Collapsin Response Mediator Protein-2 Is Associated with Neurofibrillary Tangles in Alzheimer’s Disease*

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Intraneuronal accumulation of paired helical filaments (PHF) is considered to be closely related to the neuronal loss observed in brains of patients affected with Alzheimer’s disease. The central issue is whether PHF formation itself causes or accelerates the neuronal perikaryal and neuritic degeneration or whether they are simply the consequence of preceding degeneration. We sought to address the issue in part by characterizing the PHF-associated molecules and thus raised a number of monoclonal antibodies to neurofibrillary tangles. One monoclonal antibody, 3F4, strongly reacted with neurofibrillary tangles and some plaque neurites but few neuropil threads. This monoclonal antibody labeled a 65-kDa protein, but not tau or ubiquitin, on a Western blot of human brain extract and immunoprecipitated the same protein. The peptides released from the purified 65-kDa protein had the same sequences as those of a newly identified protein, human collapsin response mediator protein-2. Incorporation into neurofibrillary tangles may deplete soluble, cytosolic human collapsin response mediator protein-2 and lead to abnormal neuritic and/or axonal outgrowth of the tangle-bearing neuron, thus accelerating the neuritic degeneration in Alzheimer’s disease.

Paired helical filaments (PHF) are unit fibrils of neurofibrillary tangles (NFT) in neuronal perikaryon that often surround the nucleus and run into the proximal portion of apical dendrites. Much smaller bundles of PHF or individual fibrils are also found mostly in dendrites as neuropil threads (curly fibers) and in a fraction of dystrophic neurites surrounding senile plaques (senile plaque neurites or dystrophic neurites); all of these constitute one of the pathological hallmarks of Alzheimer’s disease (AD). Accumulating evidence suggests that senile plaque neurites are derived mainly from axon terminals (1, 2), whereas neuropil threads are derived largely from dendrites (3–6). In either case, PHF are always associated with degenerating neuronal perikarya and their processes. The current central issue therefore is whether PHF formation itself causes the neuronal perikaryal and neuritic degeneration or whether they are simply the consequence of preceding degeneration.

One approach to the issue is complete characterization of hyperphosphorylated tau (PHF-tau), a building block of PHF (7). In contrast to normal tau that promotes tubulin assembly into microtubules, PHF-tau is assembly-incompetent (8, 9) and may destabilize microtubules. In fact, some investigators claim that PHF-tau even induces disassembly of microtubules (10). This may be seemingly consistent with the morphological observations made to date: if PHF are present in a given affected neuronal process, there are no normal cytoskeletons including microtubules and neurofilaments (11, 12). However, the weak point of the hypothesis is that there is no definitive evidence for the presence of cytosolic PHF-tau that should destabilize microtubules; PHF-tau is always recovered in the detergent-insoluble fraction. This may be because cytosolic PHF-tau is rapidly dephosphorylated during the post mortem (13).

We took a different approach to the issue: to search for a particular PHF-associated molecule that should provide insight into the microenvironmental alterations in NFT-bearing neurons (14, 15). For this purpose we raised a number of monoclonal antibodies against crude PHF and determined whether as yet unidentified molecules are associated with PHF. We obtained one monoclonal antibody (mAb), 3F4, which did not label tau but a 65-kDa protein in the soluble fraction of human brain. Sequencing and mass spectrometric analyses of the 65-kDa protein revealed that it is human collapsin response mediator protein-2 (hCRMP-2), a human homologue of CRMP-62 that was originally identified in chick brain (16). The protein is assumed to be involved in mediating an intracellular response to collapsin through a signal transduction cascade involving G protein (17) and has significant homology with a 

EXPERIMENTAL PROCEDURES

Materials—Unless otherwise indicated, all reagents were obtained from Nacalai Tesque (Kyoto, Japan) or Wako Pure Chemical Industries (Osaka, Japan). Trizma base, phenylmethylsulfonyl fluoride, N"-p-tosyl-L-lysine chloromethyl ketone, diisopropyl fluorophosphate, alkaline phosphatase (Escherichia coli, type III), Nonidet P-40, and polyvinylpyrrolidone-40 were obtained from Sigma. Culture medium and supplements were obtained from Life Technologies, Inc. Anti-papain, pepstatin, and leupeptin were obtained from Peptide Institute (Osaka, Japan). The Affi-gel Hz Immunoaffinity kit, protein assay kit, and horseradish peroxidase-conjugated anti-mouse IgG antibody were obtained from Bio-Rad.

