Short report

Immunohistological study of granulovacuolar degeneration using monoclonal antibodies to neurofilaments

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SUMMARY Neurons undergoing granulovacuolar degeneration have been examined in paraffin sections with three monoclonal antibodies to neurofilaments. Two of these antibodies (BF10, RT97) had been shown previously to react with neurofibrillary tangles. One of these antibodies (RT97) reacted with granular component of granulovacuolar degeneration. BF10 sometimes reacted with the surrounding neuronal cytoplasm. Granulovacuolar degeneration was also detected in a few cells of the anterior olfactory nucleus.

Granulovacuolar degeneration was first described by Simchowicz¹ in the hippocampus in cases of senile dementia in 1911. Subsequent studies have confirmed its close association with Alzheimer's disease although it occurs to a slight extent also in undemented elderly subjects²⁻⁵ and in amyotrophic lateral sclerosis and the Parkinson dementia complex of Guam.⁶ Pyramidal neurons in the subiculum and H1 zone of the hippocampus are those most susceptible to the development of granulovacuolar degeneration according to Ball.⁷ Relatively little is known about this form of neuronal degeneration. Affected neurons contain one or several clear cytoplasmic vacuoles within which lies a central granule stainable with silver or haematoxylin. Electron microscopy performed on neurons showing granulovacuolar degeneration in cases of amyotrophic lateral sclerosis and Parkinson dementia complex of Guam⁶ showed membrane-bound cytoplasmic vacuoles with an electron lucent matrix and an electron dense granular body. In Alzheimer's

disease, granulovacuolar degeneration occurs in cells with or without neurofibrillary tangles.

We have examined granulovacuolar degeneration in sections of hippocampus and olfactory bulbs from patients with Alzheimer's disease and controls using three monoclonal antibodies to neurofilaments.

Material and Methods

Five cases of Alzheimer's disease and five age-matched controls were investigated. Diagnosis was based on clinical evidence of advanced dementia and typical histopathological changes of the brain. For histology, we used paraffin-embedded coronal sections of hippocampus at the level of the lateral geniculate body, and horizontal sections through the anterior olfactory nucleus.

The ascites forms of three monoclonal antibodies (Mab-BF10-RT97 and -147) were used. BF10 recognises an epitope on the 155K neurofilament polypeptide; RT97 and 147 recognise different epitopes on the 210K neurofilament polypeptide. All three monoclonal antibodies have been previously described and the antigenic determinants for RT97 and BF10 have been shown previously to be shared by neurofibrillary tangles.⁸

Staining Schedule:

- (1) After dewaxing sections are treated for 30 minutes with 0.3% H₂O₂ in absolute methanol.
- (2) Take through alcohols (96, 70, 50, 30%) then 10

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Fig 1 Section of hippocampus (Alzheimer's disease) stained with Mab-RT97. High power view is focused on two granulovacuolar degeneration lesions in pyramidal cells, (▲) unstained granule; (▲) stained granule. $\times 2600$

- (10) 6 minutes DAB: (0.05% w/v DAB (Sigma) + 0.075% v/v H_2O_2 in TB).
- (11) Rinse in TB, transfer to distilled water.
- (12) Counterstain 20 seconds in Harris's haematoxylin (diluted 1:5). Adjacent sections were examined with a silver stain⁹ and haematoxylin and eosin.

Results

Hippocampus

Granulovacuolar degeneration was seen in many hippocampal pyramidal neurons from cases of Alzheimer's disease and in occasional similar neurons in elderly controls. The intravacuolar granules were readily identified when stained with silver (black) or with haematoxylin (blue). With Mab-RT97, the majority of granules were clearly positively stained (fig 1). In contrast, Mab-BF10, which frequently stained the cytoplasm surrounding the lesion, failed to stain the granules of granulovacuolar degeneration (fig 2). On a few rare occasions a large granule appeared to have reacted with BF10 (not illustrated). Some neurons containing granulovacuoles contained neurofibrillary tangles which were also strongly stained with BF10 and RT97 as described previously.⁸ Other cells undergoing granulovacuolar degeneration did not contain neurofibrillary tangles, their cytoplasm frequently showed little or no staining with either antibody. The intensity of granulovacuolar degeneration staining with RT97 was not related to presence or absence of neurofibrillary tangles in the same neuron. Mab-147 failed to stain neurofibrillary tangles or cells undergoing granulovacuolar degeneration. (We also re-examined a set of older sections which were stained with *antisera* to neurofilaments proteins 210K and 150K; no granulovacuolar degeneration staining was found (not illustrated).)

Olfactory Bulbs

Four of these cases of Alzheimer's disease examined in the present study had tangles in neurons of the anterior olfactory nucleus.¹⁰ In the fifth case of Alzheimer's disease, the sections treated for immunohistochemistry unfortunately no longer contained elements of the anterior olfactory nucleus.

The staining characteristics of the Mabs were as for hippocampus; RT97 and BF10 stained tangles (not illustrated), but only RT97 granulovacuolar degeneration granules (fig 3). The third Mab, 147, did not react with neurofibrillary tangles nor neurons undergoing granulovacuolar degeneration.

Discussion

Mabs BF10 and RT97 react with neuronal

- minutes wash in tris buffer saline pH 7.6 (TBS).
- (3) Sections are layered with normal horse serum (1:6 in TBS + 1% N horse serum-TBS/NH) 10 minutes.
- (4) Mab 60 minutes: BF10 and RT97 1:1000; 147 1:500 in TBS/NH.
- (5) 2×15 minutes wash in TBS.
- (6) 30 minutes rabbit anti mouse Ig (RAM/Ig Nordic) 1:200 TBS/NH.
- (7) Repeat (5).
- (8) 30 minutes goat anti rabbit IgG conjugated with peroxidase (GAR/IgG (H + L)/PO - Nordic) 1:400 TBS/NH.
- (9) 15 minutes wash in TBS; 5-10 minutes in tris buffer (TB).

Fig 2 Section of hippocampus (Alzheimer's disease) stained with Mab-BF10. High power view shows pyramidal cell with several granulovacuolar degeneration lesions. (▲) Largest lesion in focus. All the granules failed to stain. $\times 2600$

Fig 3 A cell from the anterior olfactory nucleus (Alzheimer's disease) stained with Mab-RT97. (↑) Granulovacuolar degeneration with positively stained granule. $\times 2600$

perikarya, which have accumulated neurofilaments as well as with neurofibrillary tangles. Only RT97 reacts unequivocally with a majority of granules in granulovacuolar degeneration. Mab-147 stains neither neurofibrillary tangles nor granulovacuolar degeneration. As far as we know, this is the first study in which one such antibody (RT97) has been shown additionally to react with granules in granulovacuolar degeneration. Most of the neurons undergoing granulovacuolar degeneration and studied with the above antibodies were found in the hippocampus but a few were also identified in the anterior olfactory nucleus. The antibodies' specificities with regard to neurofibrillary tangles and granulovacuolar degeneration both in the hippocampus and the anterior olfactory nucleus were the same.

At ultrastructural level neurofibrillary tangles consist of paired helical filaments. Since granules in granulovacuolar degeneration are not described as containing filaments,⁷ further work is now necessary

to establish more firmly a link between neurofilaments and granules. The results do suggest the intriguing possibility that granulovacuolar degeneration is a component of the disrupted cytoskeletal metabolism in Alzheimer's disease. Compared with neurofibrillary tangles, granular vacuoles have been neglected in immunohistochemical studies of Alzheimer's disease; our findings emphasise the need for more research in this area.

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