Immunolocalization and Redistribution of the FAC1 Protein in Alzheimer's Disease

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Abstract. The presence of senile plaques and neurofibrillary tangles are hallmark neuropathologic features of Alzheimer’s disease (AD). Many proteins have previously been immunolocalized to amyloid-containing plaques in AD brain. Using a monoclonal antibody to a recently described developmentally regulated gene product, we demonstrate the presence of FAC1 protein in a subset of diffuse and neuritic plaques in AD brain. FAC1 is not observed in neurofibrillary tangles common in the hippocampus or entorhinal cortex, nor is it localized in diffuse plaques of nondemented elderly control subjects. FAC1 protein is also immunolocalized in swollen dendrites of hippocampal pyramidal cells observed in some cases of early stage AD. Therefore FAC1 is a novel protein localized in early pathologic features of AD and in a subset of plaques.

Key Words: Alzheimer’s disease; Amyloid plaques; Dendrites; Hippocampus; Immunolocalization; Neocortex; Neurofibrillary tangles.

INTRODUCTION

The FAC1 gene was identified by immunoscreening a human fetal cDNA expression library with the monoclonal antibody Alz-50 (1). Alz-50 recognizes neurofibrillary pathology associated with Alzheimer’s disease (AD) (2). Within the AD brain Alz-50 recognizes hyperphosphorylated tau proteins contained in neurofibrillary tangles (NFTs), a neuropathologic characteristic of the disease. While Alz-50 displays little immunoreactivity in normal brain tissue sections it does recognize all tau isoforms by immunoblot (3, 4). The FAC1 gene encodes for a protein of 125 kDa that is widely expressed during brain development and contains very limited sequence homology to the amino terminus of tau. Alz-50 recognizes a subset of fetal brain FAC1 protein as shown by immunoblot (1). Monoclonal antibodies have been developed against FAC1 protein and used to immunolocalize FAC1 in the developing human cerebral cortex. Whereas FAC1 protein is localized throughout the cell cytoplasm and in the nucleus of neurons in the developing brain, at postnatal times FAC1 is observed predominately in neuronal nuclei (1). Since FAC1 represents a novel fetal brain Alz-50 antigen, it is important to determine the immunolocalization of FAC1 throughout the adult brain and in patients with Alzheimer’s disease.

The vast literature on AD still has not uncovered the molecular mechanisms leading to dementia. While AD appears to be heterogeneous at the genetic level (5), the associated tissue lesions appear to be more consistent. Much has been learned about the major neuropathological hallmarks of the disease, neuritic plaques (NPs) and NFTs. Many proteins have been identified as constituent proteins of plaques, including AB (6), GAP-43 (7), ubiquitin (8), α1-antichymotrypsin (9), Apo E lipoprotein (10, 11), interleukin-1 (12), and heparn sulfate proteoglycans (13). The precise sequence of events leading to amyloid deposition and formation of plaques, and the contribution of plaques to the progression of AD, remain in debate. While continued exploration on the formation of both NPs and NFTs will yield new information about their generation, additional studies on novel molecules that exhibit alterations during the course of the disease, especially at early stages, may lead to new insights into the molecular changes that occur in neurons during AD.

In this study we have determined the immunolocalization and expression patterns of FAC1 protein in multiple brain regions of nondemented controls, patients with no evidence of clinical dementia but containing sufficient neuropathological findings to fulfill the criteria of possible AD and cases of definite AD. Within the neocortex of nondemented control subjects FAC1 protein is localized primarily in neuronal nuclei; however, in the neocortex of many AD cases FAC1 protein is detected in diffuse or neuritic plaques. By immunoblot analysis increased levels of FAC1 protein are detected in soluble fractions of AD neocortical tissue homogenates. Within the hippocampus FAC1 is contained in nuclei and cytoplasm of pyramidal neurons in CA2/3 and the subiculum, but is localized predominately in nuclei within CA1 and entorhinal cortex. In cases of possible AD swollen dendrites containing FAC1 are observed in the hippocampus. In cases of definite AD plaques within the molecular layer of the hippocampus are immunoreactive for FAC1. Few additional alterations of FAC1 protein distribution are detected in the AD hippocampus. Tangle-bearing neurons do not contain aberrantly expressed or localized FAC1 protein and NFTs are devoid of FAC1 protein. These results indicate that FAC1 protein redistribution

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occurs during the early stages of AD and FAC1 is contained in neuritic and extracellular components of some plaques.

