The Pathogenesis of Senile Plaques

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Abstract. Senile plaques (SP) are complicated lesions composed of diverse amyloid peptides and associated molecules, degenerating neuronal processes, and reactive glia. Evidence suggests that diffuse, neurofi brillary amyloid deposits evolve over time with formation of discrete niduses that eventually become neurotic SP. The evidence for differential amyloid precursor protein metabolism that may favor deposition of Aβ1-42 in this early, possibly aging-related lesion is discussed. This latter molecule, also known as P3, may represent a benign form of amyloid, since it lacks domains associated with activation and recruitment of glia to SP. Subsequent to deposition of Aβ1-42 and then growth of the amyloid with precipitation of soluble Aβ1-40, in an Alzheimer disease-specific process, SP increasingly become associated with activated microglia and reactive astrocytes. In response to interaction with amyloid peptides and possibly glycated proteins, microglia and astrocytes produce a number of molecules that may be locally toxic to neuronal processes in the vicinity of SP, including cytokines, reactive oxygen and nitrogen intermediates, and proteases. They also produce factors that lead to their reciprocal activation and growth, which potentiate a local inflammatory cascade. Paired helical filament (PHF) type neurites appear to be associated with SP only in so far as neurofi brillary degeneration has progressed to affect neurons in those regions where the plaque forms. Thus, PHF-type neurites are readily apparent in SP in the amygdala at an early stage, while they are late in primary cortices and never detected in cerebellar plaques, where only dystrophic neurites are detected. If the various stages of SP pathogenesis can be further clarified, it may be possible to develop rational approaches to therapy directed at site-, cell type-, and stage-specific interventions. Although controlling the local inflammatory microenvironment of SP may hold promise for slowing lesion pathogenesis, it still remains a fundamental challenge to determine the mechanism of neurodegeneration that results in widespread neurofi brillary degeneration and eventual synaptic and neuronal loss, which is considered to be the proximate cause of the clinical dementia syndrome.

Key Words: Aging; Alzheimer; Amyloid; Astrocytes; Microglia; Neurites.

INTRODUCTION

The essential histopathological feature of Alzheimer disease (AD) is selective neuronal loss associated with neurofi brillary degeneration and extracellular amyloid deposition. The relationship of the latter 2 lesions to neuronal loss and to each other is unclear. The relationship of these changes to aging is likewise an area of current interest. The focus of this review will be on senile plaques (SP) in aging and AD. A word about nomenclature is warranted before exploring the various components of SP. The term “senile plaque” is used in this review in a general sense to encompass a wide array of lesions that contain extracellular amyloid. In the brain the most common form of amyloid is one that contains a 4 kDa protein referred to as Aβ (1). Plaques with large or poorly circumscribed amyloid deposits are frequently referred to as “diffuse plaques,” those with prominent neuritic elements as “neuritic plaques,” and those considered to be composed exclusively of amyloid as “amyloid plaques.” It must be emphasized that no single staining method permits one to recognize all elements of SP. A simple classification that takes into account only the appearance of amyloid and the presence and type of neurites, as illustrated in Figure 1, demonstrates the complex nature of the SP. As one adds more elements to the classification scheme, the number of possible combinations rapidly becomes mind boggling. Most classification schemes are inherently unsatisfactory since they ignore this complexity. The challenge to the neuropathologist is to determine which components are clinically significant.

All plaques referred to in this review have extracellular amyloid deposits and are characteristic of aging or AD. The unusual plaque-like lesions of uncommon non-Alzheimer degenerative dementias, which lack amyloid (2), and amyloid plaques associated with prion diseases (3) are not considered further. Parenchymal amyloid deposits have a wide range of appearance and are associated with cellular changes to varying degrees. They are best appreciated with immunocytochemistry (4–6), but similar diversity can also be detected with routine amyloid stains, such as thioflavin-S-fluorescent microscopy (7) (Fig. 2). The clinical significance of the different types of SP is increasingly understood as evidence accumulates from detailed studies of SP, not only in AD, but also from prospective longitudinal studies of elderly humans and primates, and from cross-sectional studies of Down’s syndrome subjects of different ages (8–11). The recent discovery that transgenic mice overexpressing the gene for human amyloid precursor protein predictably develop amyloid deposits offers great promise that at least some of the pathogenic cascade that produces SP will be amenable to

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rigorous analysis (11). Immunological and biochemical methods have contributed greatly to understanding the molecular composition of SP, and recent findings in these areas will be emphasized in this review.

Neuritic Heterogeneity of SP

Dystrophic-type Neurites: Clinically, among the most important types of SP are those associated with degeneration of nearby neuronal cell processes (neurites). These are sometimes referred to as neuritic plaques. Neurites in SP are heterogeneous—some neurites contain degenerating synaptic elements with accumulation of membranous material and lysosomal dense bodies (dystrophic type) and other neurites contain paired helical filaments (PHF-type) (major properties of these 2 types of neurites are summarized in Table 1). Dystrophic-type neurites are found in SP in AD, as well as in aged humans and other mammals (12) (Fig. 3), and in transgenic mice (13). On the other hand, PHF-type neurites are specific to humans and highly characteristic of AD (14).