PHF for Immunization—Frozen AD cortices were homogenized in 10 volumes of Tris-saline (TS; 50 mM Tris-HCl, pH 7.6, 0.15 M NaCl) containing 0.32 mM sucrose, 0.5 mM NaCl, and protease inhibitors (1 mM
EGTA, 0.5 mM diisopropyl fluorophosphate, 0.5 mM phenylmethylsulfonyl fluoride, 1 μM MgCl₂, 1 μM phenylmethylsulfonyl fluoride, 1 μg/ml antipain, 1 μg/ml pepstatin, and 1 μg/ml leupeptin), and the homogenates were centrifuged at 10,000 × g, for 30 min at 4 °C in a refrigerated centrifuge (himac CR20B2, Hitachi, Ibaraki, Japan). The obtained supernatants were supplemented with Sarkosyl powder to final concentration of 1% and stirred at room temperature for 1 h. The suspension was centrifuged at 543,000 × g for 30 min at 4 °C, and the resultant pellets (Sarkosyl-insoluble fraction; Sarkosyl PHF) were suspended in TS buffer, and the densities of the suspensions were adjusted to 1.3 g/ml.

The TS-soluble fraction from human brain was centrifuged at 543,000 × g for 30 min at 4 °C. This centrifugation gave a density gradient from 1.23 to 1.30 g/ml, for the contents to float to the bottom of the tube (fractions 1–8), and after dilution with 2 volumes of TS, each fraction was centrifuged at 543,000 × g, for 30 min at 4 °C. Each of the pellets was solubilized in Laemmli’s sample buffer at 4 °C. Each specimen, after blocking with 4% skim milk in phosphate-buffered saline by the avidin-biotin-peroxidase method, was subjected to Western blotting. Protein assay was performed by Lowry method.

The TS-soluble fraction prepared from human brain was applied to SDS-polyacrylamide gel electrophoresis on a 10% polyacrylamide gel. Separated proteins were transferred to a polyvinylidene fluoride (PVDF, Immobilon, Nihon Millipore Ltd, Yonezawa, Japan) membrane and subjected to Western blotting with Alz50, AT8, C5, or 3F4 (see below).

Preparation of Monoclonal and Polyclonal Antibodies—Monoclonal antibodies were raised against the high density (“heavy”) PHF fraction (fractions 4–7) or the low density (“light”) PHF fraction (fractions 1–3) of the pellets (Sarkosyl-insoluble fraction; Sarkosyl PHF) isolated from the TS-soluble fraction from human brain. The supernatants were concentrated by ammonium sulfate fractionation (33–50%) and were subjected to SDS-polyacrylamide gel electrophoresis. Two antibodies raised against the high density fraction were selected and used for the experiments; one was highly reactive against the human and mouse PHFs, and the other against the human and rat PHFs.

To establish a registry for Alzheimer’s disease (31), aliquots of TS-soluble fraction from the frontal cortices of the above cases were stored at −80 °C. Aliquots (10 μl) of each sample were subjected to Western blotting with 3F4 using the ECL system (Amersham Pharmacia Biotech). The relative intensity of the 65-kDa band was quantitated using one standard with a model GS-700 imaging densitometer on Molecular Analyst® Software (Bio-Rad). In this experiment, 2, 5, 10, and 15 μl of aliquots of the ammonium sulfate (33–50%)–concentrated fraction from the authentic specimens were used as a standard. One arbitrary unit is defined as the ECL signal resulting from use of 1 μl of the specimen. The standard was linear in this range (2–15 μl of the standard aliquots). Each TS-soluble specimen was diluted 1, 5, 1, 3, 2, 3, or 1:1 and subjected to Western blotting in conjunction with the standard specimens.