METHODS

Case Material

The cases studied included both control adults and subjects who came to autopsy with a clinical diagnosis of Alzheimer-type dementia. The average age at death was 75 years for controls (range of ages from 49 to 90 years [yr]) and 74 years for possible AD and definite AD cases (ages range from 63 to 92 yr). The average postmortem interval for all cases was 6.6 hours (h) (range of 1 to 18 h). Neuropathologic studies to confirm the clinical diagnosis included examination of neocortical, hippocampal, basal forebrain and cerebellar sections with thioflavine-S fluorescence microscopy and Bielschowsky silver staining for the identification of senile plaques and neurofibrillary tangles. Alzheimer disease cases were classified according to the stages of the disease (I–VI) as designated by Braak and Braak (14). Three additional autopsy cases had no clinical evidence of dementia but contained sufficient neurofibrillary tangles in the hippocampal formation to allow a diagnosis of possible AD and be classified as Stage II by the Braak scoring criteria.

Immunoblot

For Western blot analysis of brain homogenates, superior temporal cortex of adult human brain previously stored at −70°C were homogenized in ice-cold lysis buffer (1X TBS containing 1 mM PMSF (phenylmethanesulfonyl fluoride, Boehringer Mannheim, IN) and 25 µM leupeptin (Sigma, St Louis, MO) at 10% w/v. The average age of these cases was 69 yr and the average postmortem interval 8 h. After 10 strokes with a motorized Thomas tissue homogenizer, the homogenate was centrifuged at 27,000 rpm for 20 minutes (min) at 4°C to remove nuclei, large organelles, and unbroken cells. The supernatant was removed and the protein concentration determined by Bradford analysis (15). The pellets were resuspended in a volume of lysis buffer equal to the supernatant. Equal protein amounts of each sample were analyzed on 8% SDS-polyacrylamide gels and the resulting blots immunolabeled with the monoclonal antibody FA2 at a 1:10 dilution in 5% milk/TBS. An isotype specific goat anti-mouse secondary antibody conjugated with HRP (Fisher Scientific, Pittsburgh, PA) was then incubated with the blot at a 1:500 dilution for 1.5 hours. After extensive washes in TBS, FAC1 protein was visualized using the enhanced chemiluminescence technique (Amersham Life Science, Arlington, IL).

To quantitate FAC1 protein levels in each lane, the FAC1 immunoblot was digitized at 600 dpi on an automated color bed scanner (Scannaker II; Microtek), and exported to a Professional PC DXII workstation employing Optimas image processing software (BioScan, Inc, Edmonds, WA). A region of interest (ROI) box was constructed that exactly matched the digitized FAC1 immunoreactive band; this single box was moved to each additional band site when acquiring data to ensure that the same number of pixels was used for each analysis. Data was collected as grayscale counts by the image processing software and expressed in arbitrary units. Prior to data acquisition, the digitized image was standardized for brightness and contrast to ensure that gray levels were below saturation. Membrane and primary deplete gray level values were digitally subtracted from the entire blot to control for nonspecific background values.

Immunocytochemistry

Formalin-fixed human brain tissue was obtained from adults between the ages of 49 and 92 yr. The average postmortem interval was 6.6 h. 50 µm vibratome sections from the midfrontal cortex, superior and middletemporal cortex, and hippocampus were examined as follows. Beta-amyloid (Aβ) immunocytochemistry sections were first incubated in 90% formalin acid for 30 min and washed in TBS for 15 min. All sections were treated with 3% H2O2 + 0.25% Triton X-100 in TBS for 30 min. The sections were then blocked in 5% milk/TBS for 1 h.

