Fleecy Amyloid Deposits in the Internal Layers of the Human Entorhinal Cortex are Comprised of N-terminal Truncated Fragments of Aβ

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Abstract. The deposition of amyloid in the brain is a hallmark of Alzheimer disease (AD). Amyloid deposits consist of accumulations of β-amyloid (Aβ), which is a 39–43 amino-acid peptide cleaved from the Aβ-protein precursor (APP). Another cleavage product of APP is the P3-peptide, which consists of the amino acids 17–42 of the Aβ-peptide. In order to study the deposition of N-terminal truncated forms of Aβ in the human entorhinal cortex, serial sections from 16 autopsy cases with AD-related pathology were immunostained with antibodies against Aβ1-40, Aβ1-42, Aβ1-38, and Aβ1-39, as well as with the Campbell-Switzer silver impregnation for amyloid. In the external entorhinal layers (pre-β and pre-γ), sharply delineated diffuse plaques were seen. They were labeled by silver impregnation and by all Aβ-antibodies used. By comparison, in the internal layers (pre-α, pre-β, and pre-γ) blured, ill-defined clouds of amyloid existed, in addition to sharply delineated diffuse plaques. These clouds of amyloid were termed “fleecy amyloid.” Immunohistochemically, fleecy amyloid was stained by Aβ1-21 and Aβ1-29 antibodies, but not with antibodies against Aβ1-40 and Aβ1-42. Using the Campbell-Switzer technique, the fleecy amyloid deposits were found to be fine argyrophilic amyloid fibrils. Thus, the internal entorhinal layers are susceptible to a distinct type of amyloid, namely fleecy amyloid. This fleecy amyloid obviously corresponds to N-terminal truncated fragments of Aβ1-39, probably representing the P3-peptide. These N-terminal truncated fragments of Aβ are capable of creating fine fibrillar “amyloid.”

Key Words: Alzheimer disease; β-amyloid; Entorhinal cortex; P3-peptide: Pigmentoarchitectonic.

INTRODUCTION

The deposition of β-amyloid (Aβ) plays an important role in the pathogenesis of Alzheimer disease (AD) (1). Aβ is a 39–43 amino acid peptide which is derived from the Aβ-protein precursor (APP) by cleavage of putative β- and γ-secretases (1–7). An N-terminal truncated fragment of Aβ is the P3-peptide, which consists of the amino acids Aβ17-42 (Fig. 1). It is possibly the product of an α- and γ-secretase cleavage of APP, and is considered to precede the deposition of Aβ (8–10). The detection of Aβ1-40 and Aβ1-42, as well as truncated fragments of Aβ, is possible using specific antibodies (8–14).

In Alzheimer disease, Aβ-deposits are found histopathologically in senile plaques (1). Senile plaques can be divided into 4 categories: diffuse non-neuritic, diffuse neuritic, dense core neuritic, and dense core non-neuritic plaques (11). Aβ1-40 and Aβ1-42 are the major components of these plaques (12). P3 deposits and various N-terminal truncated Aβ fragments can be detected either in the presence or in the absence of full-length Aβ in another subset of plaques (10, 12–14).

The question arises whether plaques with different types of amyloid fragments have a layer-specific anatomical distribution. To this end, we investigated the entorhinal cortex for the distribution of plaques using 4 different antibodies and the Campbell-Switzer method.

Together, they allow the discrimination and C-terminal determination of N-terminal truncated fragments of Aβ.

Our data demonstrate blurred morphologically ill-defined clouds of amyloid in the internal entorhinal layers, which appear to contain N-terminal truncated Aβ forming argyrophilic fibrillar structures.