Other Methods—Rat brains at embryonic days 13 and 19, postnatal day 1, and postnatal weeks 1, 2, 4, 10 and 10 and several organs apart from brain, including heart, thymus, spleen, liver, and kidney were homogenized in 4 volumes of TS buffer containing protease inhibitors. After high speed centrifugation at 543,000 × g, for 30 min at 4 °C, the supernatants were obtained, and small aliquots (20 μg of protein) of them were subjected to Western blotting. Alkaline phosphatase treatment was performed with alkaline phosphatase (10 units/ml) in 50 mM Tris-HCl, pH 9.5, containing protease inhibitors with or without 0.1 mM sodium phosphate at 37 °C for 2 h. After the treatment, the reaction mixture was concentrated by ammonium sulfate fractionation (33–50%) and was subjected to Western blotting. Protein assay was performed using bichoninic acid protein assay kit (Pierce) or Bio-Rad protein assay kit using bovine serum albumin as a standard.

RESULTS

Heavy PHF as an Immunogen—It was previously shown that two distinct PHF fractions were separated by sucrose density gradient centrifugation (32, 33). One fraction consisted mostly of short and nonaggregated PHF, the majority of which are readily soluble in SDS, and the other contained primarily aggregated PHF in bundles, which are less soluble in SDS. We...
similarly fractionated crude PHF (Sarkosyl-PHF) by CsCl isopycnic density gradient centrifugation and obtained two distinct PHF fractions; one is “light” PHF in fraction 1 at a density of 1.23 g/ml, and the other is heavy PHF in fraction 3 at a density of 1.3 g/ml (Fig. 1). Because most proteins were recovered in fraction 1, the heavy PHF fraction was very pure (Fig. 1A). These fractions were tested for their reactivities to several well characterized monoclonal antibodies, Alz50 (epitope: residues 1–10; Ref. 23, see also Refs. 24 and 25), AT8 (phosphorylated Ser-202/Thr-205; Ref. 21), and C5 (phosphorylated Ser-396; Ref. 22). Light PHF are characterized by loss of Alz50 immunoreactivity and smear staining especially with C5 (Fig. 1, B and C). In contrast, heavy PHF are characterized by much less extents of smears and by the presence of discrete, often triplet, bands exhibiting Alz50 reactivity on Western blots (Fig. 1B). Thus heavy PHF are exclusively composed of hyperphosphorylated full-length tau, whereas light PHF are composed of more processed tau (data not shown). These results indicate that heavy PHF are less processed and so can be regarded as “younger” ones.

A Monoclonal Antibody to NFT That Shows No Reactivity with Tau—We used heavy PHF as an immunogen to raise monoclonal antibodies because they are pure to a much greater extent than light PHF. Primary screening for positive clones was based on the staining of isolated (nondenaturing) tangles (27), and the secondary screening was based on the reactivities of hybridoma media with fetal rat tau, which is known to be highly phosphorylated at sites similar to those phosphorylated in PHF-tau (34, 35). Although most positive clones reacted with phosphorylated tau or microtubule-associated protein 1B, one mAb, 3F4, was found to have no reactivity with phosphorylated tau on the Western blot. On the tissue section, 3F4 intensely reacted with NFT and occasionally with senile plaque neurites but very slightly with neuropil threads, whereas AT8 reacted with NFT and occasionally with senile plaque neurites, and innumerable neuropil threads (Figs. 2, A and B). By comparing tau- and 3F4-stained serial sections, we thought that most of intracellular tangles...
The 65-kDa Protein, but Not Tau, Is Immunoprecipitated with 3F4—To further characterize the 3F4 antigen, we immunoprecipitated the antigen with 3F4 using the TS-soluble fraction from human control aged brain. mAb3F4 precipitated the 65-kDa protein that is labeled with 3F4 but not with anti-tau, whereas anti-tau precipitated tau that is labeled with anti-tau but not with 3F4 (Fig. 4A). This result clearly indicates that the 65-kDa protein is distinct from tau. In addition, the 65-kDa protein was heat- and acid-labile and did not bind to the phosphocellulose column, properties incompatible with those of tau (data not shown).