Primary antibodies (listed above) were added and sections incubated overnight at 4°C. After five washes in TBS for 5 min each, the sections were incubated in biotinylated goat anti-mouse IgG (Fisher Scientific) at a 1:200 dilution for 3 h. After additional washes streptavidin-HRP (Fisher Scientific) was added at a 1:500 dilution in milk/TBS for 1.5 h. The reaction product was visualized with 3,3’-Diaminobenzidine (Sigma).

Immunofluorescence

Formalin-fixed superior temporal cortex and hippocampal sections were treated with 3% H2O2 + 0.25% TX-100 for 30 min and blocked in 5% milk/TBS for 1 h at RT. All antibody incubations were performed in 5% milk/TBS as a blocking agent. Primary antibodies (listed above) were added and the sections incubated overnight at 4°C. After three washes in TBS the sections were incubated in biotinylated goat anti-mouse IgG (for FA2 labeled sections) (Fisher Scientific) at a 1:250 dilution for 3 h. After TBS washes a 1:400 dilution of streptavidin-Cy5 (Jackson ImmunoResearch Labs, Inc West Grove, PA) and a 1: 250 dilution of goat anti-IgM-FITC (Fisher Scientific) (TG3) or anti-IgGn-FITC (4G8) was added to the sections for 1.5 h at room temperature in the dark. After washes in TBS the sections were mounted in crystal mount (Sigma) and analyzed on a Molecular Dynamics Model 2001 laser scanning confocal microscope. Omission of the primary antibody resulted in absence of fluorescent signal. Crossover control experiments were also performed for each primary antibody against the secondary antibody from the other label in order to determine the specificity of the secondary antibody.

RESULTS

The immunocytochemical data of FAC1 localization in multiple brain regions of nondemented controls, cases of possible AD, and definite AD are summarized in Table 1. Numerical values between 0 and 3 ("0" for no staining and "3" for most robust FAC1 immunostaining) were scored for nuclear and cytoplasmic FAC1 immunostaining profiles in each brain region. While only semiquantitative, this analysis revealed an overall increase in FAC1 immunostaining intensity in the AD cases. We next directly compared FAC1 immunolocalization characteristics in adult control and AD subjects.
TABLE 1

Antibodies Used in This Study

<table>
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<th>Antibody</th>
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<th>Isotope</th>
<th>Dilution</th>
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<td>IgG</td>
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Distribution of FAC1 Immunoreactivity in Control Adult Brain

We have previously demonstrated that the FAC1 protein is expressed at high levels in the human developing cortex and at lower levels in adult midfrontal and temporal cortex (1). The subcellular localization of FAC1 within the neocortex is also developmentally regulated. In the developing fetal brain, FAC1 protein is observed throughout the cytoplasm and nucleus of cells. In the neocortex of nondemented individuals, FAC1 protein is contained predominately in nuclei (1). To further characterize the distribution of FAC1 in the adult brain, we performed FAC1 immunocytochemistry in multiple brain regions of 8 nondemented adult autopsies (average age of 75 yr) with an average postmortem interval of 6.4 h.

A. Midfrontal and Temporal Cortex: As previously shown (1), FAC1 was observed predominately in nuclei in both the midfrontal and superior temporal cortex in 7 of 8 nondemented aged adults. Nondemented elderly individuals exhibit somewhat variable intensity of FAC1 immunostaining (Table 2), which may result from diminished expression of FAC1 during aging, loss of FA2 immunoreactivity due to post-translational modifications or postmortem proteolysis of the protein. Phosphatase treatment of tissue sections did not enhance FA2 immunostaining, indicating that phosphorylation of the FA2 epitope is not responsible for diminished FA2 immunoreactivity.

In 7 of 8 nondemented adults and 7 of 8 AD cases we also detected FAC1 protein within cells of the white matter that often resemble cell of glial origin by morphological characteristics (Fig. 1A, B). Both stellate-shaped, process-rich cells considered to be astrocytes and spiderlike and bipolar cells considered to be microglia were FAC1 immunoreactive. FA2 immunostaining of nuclei was observed at the pial surface in 4 of 8 cases. Nondemented controls containing diffuse amyloid plaques in the neocortex (as detected by Aβ immunostaining) failed to exhibit FAC1 immunostaining in any plaque structures (Fig. 1C, D). These data indicate that additional nonneuronal cell types have the potential to express FAC1, and that diffuse plaques present in nondemented adults fail to contain FAC1 protein.