Although dystrophic neuronal processes occur independently of SP in the aged brain, there is no disputing the fact that they are concentrated in the vicinity of SP, especially SP with dense amyloid cores. The close proximity of dystrophic processes to SP suggests that they may be produced by local neurotoxic factors. Among the neurotoxic factors to be considered are those derived from glial cells reacting to amyloid and amyloid itself. A novel direct assay of the toxic properties of the local microenvironment of SP has been demonstrated by culturing primary neurons on unfixed tissue sections from Alzheimer brain. Neurite outgrowths tended to divert from the SP (15). A less popular theory holds that neuritic dystrophy precedes amyloid deposition. Evidence in favor of this hypothesis is that dystrophic neurites can be seen in primates (16) and in young Down’s syndrome.
brains (9) before amyloid is detected. On the other hand, it is also clear that neuritic changes similar to those in SP can be detected in the aged brain in certain locations completely independent of amyloid. These sites include the dorsal column nuclei in the lower medulla, the pars reticularis of the substantia nigra, and the ventral pallidum. There are also dystrophic neurites in the cortex of the limbic lobe and in the amygdala in the aged brain (17).

Dystrophic neurites can be detected with a number of staining methods. Methods that detect lysosomal enzyme activity (e.g. acid phosphatase [18]) or molecules (e.g. cathepsin [19]) readily stain dystrophic neurites. Other markers that have been used to detect dystrophic neurites recognize specific glycosyl groups (e.g. concanavalin A [20]) (Fig. 4) or components of the degenerating synaptic terminals (e.g. antibodies to chromogranin A [21, 22]). Antibodies to ubiquitin also consistently detect dystrophic neurites (Fig. 4), probably because they recognize abnormal ubiquitinated proteins that have been taken up by autophagy into membranous and lamellar dense bodies. Despite the fact that ubiquitin is involved in energy-dependent, non-lysosomal proteolytic degradation and is
TABLE 1
Neuritic Components of Senile Plaques

Dystrophic neurites
Electron dense lamellar and membranous cytoplasmic bodies
Aging in humans, primates, dogs and transgenic mice
Immunoreactive for APP, ubiquitin, Concanavalin A and chromogranin
Biochemistry: some of the lamellar bodies contain lysosomal enzymes, e.g. acid phosphatase

PHF-type neurites
Paired helical filaments
22 nm diameter, 80 nm half-period
Aging in humans, primates, dogs and transgenic mice
Immunoreactive for tau and ubiquitin
Biochemistry:
Altered form of tau protein: PHF-tau
Tau protein is a microtubule-associated protein that
promotes and stabilizes microtubules in its non-
phosphorylated form.
single gene on chromosome 17
alternative splicing (6 isoforms)
microtubule binding domains (3 or 4 repeats)
amino terminal inserts (exons 2 and 3)

Tau undergoes post-translational modifications that
deploy its abnormal properties in PHF
phosphorylation inhibits function
increased or abnormal activity of proline-directed
kinesins, e.g. Cdc2-related kinesins
decreased activity of protein phosphatases, e.g.
calcineurin (PP2a)
ubiquitination
glycosylation and glycation proteolysis, especially in extracellular PHF

Within SP (28). On the other hand, since amyloid deposits very likely occur initially in the absence of any detectable
neuritic degeneration, based upon studies of Down’s syn-
drome patients of different ages and prospectively studied,
clinically normal elderly humans, the significance of
APP in dystrophic neurites is unclear. If APP is a synaptic
protein involved in maintenance of synaptic integrity (29,
30), then increased expression of APP in dystrophic neu-
rites may be an adaptive or secondary response to syn-
aptic disruption or degeneration. At least some dystrophic
neurites also contain neurofilamentous aggregates and are
immunoreactive with antibodies to neurofilament,
especially nonphosphorylated neurofilaments (14, 31).

Dystrophic neurites are not derived from neurons of a
particular type. Many different neurotransmitters have
been localized to neurites in SP (32, 33). Most evidence
suggests that specific neurotransmitters are not vulnerable
to neuritic degeneration, but that the properties of the
neurites in SP are determined by the type of neuronal
processes in the vicinity of the plaque (34). In studies of
elderly individuals with graded clinical and pathological
changes, dystrophic neurites were shown to precede PHF-
type neurites (35). A similar conclusion has been drawn
from studies of Down’s syndrome (9). In double labeling
studies, axons in close proximity to SP undergo focal
dystrophic swelling and possibly sprouting in the plaque
(14). These observations are also compatible with a toxic
local microenvironment associated with SP.

PHF-type Neurites: The PHF-type neurite is highly
significant and in many ways indistinguishable from cy-
oskeletal pathology that occurs within neurons vulnera-
tle to degeneration and cell death in aging and AD. Ev-
dence from several clinicopathological studies based
upon both qualitative (14) and quantitative markers of
neurofibrillary pathology (36-39), as well as with enzyme-
linked immunoassays for PHF proteins (40) have indi-
cated that PHF-type neuritic degeneration correlates well
with cognitive dysfunction. Less is known about the clin-
ical significance of dystrophic-type neurites. Since they
can be detected in SP of clinically normal people (17), it
is reasonable to postulate that dystrophic neurites in and
of themselves contribute to only a minor way to age-
associated cognitive impairment.

