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Alzheimer's disease: microtubule-associated proteins 2 (MAP 2) are not components of paired helical filaments

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In Alzheimer's disease, the most characteristic neuropathological changes are the formation of neurofibrillary tangles (NFT) and neuritic plaques (NP) characterized by the presence of bundles of paired helical filaments (PHF) that accumulate in the degenerating neurites and neuronal cell bodies. Although the protein composition of the PHF is ill-defined, a number of microtubule-associated proteins have been implicated in these lesions. Here we report results with an antiserum monospecific for the microtubule-associated protein MAP 2 which does not cross-react with any other microtubular protein. Immunostaining with this antibody of sections from an Alzheimer's brain show a strong reactivity with NFT but no reactivity at the level of the NP. On the other hand, immunostaining of Alzheimer's brain sections with another antibody specific for the microtubule-associated protein τ shows strong staining of PHF on both NFT and NP. These findings confirm the presence of the τ proteins in the PHF and strongly suggest that MAP 2 may not be a main structural component of the PHF. Labelling of NFT with the anti-MAP 2 antiserum suggests a non-specific binding of MAP 2 to the PHF during the process of NFT formation.

Alzheimer's disease; Neurofibrillary tangle; Microtubule-associated protein 2; Paired helical filament

1. INTRODUCTION

People with Alzheimer's disease show neurodegenerative abnormalities characterized by the presence of cortical lesions consisting mainly of intraneuronal NFT and senile NP [1]. Electron microscopic studies have shown that both NFT and NP contain PHF [2] formed by eight protofilaments slightly larger in diameter than normal neurofilaments and which are composed of proteins arranged in β-pleated sheets [3]. The identification of the components of the PHF has been the subject of a number of investigations but these studies have been hampered by the difficulties in isolating and solubilizing these lesions. Different studies using chemical and immunochemical methods indicate that τ proteins, a heterogenous group of microtubule-associated proteins, could be one of the main constituents of the PHF [4–7]. More recently molecular genetic studies have confirmed the presence of τ in PHF [8,9]. On the other hand, there is a controversy as to whether the high-molecular-mass protein MAP 2 is an actual component of PHF. Using a polyclonal antibody which specifically reacts with MAP 2, the present study demonstrates no reactivity at the level of the NP while an antibody specific for τ shows strong labelling of these lesions supporting the idea that τ forms part of the PHF but that MAP 2 does not.

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Abbreviations: NFT, neurofibrillary tangles; PHF, paired helical filaments; NP, neuritic plaque; mAb, monoclonal antibody; PMSF, phenylmethylsulfonyl fluoride; MAPs, microtubule-associated proteins

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2. MATERIALS AND METHODS

2.1. *Brain extracts*

Rat brain soluble extracts or thermostable MAPs were prepared as described previously [10]. To prepare soluble extracts from normal or Alzheimer's human brains, approximately 10 g of tissue from the frontal cortex were frozen and reduced to powder under liquid nitrogen. After resuspending the powder in buffer D (see [10]) at 37°C a soluble extract was prepared following the procedure used to prepare the rat brain extract [10].

2.2. *Gel electrophoresis*

Approximately 150 μg of protein were loaded on 7.5% SDS-polyacrylamide gels and electrophoresed using the system described by Laemmli [11]. Molecular masses were determined using a standard mixture obtained from Pharmacia. The gels were fixed with 30% methanol, 7% acetic acid and stained with 0.15% Coomassie brilliant blue.