TABLE 2

Clinical Characteristics and Data Summary of FAC1 Immunolocalization in Patient Population used for this Study

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/Gender</th>
<th>Neuropathologic diagnosis</th>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N</td>
</tr>
<tr>
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<tr>
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<td>90/F</td>
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<td>—</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
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<td>Control</td>
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<td>2</td>
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<tr>
<td>4</td>
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<td>2</td>
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<tr>
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<td>2</td>
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<td>2</td>
</tr>
<tr>
<td>8</td>
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<td>Control</td>
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<td>2</td>
</tr>
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</tr>
<tr>
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</tr>
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</tr>
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<td>63/F</td>
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<td>3</td>
</tr>
<tr>
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<td>73/F</td>
<td>AD IV</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>79/M</td>
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<td>3</td>
</tr>
<tr>
<td>17</td>
<td>66/F</td>
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<td>3</td>
</tr>
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<td>80/F</td>
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<td>19</td>
<td>79/M</td>
<td>AD V</td>
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Abbreviations: AD = Alzheimer's disease, MFCtx = midfrontal cortex, STctx = superior temporal cortex, HP = hippocampus, N = nucleus, C = cytoplasm, ND = not determined.

B. Hippocampus: Within the hippocampus FAC1 protein was immunolocalized to the nucleus of dentate granule cells (Fig. 2A). Pyramidal neurons within the CA2/3 and CA4 hippocampal subfields contain abundant FAC1
FAC1 immunolocalization in white matter. Cells of glial morphology are labeled in nondemented controls (A) and AD cases (B). Arrow in (A) denotes cell with morphologic characteristics of an astrocyte. Consecutive sections from nondemented adult midfrontal cortex (case 3 from Table 2) labeled with antibodies to Aβ (C) or FAC1 (D). Anti-FAC1 antibodies fail to detect plaquelike structures in nondemented adult brain. Magnification is ×100 in (A), ×400 in (B), and ×200 in (C) and (D).

Fig. 1. FAC1 immunolocalization in white matter. Cells of glial morphology are labeled in nondemented controls (A) and AD cases (B). Arrow in (A) denotes cell with morphologic characteristics of an astrocyte. Consecutive sections from nondemented adult midfrontal cortex (case 3 from Table 2) labeled with antibodies to Aβ (C) or FAC1 (D). Anti-FAC1 antibodies fail to detect plaquelike structures in nondemented adult brain. Magnification is ×100 in (A), ×400 in (B), and ×200 in (C) and (D).

protein in the nucleus and weak FAC1 immunoreactivity in the cytoplasm (Fig. 2B). Within the cytoplasm of these cells the FAC1 staining is sometimes punctate. Neurons within CA1 contain decreased FAC1 immunostaining within the cytoplasm, indicating different patterns of FAC1 distribution within different sectors of the hippocampus (Fig. 2C).

Abundant FAC1 protein was detected in the cytoplasm of neurons in the subiculum (Fig. 2D). All layers of the subiculum contain FAC1 immunolabeled nuclei, cell bodies, and neuritic processes. The proximal portion of the subiculum adjacent to CA1 displayed the most intense FAC1 immunoreactivity. Within the entorhinal cortex all cell layers, including the layer II projection neurons (those most severely devastated in AD), contain FAC1 predominately within the nucleus (Fig. 2E). The hippocampal and entorhinal cortex FAC1 immunostaining was similar in all 8 nondemented control brains analyzed.

FAC1 Immunolocalization in Cases of Possible AD

Upon neuropathologic examination, three nondemented adults (average age 74 yr) had significant numbers of NFTs in the CA1 subfield of the hippocampus and layer II projection neurons of the entorhinal cortex to warrant the classification of possible AD. By the AD classification criteria of Braak and Braak (14), these cases represent Stage II. NFTs were absent in the neocortex of these individuals.