MATERIAL AND METHODS

Human brains of 16 cases, aged 63–90 yr, with varying degrees in severity of Alzheimer-related pathology (Table) were obtained at autopsy from 4 different departments of Pathology, 12–72 hours (h) postmortem. The brains were fixed in a 4% aqueous solution of formaldehyde. Samples of the anterior entorhinal cortex were dissected coronally and embedded in paraffin or in polyethylene glycol (PEG) (15). The PEG blocks were micromachined at 100μm, while 10-μm sections were cut from the paraffin embedded tissue. Aldehyde-fuchsins-Darrow red staining of PEG and paraffin sections was used for topographical orientation. The entorhinal layers, stratum molecularae, pre-α, pre-β, pre-γ, lamina dissecans, pri-α, pri-β, and pri-γ, were identified by cyto- and pigmentoarchitectonical criteria (Figs. 3e, 4) (16, 17). The presence of neurofibrillary pathology was assessed using the Gallyas-silver impregnation method, and staged according to Braak and Braak (18). Amyloid deposits were detected with the Campbell-Switzer-silver impregnation method using 100-μm PEG- and 10-μm paraffin sections (15). Additionally, immunohistochemical detection of amyloid was performed after formic acid pretreatment with antibodies against Aβ (Aβ1-41, Novocastra: 6F3D, 1/50, 72 h at 4°C; Aβ1-23, Senetek: 4G8, 1/5000, 72 h at 4°C; Aβ1-39, Sigma, polyclonal rabbit, 1/100, 48 h at 4°C; Aβ1-42, [19], polyclonal rabbit, 1/750, 48 h at 4°C). Antibodies against Aβ1-40 detect Aβ as well as the P3-peptide, while the Aβ1-41 antibody does not react with this peptide or with other N-terminal truncated fragments of similar length (14, 20). The C-terminal specific antibody against

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Fig. 1. Schematic representation of APP and its cleavage products. Full-length Aβ results from putative β- and γ-secretase cleavage of APP, whereas P3 is considered to be a product of α-secretase and γ-secretase cleavage. The antibody against Aβ_{1-42}, detects both full-length Aβ and P3, whereas antibodies directed against Aβ_{1-40} do not cross-react with P3. Two types of Aβ can be generated by γ-secretases with differences in the C-terminus, Aβ_{1-40} and Aβ_{1-42}. Aβ_{1-40} is detected selectively by C-terminal specific polyclonal antibodies. The antibody is cross-reactive with shorter fragments of Aβ_{1-40} (Aβ_{10}). The antibody against Aβ_{1-40} (Aβ_{10}) cross-reacts with Aβ fragments of various lengths including Aβ_{1-40} and P3.

Aβ_{1-40} was applied to determine the C-terminal configuration of Aβ (Fig. 1). The antibody against Aβ_{1-40} is cross-reactive with Aβ_{1-40} and various peptides of Aβ including P3 (19). The primary antibodies were detected using biotinylated secondary antibodies and the ABC-method with 3,3 diaminobenzidine-HCl (21). In paraffin sections, counterstaining was performed with hemalum. PEG-sections were not counterstained.

RESULTS

The amyloid deposits in the entorhinal region exhibited a layer-specific pattern throughout the molecular layer, the external layers (pre-β and pre-γ) and the internal layers (pri-α, pri-β, and pri-γ) (Figs. 2, 3, 4).

The amyloid deposits in the molecular layer were confined to the subpial zone and were positive with the Campbell-Switzer staining and all 4 antibodies against Aβ (Figs. 3, 4). Subpial amyloid was diffusely distributed (Fig. 2) and noted only in cases of moderate to severe β-amyloidosis.

Within the pre-α layer, Aβ-deposits were noted only in end-stage β-amyloidosis. In the external layers, scattered Aβ-deposits of various size formed sharply delineated diffuse plaques (Figs. 2, 3, 4). They were labeled with all 4 antibodies and the Campbell-Switzer method. Only in 2 cases of mild β-amyloidosis, they were not immunoreactive with antibodies against Aβ_{1-40} and Aβ_{1-42}. The diffuse plaques of the exterior entorhinal layers displayed a tight network of aggregated and frequently branched argyrophilic fibrils when visualized by the Campbell-Switzer method. The lamina dissecans was devoid of amyloid deposits.

