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An extensive network of PHFτ-rich dystrophic neurites permeates neocortex and nearly all neuritic and diffuse amyloid plaques in Alzheimer disease

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Abstract

Previous studies demonstrated paired helical filament τ (PHFτ) in neuritic but not diffuse β-amyloid (Aβ) plaques in Alzheimer's disease (AD). Re-examination of amyloid deposits with antibodies to Aβ and PHFτ by conventional and confocal microscopy using double label immunohistochemistry showed that PHFτ is a component of both diffuse and neuritic plaques in AD. Unlike controls, a dense network of PHFτ positive dystrophic neurites extended throughout the AD neocortex permeating nearly all neuritic and diffuse plaques. Thus, PHFτ-rich dystrophic neurites are common components of neuritic and diffuse plaques in AD neocortex.

Key words: Alzheimer's disease; Senile plaque; Neuronal cytoskeleton

1. Introduction

β-Amyloid peptides (Aβ) derived from one or more amyloid precursor proteins accumulate in plaques throughout the Alzheimer disease (AD) brain [1,2]. Aβ is produced in the normal and AD brain, and it is secreted by cultured cells including human neurons [3–6]. Although the mechanisms leading to the aggregation and fibrillogenesis of secreted Aβ are unknown, amyloid plaques contain several components that have been implicated in the genesis of these lesions [7–14]. However, two major types of Aβ deposits, i.e. the neuritic and diffuse plaque, differ with respect to composition and structure [7,8,11,12,14]. For example, aberrantly phosphorylated τ proteins (A68 or PHFτ) that form paired helical filaments (PHFs) in neurofibrillary tangles (NFTs) have been found in dystrophic processes associated with neuritic plaques (NPs), but a number of studies have failed to detect PHFτ in diffuse plaques [14]. Since this might signify that diffuse and neuritic plaques arise from different mechanisms, we re-examined this issue by conventional and confocal microscopy using double label immunohistochemistry.

To determine if PHFτ is a common or inconstant component of different types of Aβ deposits, we re-examined the association of PHFτ with Aβ in NPs and diffuse plaques using several monoclonal (MAbs) and polyclonal antibodies to defined epitopes in Aβ, normal τ and PHFτ. A recently developed MAb (PHF1) to an aberrantly phosphorylated epitope in PHFτ [15–18], was included here because this MAb labeled a more extensive network of dystrophic neurites in the AD brain than other antibodies to normal fetal and adult τ or PHFτ (see below). Indeed, unlike other antibodies to normal τ and PHFτ, PHF1 labeled PHFτ-rich dystrophic neurites in nearly all of the Aβ deposits in AD neocortex including diffuse plaques. In contrast, few neuritic and diffuse plaques were labeled by PHF1 in control neocortex.

2. Materials and methods

2.1. Tissue collection

Postmortem samples of neocortex obtained from 12 patients with AD ranging in age from 56 to 89 (average = 77) and 4 elderly controls ranging in age from 71 to 91 (average = 81) without evidence of a neurological disease were used in this study. The average postmortem interval was 11 h for the AD patients and 12 h for the controls. The diagnosis of AD was based on consensus criteria [19] as described [7,11,12]. None of the AD cases had cortical Lewy bodies [14,20]. Five cortical areas were studied, i.e. superior temporal gyrus (area 22), midfrontal gyrus (area 9/10), gyrus rectus (area 11), and occipital cortex (area 17). All tissue samples were immersion fixed in 70% ethanol with 150 mM NaCl and embedded in paraffin as described [7,11,12].