We observed variable numbers of FA2 immunostained swollen fibers in the hippocampus from cases of possible
Fig. 3. FAC1 localization in the hippocampus of possible AD. (A) Low power (×40) magnification of swollen dendrites labeled for FAC1 above the hippocampal fissure in case 10. This case exhibited the greatest number of swollen dendrites in the hippocampus. (B) Higher magnification (×100) displaying shape and orientation of the fibers. Colocalization of FAC1 (C) and MAP2 (D) proteins as shown by double-label confocal microscopy. Bar = 50 μm. DG = dentate gyrus.

AD (Fig. 3A, B). Swollen neurites were not observed in other brain regions examined in these cases. The abnormal, swollen neurites were not immunoreactive with antibodies that recognize pathologic features of AD (Alz-50 or anti-Aβ) or with antibodies to axonal or synaptic elements such as tau or GAP-43. These structures were also not immunoreactive with an antibody to actin, suggesting that they are not Hirano bodies (16, 17). However these structures were labeled with the anti-MAP2 monoclonal antibodies AP14 and AP18 (18), indicating that the fibers represent MAP2 containing swollen dendrites (Fig. 3C, D). From the location of the fibers (adjacent to the hippocampal fissure nearest the pyramidal cell layer in the stratum lacunosum) they likely arise from the pyramidal cells and may signify a regenerative response within the hippocampus due to damage of the perforant pathway (19, 20). The swollen fibers were most prominent in the CA1 sector. The shape of these fibers is similar to swollen neurites observed by McKee et al in the AD hippocampus (21). We also identified a small number

Fig. 2. FAC1 localization in nondemented adult hippocampus. (A) FAC1 protein is contained in dentate granule cell nuclei. Nuclei of cells in CA4 (lower left) are also FAC1 immunoreactive. (B) Pyramidal neurons of CA3 exhibit FAC1 immunoreactivity in the nucleus, cell body, and neurites. (C) CA1 neurons contain FAC1 predominately in the nucleus, with little FAC1 in the cell cytoplasm. (D) Within the subiculum abundant FAC1 protein is visualized in the nucleus, cell body, and neurites of pyramidal neurons. (E) In the entorhinal cortex FAC1 again is localized predominately in cell nuclei. The top of the panel is towards the pial surface, and layer II is marked with (+). All panels are from case 2. (A) ×100; (B) and (C) ×400; (D) and (E) ×200.
Fig. 4. FAC1 immunolocalization in AD brain neocortex. FAC1 immunostained diffuse (A) and neuritic (B) plaques are evident in the AD temporal cortex. However case to case variability was present in the number and type of plaque structure labeled by antibodies to FAC1. Double-label confocal microscopy using antibodies to FAC1 (C) and Aβ (D) FAC1 protein colocalizes to amyloid containing plaques. Bar = 50 μm and the section is from case 15. Panels A and B are from cases 16 and 13 respectively, and at ×200.

Fig. 5. FAC1 localization in AD hippocampus. (A) FAC1 expression in dentate granule cells. The staining pattern is sometimes less intense than in control subjects. (B) FAC1 immunoreactive plaques within the molecular layer in case 16. (C) CA3 pyramidal neurons continue to display FAC1 immunoreactivity in the nucleus and cytoplasm but FAC1 is not contained in plaques and tangles. FAC1 immunostaining of CA1 (D), the subiculum (E), and entorhinal cortex (F) is also identical to nondemented controls. (A) and (B) ×100; (C), (E), (F) ×200; (D) ×400. DG=dentate gyrus. In panel (E), the pial surface is near the top and (+) marks layer II cells. Panels A, D, E, and F are from case 13, panel B from case 16, and panel C from case 14.

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of similar FAC1 labeled structures within the hippocampal pyramidal cell layer (stratum pyramidale) in 2 definite AD cases.