2.3. *Preparation and characterization of the antibodies*

The anti-MAP 2 antibody was prepared as described [12]. Briefly, microtubule proteins [10] were submitted to electrophoresis and stained. The band corresponding to MAP 2 was excised, homogenized in PBS and used for the production of MAP 2 antiserum by immunizing New Zealand White rabbit. The preparation and characterization of the anti-τ antibody has been described elsewhere [13,14]. Analysis of the specificity of the antibodies was achieved after improved transfer of MAP 2 as well as other proteins to nitrocellulose sheets by diffusion blots as follows. After the brain extracts had been electrophoresed, the gel was sandwiched between two sheets of nitrocellulose paper followed by 3 sheets of Whatmann 3MM paper. The entire sandwich was placed between sponges and held together by perforated stainless steel plates firmly clamped and immersed for 48 h in a pH 8.0 buffer containing 10 mM Tris-HCl, 50 mM NaCl, 2.0 mM EDTA, 0.1 mM PMSF and 10 mM β-mercaptoethanol. In this manner, we obtained a more effective transfer of the high-molecular-mass proteins. Two nitrocellulose sheets containing the blotted proteins can be obtained from a single gel. Immunostaining was done as described [15]. For competition experiments the anti-MAP 2 antibody was preincubated with purified (rat) MAP 2 for 1 h before incubation of the blot.

2.4. *Immunolabelling of brain sections*

The immunohistochemistry was done on adjacent sections of Alzheimer's brain as previously described [7] on paraffin embedded samples.

3. RESULTS AND DISCUSSION

Given that a number of reports have shown that mAb [17] prepared against neurofilament proteins [16,17] show varying degrees of cross-reaction with microtubular proteins, we investigated, by immunoblot analysis, the specificity of our anti-MAP 2 antibody. For this we used heavily loaded gels containing whole brain extracts from rat and Alzheimer's brains. As seen in fig.1B, the anti-MAP 2 antiserum shows a strong reaction with a band present in rat brain MAPs (fig.1A) corresponding in size to MAP 2 (a doublet corresponding to MAP 2 a and b can be seen in non-overloaded gels, data not shown) and shows no reactivity with the proteins in the τ region.

Several reports have shown that human MAP 2

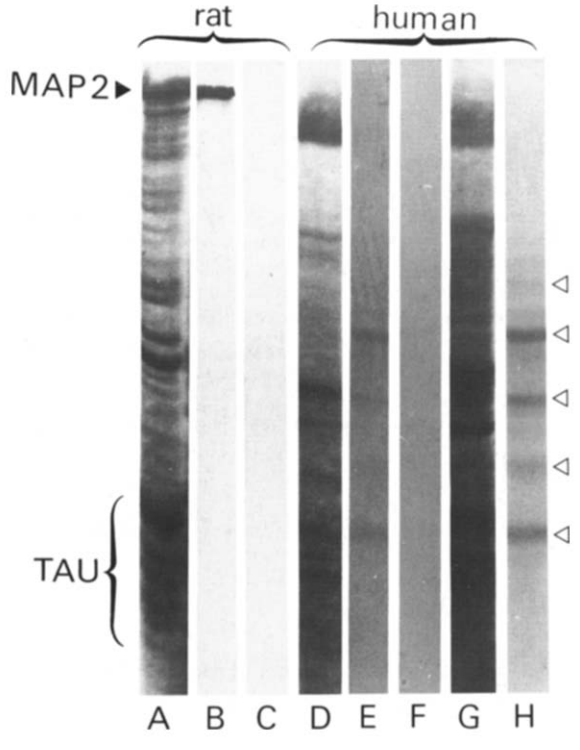

Fig.1. SDS-polyacrylamide electrophoresis and immunoblots of various brain extracts. (A) 50 μg of rat brain thermostable MAPs stained with Coomassie blue; (B) immunoblot of the same extract shown in A after reaction with the anti-MAP 2 antibody; (C) immunoblot of the same extract shown in A after absorption of the anti-MAP 2 antibody with 50 μg/ml of purified rat brain MAP 2; (D) 150 μg of brain soluble extract from an Alzheimer's brain stained with Coomassie blue; (E) immunoblot of the same extract shown in D after reaction with anti-MAP 2 antibody; (F) immunoblot of the same extract shown in D after absorption of the anti-MAP 2 antibody with 50 μg/ml of purified rat brain MAP 2; (G) 150 μg of brain extract from an 85-year-old woman brain stained with Coomassie blue; (H) immunoblot of the same extract shown in G after reaction with anti-MAP 2 antibody; arrowheads point to either the 300 kDa MAP 2 protein (►) or peptides of smaller size produced by MAP 2 proteolysis (◅). All immunoblots were done with the antiserum diluted 1:500 in PBS.

