A4 Protein in Alzheimer’s Disease: Primary and Secondary
Cellular Events in Extracellular Amyloid Deposition

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Abstract. This study was designed to investigate the role of serum proteins, microglia, glial fibrillary acidic protein (GFAP) positive cells and dystrophic neurites in the genesis of cerebral amyloid. Using A4 protein antisera, we found an amorphous non-congophilic, form of plaque, which was not seen in Bielschowsky silver staining or Bodian impregnations. GFAP-positive glial cells, cells immunolabelled for some macrophage markers and dystrophic neurites were detected in congophilic plaques with crystalline amyloid, but not in the amorphous, non-congophilic plaques. The presence of α1-antichymotrypsin, complement factors and P component, but not of common serum proteins in both the amorphous and congophilic plaques, indicates that these three proteins may have a pathogenetic role in amyloid formation. Amorphous plaques may be the earlier forms of plaque and consequently, the presence of reactive cells and dystrophic neurites may be secondary phenomena.

Key words: A4 protein; Alzheimer’s disease; Amyloid, cerebral; Macrophages; Serine protease inhibitor; Serum proteins.

INTRODUCTION

Cerebral amyloidosis is a pathologic feature of Alzheimer’s disease (AD). Extracellular amyloid deposits are found as plaques in the neuropil, and in the walls of leptomeningeal and parenchymal cortical arteries and arterioles (congophilic angiopathy) (1–3). The plaques are of varied morphology, depending to a large degree on the amount of congophilic material and associated peripheral neuritic change (4–6). A special form of vascular amyloid deposition is the dyshoric angiopathy (2), or “drusige Entartung” (7) showing infiltration of vascular amyloid into the neuropil. Another important aspect of Alzheimer’s disease is neurofibrillar degeneration. Neurofibrillary tangles (NFT) are found in the perikarya of large pyramidal neurons and free in the neuropil (ghost tangles). In addition neurites with neurofibrillary degeneration are found around amyloid plaque deposits and dispersed in the neuropil. The relationships between NFT, neurites, plaques and vascular amyloid are not yet understood, but clearly are important in unravelling the nature of AD and related conditions in which cerebral amyloidosis occurs.

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The amyloid fibrils in both plaques and in vascular deposits consist of polymers of a 4 kDa protein subunit (8–13), the A4 protein (also referred to as β-protein). The N-terminal sequences of the A4 protein in plaques and vascular amyloid are almost identical (8–10). The A4 protein is a cleavage product from a precursor membrane glycoprotein (PreA4) (14, 15). At least three alternatively spliced forms of PreA4 are now known (14, 16–18). The gene for PreA4 is expressed in many tissues including the brain. The PreA4 mRNA is particularly well seen in larger neurons (19–23).

The cellular origin of the amyloid in AD is controversial. While the relative abundance of PreA4 in neurons is consistent with the postulated neuronal origin of the amyloid (11) other cellular events may well take place in the processing of the PreA4 molecule into the A4 amyloid aggregates. It has been suggested that macrophages or microglia may contribute to the proteolytic cleavage of PreA4, analogous to processing events which may occur in systemic amyloidogenesis (24, 25). Some evidence was found that plaque formation starts around microglial cells because these cells were found in the center of possibly early plaques (26). Astrocytes, the other cellular element readily recognizable in the plaque, may also be implicated in the deposition of amyloid. Some investigators have argued that the extracellular amyloid arises from a hematogenous precursor (9, 12, 27) or that the major pathogenic process occurs at the level of fibroblast, pericyte or endothelial cell of the blood-brain barrier (24, 28, 29) through mechanisms which involve leakage of neurotoxic factors or serum proteins.

In this study we used antisera to the A4 protein to demonstrate the broad spectrum of extracellular amyloid deposition, and used immunocytochemical markers of NFT, macrophages, microglia, astrocytes and serum proteins to address some of the issues concerning the events associated with amyloid deposition in AD and related conditions.