FAC1 Immunolocalization in AD Brain

A. AD Midfrontal and Temporal Cortex: We next determined the subcellular distribution of FAC1 within the same brain regions in 8 definite AD cases (Table 2). In the midfrontal cortex of 6 of 8 AD cases we observed FAC1 immunostaining of the cytoplasm and processes of some neurons, in addition to the nuclear staining observed in normal brain. In 5 of 8 cases we detect FAC1 staining in diffuse or neuritic elements of plaques (Fig. 4A, B). Diffuse FAC1 immunoreactivity often appears throughout the extracellular plaque structure. By labeling adjacent sections of the midfrontal cortex with antibodies to either the Aβ peptide or FAC1 we determined that in all subjects the number of FAC1-immunoreactive plaques was less than the number of amyloid-containing plaques; however, double-label confocal laser microscopy demonstrated that FAC1 protein is localized to amyloid-containing plaques (compare Fig. 4C and D). In this particular field FAC1 (depicted in panel C) is contained in all Aβ containing plaques, though the immunostaining is not 100% coincident with Aβ immunostaining. A similar FAC1 staining pattern was observed in both the superior and midtemporal cortex in AD subjects.
B. **AD Hippocampus:** The subcellular localization of FAC1 within the hippocampus exhibits few alterations in AD brain. Cells of the dentate gyrus continue to display FAC1 immunoreactivity (Fig. 5A), although in two AD cases an overall decrease of dentate FAC1 immunostaining was noted. In 4 of 8 AD cases plaques within the molecular layer of the dentate gyrus were immunoreactive for FAC1 (Fig. 5B). Cells within CA4, CA2/3 and the subiculum continue to display cytoplasmic staining of FAC1 in neurons (Fig. 5C, E). While we observed occasional FAC1-containing neurites in CA1, no plaques or tangles were labeled for FAC1 in this sector (Fig. 5D). FAC1 immunoreactivity within the entorhinal cortex also remains unchanged in AD (Fig. 5F). We detected no FAC1 protein within the plaques or NFTs that are quite abundant in these brain regions (see below). Therefore FAC1 is not a general marker for neurodegeneration. A small number of swollen FAC1-containing neurites were observed in the hippocampus in 2 of 8 definite AD cases.

**FAC1 Fails to Colocalize with NFTs in Hippocampal Neurons**

It is possible that cytoplasmic FAC1 protein colocalizes with NFTs in degenerating neurons in AD brain. Since neurofibrillary damage is often most prevalent in the hippocampus and entorhinal cortex, we determined the localization of FAC1 in hippocampal neurons containing NFTs by double-label confocal laser microscopy. To detect NFT-containing cells we utilized TG3, a monoclonal antibody produced against purified paired helical filaments (PHF) that recognizes NFT's (22). Confocal microscopy demonstrates that FAC1 is not localized in the cytoplasm of neurons containing intracellular NFTs, but instead is localized only in the nucleus (Fig. 6, top). FAC1-positive neuritic processes do not possess TG3 immunoreactivity (Fig. 6, bottom), indicating neurofibrillary pathology is absent in FAC1-containing neurites. The large number of neurons within the hippocampus and subiculum that have abundant cytoplasmic FAC1 protein fail to exhibit characteristics of AD pathology.

**Increased FAC1 Protein Levels in Soluble Fractions from AD Brain**

We also evaluated the expression levels of FAC1 protein in nondemented control and AD brain tissue by immunoblot analysis. Since FAC1 was observed in plaques and occasionally in neurites, we quantitated FAC1 protein levels in soluble protein fractions from the superior temporal cortex from nondemented and AD brain (see Methods). Equal amounts of protein from each fraction were loaded onto the gel and the resulting blot immunostained for the 125kDa FAC1 protein. All AD samples were classified as Stage III or IV by the Braak scoring criteria.

Figure 7 demonstrates that increased levels of FAC1 protein are detected in soluble fractions from AD brain when compared to similar fractions from control nondemented brains. Quantitative densitometric analysis revealed a 7-fold average increase in FAC1 protein within the AD samples in comparison to the control samples; however, Braak Stages V and VI (the end points of the disease) often failed to exhibit increased FAC1 protein levels by immunoblot (data not shown).