2.2. Antibody probes

The ability of PHF1 to detect PHFτ-rich NFTs and dystrophic neurites was compared with that of 11 other previously characterized antibodies specific for normal τ and/or PHFτ (Alz50, T60, Tau2, T46, T14, T3P, 133, 304, 189, 135, 134) all of which label neurofibrillary lesions in situ [7,11–14,17,21–31]. Based on preliminary studies, three of these antibodies that most intensely labeled abundant NFTs and

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dystrophic neurites were selected for detailed comparison with PHF1 in the quantitative double label studies described here. These antibodies included: T14, an MAb that recognizes a phosphate-independent epitope within amino acids 141–178 [25] according to the numbering system for the 441 amino acid long τ isoform [31]; T46, an MAb to a phosphate independent epitope within amino acids 404–441 [25], and T3P, a rabbit antiserum that binds to a phosphate dependent epitope within amino acids 389–402 only when serine-396 is phosphorylated [27]. The phosphate dependent τ epitopes recognized by T3P and PHF1 are nearly identical [17,18,27], and both epitopes are found in PHFτ and normal fetal CNS τ, but not in any of the six alternatively spliced isoforms of τ found in the postmortem adult CNS [17,23,27]. To label deposits of Aβ, polyclonal antisera to Aβ_{1-40} (UP107) and Aβ_{1-42} (2332) were used [11–13]. Finally, a MAb (2.2B10) to glial fibrillary acidic protein (GFAP; [32]) also was used to identify NPs in double stained preparations since reactive astrocytes infiltrate NPs but not diffuse plaques [11–14,33].

2.3. Immunohistochemical procedures

Immunohistochemistry was carried out with the peroxidase antiperoxidase procedure for both single and double label studies [11–13,34]. Double labeling also involved immunohistochemistry followed by Thioflavine S staining as described [12,34,35]. To retard Thioflavine S fading, the sections were mounted in medium containing 0.25% *p*-phenylenediamine, 5%*N*-propyl gallate, and 0.0025% 1,4-diazabicyclo-(2,2,2)-octane [35,36] and viewed with brightfield and fluorescent illumination. Additionally, selected alcohol fixed frozen and paraffin embedded sections were probed with antibodies using a Texas red and fluorescein isothiocyanate detection systems, and these sections were viewed with a conventional fluorescence microscope and a LEICA confocal microscope [14,20,35]. The positive and negative controls for these studies were as described [11–13,20,22,25–27,29,32–35].

