Fibrous Astrocytes in Senile Dementia of the Alzheimer Type

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Abstract. Fibrous astrocytes were stained by the Sternberger (peroxidase-antiperoxidase) method, using paraffin sections of mid-frontal cerebral cortex of patients with senile dementia and of normals of similar age. The populations of fibrous astrocytes were similar in the molecular layer, but were widely divergent in layers II through VI. Here the mean count of fibrous astrocytes in senile dementia of the Alzheimer type was more than four times that of the normal aged cortex.

INTRODUCTION

Neurofibrillary tangles and neuritic plaques are the neocortical, histological hallmarks of Alzheimer's disease and of senile dementia of the Alzheimer type (SDAT). Less well appreciated are alterations in the glial population of this tissue. The purpose of this report is to present data which demonstrate a major increase in the population of fibrous astrocytes in the neocortex of patients with SDAT, as compared with that of normal aged people.

MATERIALS AND METHODS

The tissue selected for study was taken from the mid-frontal region of autopsy specimens removed within 24 hours of death. The sixteen patients of the SDAT group had been studied clinically and were found to have a syndrome characteristic of this type of senile dementia, without evidence of other neurological disorders. Post-mortem neuropathological study confirmed the presence of moderate to severe Alzheimer's disease, without other gross or histologic abnormalities. The fourteen age-matched normal (AMN) control specimens came from patients with a history of neither dementia nor other neurological disease. Neuropathological study confirmed the absence of any significant disorder in the brain. In particular, both groups were free of cerebrovascular disease and of histologic evidence of anoxia. The AMN group was 79% female (11/14), and the mean age was 79 ± 6.4 (standard deviation) years. The SDAT group was 69% female (11/16), and the mean age was 85 ± 7.7 years.

The left cerebral hemisphere from which this tissue came was fixed in buffered formalin for one to four months prior to dissection. A block of cerebral cortex from the mid-frontal region was then dissected so that it was perpendicular to the gyrus. This

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block was embedded in Paraplast by the usual method. Serial sections were stained with cresyl violet for counting of neuroectodermal cells (17), and with thioflavine S and Bodian techniques for enumeration of plaques and tangles. Sections 8 microns thick were used for immunocytochemical preparations for counting the fibrous astrocytes. The same area of the same gyrus was analyzed by these several techniques.

The antiserum was prepared as follows: Brain filaments were isolated from normal human white matter by the axonal flotation procedure of Liem et al. (11). The resulting pellet was dissolved in Tris buffer containing SDS, mercaptoethanol, and EDTA. This solution was electrophoresed on a polyacrylamide slab gel, and the band with a molecular weight of 49,000 daltons was excised, mixed with Freund's complete adjuvant, and injected subcutaneously in a rabbit. Two booster doses were subsequently administered, and the animal was bled one week after the final immunization. The antiserum was characterized by double immunodiffusion against the whole dissolved pellet and against the 49,000 dalton band. It was also tested with rocket immunoelectrophoresis (10, 12), and finally by immunofluorescence on cryostat sections of rat brain and on dissociated rat cerebellar cultures. All tests indicate that this antiserum is specific for astrocytic glial filaments (18).

The peroxidase-antiperoxidase (PAP) immunocytochemical method of Sternberger (16) proved to be very satisfactory for recognition of fibrous astrocytes in the autopsy tissue. The antiserum against glial fibrillary acidic protein was diluted 1:300 or 1:500, and was incubated with rehydrated tissue sections for 45 minutes at room temperature. Following reaction with diaminobenzidine and osmic acid, the PAP technique resulted in diffuse, dark brown cytoplasm in the fibrous astrocytes and their processes. Bundles of glial filaments were even darker than the cytoplasm. Other tissue constituents were not significantly stained. Three controls were used: (a) omission of the step involving the specific antiserum, (b) substitution of normal rabbit serum for the specific anti-glial filament serum, and (c) substitution of the specific antiserum with antiserum which had been adsorbed against the filament pellet. All these controls were negative.

The cell bodies of fibrous astrocytes were counted at a magnification of 125×. The molecular layer was examined over a length of 4.2 mm. The underlying cortex was assayed in a rectangle 1.4 mm wide through the full thickness of layers II through VI.

RESULTS

Fibrous astrocytes were particularly prominent in the subpial layer of both groups, where their processes formed a dense mesh (Fig. 1). In the cellular layers, these cells were randomly distributed, but pairs were not infrequent. One or two of these cells often lay at the edge of a neuritic plaque (Fig. 2), the substance of which was infiltrated by glial processes (Fig. 3). The dense amyloid cores were usually not penetrated. Many plaques did not appear to have a significant glial component, so that more plaques were seen with thioflavine S than with the PAP method.

Figure 4 illustrates the fact that there were no significant differences between the average number of fibrous astrocytes in the molecular layer of the normal aged cortex and that of patients with SDAT. The mean number of these cells in the molecular layer of the normal specimens was 27, with a standard deviation of 7.3. In the SDAT cases, fibrous astrocytes averaged 29 ± 6.8.

The relative glial population in the cellular portion of the cortex, layers II through VI, was very different from that of the molecular layer, as also illus-
Fig. 1. The molecular layer of two closely apposed mid-frontal gyri contain many fibrous astrocytes, which are especially prominent in the subpial area. PAP preparation. ×200.

Fig. 2. The relationship between fibrous astrocytes and neuritic plaques is seen in this PAP preparation from an SDAT case. ×200.