MATERIALS AND METHODS

Brain tissue was obtained from eleven patients with AD (one parietal biopsy) and three patients with Down’s syndrome (DS) (Table 1). Autopsy was performed 4–23 hours after death. Frontal, temporal, parietal, occipital and hippocampal cortex was taken after 10% formalin fixation and embedded in paraffin. In seven patients with AD (postmortem delay less than eight hours) pieces of the same cortical regions were frozen in liquid nitrogen for cryostat sections. Hematoxylin and eosin stains, Bodian or Bielschowsky silver impregnation, Congo red, and the Klüver-Barrera Luxol fast blue stains were performed on all paraffin blocks. The silver technique of Braunmühl (30) was performed on selected formalin-fixed frozen sections. The clinical diagnosis and neuropathological findings are summarized in Table 1. Fresh frozen or formalin-fixed brain tissues of four nondemented aged patients without cerebral disorders (postmortem delay less than six hours) were used as normal control tissues for the study of serum proteins and macrophages. In addition fresh frozen tissue of six aged-matched controls and two controls under the age of 50 years were studied for the presence of A4 positive plaques.

Immunocytochemistry

For immunocytochemical staining on formalin-fixed tissue, 8 μm thick paraffin sections were mounted on poly-l-lysine coated glass slides, dehydrated in ethanol and preincubated in 0.3% H2O2 to block endogenous peroxidase. Immunocytochemical staining for the A4 protein on paraffin sections was enhanced by pretreatment with 80% formic acid (31, 32). Immunocytochemical staining for macrophagemarkers on paraffin sections was enhanced by trypsinization (0.5% trypsin in 0.5% CaCl). For immunocytochemistry with cryostat sections, 8
<table>
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<th>Case</th>
<th>Sex</th>
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<th>Clinical diagnosis</th>
<th>Postmortem delay (hours)</th>
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<td>5</td>
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<td>84</td>
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<td>&lt;23</td>
<td>1,500</td>
<td>-</td>
<td>-</td>
<td>yes</td>
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</table>

--: none.
(+): a few.
?: unknown or questionable.
+ moderate number; ++ numerous congophilic vessels, compact plaques or tangles.
AD: Presenile Alzheimer's disease or senile dementia of the Alzheimer type.
AD-FAM: Familial case of presenile AD, or familial senile dementia of the AD type.
DS: Down's Syndrome.
ACA: Alzheimer's congophilic angiopathy.
DA: Dyschoric angiopathy.
NFT: Neurofibrillary tangles.
μm thick sections were mounted on poly-L-lysine coated glass slides, air-dried and fixed in acetone for ten minutes (min) before use.

The primary antibodies used in this study are listed in Tables 2 and 3, together with their specificity, sources, selected references and the immunocytochemical technique which was used in this study (33–47). The specificity of each antibody against macrophages was evaluated in lymphoid tissue (spleen, tonsil). Macrophages in brain tissue were studied with the same technique as usual for each antibody on lymphoid tissue. All antibodies were appropriately diluted in phosphate buffered saline (PBS), pH 7.4, containing 1% bovine serum albumin. Each incubation was performed at room temperature and followed by repeated washes in PBS. The antisera to the A4 protein were generated by immunizing rabbits with synthetic peptides corresponding to residues 1–28 or 1–42 of the A4 sequence (10, 11, 14, 32).

Secondary antisera and reagents were tested for lack of cross-reactivity and nonspecific staining. Some sections were preincubated with normal human serum to prevent nonspecific staining.

Peroxidase activity was visualized using 3,3-diaminobenzidine (DAB) (5 mg DAB in 10 ml PBS, pH 7.4, containing 0.02% H2O2 for 3–5 min). Cryostat sections were darkened with copper sulphate (0.5% in 0.9% NaCl for five min (48). All sections were counterstained with Congo red (49) to visualize the amyloid. After Congo red staining sections were dehydrated and mounted in nailon.

The indirect technique for mouse monoclonal antibody (MAb) was as follows: acetone-fixed cryostat sections were washed in PBS and incubated with the primary MAb for 60 min and incubated in the second step with peroxidase labelled rabbit anti-mouse antisera (DAKO) for 30 min. Peroxidase activity was revealed by the DAB method.