2.4. Quantitation of immunohistochemical results

Areas of highest plaque density were selected for quantitative analy-

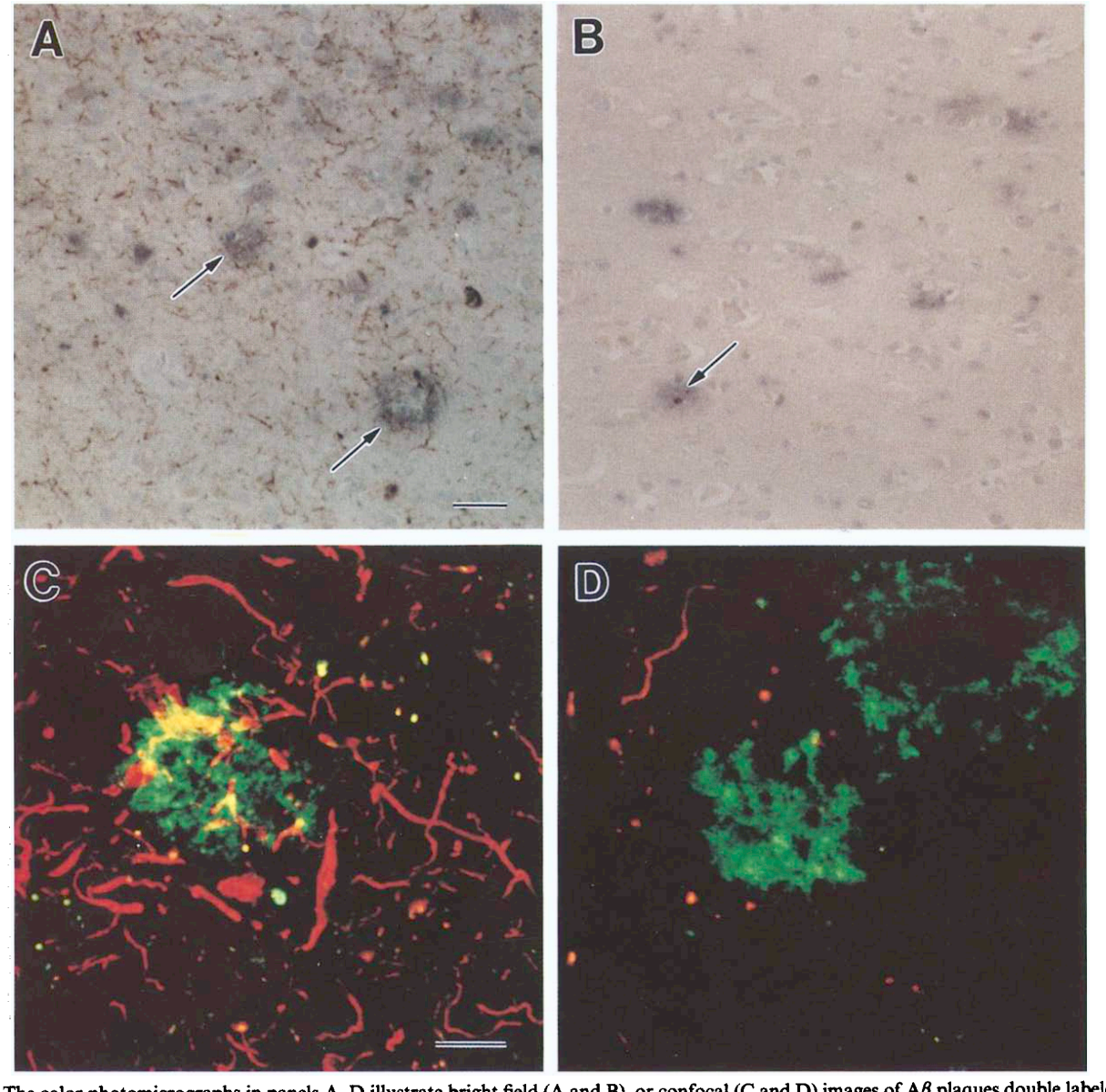

Fig. 1. The color photomicrographs in panels A–D illustrate bright field (A and B), or confocal (C and D) images of Aβ plaques double labeled with the anti-Aβ antiserum 2332 (gray-blue reaction product in A and B; green fluorescence in C and D) in combination with either the anti-PHFτ antibody PHF1 (brown reaction product in A and B; red fluorescence in C) or with the anti-τ antibody T46 (red fluorescence in D). Neocortical samples from 2 different AD patients are shown in A, C and D while B shows neocortex from an elderly control subject. PHF1 labeled dystrophic process (arrows) are more prominent in A and C than in B and D. Panels A and B are at the same magnification and the scale bar in A = 25 μm, while C and D are at the same magnification and the scale bar in C = 10 μm. In C and D, autofluorescent lipofuscin was edited out using Adobe Photoshop.

sis. Plaques within a one mm^2 area of a cortical region were counted [7,11,12], and a Student's t-test was used to assess the statistical significance of differences between single and double labeled amyloid plaques in the AD and control brains.

3. Results

Neocortical samples from all of the AD patients (Figs. 1A,C,D and 2A–C) and three control cases (Figs. 1B and 2A–C) contained amyloid plaques labeled by antibodies to $A\beta_{1-40}$ (UP107) and $A\beta_{1-42}$ (2332). As reported, [1,11–14,33], anti-$A\beta$ antibodies labeled many more plaques than Thioflavine S or other amyloid stains, and the number of $A\beta$ labeled plaques/mm^2 was similar in some of the AD and control neocortical samples (Fig. 2A). In contrast, a far greater number of $A\beta$ plaques in the AD neocortex was associated with dystrophic neurites labeled by the anti-τ (T14, T46) and anti-PHFτ (T3P, PHF1) antibodies (Fig. 2B,C). Indeed, no T14 positive plaques double labeled with anti-$A\beta$ antibodies were seen in the controls, and a significantly greater number ($P < 0.001$) of plaques double labeled with the PHF-1 MAβ and the anti-$A\beta$ antibodies was found in the AD neocortex compared to control samples. NPs were readily distinguished from diffuse plaques in sections double labeled with antibodies to $A\beta$ in conjunction with antibodies to normal adult τ, PHFτ and GFAP (data not shown).