Paired helical filaments are composed of 22 nm di-
ameter filaments with a 80 nm half-period (41). They
have been the subject of a recent review (42). PHF are
relatively specific to AD, although a few non-AD de-
mentias (e.g. Guam Parkinson-dementia complex) and
rare storage diseases (e.g. Niemann-Pick type C) have
filaments similar to PHF (43). Although filaments in oth-
er disorders are immunocytochemically similar, recent
structural and biochemical studies suggest that abnormal
filaments in these non-AD dementias, including progressive
supranuclear palsy, corticobasal degeneration, and
Pick’s disease, are distinct from those found in aging and

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AD (44). Immunochemical studies provided the first evidence that PHF contained tau protein (45–47), which is a low molecular weight microtubule-associated protein that promotes microtubule polymerization and stabilization when it is dephosphorylated. Molecular cloning and direct biochemical studies confirmed these initial observations (49, 50). In the normal brain tau exists as 6 isoforms derived from alternative splicing of a gene on chromosome 17 and also post-translational modification. In PHF, tau protein has a simpler isoform pattern (3 bands of 62, 64 and 68 kDa) and a higher molecular weight due to increased and abnormal phosphorylation (51). The pathogenesis of tau alterations in PHF is unknown, but increased activity of specific kinases and decreased activity of specific phosphatases (e.g. calcineurin or protein phosphatase 2A) have been proposed (52). Among the kinases that have been implicated, proline-directed kinases have attracted the most attention, and Cdc2-related kinases (53) are of particular interest since they co-localize with PHF in neurons and neurites of SP.

In addition to phosphorylation, PHF also have other covalent modifications. The filaments in PHF-type neurites are ubiquitinated (54) and glycosylated (55). Their aggregation appears to be critically dependent on association with glycosaminoglycans (56). PHF also undergo progressive glycation (57), a type of non-enzymatic covalent modification that produces cross-linked and insoluble proteins, and which has been implicated as a stimulus to oxidant cell stress that may contribute to neurodegeneration (58). Finally, when neurons or their processes die, the PHF are released into the extracellular space where they undergo proteolysis (59).

PHF-type neurofibrillary pathology has a predilection for certain neurons in an hierarchical pattern of susceptibility in aging and AD. The stereotypic pattern of vulnerability of neurons to NFT in aging and AD is clearly recognized (60–62) and is the basis for a proposed staging of Alzheimer type pathology (63). The initial stages (transentorhinal and early limbic) of neurofibrillary degeneration are compatible with a normal clinical cognitive state, while advanced stages are correlated with dementia of the Alzheimer type (64). Neurofibrillary tangles are accumulations of PHF within neuronal cell bodies, while similar filaments in neuronal cell processes are referred to as “neuropil threads” (65). In normal elderly brains, neuropil threads are either absent or very few.

Fig. 3. Electron micrographs from SP in AD (from department files at Albert Einstein College of Medicine from work of Robert D. Terry). The amyloid deposits (am) in (A) are closely associated with a cell with polymorphic and dense cytosomes consistent with a microglia. In the vicinity of the microglial reaction to the amyloid are dystrophic neuronal processes (arrow) that contain membranous and lamellar dense bodies derived from degenerating pre- and postsynaptic elements. At a higher magnification (B), the amyloid fibrils are in direct contact with cell processes of microglia, with patchlike areas of membrane densification suggestive of receptor-mediated phagocytosis. In (C) a neurite associated with a plaque of AD contains both dystrophic and filamentous elements.
Although PHF-type neuritic pathology is best appreciated with immunochemical methods, PHF-type neurites can also be detected with simpler methods, such as thioflavin-S fluorescent microscopy (Fig. 6). The importance of distinguishing neuritic from non-neuritic SP cannot be overemphasized since it is the most readily accessible significant differentiating feature of SP. Even the presence of a few neuritic plaques in the neocortex is significant, and is almost invariably associated with cognitive impairment (36). Using silver stains, Gibson was one of the first to make a distinction between neuritic plaques of AD and non-neuritic, amyloid plaques of aging (69). Probst also drew attention to the relationship of PHF-type neurites in SP with reference to brains with NFT (54). These observations have been confirmed in several independent studies (70–74), all of which point to essential differences between SP in aging and AD. These results are again consistent with the hypothesis that neurites that are incorporated into SP reflect the nature of the neurons in the vicinity of the plaque. In AD, where neuronal processes throughout the gray matter have PHF-type pathology, neuritic elements in SP frequently contain PHF; in contrast, dystrophic-type neurites predominate in aging.

Amyloid Heterogeneity in SP: Morphology and Anatomy

Amyloid deposits occur in a bewildering array in the aged human brain. Amyloid in SP may take the form of indistinct wispy deposits that may be only a few microns in diameter, or it may spread diffusely over many hundreds of microns (Figs. 2 and 5). Discrete amyloid deposits have amorphous or reticular appearances. Dense central amyloid deposits sometimes have radiating fibrils similar to plaques in prion diseases, such as kuru or Gerstmann-Straussler-Scheinker syndrome (Fig. 5).