The indirect technique for antisera against lysozyme and α1-antichymotrypsin was as follows: deparaffinized, rehydrated sections were trypsinized and preincubated with normal swine serum for ten min followed by an incubation with the primary antisera. In the second step sections were incubated with peroxidase labelled swine anti-rabbit antibodies (DAKO).

In the peroxidase-anti-peroxidase (PAP) technique, deparaffinized and rehydrated sections or acetone-fixed cryostat sections were washed in PBS, pH 7.4, preincubated with normal swine serum and incubated with the primary rabbit antisera for 60 min. After a second preincubation with normal swine serum, sections were incubated with swine anti-rabbit immunoglobulins (DAKO) for 30 min. In the third step sections were incubated with rabbit peroxidase-anti-peroxidase (PAP) complex (DAKO) for 30 min. Peroxidase activity was revealed by the DAB method.

Using the ABC (avidin–biotin–peroxidase) technique, deparaffinized, rehydrated sections (for demonstration of DAKO LC, Mig T4 and Alz 50) or acetone-fixed cryostat sections (for demonstration of EBM11) were preincubated with normal horse serum for 20 min followed by incubation with the primary MAb for 60 min (DAKO LC and Mig T4) or overnight incubation (Alz 50).

After a second preincubation with normal horse serum, sections were incubated with biotinylated horse anti-mouse immunoglobulin for 30 min and the avidin–biotin–peroxidase complex (Vectorlab) for 60 min.

Double staining for EBM11 and GFAP was performed as previously described (50).

Fresh frozen lymphoid tissues were used as positive controls for the presence of macrophages in cryostat sections. A case of neuronal ceroid lipofuscinosis with many macrophages in the cortex was used as a positive control for formalin-fixed, paraffin-embedded tissue.

RESULTS

The results of this immunocytochemical study are summarized in Table 4.

A4 Protein: Immunocytochemistry for the A4 protein in frozen sections and in paraffin sections pretreated with formic acid displayed two basic types of plaques, which were found in all patients with AD and DS:

1. Amorphous, cloudy plaques which were not stained by Congo red (Figs. 1, 2A).
<table>
<thead>
<tr>
<th>Antibody (antigen)</th>
<th>Source</th>
<th>Raised in</th>
<th>CD number</th>
<th>Specificity</th>
<th>Reference</th>
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</thead>
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<td>Dako LC</td>
<td>DAKO</td>
<td>mouse</td>
<td>CD 45</td>
<td>Leucocyte lineage (T200)</td>
<td>Warnke et al (33)</td>
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<tr>
<td>OKIa</td>
<td>DAKO</td>
<td>mouse</td>
<td>--</td>
<td>HLA-Dr (class II MHC)</td>
<td>Reinherz et al (34)</td>
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<td>α1-antichymotrypsin</td>
<td>DAKO</td>
<td>rabbit</td>
<td>--</td>
<td>Monohistiocytic cell series</td>
<td>Papadimitriou et al (35)</td>
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<td>Lysozyme</td>
<td>DAKO</td>
<td>rabbit</td>
<td>--</td>
<td>Myeloid cells, monocytes</td>
<td>Mason et al (36)</td>
</tr>
<tr>
<td>C3b-rec. (To5)</td>
<td>DAKO</td>
<td>mouse</td>
<td>CD 35</td>
<td>CR1 (complement rec.)</td>
<td>Gerdes et al (37)</td>
</tr>
<tr>
<td>MO 1</td>
<td>C.I.</td>
<td>mouse</td>
<td>CD 11b</td>
<td>CR3 (complement rec.)</td>
<td>Todd et al (38)</td>
</tr>
<tr>
<td>Bear-1</td>
<td>gift</td>
<td>mouse</td>
<td>CD 11b</td>
<td>CR3 (complement rec.)</td>
<td>Keizer et al (39)</td>
</tr>
<tr>
<td>OKM1</td>
<td>Ortho</td>
<td>mouse</td>
<td>CD 11b</td>
<td>CR3 (complement rec.)</td>
<td>Wright et al (40)</td>
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<td>mouse</td>
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<td>Monocytic cells, platelets</td>
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<td>CD 14</td>
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<td>Sanb.1</td>
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<td>CD 11c</td>
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<tr>
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<td>B&amp;D</td>
<td>mouse</td>
<td>CD 11c</td>
<td>Monocytes/macrophages</td>
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<td>B&amp;D</td>
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<td>FcR&lt;sub&gt;low&lt;/sub&gt;</td>
<td>Bliss et al (45)</td>
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<td>mouse</td>
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<td>mouse</td>
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DAKO: DAKOPATTS, Denmark.
Ortho: Ortho-mune.
Sanb.: Sanbio.
B&D: Becton & Dickinson, Inc.
C.I.: Coulter Immunology.
gift<sup>3</sup>: K. Lennert, Klinikum der Christian-Albrechts-Universität, Kiel.
gift<sup>4</sup>: L. Poulter, Royal Free Hospital, London.
CD number: Cluster of Designation.
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<td>Innog</td>
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<td>Tangles, dystrophic neurites (non-tau, non-ubiquitine epitope)</td>
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<td>mouse</td>
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DAKO: DAKOPATTS, Denmark.
Innog: NV Innogenetics sa, Belgium.
Pharm: Pharmacia Fine Chemicals (Sweden).
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<tr>
<td>A4 protein</td>
<td>(perivascular cells)</td>
<td>(small glial cells)</td>
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<tr>
<td>AP amorphous</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>AP congophilic</td>
<td>+</td>
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</tr>
<tr>
<td>ACA -DA</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ACA +DA</td>
<td>+</td>
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- : no immunoreactivity.
+/-: immunoreactivity often seen.
+: strong immunoreactivity.

