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Short original communication

Phagocytosis of β/A_4 amyloid fibrils of the neuritic neocortical plaques

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Summary. Immunocytochemistry using monoclonal antibodies to β/A_4 protein was applied to study the macrophages involved in the removal of amyloid deposits. The material examined included necrotic brain tissue areas with abundant amyloid deposits collected from 32 autopsy cases. The β/A_4-immunoreactive products were found in numerous macrophages, appearing as early as 24 h after the onset of stroke. Immunogold electron microscope studies allowed us to localize the reaction product to the secondary lysosomes. Our study clearly demonstrates the differences between macrophages engaged in amyloid removal and microglial cells associated with amyloid deposits, which according to previous observations contain β/A_4 material within endoplasmic reticular channels.

Key words: Alzheimer disease – Amyloid – Phagocytosis – Microglia – beta protein

One of the unanswered questions in the study of the pathogenesis of neuritic and amyloid plaque formation is the role of the microglia/macrophages, whose presence is associated with the plaques' amyloid deposits [1, 2, 4, 5, 6]. These cells have the appearance of being reactive. The question then arises as to whether they function as phagocytes removing the amyloid deposits or are engaged in the production of amyloid. On the basis of three-dimensional ultrastructural reconstruction of the microglial cells in the amyloid deposits as well as ultrastructural immuno- and histochemical studies of the microglia-amyloid star complex, we concluded that these cells are the place of origin of the amyloid fibers [7, 8]. We describe here the morphology of the macrophages that remove the β/A_4 amyloid deposits of the neuritic and amyloid plaques in people with stroke and show the differences between the cells that make and those that remove the amyloid fibers.

Materials and methods

From a collection of brains of individuals aged 71–96 years, who died because of stroke due to middle cerebral artery occlusion, 32 cases were chosen with numerous (more than 40 in 1 mm^2) neuritic and amyloid plaques. The time between the onset of the stroke and death varied from 24 h to 68 days. For light microscopic and electron microscopic (EM) studies, samples of tissue were taken from the borderline between the infarcted and the unaffected areas of temporal and parietal cortex. The material was fixed in 10% neutral formalin, routinely processed, and embedded in paraffin. The paraffin blocks were cut and the serial sections stained with hematoxylin and eosin, Bielschowsky, and thioflavin S. Immunostaining with monoclonal antibody (mAb) 4G8 [3] (dilution 1:1 000) was performed using the avidin-biotin peroxidase complex and diaminobenzidine as a chromogen. For EM immunocytochemistry, small tissue blocks (2 × 2 mm) were dissected out from the paraffin blocks from the cases with large numbers of macrophages positively stained for β/A_4 (3-week-old infarcts). Tissue was deparaffinized, hydrated through the gradient of alcohols, washed for 2 h in phosphate-buffered saline, pH 7.4, and refixed in the mixture of 2% paraformaldehyde and 0.25% glutaraldehyde. After dehydration, the material was embedded in LR white. Ultrathin sections were cut, put on nickel grids, and stained using the protein A immunogold method [6]. Dilution of mAb was 1:5 (tissue culture supernatant). The diameter of gold particles conjugated with protein A was 10 nm. Control of the method included replacement of mAb 4G8 by mAb to cytomegalovirus of the same subclass of IgG (2b) and by specific buffer. Immunostained sections were counterstained with uranyl acetate and examined with the Hitachi 7 000 electron microscope.

Results

The β/A_4-positive macrophages were already seen in 24-h-old infarcts. Usually, they were found in close association with the primitive and classical plaques. Their number was low in the 24-to 72-h infarcts and increased with time, reaching the largest number in 3-week-old infarcts (Fig. 1). Later on their number

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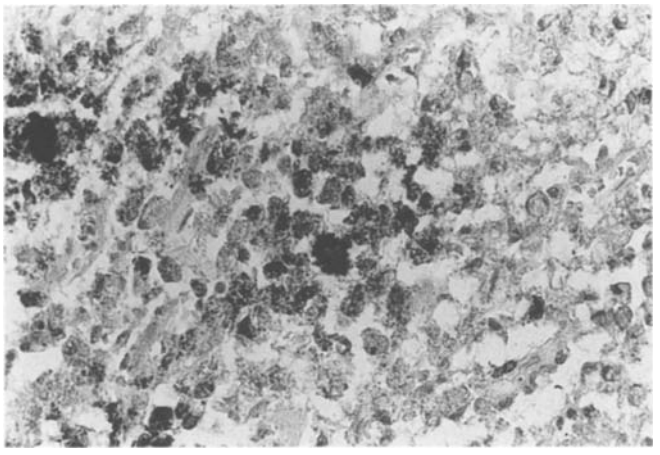