Previous studies showed that several phosphorylation-dependent antibodies labeled NFT, and phosphorylated species may be characteristic of NFT-associated proteins (14). Thus, we determined the effect of alkaline phosphatase treatment on 3F4 immunoreactivity. The 65-kDa protein immunoreactivity-purified from the human brain soluble fraction (see below) was dephosphorylated with alkaline phosphatase. After the treatment, the 3F4-reactive band migrated faster on the gel (Fig. 4B), suggesting that the 65-kDa protein is a phosphoprotein and mAb3F4 is a phosphorylation-independent antibody.

The 65-kDa Protein Is Brain-specific and Its Expression Is Developmentally Regulated—To obtain more information about the 65-kDa protein, we investigated its expression levels in rat brains at various ages and in various organs apart from the brain. First, we determined its expression in brain during development from embryonic day 13 to postnatal week 10. The 65-kDa protein was detectable from embryonic day 19, reaching a peak at postnatal weeks 1–2, and became barely detectable at postnatal week 10 (Fig. 5A). Initially the 3F4-reactive protein appeared as doublets at embryonic day 19 and postnatal day 1, and the upper band predominated the lower one, disappearing at postnatal week 1 (Fig. 5A).

We next determined the level of expression of the 3F4 antigen in various organs from rat at postnatal week 2, when the 3F4 antigen expression in the brain had peaked. TS-soluble fractions from various organs including the brain, heart, thymus, spleen, liver, and kidney, were subjected to Western blotting with 3F4 using an ECL system.

Identification of the 3F4 Antigen as hCRMP-2—We purified the 65-kDa protein by immunoaffinity chromatography (see
Unexpectedly, two major bands at 65 and 60 kDa, roughly at the same stoichiometry, were observed upon CBB staining of the Western blot, but only the upper band was 3F4-reactive (Fig. 4B). The 3F4-reactive band was carefully excised and digested with API. The digest was separated by HPLC, and numbered peaks were subjected to protein sequencing and time of flight mass spectrometry. In the lower panel are shown the amino acid sequences of hCRMP-2 (16) and the peptides (boxed) released from the 3F4 antigen. The sequences selected for raising peptide antibodies are underlined (see Fig. 7).

We similarly analyzed the peptides released from a digest of the protein represented by the lower (3F4-nonreactive) band for their mass numbers. Most interestingly, the HPLC profile for API peptides from this protein was very similar to that from the upper (3F4-reactive) band (data not shown). Consistent with this, peaks 1, 3, 4, and 5, eluting at the same positions as those in the profile of the 3F4-reactive 65-kDa protein, provided the same mass numbers as corresponding peaks generated from the 65-kDa protein (peak 2 gave no significant signals). This result raises the possibility that the 3F4-reactive protein is a modified 60-kDa protein and 3F4 recognizes the modification, but the epitope was refractory to alkaline phosphatase (Fig. 4B). Another possibility is that the proteins are two closely related isoforms and 3F4 recognizes only the larger one. The peptide antibodies to hCRMP-2 (see below) labeled both bands similarly (data not shown), thus excluding the possibility that the lower band excised for sequence analysis was contaminated with the upper 65-kDa protein. Although the above data may point to the possibility that hCRMP-2 exists as hetero- or homodimers, much work is required to clarify this.

**Several Peptide Antibodies to hCRMP-2 React with NFT**—Although 3F4 has been shown to recognize hCRMP-2, we cannot rule out that the mAb recognizes similar sequences in other (related) molecules. To confirm that hCRMP-2 itself is present in PHF, we raised polyclonal antibodies against seven synthetic peptides homologous to the hCRMP-2 sequences (Fig. 6, underlined sequences). All these antibodies labeled both the 60-
and 65-kDa bands on the Western blot of the immunoaffinity-purified fraction (data not shown). Four antibodies, anti-MA52, anti-MA53, anti-A661, and anti-A666, labeled some isolated tangles and/or NFT in vibratome sections (Fig. 7, A–D). Only anti-A666 labeled both NFT in vibratome sections and isolated tangles. Other antibodies stained either NFT in vibratome sections or isolated tangles. The amino acid sequences that generated NFT-reactive antibodies spanned the hCRMP-2 molecule (residues 44–510). Two antibodies, anti-A660 and anti-A662, which were raised against amino-terminal (residues 30–48) and carboxyl-terminal portions (residues 401–418) of hCRMP-2, respectively, stained neither NFT in vibratome sections nor isolated NFT. This result suggests that the amino-terminal portions of the molecule might be lost in vivo or structurally hidden in NFT. Although A662 is the sequence of the peptide identified in the digest of the 65-kDa protein (peak 4), anti-A662 was unable to stain any NFT in the AD brain. Based on the above results, we concluded that hCRMP-2 or a closely related molecule is associated with NFT.