Panel I: Dako LC, α1-antichymotrypsin, Lysozyme, C3b-rec, MO 1, OKM1, OKM5, LeuM3, 5D2, IL2-rec, KiM6, My 7 and RFD7.
Panel II: OKIa, Bear-1, FK 24, My 4, LeuM5 and EBM11. (See Table 2.)
AP: Amyloid Plaque.
ACA -DA: Alzheimer's congophilic angiopathy without dyschoric angiopathy.
ACA +DA: Alzheimer's congophilic angiopathy with dyschoric angiopathy.
Fig. 1. Parietal cortex of a 55-year-old man with familial Alzheimer's disease. Frozen section stained for Aβ protein and showing amorphous non-cogophilic plaques. Note the absence of compact amyloid. The PAP technique counterstained with Congo red. ×264.

Fig. 2. Cerebral cortical biopsy from a 46-year-old man with Alzheimer's disease. A) Aβ-positive, amorphous plaques in frozen cortical tissue. ×132. B) Bodian silver impregnation on formalin-fixed tissue. Amorphous plaques are not visible. ×132.
These amorphous, noncongophilic plaques could not be visualized by the silver techniques of Bodian or Bielschowsky (Fig. 2B). Using Braunmüll's technique on formalin fixed, frozen sections, similar amorphous plaques could be demonstrated (Fig. 3).

2. Classical plaques with an A4 positive central core and an A4 negative corona.

In adjacent sections stained for Congo red, the central A4 positive core appeared to be congophilic and showed the well known green birefringence when viewed by polarized light.

Besides these two basic types of plaques an intermediate form was occasionally found: an amorphous deposit with a congophilic center.

The amorphous plaques outnumbered the classical types. In the parietal and temporal lobes of Alzheimer patients we found 0–20 amorphous plaques per high power field (×250). In aged controls 0–7 amorphous plaques were found. Classical plaques were 0–8 per high power field in AD patients and 0–1 in aged controls. Amorphous plaques were not seen in controls under the age of 50. Amorphous plaques were seen in all cortical layers and could be detected in all cortical regions.

A4 positivity was also found in the walls of all congophilic vessels and in the amyloid deposits around dyshoric vessels (Fig. 4).