Fig. 3. At higher magnification of a PAP preparation, a neuritic plaque is seen to be invaded by several processes from the adjacent astrocyte. The dense amyloid core is free of glial material, but the halo around it contains numerous glial processes. ×790.
FIBROUS ASTROCYTES

- MOLECULAR LAYER
- LAYERS II-VI

Fig. 4. This scattergram demonstrates the number of astrocytes in the molecular layer (closed circles) and in the cellular layers (open circles) of the mid-frontal region of each of the 14 normal patients (control) and each of the sixteen patients with SDAT. The counts in the molecular layer are essentially identical, but in the cellular layers there are obviously far more fibrous astrocytes in the group with SDAT.

trated in Figure 4. In this location, the average number of fibrous astrocytes in SDAT was four times greater than that in the normal specimens. The mean count in the former was 36.1 ± 11.5, and in the latter 9.0 ± 4.6. There was no overlap between the two groups. The difference of means is statistically significant, with \( p < .002 \). Since there is no statistical difference between the two groups as to the average cortical thickness measured in these sections (17), this is not the result of condensation of diseased tissue.
The age range of the normal elderly groups was largely from 72 to 89, with just one patient who was 65 at death. The number of fibrous astrocytes within the cellular layers in specimens from this group ranged between 1 and 17. A statistically significant correlation could not be found between these two parameters.

In the group of sixteen patients with SDAT, the mean number of astrocytes ranged from 21 to 61, while the average number of plaques in the same region varied from 9 to 45 per field, as visualized by thioflavine S. Again, there was no significant correlation between the number of plaques and the number of astrocytes in individuals of this group.

DISCUSSION

Histologic techniques other than glial fibrous acidic immunocytochemistry are available for the identification of astrocytes. The Cajal method of impregnation with gold sublimate (3) requires frozen sections, which would have necessitated a block of tissue more distant from the paraffin section used for the cell counts (17). The paraffin modification of the Cajal method (13) requires shorter fixation than was often possible for the current material. The anti-GFA serum used in this current work was effective on tissue fixed as long as several months. This indicates that the antigen is well preserved in the formalin-fixed tissue. Both protoplasmic and fibrous astrocytes are stained by the metallic impregnations. The Holzer method (9) for fibrous astrocytes is less sensitive than the PAP technique (4).

Fibrous astrocytes are sparse in the cerebral cortex of the normal, young human adult. This type of glial cell is far more common in the white matter, and to some extent in the deeper gray areas of the cerebral hemispheres. Quantitative data are not available concerning the increase in fibrous astrocytes in any part of the human brain in the course of normal aging. There would seem to be, however, little doubt that the number of such cells in both neuronal and molecular layers of our control group is increased beyond that of the younger human brain. This might be a reaction to the loss of neurons and dendrites reported by Brody (2), by Henderson et al. (8), and by Scheibel (15) as occurring in the course of normal human aging.

Three possibilities exist for the source of the increased fibrous astrocytes in the SDAT group. First, they might have migrated from deeper portions of the brain, but this seems unlikely, especially since there was not an increased concentration in the deepest layers of the cortex, although the white matter does indeed have many such cells. Rose et al. (14) suggested that such a migration from adjacent areas accounts for the gliosis found in the experimentally deafferented dentate gyrus of the rat. The second alternative is that these fibrous astrocytes proliferated from pre-existing fibrous astrocytes. Although mitotic figures among astrocytes are very rare, the presence of occasional pairs of closely apposed cell bodies and rare double nuclei among individual cells would seem to indicate that cell division remains a possibility. Protoplasmic astrocytes do not react to anti-GFA, although they usually have a few filaments, as seen by electron microscopy. It is to be noted that counts of
neuroectodermal cells in the mid-frontal region in patients with SDAT do not reveal a statistically significant increase in the number of small cells when compared with similar counts from age-matched normals (17). These cells, each with an area less than 40 microns square, are almost all glia. Therefore, cellular hyperplasia is probably not a major phenomenon in this disorder. Most likely is the third alternative: that these fibrous astrocytes derive by transformation of protoplasmic astrocytes. This is, of course, a common and well-recognized change in central nervous system injury of all sorts. Their structural differences are few. Protoplasmic astrocytes have shorter, more highly branched processes and fewer filaments within their cytoplasm. The change from protoplasmic to fibrous type is apparently readily accomplished throughout the lifespan, is increased in normal aging, and is exaggerated in SDAT.

The functional effect of increased number of fibrous astrocytes in the cortex cannot be assayed at this time. These cells seem to be larger than protoplasmic astrocytes, and their processes are longer. Therefore, glia probably take up a relatively greater portion of the volume of the neuropil than in the normal controls, even without significant increase in their numbers. This hypertrophied glial component might well account for the relatively increased amount of neuropil we have found in our quantitative assays of the cortex in SDAT (17). Glial hypertrophy was reported by Geinisman et al. (7) in the dentate gyrus of the normal aged rat. The gliosis undoubtedly contributes to disorganization of the pattern of the neurites. Presumably, the chemical micro-environment of the neuronal components is also altered.

No explanation can be offered for the fibrous gliosis of normal aging, but it must be an individual process for each patient, since the number of these cells does not correlate with the simple chronologic age. Neither can one cite which aspect of the pathogenesis of SDAT causes the marked increase in fibrous astrocytes, since their number does not correlate with the number of senile plaques or of neurofibrillary tangles, which are the most striking of the several histologic abnormalities.

A number of previous authors have observed an increased astrocytosis in the cortex of Alzheimer’s disease (1, 6). Duffy et al. (5) applied the immunohistochemical method to this tissue and recognized an increase in glial fibrillary acidic protein. Quantitation of these astrocytic changes has not previously been published.

In the absence of any firm information about the source of these fibrous astrocytes or their functional role, we can only surmise that they probably derive by a reactive process from protoplasmic astrocytes, and that they represent a form of diffuse scarring of the cerebral cortex in Alzheimer’s disease of the senile type.

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