Levels of the 3F4-immunoreactive 65-kDa Protein in AD Brain Are Significantly Higher than in Control Brain—It is now known that a number of cytosolic proteins are tightly associated with NFT (see Refs. 14 and 15), but its significance and their roles in PHF formation remain unclear. The levels of the two major components of PHF, tau and ubiquitin, are significantly decreased in the soluble fraction from AD brains.2 Thus we have determined whether the 65-kDa protein shows a similar tendency.

The levels of the 65-kDa protein in TS-soluble fractions from the temporal or frontal cortices of 24 AD and 37 control brains were quantitated by Western blotting. In 4 AD and 22 control brains the levels of the protein were below the detection limit and thus these cases were excluded from statistical analysis.

By examining several cases, we confirmed that in a given brain the 65-kDa protein levels in the soluble fraction do not significantly differ between temporal and frontal cortices (data not shown). The 65-kDa protein from the soluble fraction of AD brain showed a distinct band but not smearing that presumably represents its tight association with NFT in the insoluble fraction (Fig. 1D). Thus, one may assume that the soluble 65-kDa protein originates largely from the cytosol of neurons in AD brain. Although the levels of the soluble 65-kDa protein varied greatly, its mean level in AD brains is significantly higher than that in control brains (Fig. 8, A and B; \( p < 0.005 \), two-tailed Student’s \( t \) test).

Fig. 8. The levels of 3F4 antigen in control and AD brains. Equal amounts of TS-soluble fractions from control and AD brains were subjected to Western blotting with 3F4 using an ECL system (A). The relative intensity of the 65-kDa band was quantitated using a model GS-700 imaging densitometer with Molecular Analyst® Software (Bio-Rad). The levels of the 65-kDa protein in TS-soluble fractions from 20 AD and 15 control cases were plotted (B). The mean levels are statistically significant between control and AD brains (\( p < 0.005 \), two-tailed Student’s \( t \) test).
with the density of NFT. Sarkosyl-insoluble fractions were prepared from the same cortical pieces as were used for quantification of the soluble 65-kDa protein and subjected to Western blotting with C5. No correlation was found between the levels of the soluble 65-kDa protein and the extents of C5 immunoreactivities, strongly suggesting that the levels of soluble 65-kDa protein have no relationship with NFT densities (data not shown).

**DISCUSSION**

In the present work we raised mAbs against the heavy PHF fraction prepared by CaCl2 centrifugation and obtained one mAb to NFT, 3F4, which recognizes hCRMP-2, a human homologue of unc-33 protein in *C. elegans* (16). Because CRMP-2 is associated only with a fraction of PHF and straight tubules, it cannot be referred to as their integral component. Thus CRMP2 itself is supposed not to be involved in the formation of PHF or straight tubules, which are extensively distributed throughout AD cortex.

The association of CRMP2 with PHF or straight tubules raises the possibility that CRMP2 and tau interact with each other in human brain. However, the immunoprecipitation experiment clearly showed that the two molecules are not associated with each other under normal conditions (Fig. 4). This should be also the case in AD brain. First, when soluble fractions from AD brain were applied to the 3F4 column, CRMP2 did not coelute with tau (data not shown). Second, our previous study (36) showed that tau prepared from soluble fraction of AD brain is biochemically indistinguishable from normal tau from control brain.