Fig. 1. Fragment of 20-day-old infarct area stained with monoclonal antibody (mAb) 4G8. Intact plaque core surrounded by β/A_4-positive macrophages. × 408

slowly started to decrease. In the early infarcts, the macrophages were seen mostly around the plaques; afterwards, they were distributed more diffusely. Vessels with perivascular plaques showed early accumulation of β/A_4-positive macrophages. Vessels with amyloid deposits limited only to the vessel wall did not show early β/A_4-positive macrophages. However, in infarcts older than 5 days, β/A_4-positive macrophages were seen around both amyloid-positive and amyloid-negative vessels. The proportion of β/A_4-positive and β/A_4-negative macrophages changed with the age of the infarct. During the first 24–48 h of tissue necrosis, there were few β/A_4-positive macrophages. In 3 week-old infarcts more than 50% of the macrophages showed immunostaining. In the necrotic white matter almost none of the many macrophages were immunoreactive.

EM immunocytochemical studies revealed many immunogold-labeled macrophages. Within the large

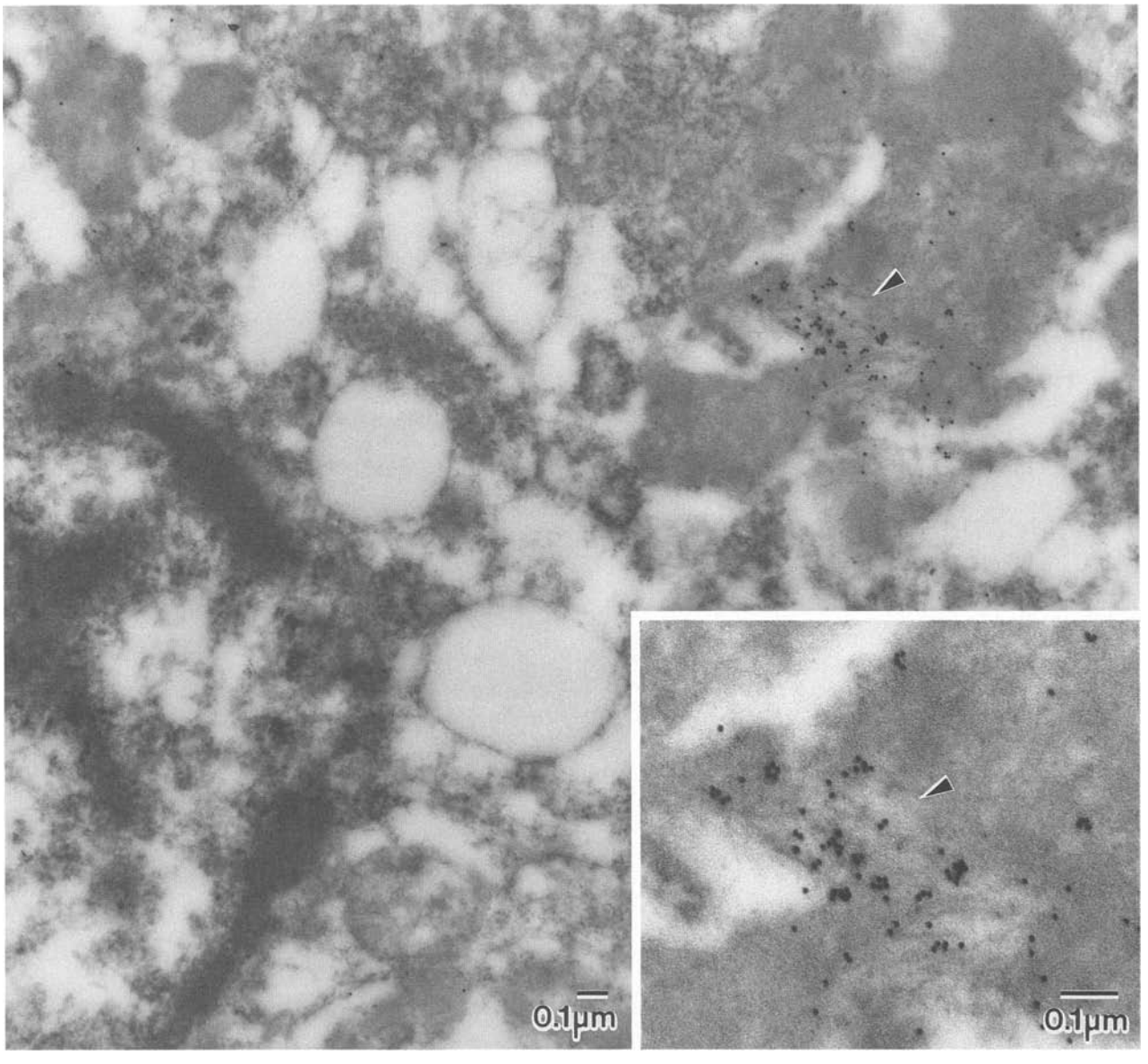

Fig. 2. Immunogold-labeled (mAb 4G8) amyloid fibrils (*arrow*) within the secondary lysosome of the macrophage. × 44,000; *inset*, × 86,000

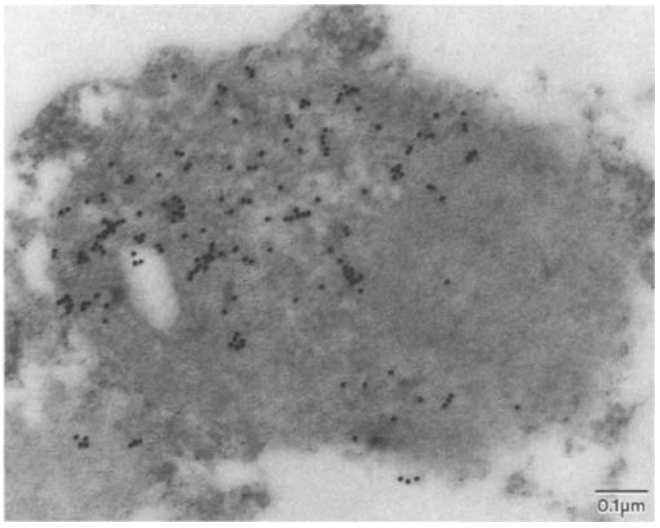

Fig. 3. Immunostaining of disintegrating amyloid fibrils embedded in the residual body (mAb 4G8). × 69,360

conglomerates of secondary lysosomes immunogold particles were frequently, found in wisps of fibril profiles (Fig. 2). Also, in many secondary lysosomes, numerous immunogold particles were seen in areas occupied by fuzzy, disintegrating fibrillar material (Fig. 3) as well as in homogeneous structureless profiles. However, only a few and scattered immunoreactive gold particles were seen in the electron-dense profiles of secondary lysosomes without any evidence of fibrillar or blurred fibrillar profiles.