Amyloid deposits of specific types occur consistently in certain anatomical locations (Fig. 2). Diffuse deposits tend to be more abundant in upper cortical layers, while dense deposits are more common in lower cortical layers. Primary visual and motor cortices are vulnerable to SP with dense amyloid cores and a paucity of neurites, while neuritic SP containing reticular or diffuse amyloid deposits are common in higher-order-association cortices. In the cerebellum, pale, diffuse amyloid deposits are most often detected in the molecular layer of the hemispheres, while dense, often multicentric amyloid deposits are found in the Purkinje and internal granular cell layers of the vermis. In the basal ganglia, SP in the striatum in AD are usually numerous, pale diffuse deposits, while dense deposits are sparse in the globus pallidus. In neither location are there prominent neuritic changes. Few biochemical studies have addressed the regional differences in amyloid. Clearly, functional and structural differences in anatomical regions contribute to the diversity in amyloid deposits in a way that is largely unexplored.
Amyloid may form diffuse deposits beneath the pial surface (Fig. 6). These lesions are much more numerous with immunostaining methods than with thioflavin, suggesting that many of them may be composed of pre-amyloid (see below). Neuritic processes are usually sparse or absent in this type of plaque. Some amyloid deposits appear to arise from the basal layer of blood vessels to spread into the surrounding brain parenchyma as discrete perivascular "caps," radiating fibrils ("dysphoric angiopathy"), or as juxtavascular SP (Fig. 6).
All the deposits illustrated in these figures can be detected with classical histochemical methods for amyloid, such as Congo red birefringence or thioflavin-S fluorescent microscopy, but some deposits are only visible with immunocytochemical methods. The latter are called “preamyloid” deposits (75). Amyloid refers to a substance that has characteristic biophysical properties, including high content of cross beta-pleated sheet and twisted fibrils 7.5 to 10 nm in diameter at the ultrastructural level (76). Pre-amyloid deposits lack these properties, but nevertheless can be visualized with immunocytochemical methods for amyloid peptide. Pre-amyloid presumably represents precursor protein or amyloid protein whose physical properties are modified by the local environment or by associated proteins. Diffuse deposits of pre-amyloid cannot be distinguished from diffuse amyloid with most antibodies to Aβ. It is therefore not surprising that there should be controversy about whether diffuse amyloid contains fibrillar material at the ultrastructural level depending upon whether amyloid- or pre-amyloid-type diffuse deposits were studied (77, 78).

**Amyloid Heterogeneity in SP: Aging vs AD**

Application of sensitive methods for detecting amyloid have not only demonstrated a wide array of amyloid deposits and amyloid deposits in regions of the central nervous system (CNS) that were not previously suspected of having pathology in AD, but they have also shown that amyloid deposition is very frequent, if not inevitable, in aging (79, 80). In virtually every published prospective study of normal aging, postmortem studies have revealed some elderly people with a form of pathological aging associated with extensive cerebral amyloid deposits, but who were nevertheless cognitively intact (81–84). Subjects with this form of pathological aging cannot be readily distinguished from elderly people with few or no amyloid deposits by most clinical criteria. It is of some interest that most of the amyloid deposits in pathological aging are noncompact or diffuse, lack PHF-type neurites, and display only minimal glial reaction (Fig. 4).

In contrast, in AD more SP have dense reticular or cored amyloid (Fig. 5) and are surrounded by degenerating neuronal processes and clusters of microglia and astrocytes. The basis for these differences in plaque morphology between aging and AD is a focus of intense research efforts. Recent studies suggest that there may be differences in the composition of amyloid in aging and AD that goes beyond the difference noted above with respect to neuritic elements.
Amyloid Heterogeneity in SP: Biochemistry

Amyloid Precursor Protein Metabolism in Aging and AD: Although initial biochemical studies suggested that the major constituent protein of amyloid in the brains of elderly subjects was biochemically similar to that of AD (85) and derived from the same amyloid precursor protein (APP) (29), more recent observations suggest that there may be subtle differences between amyloid in aging and AD. It must be stated from the beginning that APP biology has become complex with the discovery of multiple alternative splice forms of APP, including forms that lack the Aβ domain (APP-L) and forms relatively specific to glia or neurons, as well as the discovery of APP-like molecules (86–87). A detailed review of the molecular biology of APP is beyond the scope of the current discussion and can be found in recent reviews (88, 89). For the purpose of this discussion only APP that contains the Aβ domain will be considered. Where relevant to functional considerations, the form of APP containing a protease inhibitor domain (KPI domain) is also noted. The latter form also appears to be more abundant in non-neural cells (87).

With respect to functional domains, several discrete regions in the APP have been defined. Select functional domains of relevance to SP pathogenesis are illustrated schematically in Figure 7. Within the ectodomain of APP are several regions that have relevance to the normal function of APP, which today still remains largely unknown. The best defined of these regions is the KPI domain. The secreted APP generated within platelet α-granules by the action of α-secretase is also known as protease nexin-II (90). Relevant to the present discussion of SP are several domains within the Aβ region. An apolipoprotein-E (apo-E) binding domain has been mapped to the amino terminal region of Aβ and a complement activation domain likewise resides in the first 16 amino acids (91, 92). The 2 lysine residues in Aβ are located at 16 and 28. These are peptides with free amine groups that may be the target for non-enzymatic glycation.