*Tangles and Neuritic Degeneration:* Using Mig T4 and Alz 50 as markers for NFT and neuritic changes, intra- and extracellular tangles were found in AD and DS cases. Moreover, many swollen fragments of nerve fibers in the neuropil were stained. The well known neuritic profiles in the corona of classical plaques stained intensely. Neuritic plaques were seen especially in upper cortical layers. In contrast neuritic profiles were not seen around the amorphous plaques. Swollen Mig T4-positive and Alz 50-positive fiber fragments were also found around amyloid deposits radiating from dyshoric vessels (Fig. 5). Congophilic vessels without amyloid sleeves were not surrounded by Mig T4-positive or Alz 50 positive fiber fragments. Adjacent sections stained either using Mig T4 or Alz 50 showed identical results.

*Glial Fibrillary Acid Protein (GFAP):* Amorphous plaques were not associated with GFAP positive glial cells. In classical plaques, however, GFAP positive fibers were often found in the corona and labeled cells could be found nearby. An increase of GFAP-positive cells was not observed around congophilic vessels compared to other vessels. However, near the perivascular deposits around dyshoric vessels there often was a vigorous astrocytic response in the surrounding neuropil (Fig. 6).

*Small Glia and Macrophage/Microglia Markers:* In classical plaques, variable numbers of small glial cells were labelled by OKIa, Bear-1, LeuM5, FK24, My4 and EBM11 markers (Fig. 7). These cells were especially found at the border between the congophilic center and the corona. In contrast, these immunostained cells were not found in association with non-congophilic amorphous deposits. Throughout the cortex, dispersed immunolabelled small glial cells were found. In the subcortical white matter these immunolabelled cells were frequent and were similar to the branched forms of microglia described by Del Rio-Hortega (51). The pattern of staining of these cortical and subcortical cells for macrophage markers was similar to that of the cells around the amyloid core of the classical plaques. There was no increase of immunolabelled cells around congophilic vessels. Some OKIa positive cells were seen at the edge of the dyshoric vessels. No labelling was found using other macrophage markers except for cells within intracerebral blood vessels. Labelling in lymphoid tissues was according the specificity of each antibody.

*Serum Proteins:* In frozen sections, complement Clq and C3c, α1-antichymo-
Fig. 3. Same patient as Figure 1. Cryostat section of formalin-fixed parietal cortex stained according to Braunmühl (30). Amorphous plaques with this silver technique have the same morphology as the Aβ-positive plaques in Figure 1. ×252.

Fig. 4. Parietal cortex of a 71-year-old man with Alzheimer's disease. Paraffin-embedded section stained for Aβ-protein (after formalin fixation for 48 h). This section demonstrates immunoreactivity of vascular amyloid and dystrophic angiopathy (arrow). ×330.
Fig. 5. Parietal cortex of a 70-year-old man with congophilic angiopathy and dyshoric angiopathy. Paraffin-embedded section stained for Mig T4; Mig T4-positive structures (arrows) around the dyshoric vessels. Mig T4, a mouse MAb against tangles; avidin–biotin–peroxidase technique. Amyloid is demonstrated with Congo red stain. ×330.

Fig. 6. Paraffin section stained for GFAP of the same area as Figure 5. GFAP is demonstrated with a polyclonal antibody; PAP-technique. Note GFAP-positive processes around dyshoric vessels (arrows). ×330.
Fig. 7. Temporal cortex of an 87-year-old man with AD. Frozen section stained for the macrophage/microglia marker EBM/11 (mouse MAb); avidin–biotin–peroxidase method; positively stained cells in and around a classic amyloid plaque. One cell is indicated with an arrow. Amyloid plaque is counterstained with Congo red stain. ×208.

Fig. 8. Same patient as Figure 1. Frozen section stained for α1-ACT; PAP method. Plaques have the same amorphous morphology as plaques in Figure 1. ×252.
trypsin (α1-ACT) and serum amyloid P component (SAP) were demonstrable in all amorphous plaques (Fig. 8) and in the central amyloid core of the classical plaques. Some tangles also stained for SAP. In the vascular amyloid deposits, the complement factors and α1-ACT were variably present, but staining for SAP was always seen. Other serum proteins (albumin, IgG, fibrinogen and prealbumin) could not be detected in any of the amyloid deposits in frozen sections.