Discussion

To the best of our knowledge, phagocytosis of β/A_4 amyloid fibrils has not been previously reported. The process of removal of the amyloid fibrils was seen in many macrophages. This was because we chose for this study cases with numerous neuritic and amyloid plaques in neocortices that were affected by ischemic or hemorrhagic necrosis. At the EM level in all the macrophages examined, the immunoreactive products were shown to be confined to the large conglomerates of secondary lysosomes. The immunolabeling of amyloid gold particles was especially intensive in areas where amyloid was embedded in electron-dense profiles revealing clear fibrillar structure. However, pronounced β/A_4 immunoreactivity was also retained even in the areas where disintegration of the amyloid fibrils within the lysosomal profiles occurred.

In summary, β/A_4 amyloid fibril degradation in areas of neocortical necrosis with numerous neuritic and amyloid plaques was evidenced within the macrophages' secondary lysosomes. This picture contrasted with the recently described microglia/macrophage cells associated with amyloid deposits in classical plaques. In the microglial cells associated with the classical plaques, the amyloid fibrils were never observed in the secondary lysosomes; instead, they were seen in the endoplasmic reticulum systems and the infoldings of cytoplasmic membranes [7, 8]. In conclusion, this study supports our hypothesis that the place of origin of amyloid fibrils is the microglial cell.

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