Levels of FAC1 protein in the membrane fraction (nuclear-containing pellet) were not significantly altered in AD brain preparations, with the pellet fractions from AD brain containing 106% the FAC1 protein as the nondemented control pellets (data not shown). As a control we immunolabeled a blot with anti-actin or anti-GFAP antibody and observed at least 60% of the total cellular immunoreactivity in the corresponding supernatant fraction. Therefore a majority of cytoplasmic and soluble proteins remain in the supernatant under these fractionation conditions.

**DISCUSSION**

We have presented data characterizing the localization of FAC1 protein in multiple brain regions of nondemented adults, possible AD, and definite AD cases. In the neocortex of nondemented adults FAC1 protein is localized predominately to nuclei. However in the hippocampus and subiculum FAC1 is located in both the nucleus and cytoplasm of neurons. In the entorhinal cortex, layer II stellate neurons exhibit only nuclear FAC1 immunoreactivity and neurons within other cell layers display predominately nuclear FAC1 immunostaining. In cases of possible AD FAC1 immunoreactive swollen dendrites are present in the hippocampus. These dendrites likely originate from pyramidal cells. In AD brain FAC1 is contained in a subset of plaques in the neocortex and increased levels of FAC1 protein are localized in soluble fractions from brain homogenates. FAC1 immunoreactive plaques are also present in the molecular layer of the hippocampal formation but few additional changes are observed in the hippocampus or entorhinal cortex.

FAC1 protein is observed in a subset of diffuse and neuritic plaque structures labeled for Aβ or tau proteins. Whereas other nuclear antigens such as p105 and Ki-67 have recently been directly associated with neurofibrillary degeneration in AD (23, 24), FAC1 is not generally observed in NFTs. Therefore neuronal nuclear changes occur during AD that may directly contribute to degeneration (expression and redistribution of cell proliferation antigens such as p105) or play another role in the disease, such as a regenerative response. The presence of FAC1 in extracellular plaque components implies that FAC1 localization within plaque structures can occur prior to or independent of neuritic pathology. However it is important to note that amyloid-containing diffuse plaques present in nondemented adults exhibit no FAC1 immunoreactivity, suggesting that accumulation of FAC1 protein
Fig. 6. FAC1 does not colocalize with neurofibrillary tangles in the hippocampus. Hippocampal sections were labeled with monoclonal antibodies to FAC1 (Cy5 label) and NFTs (FITC) and analyzed by laser confocal microscopy. CA1 pyramidal neurons containing NFTs fail to localize FAC1 in the cytoplasm (Top panel). FAC1 remains in the nucleus of NFT-containing neurons. In the bottom panel, FAC1-containing neurites (red) are shown to be distinct from NFT containing dystrophic neurites (green label) within the subiculum. Bar is equivalent to 10 μm in both panels.
cases FAC1 protein is also observed in white matter cells with a morphology of microglia or astrocytes. Further experiments are required to determine all cell types that express FAC1 protein.

FAC1 immunolocalization and distribution is not a definitive measure of AD but it does underscore the complexity of the disease. Since FAC1 immunostaining is not identical in all AD cases, specific AD subtypes may be defined by FAC1 immunoreactivity. FAC1 protein is contained in a subset of plaques in the neocortex and hippocampus, but is absent from plaques in the subiculum or entorhinal cortex. Therefore regional differences occur within the protein components of Aβ containing plaques. The presence of FAC1 in a subset of Aβ plaques may also be explained by varying stages of plaque evolution. Further studies of FAC1 in AD and other neurodegenerative diseases will help clarify the role of FAC1 in neurologic diseases. Additional studies of FAC1 expression and localization in normal aging and AD may also lead to novel insights into neurodegenerative disease mechanisms. In summary, FAC1 is localized in swollen neurites in the hippocampus of early stage AD and also in diffuse and neuritic plaques in specific regions of the AD brain. FAC1 protein is not contained in neurofibrillary tangles nor in diffuse plaques in nondemented adults.

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