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Neurofibrillary changes and amyloid pathology are staged according to Braak and Braak [18]. Staining pattern of the deposits in the entorhinal cortex with anti-Aβ_{1-40}, anti-Aβ_{1-42}, antibodies and the Campbell-Switzer silver impregnation separately in the external and internal entorhinal layers. (m = male, f = female, age in years, roman number = stage for neurofibrillary tangles [18], − = negative, + = positive, (+) = single positive plaques, FA = fleecy amyloid.)
Fig. 2. Entorhinal region of an AD-brain with advanced β-amyloidosis immunostained with anti-Aβ1-23. The typical distribution pattern consists of sharply delineated, diffuse amyloid deposits in the external layers pre-β and pre-γ and the internal layers pri-α and pri-β. By contrast, morphologically ill-defined, blurred clouds of fleecy amyloid (arrows) are localized in the layers pri-β and pri-γ. Subsial amyloid deposits are found in the molecular layer. (PEG, 100-μm section, case No. 12, Magnification: 20/1)

A distinct type of amyloid was found in the internal entorhinal layers (Figs. 2, 4). It consisted of morphologically ill-defined, blurred amyloid clouds, gradually merging into the neuropil. This particular type of amyloid was termed fleecy amyloid. Fleecy amyloid was immunoreactive with antibodies directed against Aβ1-23 and Aβ1-42, but was not labeled with anti-Aβ1-7 and anti-Aβ1-40 antibodies (Fig. 3). Using the Campbell-Switzer technique, fleecy amyloid showed fine and rarely branching argyrophilic fibrils that were only slightly aggregated (Fig. 5). In addition to fleecy amyloid, sharply delineated diffuse plaques of various sizes were also found in the

Fig. 3. Serial sections of the same case illustrated in Figure 2 stained with Campbell-Switzer (a), anti-Aβ1-23 (b), anti-Aβ1-42 (c), anti-Aβ1-40 (d) and Aldehydefuchsin-Darrow red (e). a) The entorhinal cortex shows the typical pattern of amyloid deposits as visualized by the Campbell-Switzer method. A band of scattered, sharply delineated, diffuse amyloid plaques characterizes the layers pre-β and pre-γ and is also seen in the layers pri-α and pri-β. In the layers pri-β and pri-γ, morphologically ill-defined, blurred amyloid deposits with a fleecy appearance are evident (arrows). The pre-α layer does not show amyloid deposits. Subsial amyloid deposits are localized in the molecular layer. b) Corresponding to Figure 2, a pattern similar to that described with the Campbell-Switzer method can be detected with the antibody against Aβ1-23. The fleecy amyloid in the internal layers is blurred throughout the layers pri-β and pri-γ (arrows). c) Immunolabeling with anti-Aβ1-42 antibodies detecting the C-terminus of Aβ displays the same pattern as Aβ1-23 (b). The fleecy amyloid is indicated by arrows. d) The sharply delineated diffuse plaques in the external and internal layers are clearly stained with anti-Aβ1-40. The fleecy amyloid seen in Figures 3a, b and c remained unstained. e) A serial section stained with Aldehydefuchsin-Darrow-red shows the layering of the entorhinal cortex for topographical orientation (PEG, 100-μm sections, case No. 12, Magnification: 25/1).
Fig. 4. Schematic drawing of amyloid deposition in the entorhinal cortex in moderate to severe β-amyloidosis. Amyloid detectable with antibodies against all 4 Aβ-epitopes is localized in the molecular layer and in the layers pre-β, pre-γ, and pri-α. Fleecy amyloid detectable only with antibodies against Aβ_{12-23} and the C-terminus of Aβ_{1-14} and by the Campbell-Switzer method is deposited in the internal entorhinal layers pri-β and pri-γ.

internal entorhinal layers, mainly in the layers pri-α and pri-β, in cases of moderate to severe β-amyloidosis. In end stage β-amyloidosis, sharply delineated plaques were also seen in the layer pri-γ in the absence of fleecy amyloid in 3 cases. The plaques in the layers pri-α, pri-β, and pri-γ were labeled with all 4 antibodies against Aβ and the Campbell-Switzer method.