Since Aβ is an integral component of a larger precursor protein, proteolytic events are obligatory for its release. These pathways are shown schematically in Figure 8. The major metabolic pathways have been arbitrarily referred to as amyloidogenic and “non-amyloidogenic,” the former being associated with activity of at least 2 putative enzymes, β-secretase and γ-secretase. The non-amyloidogenic pathway is the best defined pathway and is also known as the secretory pathway. It is associated with activity of α-secretase. The precise cellular location and functional properties of these enzymes are clearly of great significance in terms of eventually understanding the cellular biology of amyloid deposition, but from a purely phenomenological perspective the by-products of these pathways can be discussed as they relate to lesions in aging and AD independent of this more basic knowledge.

With respect to the nonamyloidogenic pathway of APP metabolism it is noteworthy that the final product, Aβ17-42, has a molecular weight of about 3 kDa and has been referred to as P3. P3 lacks the complement activation domain and the apo-E binding domain. It also has only 1 free lysine. Biochemical studies of brain tissue have suggested that P3 does exist in vivo (94). Moreover, physicochemical studies have suggested that P3 is relatively insoluble (95). One might speculate, however, that it might form relatively “benign” amyloid fibrils since it lacks crucial functional domains of Aβ1-42. The amyloidogenic pathway is currently considered to be located in the endosomal-lysosomal compartment of the cell. It generates an insoluble, fibrillogenic Aβ1-42, and upon further metabolism, a more soluble Aβ1-40. The former is of particular importance since it has domains that activate complement and bind apo-E. It should also be more vulnerable to non-enzymatic glycation. The major form of Aβ produced during normal physiological metabolism of most cells in Aβ1-40 (96, 97). This is the most soluble of the peptides under consideration. A shift to relatively more production of Aβ1-42 is apparently associated with significant pathology. This shift has been detected in cells of subjects with early onset AD (98) and in genetically engineered cells containing genes not associated with direct mutations of APP (99), which suggests that changes in the ratio of Aβ1-42/Aβ1-40 may be of fundamental importance to AD pathogenesis (99–100).

Amyloid Beta Peptide Heterogeneity

Recently, monoclonal antibodies have been developed that are specific to the carboxy terminal regions of Aβ.

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The available antibodies, however, do not distinguish Aβ1-42 from Aβ17-42 in immunocytochemical studies. In this discussion they are referred to as recognizing Aβx-42. The same applies for antibodies to Aβx-40. Immunocytochemical studies with these antibodies demonstrate that more diffuse SP are positive with antibody to Aβx-42 than to Aβx-40, while dense core SP were equally stained by both antibodies (101–104). The interpretation of these results is that diffuse SP represent an earlier stage of amyloid deposition and that additional proteolytic events occur in dense core SP. Alternatively, initial deposits of Aβx-42 serve as a nidus for co-precipitation of the more soluble Aβx-40. The studies do not make it clear whether Aβ1-42 or Aβ17-42 is the initial nidus.

To get at an answer to this question requires antibodies that recognize specifically the P3 molecule or a double-staining approach using antibodies to epitopes on either side of the α-secretase site. Antibodies are commercially available for detecting the epitopes in the Aβ1-16 domain (6E10) and in the Aβ17-28 domain (4G8) (105). Application of these antibodies to tissue sections using double labeling demonstrated that in aged and AD brains, all SP were stained for Aβ17-28 (which would recognize both P3 and Aβ), including diffuse and compact deposits (Fig. 9). On the other hand, only a fraction of the SP were positive for Aβ1-16, with the stained plaques being those with Aβ. Most of the deposits that were stained for Aβ1-16 had dense compact or reticular amyloid, while diffuse amyloid deposits, especially in pathological aging, were largely negative. These results suggested that there may be differential APP metabolism in aging and AD, with the amyloidogenic pathway preferred in AD and the secretory pathway (producing P3) favored in aging. Clearly, additional studies are needed to address this issue.

Biochemical studies by the laboratory of Roher et al. support these morphological observations. Direct protein analysis of amyloid peptides from AD revealed the most abundant peptide to be Aβ1-42 (106, 107), while similar analyses from brains with mostly diffuse amyloid revealed abundant Aβ17-42 (P3) (94).