is highly sensitive to proteolysis giving rise in gels to several small size peptides [18]. In agreement with this, Alzheimer's brain extracts (fig.1D) and normal human brain extracts from a 85 year old woman (fig.1G), present no major protein band corresponding to MAP 2. The corresponding immunoblots show only positive reactivity with a number of smaller peptides (fig.1E). The high level of MAP 2 proteolysis is mainly due to the time elapse between the death of the patient and the autopsy. In order to insure that the bands recognized by this serum actually derive from MAP 2 and not from cross-reacting peptides we performed absorption experiments of the antiserum with purified rat MAP 2. As seen in fig.1, lanes C and F, the reactivity of the serum against both the rat and Alzheimer's proteins is almost completely abolished by this treatment. Taken together these data confirm the highly specific nature of the anti-MAP 2 antiserum as well as the fact that it is capable of recognizing a number of different MAP 2 epitopes in Alzheimer's brain. This antibody thus represents an excellent tool for studying the presence of MAP 2 determinants in PHF.

The knowledge of the protein composition of

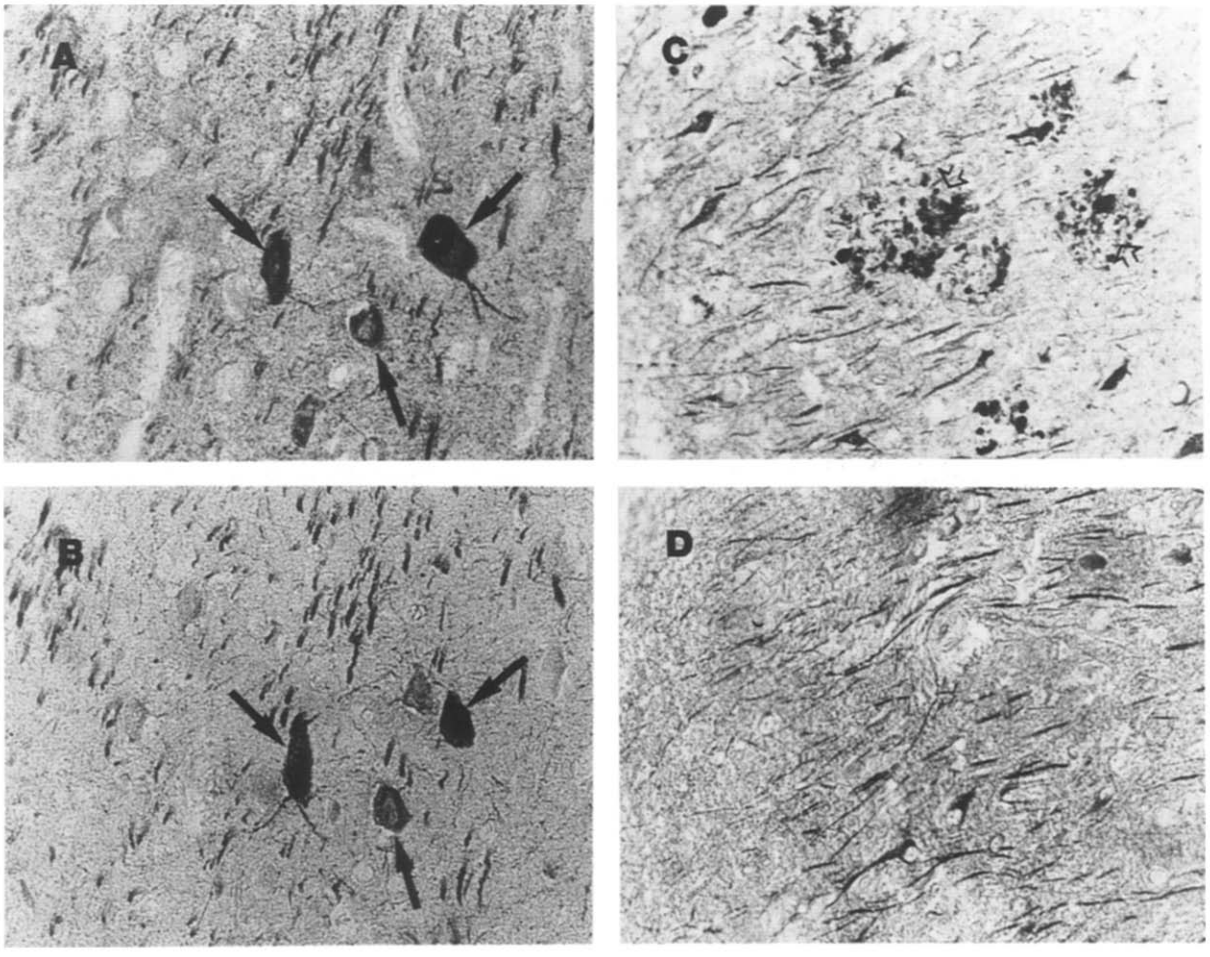

Fig.2. Immunostaining of sections of Alzheimer's brain hippocampus. Staining of NFT on two adjacent sections: (A) with the human anti-τ antibody; (B) with the anti-MAP 2 antibody. Staining of NP on two adjacent sections: (C) with the anti-τ antibody; (D) with the anti-MAP 2 antibody. Arrows in A and B show labelled NFT. Arrows in C show the labelled neuritic plaques. In all experiments antibodies were diluted 1:1000 in PBS.

the PHF, the main component of both the NFT and NP in Alzheimer's patients, should be the major aim if one wishes to understand the etiology of this disease. Immunological studies have identified several microtubular proteins as components of the PHF [5,6,8,18]. Among these, both MAP 2 [17] and τ have been signalled as