In formalin-fixed paraffin-embedded sections the immunoreactivity for serum proteins of the amyloid core of the classical plaques was variable; complement factors could not be detected whereas antisera raised against α1-ACT, SAP, fibrinogen IgG and albumin showed inconsistent results. Neuronal and glial cells adjacent to some plaques were often stained for serum proteins in routinely fixed tissue, both in demented and in control cases.

DISCUSSION

In this study we have described a “new” type of A4 positive plaque, the amorphous plaque, which is not congophilic and can not be visualized in silver impregnations of Bodian and Bielschowsky in contrast to the A4 positive classical plaques. Both amorphous plaques and classical plaques were stained for a selective group of serum proteins in frozen sections. Amorphous plaques were more numerous than congophilic plaques and could be found in all cortical layers. Many amorphous plaques were seen in brains of patients with AD and DS while these amorphous plaques were less numerous in the brains of nondemented aged-matched controls and could not be detected in controls under the age of 50 years. These findings are in agreement with a recent study which shows that in a normal aging population A4 deposition is a strongly age related phenomenon (32). Age related deposition of classical plaques in controls has been also demonstrated (52).

The relationship between these amorphous plaques and the well-known congophilic plaques is still an open question. In addition differences in congophilia and frequency of occurrence the following differences between these two types of A4-positive deposits were found:

a) Dystrophic neurites appear only around compact forms of amyloid but are absent in amorphous plaques.

b) Gliosis is found only in classical plaques, not in amorphous plaques.

c) Microglial cells appear only in classical plaques, not in amorphous plaques.

Based on these observations, we suggest that the amorphous plaques may be the earliest forms of extracellular amyloid deposition. We propose an outline of the plaque formation as shown in Table 5. A similar evolution is suggested for plaques in monkeys (53).

In our study, dystrophic neurites were found around classical plaques and dyshoric vessels but not around noncongophilic, amorphous plaques, or around congophilic vessels without a dyshoric sleeve; this suggests that the dystrophic neurites may be reactive and are secondarily involved in amyloid formation. This concept, originally suggested by Cajal (54), was later supported by others (55). Little or no neuritic change is seen around the amyloid core in the unconventional virus encephalopathies such as scrapie (56) and Creutzfeldt-Jakob disease (57). Amyloid plaques in scrapie affected mice (58) and Creutzfeldt-Jakob disease (59) also lack immunoreactivity for complement factors and α1-ACT (60). Therefore these factors may be toxic for the surrounding neuropil and may play a role in the formation of dystrophic neurites.

The amorphous A4 positive plaques in this study are morphologically similar to
TABLE 5
Proposed Evolution of the Amyloid Plaque in Alzheimer’s Disease

<table>
<thead>
<tr>
<th>Light microscopy</th>
<th>Electron microscopy (after Wisniewski and Terry (6))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I: Infiltration with amorphous, non-congophilic Aβ protein. No reactive cells or neurites.</td>
<td>Premature plaque: amyloid fibrils with negative and positive stained neurites. Few reactive cells.</td>
</tr>
<tr>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Stage II: Condensation of Aβ protein into congophilic masses; neuritic changes at periphery with cellular (microglial and astrocytic) reaction.</td>
<td>Classical plaque: a central core of amyloid with neuritic and reactive cell changes.</td>
</tr>
<tr>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Stage III: Crystallization of Aβ protein into central core of congophilic amyloid (eventual resolution of reactive changes).</td>
<td>Compact (burned out) plaque: amyloid core with few neuritic or reactive cell changes.</td>
</tr>
</tbody>
</table>

The amorphous plaques described earlier by von Braunmühl (30). He pointed out that these amorphous plaques were not congophilic and could only be visualized by his own silver impregnation technique on frozen sections. We have confirmed his findings. In this study the amorphous plaques were not found using the silver impregnation of Bodian and Bielschowsky. It is possible that in these silver methods only dystrophic neurites are visualized, while in the modified silver staining extracellular protein is visualized also. Amorphous plaques are different from the well-known primitive plaques which can be found with the usual silver impregnation techniques or using the antibodies A150 and Mig T4. Primitive plaques contain neurofibrillary material, abnormal neurites and reactive cells (6). Primitive plaques were seen especially in the upper cortical layers whereas amorphous plaques were seen in all cortical layers in this study.