DISCUSSION

This study demonstrates distinct, morphologically ill-defined clouds of amyloid in the internal layers of the entorhinal cortex (pri-α, pri-β, and pri-γ). This type of amyloid, identified by its unique morphology, is called fleecy amyloid. Fleecy amyloid is immunoreactive with anti-Aβ_{12-23} and anti-Aβ_{1-14} antibodies, but not with antibodies directed against Aβ_{8-14} and Aβ_{1-40}. This immuno-histochemical profile suggests that fleecy amyloid consists of N-terminal truncated fragments of Aβ_{1-14}, such as P3, in the absence of full length Aβ. Interestingly, these deposits were detectable as fine, fibrillar argyrophilic structures. This demonstrates that N-terminal truncated Aβ fragments are capable of generating fibrillar amyloid deposits.

Amyloid deposits, consisting of N-terminal truncated Aβ-fragments including P3, were described in the cortex, in the molecular layer of the cerebellum (8-10, 12-14), and in cases of mild β-amyloidosis in the external entorhinal layers. Morphologically, they appeared as sharply outlined diffuse plaques (10, 12-14). In contrast to these plaques, fleecy amyloid can morphologically be distinguished by its ill-defined, cloud-like appearance. This finding suggests that N-terminal truncated Aβ-peptides form deposits that vary in their morphological appearance in different locations.

Fleecy amyloid seems to be restricted to a distinct anatomical region; the internal entorhinal layers. This suggests a layer-specific susceptibility for the development of distinct types of amyloid deposits in the entorhinal cortex. It is tempting to speculate that the selective susceptibility for the production of N-terminal truncated Aβ in the internal entorhinal layers might be related to higher levels of β-secretase-like enzyme activity, in contrast to the external layers. The fleecy appearance may be attributable to the deposition of short N-terminal truncated fragments with a low molecular weight which eventually have the ability to spread diffusely throughout the internal layers of the entorhinal cortex, whereas longer fragments of higher molecular weight may be sequestered locally.

The aggregation of argyrophilic material in the fleecy amyloid suggests that N-terminal truncated Aβ may be able to create fibrils (22, 23). In contrast to sharply delineated amyloid plaques, the material in fleecy amyloid consists of sparsely aggregated fibrils quite similar to those found in vitro at the beginning of Aβ-fibrillation (24, 25). Therefore, the question arises whether fleecy amyloid may represent an early stage of Aβ-deposition, or whether it is a unique type of amyloid that does not show extensive aggregation.

The fact that fleecy amyloid is not seen in end stage β-amyloidosis suggests that it may either be replaced by sharply delineated, diffuse plaques, or may develop into such plaques. Such a replacement of fleecy amyloid by sharply delineated diffuse plaques or the morphological change into such plaques seems to begin in the layer pri-α, where the first sharply delineated plaques are found in moderate to severe β-amyloidosis of the entorhinal region. These morphological changes are combined with the beginning of Aβ_{1-14} immunoreactivity in such plaques, whereas fleecy amyloid does not react with this antibody. Therefore, it is tempting to speculate that the deposition of N-terminal truncated Aβ fragments in the fleecy amyloid precedes the deposition of full-length Aβ in the internal entorhinal layers. The fact that N-terminal truncated Aβ is also observed in the external entorhinal layer of 2 cases supports the hypothesis that the transformation of N-terminal truncated Aβ deposits into full-length Aβ containing plaques is not limited to fleecy amyloid deposits (8, 9).

In conclusion, fleecy amyloid is a morphologically distinct type of amyloid deposition which forms argyrophilic fibrils, contains N-terminal truncated Aβ, and is in the entorhinal region restricted to the internal layers pri-α, pri-β, and pri-γ.
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REFERENCES


Fig. 5. Campbell-Switzer staining of a case with moderate β-amyloidosis of the entorhinal cortex shows the aggregation of argyrophilic fibrils in the sharply delineated plaques of the external entorhinal layers (a) and in the fleecy amyloid (b). a) High power view of sharply delineated amyloid deposits in the external entorhinal layers in the Campbell Switzer silver impregnation. The amyloid fibrils are aggregated, and show irregular branching in various directions. b) The few fine amyloid fibrils of fleecy amyloid in the internal layers are sparsely aggregated and the level of fibril branching (arrows) is lower than that in external layer plaques. (Paraffin embedded tissue, 10-μm section, case No. 2, Magnification: 875X).

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