In contrast to other antibodies to normal CNS τ or PHFτ that label plaque associated dystrophic neurites [14], the PHF1 MAb demonstrated immunoreactive linear and punctate profiles throughout the AD neocortex and in nearly all diffuse plaques in the AD brains, but only a small number of PHF1 positive dystrophic neurites were seen in control brains and diffuse plaques in the controls rarely contained PHF1 positive profiles (Fig. 1 and 2). Indeed, PHF1 usually labeled dystrophic processes in > 2–3 times more $A\beta$ positive deposits than T14, T46 and T3P. This was due to the greater frequency with which PHF1 labeled dystrophic neurites in the AD neocortex and in diffuse plaques (Fig. 2B,C). This finding is provocative because previous studies performed with a large number of other antibodies to a variety of epitopes distributed over large stretches of normal adult τ or PHFτ have failed to detect normal τ or PHFτ in diffuse plaques of the AD, control or Down's syndrome brain [1,7,11,12,14,33].

Additional immunohistochemical studies were conducted to determine if the ability of PHF1 to detect PHFτ-rich profiles in diffuse plaques reflected the exquisite sensitivity and specificity of PHF1 for PHFτ-rich processes in situ. For example, T14, T46 and PHF1 were used in parallel to probe adjacent sections of frontal cortex from control and AD brains. As described earlier [29], T14 and T46 stained axons in the normal cortical neuropil, but not the somato-dendritic domain of neurons, while normal adult neocortex was almost completely negative when probed with PHF1. Further, T14 and PHF1 stained numerous NFTs and dystrophic neurites in AD neocortex, but PHF1 labeled more PHFτ-rich lesions than T14 (Figs. 2 and 3), and PHF1 continued to label these lesions at much higher dilutions than T14 (data not shown). Detailed confocal analysis of serial optical sections at 1 μm intervals over a z-axis distance of 15 μm through the AD neocortex confirmed these findings (Fig. 3). Indeed, PHF1 revealed a much denser meshwork of PHFτ positive dystrophic neurites than T14, T46, T3P or any other anti-τ or anti-PHFτ antibody used in this or previous studies [7,11–14,20,22,25,27,33,35].