CRMP-2 is also referred to as rat Ulip (unc-33-like protein) or TOAD-64 (turned on after division; Refs. 37 and 38). All of these are probably the same protein, which is expressed exclusively in the nervous system and abundantly during development. Ulip was shown to be a phosphoprotein and to have at least two isoforms, and the lower molecular weight one is the predominant species. Presently, it is unknown whether the difference in their apparent molecular masses is due to modification(s) or unidentified insertion. Three hCRMP proteins, which are highly homologous to CRMP-62, have been identified and termed hCRMP-1, hCRMP-2, and hCRMP-3 (partial sequence), and four rat (r)CRMP cDNAs have been cloned (39). RCMP-2 is almost identical to TOAD-64, most closely related to chick CRMP-62, and is the most widely expressed species within the nervous system.

Mutations of the unc-33 gene of *C. elegans* lead to severely uncoordinated movement. This is attributed to defects of neuronal processes in unc-33 mutants; axonal processes in several classes of neurons in *C. elegans* tend to terminate prematurely and often at abnormal locations and generally have swollen endings (18). The dendrites of all classes of sensory neurons in the mutant appear to contain a superabundance of microtubules. In addition, microtubules are sometimes larger than normal ones or exhibit abnormal hooks or doublet or triplet microtubules (18). Thus, it was suggested that the product of unc-33 is a component of the axonal cytoskeleton that directs axonal outgrowth and that the axonal guidance defects in the mutant are a consequence of cytoskeletal defect. In particular, it was suggested that the wild-type gene product may be a microtubule-associated protein that controls the assembly or stability of microtubules (18–20).

CRMP-62, a chick homologue of unc-33, was originally identified by expression cloning in Xenopus oocyte and is now proposed to be an intracellular component of the multimetric receptor complex that couples collapse-binding to the G protein-mediated signaling machinery (16, 17). The expression of TOAD-64 coincides with neuronal differentiation and is down-regulated when axon growth is completed (38). Following neural induction of P19 and PC12 cells, TOAD-64 expression is up-regulated, followed by the expression of several neuronal markers, such as neurofilaments and microtubule-associated protein 2. TOAD-64 is found in the lamellipodia and filopodia of growth cones in cultured dorsal root ganglia. It appears that the cytoskeletons in neurites or growth cones could be morphologically unstable.

Interestingly, whereas rCRMP-2 is almost undetectable in the adult rat brain (Fig. 5A), the 3F4 antigen is detectable in human aged brains on Western blot (Figs. 3 and 8). This suggests that even in the adult, hCRMP-2 is involved in neurite and/or axonal growth or regeneration and thus in plasticity. It is tempting to speculate that continued expression of fetal isoforms provides adult human brains with the highest plastic potential. However, in control aged brains, 3F4 provided no significant staining in vibratome sections, although the 3F4 antigen was clearly detected on the Western blot of the brain extract (Figs. 3 and 8). This may be explained by too low an amount of the 3F4 antigen in the normal aged brain for it to be detected immunocytochemically and/or unusual susceptibility of this cytoplasmic protein to formalin fixation. In contrast, the NFT-associated 3F4 antigen could be much less affected by fixation and thus can be readily immunocytochemically detected; in addition, its accumulation on NFT is one more factor contributing to its immunodetectability. Thus, the situation with immunodetectability of hCRMP-2 would be similar to that with normal tau and PHF-tau in the fixed tissue; normal tau in normal neuropil cannot be immunocytochemically detected with anti-tau, if neither a specific fixation method (40) nor enhancement procedure is employed (41, 42), whereas PHF are readily detected with the same antibody, presumably because the hyperphosphorylated tau composing PHF does not undergo profound conformation changes after fixation. Normal tau is recovered in the soluble fraction, whereas PHF-tau and processed tau are recovered in the insoluble fraction, showing triplets (PHF-tau) and a smear on the Western blot (Fig. 1C). In this context, localization of the 65-kDa protein to NFT and plaque neurites in AD brain may reflect the normal distribution of the molecule; under normal conditions the 65-kDa protein may be enriched in neuronal perikarya and axon terminals, the latter of which form senile plaque neurites (1, 2).

