined for each cell cycle protein compared with either HIV virus (p24) or macrophages (HLA-DR) staining area (A) or total fluorescence (T). The r values are indicated in the table. *p ≤ 0.05. NA, Not assessed.
caused by an alteration in the availability of the E2F1 epitope recognized by the antibody used in this study. The unique feature of this antibody is that it recognizes E2F1 through its pRb interaction domain. Thus, when E2F1 is in complex with pRb, the antibody will not bind E2F1, but when pRb is phosphorylated and no longer interacting with E2F1, the antibody detects E2F1. This implies that the observed increase in E2F1 in HIVE could be caused by an increase in “free” E2F1 and not necessarily increased E2F1 gene expression. We have tried to support this hypothesis by assessing protein levels by immunoblot analysis; however, because of variability in human tissue preservation, this was not possible. Our results in the simian model support this hypothesis (Jordan-Sciutto et al., 2000). In SIVE, we observed increased E2F1 staining (Fig. 3) but did not see changes in protein levels in SIVE midfrontal cortex. E2F1 protein levels also do not change in BDNF-, NGF-, or RANTES-treated neuralgial cultures, despite dramatic increases in E2F-1 immunostaining and altered subcellular localization (Jordan-Sciutto et al., 2001). Further support for this hypothesis will be provided by using this in vitro model to assess mechanisms regulating E2F1 subcellular localization.

Whether the increase in E2F1 staining is caused by E2F1 liberation or induced E2F1 expression, the presence of phosphorylated pRb indicates that less E2F1 will be complexed with pRb. In the nucleus, free E2F1 induces transcription of genes necessary for cell cycle control (e.g., cyclin A, cyclin E, cdc2, and p19ARF) (Bates et al., 1998; Black and Azizkhan-Clifford, 1999). Our observations indicate that the proteins binding to the E2F DNA binding site are distinct in SIV encephalitic versus control brains. These data suggest that under normal conditions, a specific set of E2F/pocket protein complexes are able to bind DNA in the nucleus; under encephalitic conditions, the E2F/pocket protein complexes bound to promoters are different. Several cell types in the brain can be contributing to the observed E2F complexes in brain extracts. In encephalitic cases, the presence of infiltrating macrophages may account for the E2F complexes observed. However, our immunohistochemistry for E2F1 suggests that neuronal, oligodendroglial, and astrocytic elements may also exhibit altered E2F complexes in response to pathology in SIVE brain. In any case, it is likely that changes in E2F/pocket protein complexes present in the nucleus represent a change in E2F target promoters being modulated. E2F-regulated gene products can be divided into distinct subsets on the basis of their function in S phase: those needed to regulate cell cycle phase progression (i.e., cyclin E, cdc2, pRb), those needed to synthesize DNA (DNA polymer-
The role of cytoplasmic E2F1 in neurons is less easily understood. One possibility is that E2F1 is shuttled into the cytoplasm for degradation by the proteosome, but this does not explain the staining in neuronal processes. Alternatively, E2F1 may serve other functions in neurons besides transcriptional regulation. Recent reports have indicated a role for E2F1 in apoptosis independent of its ability to induce transcription (Phillips et al., 1999; Hou et al., 2001). This was shown recently in cerebellar granule neurons induced to die by dopamine-evoked apoptosis. These reports are intriguing in association with our observations regarding E2F DNA binding activity in cytoplasmic extracts. We observed distinct DNA/protein complexes in normal verses encephalitic conditions. The observations in the simian model did not reflect the results in the human disease. This is because the macaques are killed before end-stage encephalitis as part of a vaccine trial. This is in direct contrast to the human disease, in which patients are at the terminal stage. In early stages seen in the simian model, the disease is more focal, and therefore its effects are localized to the initiating lesions. In the human disease, the changes have propagated to regions more distal to the site of injury. This explains why the cell cycle protein staining is more widespread than macrophage or viral staining, which is limited to deep gray matter tracts in HIVE. This would preclude our ability to correlate initiating factors of disease with increased cell cycle protein staining. Despite the discrepancy in the human autopsy tissue, results in our simian model are consistent with previous findings in our human neuroglial cultures demonstrating that both E2F1 and ppRb respond to neurotrophic factors or chemokines with increased staining intensity and altered subcellular distribution in neurons (Jordan-Sciutto et al., 2001). The role of viral-associated proteins in cell cycle protein induction remains to be investigated in this model and may also address our observed discrepancies between the simian and human disease.

Although the virus is the etiologic entity of HIVE, a number of reports indicate that the damage induced in adjacent neurons may be attributable to factors secreted by HIV-infected or activated macrophages. Additional support for a specific role for activated macrophages in neuronal damage associated in HIVE comes from studies of other neurodegenerative diseases in which inflammatory processes have been documented. Alterations in cell cycle protein staining patterns have been observed in several neurodegenerative conditions, including Alzheimer’s disease (AD), amyotrophic lateral sclerosis, and Parkinson’s disease (Arendt et al., 1996; Vincent et al., 1996; McShea et al., 1997, 1999; Nagy et al., 1997a,b). Activation of CDKs usually results in phosphorylation of pRb (Kouzarides, 1995; Pines, 1995, 1997, 1999; Nagy et al., 1997a,b). Activation of CDKs usually results in phosphorylation of pRb (Kouzarides, 1995; Pines, 1995, 1997, Whyte, 1996). Our results in HIVE are consistent with findings in AD demonstrating alteration in pRb and E2F1 staining patterns in areas of inflammatory activation surrounding β amyloid-containing plaque structures. It will be interesting to correlate changes in pRb with changes in CDKs and their regulators in HIVE, to determine whether the upstream signaling cascades are also conserved between neurodegenerative responses in multiple disease states.

Our findings in HIVE are consistent with several reports studying expression patterns of E2F1 and ppRb in neurodegenerative diseases involving an inflammatory component including SIVE, and other neurodegenerative diseases (Jordan-Sciutto et al., 2000, 2001; Ranganathan et al., 2001). Our results indicate that one neuronal response to inflammatory signaling is phosphorylation of pRb, resulting in an alteration in the state of E2F1. The impact of these changes on neuronal function and survival needs to be investigated further, although preliminary evidence suggests that these changes will have consequences for nuclear gene expression. These data also implicate a novel function for E2F1 and ppRb in neurons and potentially oligodendrocytes. By studying the activities of E2F1 and pRb in neurons responding to neurodegenerative stimuli present in HIVE, we hope to gain a greater understanding of disease progression and provide targets for therapy that are specific to degenerating neurons.

REFERENCES