Using an antibody specific to P3, Higgins and co-workers have recently found somewhat similar results (108). Specifically, they demonstrated that anti-P3 recognized only a subset of SP and that most of the SP were diffuse in type; plaques with dense amyloid cores were negative. Their results also suggest the possibility that a non-amyloidogenic pathway may contribute P3 to diffuse plaques. In contrast to studies by Higgins and those illustrated above, Iwatsubo and co-workers concluded that diffuse SP contained full-length Aβ using serial sections and a panel of antibodies to different domains of Aβ (109). Their analysis was mostly limited to AD, however, and did not include substantial numbers of cases of pathological aging. Further studies are needed to confirm
Fig. 9. Double immunostaining of SP in AD (a, b) and aging (c, d) for apo-E. In AD, SP that are visible with thioflavin-S (a) are also immunoreactive for apo-E. Diffuse SP that can be stained with antibodies to Aβ, but which are not visible with thioflavin (c), have no detectable apo-E immunoreactivity (d). Sections double stained for AGE (fluorescein, green) and Aβ (rhodamine, red-orange) show that diffuse deposits at the pial surface (arrow) and in the superficial cortex (e) have no detectable AGE-immunoreactivity, while neurofibrillary tangles (arrowheads) are readily stained. Note that many cells have double-stained (yellow) autofluorescent material. In reticular and dense cores of SP (f), amyloid double stains consistent with more advanced glycination and probably deposits with a longer lifespan (arrow). An additional degree of heterogeneity in the amyloid in SP is apparent with antibodies that recognize specific domains of Aβ. The brown chromogen is used to detect an epitope in the carboxyl terminal half of Aβ (4G8), while the blue chromogen is used to detect an epitope in the first 16 amino acid residues of Aβ (6E10). Note that diffuse deposits in (g) show 4G8 immunoreactivity and only discrete foci of 6E10 staining. The dense deposits at the lower part of the figure are double stained. In (h) the dense centers of the SP have more 6E10 immunostaining, while the periphery has more carboxyl terminal epitopes.

Fig. 10. Microglial markers stain SP in direct apposition to dense amyloid deposits. The thioflavin-positive deposits in (a) are seen to directly correspond to foci of increased HLA-DR immunoreactivity with activated microglia (b). TNFα also stains activated microglia in clusters consistent with SP in the AD cortex (c). Astrocytes in the cortex of AD are usually located at the periphery of SP as shown, with antibodies to glial fibrillar acidic proteins (d) and LN-1 (e). Double staining with antibodies to Aβ (blue chromogen) and GFAP (brown chromogen) show the peripheral relationship of reactive astrocytes to SP.

Fig. 11. A diagrammed schematic of the cellular events of SP pathogenesis shows the cortex with pyramidal neurons, microglia, and astrocytes. The initial deposits of diffuse amyloid appear around the cell bodies and processes of neurons. These deposits enlarge, and discrete foci within the deposits eventually form the nidus of the mature SP. These niduses contain amyloid that has different biochemical properties and a different mix of associated proteins. At this early stage neuritic dystrophy cannot be seen. In the aged brain SP do not advance beyond this stage. In AD there is simultaneous PHF formation within neurons and their processes. As the microglia reaction increases in the maturing SP there is increasing neuritic dystrophy and eventually the PHF-type neurites are detected in the SP. It is to be noted, however, the PHF-type neurites are also abundant in non-plaque regions and in diffuse plaques that may contain early types of amyloid.
the observation made originally by Rohrer and co-workers that Aβ17-42 (P3) is the predominant peptide in brains containing predominantly diffuse amyloid.

The significance of this observation is that P3 should be a more benign form of amyloid. Since it does not have certain important domains, it may theoretically be associated with less microglial reaction, less apo-E binding and less glycation.

**Amyloid Nidus Formation and Progressive Binding**

In vitro binding assays show that Aβ peptides bind to only a subset of SP (110). In other studies the kinetics of binding to brains with SP was different from that without SP, suggesting that initiation or nidus formation had different kinetics than growth of amyloid fibrils (111). The types of SP that bind exogenous Aβ peptides were usually those that had dense amyloid cores. Factors that influence amyloid binding to SP are incompletely understood, but several molecules likely play important roles, including apo-E, apo-J and heparin sulfate proteoglycans (HSPG) (112).

An unanswered question is what determines the nidus of the SP. Image analytical methods have demonstrated that SP are not specifically related to blood vessels (113). On the other hand, in the earliest stages SP often have a columnar arrangement, suggesting that amyloid may be derived from the functional unit of the cortex. It has also been demonstrated by image analysis that amyloid deposits are consistently neurocentric in the earliest stages of deposition and are often closely apposed to cell membranes of apparently normal neurons (114–116). This neurocentric location does not prove that amyloid is derived from neurons, since other cells are intimately associated with neurons, such as perineuronal satellite cells.

**Apolipoprotein-E and SP**

Work in several laboratories led to the discovery that apo-E is associated with increased risk for late onset familial and sporadic AD (117–119). Previous studies had also shown that apo-E was associated with SP (120), and that the apo-E genotype influenced the amount of amyloid deposition in the brain (121). In studies of non-AD dementia disorders, such as dementia with Lewy bodies, apo-E genotype also influenced the frequency of amyloid formation and amyloid angiopathy (122). In vitro studies have demonstrated that apo-E binds to Aβ and that there is differential binding with respect to apo-E isotype (123). A convergence of lines of evidence, thus, suggests that apo-E plays a significant role in SP pathogenesis. Apo-E also has a binding site for HSPG (91), which is consistently present in amyloid deposits of SP. The detection of apo-E in SP may thus reflect binding to HSPG in addition to amyloid. Apo-E may act as a "pathological chaperone," a molecule that by binding to amyloid, favors its tissue deposition (124). Other lipoprotein molecules may have opposite effects. For example, apo-J (also known as SP-40,40, clusterin or SGP-2), which is another astrocyte-derived molecule that has been localized to SP, may have a negative influence on amyloid fibrillogenesis (125).