In view of this, it is quite reasonable to postulate that hCRMP-2 recovered in the TS-soluble fractions from AD brain is largely derived from the cytosolic compartment but not from NFT-associated, presumably processed, hCRMP-2; mAb3F4 recognizes the 65-kDa protein in TS-soluble fraction both from AD and control brains but labeled a smear on the blot of Sarkosyl-insoluble fraction only from AD brain (Figs. 1D and 8). This suggests that, in contrast to immunocytochemically visualized NFT-associated hCRMP-2 that may no longer function, soluble hCRMP-2 that is not immunocytochemically detectable and increased in its level in AD brain is presumably functioning in NFT-free neuronal processes. This leads us to the speculation that there are two populations of a subset of neurons vulnerable to AD: NFT-bearing neurons in which cytoplasmic hCRMP-2 is depleted because of its entrapment by NFT and NFT-free neurons where hCRMP-2 levels are increased. In this respect, it should be noted that the AD cortex exhibits extensive abnormalities of dendrites and axons that are free from PHF (2, 43, 44) as well as those containing PHF (neuropil threads or curly fibers). Thus, depletion and abundance of hCRMP-2 may be related to such a complicated picture of degeneration.

The above view may be important because the extent of neuronal loss in AD brain exceeds by far the number of NFT-
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bearing neurons and thus cannot be explained by NFT formation (45, 46). This strongly suggests that most of neurons being lost in AD brain do not form NFT, and raises the possibility that NFT formation represents a particular response of a subset of neurons that attempt to compensate for the loss of neighboring neurons. Thus, it is possible that increased levels of CRMP2 caused by the underlying pathological process are related to extensive loss of NFT-free neurons, presumably the most important consequence of Alzheimer’s disease (46).