The presence of so-called microglial cells is usually described around the amyloid core of plaques. Some of these cells have hyperchromatic nuclei and lipofuscin in their cytoplasm. Microglial cells share some epitopes with macrophages (61, 62) and could play a crucial role in amyloid formation (25). Using a large panel of antibodies against macrophages, we have confirmed recent findings about a relationship between cells of the nervous system, expressing immune system-associated antigens and congophilic plaques (61, 63). However, we could not find immunostained cells in amorphous, non-congophilic plaques. Our data do not support the suggestion that initial plaque formation is related to microglial cells (26).

In previous studies complement factors (64, 65) and α1-ACT (60) were described in congophilic plaques. The amyloid P component was seen in classical plaques and in some tangles (66, 67). In this study we also found staining of the amorphous types of plaques for α1-ACT, complement factors and SAP in frozen tissue. In contrast, common serum proteins (IgG, albumin and fibrinogen) were not found either in
amorphous plaques or in amyloid plaques in frozen tissue. Reports about their presence in plaques seem to be related to the use of formalin fixed tissue (68). In this and previous studies, IgG, albumin and fibrinogen were found only in focal areas in neuronal and glial cells and occasionally in the core of classical plaques in fixed tissue. This pattern of staining was seen both in the brains of some demented patients and in controls with and without neuropathological disorders (69, 70). It is unlikely that these proteins play a role in formation of amyloid (71). Nor is there evidence that the presence of these common serum proteins reflects an underlying pathogenic blood-brain barrier dysfunction in Alzheimer's disease (70), although others have so contended (29). Immunoreactivity for C3c, C1q, α1-ACT and SAP was seen in all A4 positive plaques in frozen sections of all the cases studied. Positivity for these proteins does not permit one to conclude whether these proteins are synthesized locally, or are derived from serum. Some of these proteins in senile plaques maybe of endogenous origin. Complement factors can be produced by astrocytes (72) and a possible endogenous origin of α1-ACT has also been proposed (60). Using immunocytochemical techniques with antibodies against complement components and activated complement we have found that complement components are not passively bound to the amyloid plaque structure but are the result of a complement activation process (73). It is not yet understood why proteases (activated complement fragments) or protease inhibitors (α1-ACT) should be present in the plaques from an early stage, but clearly there may be a role in the basic mechanism in which the PreA4 is converted to the A4 amyloidogenic subunit. It was recently suggested that the PreA4 protein resembles a heparan sulfate proteoglycan which has a synaptic localization and can accumulate by limited proteolysis (74). However, others have demonstrated that precursors of the A4 protein are tyrosine-sulfated, O-, and N-glycosylated membrane proteins and a proteolytic cleavage could be the first step in formation A4 (75).

All these data suggest that there may be two basic plaques: the amorphous, non-cogophilic plaque and the compact, cogophilic plaque with crystalline amyloid. Reactive cells and dystrophic neurites are usually found around compact amyloid. We found no evidence for a role for macrophages, glial cells, common serum proteins or dystrophic neurites in the pathogenesis of the amorphous, A4 positive plaques. These findings are consistent with the hypothesis that the amorphous, non-cogophilic plaque is the earlier form of plaque. There may be a gradual process of infiltration, condensation and final crystallization of the A4 amyloidogenic protein subunit as the basic process underlying the plaque formation. We favor a neuronal origin of the amyloid and its deposition in the extracellular space may cause a reactive change of neuronal processes and glial cells (10).

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