forming part of the NFT. We have previously prepared an anti-NFT antiserum that reacts only with the microtubule-associated protein τ and that does not cross-react with MAP 2 [13]. Using this antibody for in situ staining of sections from an Alzheimer's brain we demonstrate here that both NFT (fig.2A) and NP (fig.2C) are marked by this antibody, confirming previous reports that show that τ or some of its fragments form part of the PHF [5,6,8,13]. In separate experiments using a monoclonal antibody specific for τ proteins we have confirmed the presence of these proteins in both PHF and NFT (data not shown). On the other hand, using our MAP 2-specific antiserum we demonstrate the absence of MAP 2 epitopes in the NP (fig.2B). However, adjacent sections show that NFT are labelled with this antibody (fig.2D). Since PHF have been clearly identified in both NP and NFT [2,3] the presence of MAP 2 epitopes in one and not in the other, as shown here, may appear difficult to reconcile. However, this can be interpreted as MAP 2 being a contaminant in the NFT rather than an actual constituent of it. This interpretation is supported by reports showing that MAP 2 may bind nonspecifically to NFT [22,23] or that it may be trapped inside the lesions due to the abundance of MAP 2 in the neuronal cell body [24–26]. This will not be the case for the NP since no MAP 2 has been described to be present in degenerating axonal neurites. Although some investigators have reported the presence of MAP 2 epitopes in PHF using mAbs [24], more recent findings seem to indicate that this could be due to cross-reaction with τ [20]. This interpretation is strengthened by the present data, also confirmed by others [4–6,9] that show that τ is part of the Alzheimer's lesions. However, one cannot rule out the possibility that the composition of PHF in NFT and NP may differ by a molecule present in NFT and characterized by a nonspecific affinity for MAP 2.

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