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REFERENCES

1. Probst, A., Brun, V. B., and Ulrich, J. (1983) Brain Res. 268, 249–254
2. Masliah, E., Mallory, M., Hansen, L., Alford, M., DeTeresa, R., and Terry, R. D. (1993) Am. J. Pathol. 142, 671–882
3. Braak, H., and Braak, E. (1988) Neuropathol. Appl. Neurol. 14, 39–44
4. Ihara, Y. (1988) Brain Res. 459, 138–144
5. Yamaguchi, H., Nakazato, Y., Shoji, M., Ihara, Y., and Hirai, S. (1990) Acta Neuropathol. 80, 368–374
6. Masliah, E., Ehlisman, M., Carragher, B., Mallory, M., Young, S., Hansen, L., DeTeresa, R., and Terry, R. D. (1992) J. Neuropathol. Exp. Neurol. 51, 484–494
7. Lee, V. M.-Y., Balin, B. J., Otteus, L., Jr., and Trojanowski, J. Q. (1991) Science 251, 675–678
8. Bramblett, G. T., Goedert, M., Jakes, R., Merrick, S. E., Trojanowski, J. Q., and Lee, V. M.-Y. (1993) Neuron 10, 1689–1699
9. Yoshida, H., and Ihara, Y. (1993) J. Neurochem. 61, 1153–1186
10. Alonso, A. D. C., Zaidi, T., Grunke-Iqbal, I., and Iqbal, K. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5562–5566
11. Perry, G., Kawai, M., Tabatou, M., Ororato, M., Balin, B. J., and Gambetti, P. (1991) J. Neurosci. 11, 1748–1755
12. Watanabe, T., Hasegawa, M., Araki, Y., and Ihara, Y. (1992) Am. J. Pathol. 140, 277–282
13. Matsuo, E. S., Shin, R.-W., Billingsley, M. L., Van deVoorde, A., O’Connor, M., Trojanowski, J. Q., and Lee, V. M.-Y. (1994) Neuron 13, 869–1002
14. Hasegawa, M., Araki, T., and Ihara, Y. (1996) Neuron 4, 909–918
15. Morishima-Kawashima, M., Araki, T., Ogawara, M., Takio, K., Titani, K., Saitoh, T., Kosik, K. S., and Ihara, Y. (1991) Brain Res. 554, 316–320
16. Goshima, Y., Nakamura, F., Strittmatter, P., and Strittmatter, S. M. (1995) Nature 376, 509–514
17. Igarashi, M., Strittmatter, S. M., Variotian, T., and Fishman, M. C. (1993) Science 259, 77–79
18. Hedgecock, E. M., Culotti, J. G., Thomson, J. N., and Perkins, I. A. (1985) Dev. Biol. 111, 158–170
19. Hedgecock, E. M., Culotti, J. G., Hall, D. H., and Ster, B. D. (1987) Development 100, 365–382
20. Desai, C., Garriga, G., Mcintire, S. L., and Horviz, H. R. (1988) Nature 336, 683–646
21. Goodart, M., Jakes, R., and Vannechemelen, E. (1995) Neurosci. Lett. 189, 167–170
22. Hasegawa, M., Watanabe, A., Takio, K., Suzuki, M., Titani, K., and Ihara, Y. (1993) J. Neurochem. 60, 2068–2077
23. Goodart, M., Spillantini, M. G., and Jakes, R. (1991) Neurosci. Lett. 126, 149–154
24. Carmel, G., Mager, E. M., Binder, L. I., and Kuret, J. (1996) J. Biol. Chem. 271, 32789–32795
25. Jicha, O. A., Browse, R., Kaazam I. G., and Davies, P. (1997) J. Neurosci. Res. 48, 128–132
26. Ihara, Y., Kondo, J., Miura, R., Nakagawa, Y., Mori, H., and Honda, T. (1990) Gerontology 35, (Suppl. 1) 15–24
27. Ihara, Y., Abraham, C., and Selkoe, D. S. (1989) Nature 340, 727–730
28. Sato-Yoshitake, R., Shiomura, Y., Miyasaka, H., and Hirokawa, N. (1989) Neuron 3, 229–238
29. Fernandez, J., DeMott, M., Atherto, D., and Mische, S. M. (1992) Anal. Biochem. 201, 254–264
30. Tierney, M. C., Fisher, H., and Lewis, A. J. (1988) Neurology 38, 356–364
31. Mirra, S. S., Heyman, A., and McKee, D. (1991) Neurology 42, 1681–1688
32. Kiefer, H., Morgan, K., and Dickson, D. W. (1994) Brain Res. 649, 159–164
33. Kiefer, H., and Wall, J. S. (1996) Neurobiol. Aging 15, 11–19
34. Watanabe, T., Hasegawa, M., Suzuki, M., Takio, K., Morishima-Kawashima, M., Titani, K., Arai, T., Kosik, K. S., and Ihara, Y. (1993) J. Biol. Chem. 268, 25712–25717
35. Morishima-Kawashima, M., Hasegawa, M., Takio, K., Suzuki, M., Yoshida, H., Titani, K., and Ihara, Y. (1995) J. Biol. Chem. 270, 823–829
36. Hasegawa, M., Morishima-Kawashima, M., Takio, K., Suzuki, M., Titani, K., and Ihara, Y. (1992) J. Biol. Chem. 267, 17047–17054
37. Byr, T., Dohrnk, T., Cifuentes-Diaz, C., and Sobel, A. (1996) J. Neurosci. 16, 638–701
38. Minturn, J. E., Frey, H. J., Geschwind, D. H., and Hockfield, S. (1995) J. Neurosci. 15, 6757–6766
39. Wang, L.-H., and Strittmatter, S. M. (1996) J. Neurosci. 16, 6197–6207
40. Pollock, N. J., and Wood, J. G. (1988) J. Histochem. Cytochem. 36, 1117–1121
41. Shin, R.-W., Iwaki, T., Kitamoto, T., and Tateishi, J. (1991) Lab. Invest. 64, 693–702
42. Shin, R.-W., Iwaki, T., Kitamoto, T., Sato, Y., and Tateishi, T. (1992) Am. J. Pathol. 140, 937–945
43. McKee, A. C., Kowall, N. W., and Kosik, K. S. (1989) Ann. Neurol. 26, 652–659
44. Benzing, W. C., Ikonomotovic, M. D., Brady, D. R., Mufson, E. J., and Armstrong, D. M. (1995) J. Comp. Neurol. 354, 176–191
45. Leuba, G., and Kraftsik, T. (1994) Neurobiol. Aging 15, 29–43
46. Gómez-Isa, T., Hollister, H., West, H., Mui, S., Growdon, J. H., Petersen, R. C., Paris, J. E., and Hyman, B. T. (1997) Ann. Neurol. 41, 17–24
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