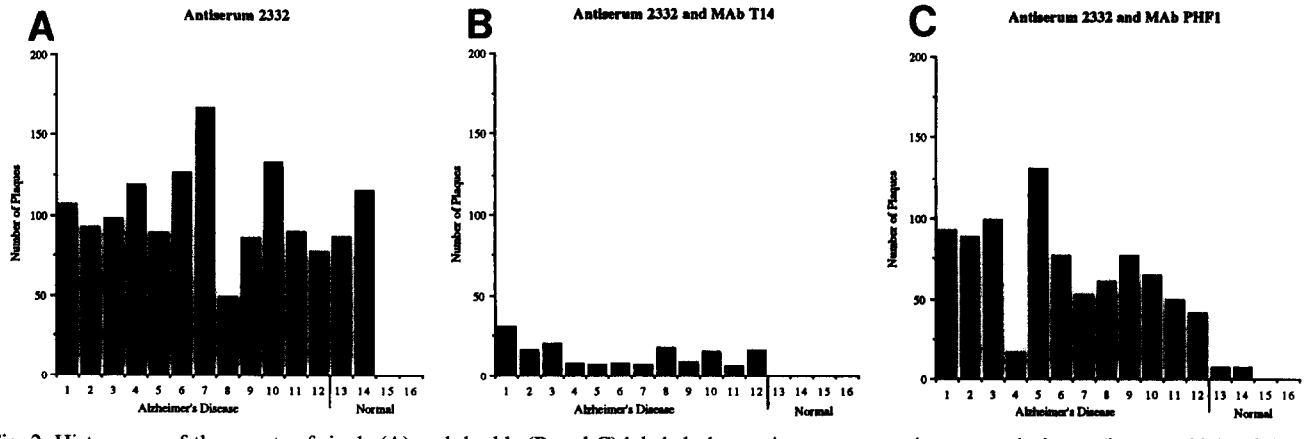

Fig. 2. Histograms of the counts of single (A) and double (B and C) labeled plaques in a representative neocortical area (i.e. area 9/10) of the AD (cases 1–12) and control (cases 13–16) brains. The rabbit polyclonal antiserum to $A\beta$ (2332) was used alone to label diffuse and neuritic plaques in A, while 2332 was used in a double label procedure with the mouse MAbs T14 and PHF1 in B and C. The absence of solid bars for some of the control cases in the histograms indicate that no single or double labeled plaques were identified. The scale for the vertical axis in all of the histograms is the same. The difference between the number of double labeled plaques in AD versus control cortical samples in C is statistically significant ($P < 0.001$). See text for further details on quantitative methods and interpretation of the results shown here.

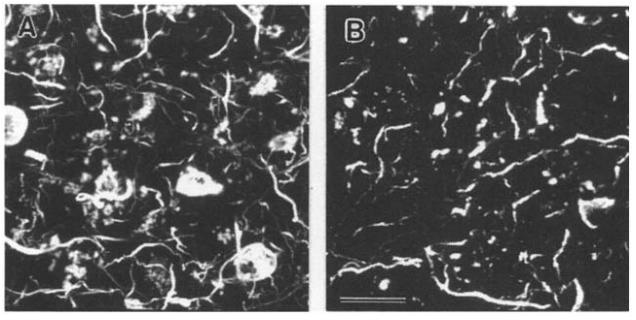

Fig. 3. Confocal images of two adjacent 30 μm thick sections of AD neocortex (area 9/10) that were optically sectioned at 1 μm intervals spanning a 15 μm thickness following immunofluorescent labeling of each section with PHF1 (A) or T14 (B). The optical sections were superimposed and these representative fields demonstrate the greater abundance of the dystrophic neurites revealed by the PHF1 MAb. The bar in A = 25 μm.

4. Discussion

We demonstrated that PHFτ positive dystrophic neurites are present throughout the AD neocortex and in nearly all NPs and diffuse plaques in these regions. The more extensive network of PHFτ positive processes revealed by PHF1 probably reflects the greater affinity of this antibody for abnormally phosphorylated PHFτ. Indeed, PHF1 binds more avidly to synthetic τ peptides phosphorylated on both Ser^{396} and Ser^{404} than to the same τ peptides with phosphates on either Ser^{396} or Ser^{404} (unpublished). Notably, both Ser^{396} and Ser^{404} are abnormally phosphorylated in the AD brain compared to the normal postmortem brain [17,18,27]. While the relationship of neuritic and diffuse plaques to each other is not known, diffuse plaques may be precursor lesions of the more complex NPs that contain abundant Aβ fibrils and other elements including dystrophic neurites, microglia and reactive astrocytes. Based on the data presented here, we conclude that PHFτ is a constituent not only of NPs, but also of most diffuse plaques in the AD neocortex. In contrast, PHFτ is infrequent in the amyloid deposits found in the control neocortex.

Although the precise role of Aβ in the pathogenesis of neuron loss and dementia in AD remains controversial [1,2,14], the demonstration that Aβ is secreted into the cerebrospinal fluid of the normal and AD brain [4,5] suggests that plaque formation in AD may depend on extracellular 'factors' which favor the aggregation and polymerization of Aβ into amyloid fibrils. Indeed, an understanding of how Aβ contributes to neuron degeneration in AD may await the characterization of factors in the extracellular space that promote the aggregation and fibrillogenesis of secreted Aβ. Although some correlative and experimental evidence suggests that proteoglycans could play a role in the assembly of Aβ into amyloid fibrils [9,37,38], other extracellular matrix proteins (e.g. laminin) also have been implicated in these events [39]. The demonstration that injections of PHFτ into the rodent brain induce co-deposits of Aβ [13], and that Aβ [40] and APP fragments associate with PHFτ [41] provide evidence for interactions between Aβ, amyloid precursor proteins and PHFτ [11–14,42] that could contribute to the formation of amyloid plaques. This notwithstanding, the evidence that soluble Aβ is a normal metabolic product of neural cells should prompt further studies of the fate of secreted Aβ and the biological significance of the convergence of Aβ with other components in amyloid plaques.

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