If apo-E binding to Aβ is mediated through a domain in its amino terminus, then Aβ17-42 (P3) should have less apo-E binding than Aβ1-42. If P3 is present in diffuse SP in aging, they should have less apo-E. Interestingly enough, this has been independently demonstrated with immunocytochemical methods in 2 different laboratories (126, 127). Specifically, diffuse SP had less apo-E immunoreactivity than dense or compact amyloid deposits and pre-amyloid deposits were negative for apo-E. As a consequence more than 90% of the SP in AD were stained with antibodies to apo-E, but less than 50% of the SP in aging were similarly double stained.

**Advanced Glycation End-products (AGE) and SP**

AGE are the consequence of nonenzymatic covalent modification of long-lived proteins (128). The extent of glycation can be used as an index of the age of the protein. The process begins with reaction of glucose and other reducing sugars to free amine groups on proteins (lipids or nucleic acids) to generate a Schiff base. Subsequently, rearrangements and additional modifications produce Amadori products and Maillard reaction products and more chemically complex substances. The end result is an insoluble cross-linked substance with characteristic fluorescence properties (93).

Recent studies by Vitek and coworkers (129) have demonstrated that there are more AGE in AD compared with controls and that glycated Aβ peptides act as effective seeds for amyloid fibrillogenesis. Immunocytochemical studies of the AD brain with antibodies to AGE reveal a pattern of immunoreactivity surprisingly similar to antibodies to apo-E (130). Specifically, compact and dense amyloid deposits have more AGE-immunoreactivity than diffuse and pre-amyloid deposits. In triple labeling studies apo-E and AGE co-localized to such a high degree that it suggested the possibility that either apo-E was itself glycated or that apo-E might be binding to glycated moieties. The latter has subsequently been demonstrated in vitro (131).

The presence of glycated substances in SP provides a possible link to cellular reactions in SP. In particular, glycation may contribute to recruiting microglia to SP and also to activating microglia (see below). Macrophages have been shown to possess specific receptors for AGE that are different from the nonspecific scavenger receptor (132). Both types of receptors have been shown to be involved in interaction of macrophages with Aβ (132).
The binding of Aβ to microglia results in the production and release of proinflammatory cytokines (IL-1β and TNFα) (134), cytokines that may be relevant in AD, due to the fact that the promoter for APP is responsive to these cytokines (135). Production of IL-1β may also be relevant to astrocytic reactions that are common in the vicinity of SP, since IL-1β is the most potent known stimulus for astrocyte activation (136).

The immunocytochemical evidence suggests not only the possibility that dense or compact amyloid is older, but also that specific forms of amyloid associated with dense deposits may be more vulnerable to glycation. In this regard Aβ1-42 has twice as many lysine residues as Aβ17-42. These results are consistent with the hypothesis that amyloid peptides may be differentially distributed in different SP subtypes.

**Amyloid-Associated Molecules**

Immunocytochemical studies have shown that amyloid in SP contain a variety of brain and serum-derived proteins in addition to Aβ, including HSPG (137), complement proteins (C1q) (138), protease inhibitors (α1-anti-chymotrypsin) (139), and apolipoproteins. Although complement proteins (C1q) have been known to be associated with SP for over a decade (140), recent studies have demonstrated that C1q is more abundant in dense amyloid deposits than in diffuse amyloid deposits and more closely associated with deposits that have Aβ1-40 immunoreactivity (141). In light of the above discussion, these observations are consistent with the idea that diffuse amyloid may have amyloid that is deficient in complement-binding domains. Dense amyloid deposits, which are lesions with the most Aβ1-40 immunoreactivity, are also lesions that have the most consistent microglial response, and microglia are the prime source of C1q in the brain (141).

**Inflammation and Microglial Involvement in SP (Fig. 10)**

Microglia are associated with most SP when sensitive methods of analysis are used for their detection (142, 143), although microglia are most clearly associated with dense amyloid deposits (144–146). More recently, double staining methods have demonstrated that microglia are most numerous in plaques containing Aβ8-40, corresponding largely to plaques with dense amyloid cores (147). A lesser proportion of SP with Aβ8-42, mostly diffuse SP, were associated with microglia.

With ultrastructural studies, microglia are seen to be closely apposed to extracellular fibrils, where they are most likely involved in a phagocytic response (148). These observations find support in experimental conditions, where macrophages have been shown to have receptors for Aβ, including Scavenger-type receptors and receptors for AGE (132, 133). In culture conditions, macrophages readily phagocytose Aβ aggregates, as well as isolated SP cores. Interaction of microglia with Aβ is associated with release of potentially toxic molecules, including pro-inflammatory cytokines, reactive oxygen intermediates, and other poorly defined neurotoxic molecules (149, 150).

In addition to their physical proximity to SP, immunocytochemical studies have demonstrated that microglia in AD have phenotypic features consistent with a state of activation. Such cells might be expected to produce a number of biologically active molecules that may contribute to later stages of SP pathogenesis (see Table 2). Microglia in the cortex of AD especially associated with SP display immunoreactivity for the proinflammatory cytokines IL-1β, TNFα and IL-6 (151). Other immunocytochemical and immunoblotting studies have shown that the AD brain has increased IL-1α immunoreactivity (152). The most consistent marker of activation of microglia, expression of the class II major histocompatibility complex antigen HLA-DR, is readily detected in AD (153–155). Other markers of microglia activation include expression of matrix metalloproteinase (156), metalotransferrin (157) and reactivity with certain antibodies to lysosomal antigens (158). Increased expression of HLA-DR in the AD is partially a function of agonal state (154), but is also independent of systemic factors. It is tempting to speculate that microglial reaction is due to response to glycated substances that are abundant in AD. On the other hand, there is also neuronal injury in AD, as well as evidence for neuronal death through an apoptotic process. Microglia may be activated in response to neuronal signals from injured neurons or during phagocytosis of apoptotic cellular debris through binding to vitronectin receptors that are expressed on their cell surface and that mediate phagocytosis of apoptotic cellular debris (159).

**Table 2**

<table>
<thead>
<tr>
<th>Microglial Products Potentially Relevant to Senile Plaque Pathogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokines, e.g. TNFα and IL-1β</td>
</tr>
<tr>
<td>Reactive oxygen intermediates</td>
</tr>
<tr>
<td>Excitatory amino acids</td>
</tr>
<tr>
<td>Proteases (e.g. matrix metalloproteinase)</td>
</tr>
<tr>
<td>Platelet activating factor</td>
</tr>
<tr>
<td>Adhesion molecules (e.g. β2-integrins)</td>
</tr>
</tbody>
</table>

These findings, along with evidence of increased expression of a host of immune-related molecules in AD lesions, suggest that later stages of SP pathogenesis have features in common with a local foreign body-type inflammatory reaction. It is of more than academic interest that epidemiological studies (160) and a pilot clinical study (161) have indicated that anti-inflammatory drugs may offer protection from AD.
TABLE 3
Astrocyte Products Potentially Relevant to Senile Plaque Pathogenesis

<table>
<thead>
<tr>
<th>Nitric oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokines, e.g. IL-6</td>
</tr>
<tr>
<td>Apolipoprotein-E</td>
</tr>
<tr>
<td>Apolipoprotein-f (SP40,40)</td>
</tr>
<tr>
<td>α2-macroglobulin</td>
</tr>
<tr>
<td>Colony stimulating factors, e.g. GM-CSF</td>
</tr>
</tbody>
</table>

Astrocytes in SP

A prominent astrocytic reaction is readily apparent in the cortex in AD, and astrocytes can be shown to be associated with many, but not all SP (162, 163). They are most closely associated with SP with a dense amyloid core and an attendant microglial reaction. Given the likely activated state of microglia in SP and their expression of IL-1β, it is hardly surprising that hypertrophic astrocytes would be associated with SP. Interestingly, the activation of astrocytes may lead to production of reciprocal growth factors, such as GM-CSF, which in turn promote microglial growth and activation (164). The interplay between the glia may contribute to the local inflammatory response of chronic SP.

Astrocytes may be the source for other important biomolecules in SP (see Table 3). Although microglia in rodents have the capacity to produce nitric oxide (NO) owing to the fact that they possess inducible nitric oxide synthase (iNOS), there is no evidence that human microglia have this property (165). On the other hand, both human and rodent astrocytes express iNOS. Primary human astrocyte cultures and glioma cell lines can be induced to produce reactive nitrogen intermediates by priming with interferon-gamma (INFγ) and then stimulating with interleukin-1β (165). In glioma cell lines it has also been possible to demonstrate production of reactive nitrogen compounds in response to Aβ (166). Since the production of NO from astrocytes is at a high concentration and is sustained over time, it may contribute to local neurotoxicity. Astrocytes also produce the pro-inflammatory cytokine IL-6, which has been shown to be elevated in AD brain and to be a mediator of the acute phase response. Astrocytes in SP also produce the cytokine-binding molecule α2 macroglobulin, and amyloid-binding proteins previously discussed, namely, apo-J and apo-E (reviewed in 156).

SUMMARY AND HYPOTHESIS

The cellular events in SP pathogenesis are complex (Fig. 11), but based upon available evidence it is possible to suggest that yet-to-be-determined age-related factors lead to production of early diffuse amyloid deposits in a neurocentric pattern. It is still too early to say whether or not this amyloid is derived from neuronal or glial processing. If P3 is a major component of this earliest amyloid, then a glial origin may be favored. A qualitatively distinct process leads to subsequent deposition of amyloid of another type, Aβ1-42, possibly due to a shift in metabolic balance or factors fundamental to AD. The nature of the injury or factor responsible for generation of

![Hypothetical Difference in APP Metabolism Between Aging and AD](http://jnen.oxfordjournals.org/)

Fig. 12. The major hypothesis to arise from recent biochemical and immunochemical studies of SP is that there are fundamental differences between the amyloid in aging and AD. In aging SP, amyloid composed of P3 may be derived from the secretory pathway. In AD, different metabolic pathways are favored that lead to increasing formation and deposition of Aβ1-42 and subsequent co-precipitation of Aβ1-40 as the SP matures. The latter types of SP may be associated with cellular alterations due in part to differences in the functional domains of Aβ compared with P3.

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