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Transmissible Cerebral Amyloidoses as a Model for Alzheimer's Disease

An Ultrastructural Perspective

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Abstract

Alzheimer's disease, a prototypic nontransmissible cerebral amyloidosis, has no adequate experimental model. Several pathogenetic events, however, may be modeled and accurately studied in the transmissible cerebral amyloidoses of kuru, Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker disease, and scrapie. The common neuropathological denominator in both types of cerebral amyloidoses is the presence of stellate kuru plaques, senile plaques, and pure neuritic plaques. These amyloid plaques consist of amyloid fibers, dystrophic neurites, and reactive astrocytes in different proportions. Microglial cells, which are regarded as amyloid producer/processor cells in Alzheimer's disease, may play the same function in the transmissible cerebral amyloidoses. In both transmissible and nontransmissible amyloidoses, the impairment of axonal transport leads to accumulation of abnormally phosphorylated cytoskeleton proteins (such as neurofilament proteins and microtubule-associated protein τ), which eventually produce dystophic neurites observed as parts of plaque or as isolated pathological structures.

Index Entries: Alzheimer's disease; cerebral amyloidoses; Creutzfeldt-Jakob disease.

Introduction

The subacute spongiform virus encephalopathies (SSVE), also known as slow virus disorders, transmissible cerebral amyloidoses, or prion diseases, are a group of neurodegenerative disorders caused by not completely characterized pathogens variously referred to as unconventional or atypical viruses, or agents, prions, or virinos *(1–5)*. The current degree of ignorance of the nature of the causative agent is reflected by the different views held by different investigators. Those who believe this pathogen is composed of only one protein, PrP, use the term "prion" *(6)*. Those who think that it is a molecular chimera composed of yet to be discovered oligonucleotide enveloped by the host-encoded protein favor the term "virino" *(1,4)*. The term "virus," used by others and by the author,

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implies only that this pathogen lies within the realm of virology because it possesses several virus-like characteristics (5). Although the nature of causative pathogen still remains obscure, it is becoming increasingly clear that the pathogenesis of the SSVE is analogous to that of other amyloidoses in general and the nontransmissible cerebral amyloidoses in particular (2,3). The last category includes: Alzheimer's disease (AD), Down's syndrome, and hereditary cerebral hemorrhage with amyloidosis, Dutch (HCHWA-D) and Icelandic (HCHWA-I) types. However, this analogy is only partial, and it reflects only the major steps in the pathogenesis of both types of brain amyloidosis, whereas the molecules involved (first of all, amyloids) are clearly different.

This article will discuss the neuropathology and pathogenesis of the SSVE to make a point of a basic similarity of neuropathology and neuropathogenesis between both types (transmissible and nontransmissible) of amyloidoses. Since several parameters, including the formation of amyloid plaques, can be modeled and manipulated with a high predictability in experimentally induced SSVE, these models may prove useful for the nontransmissible cerebral amyloidoses of AD type. Finally, I shall discuss the only structure that is unique to the transmissible brain amyloidoses, namely the tubulovesicular structures.

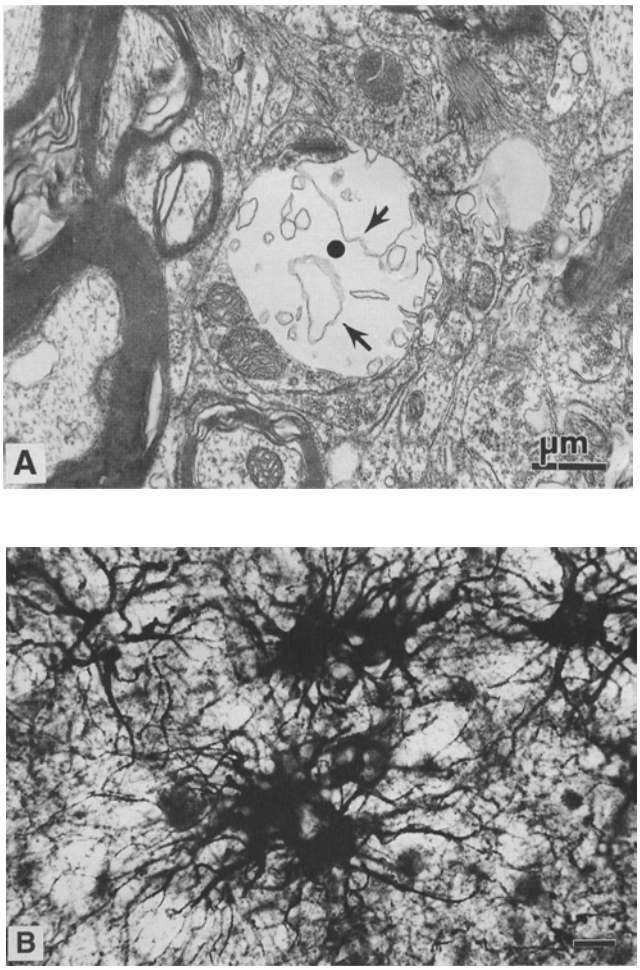


Fig. 1. Classical neuropathological findings in SSVE. **(A)** Electron micrograph of a single vacuole (filled circle) in squirrel monkey brain infected experimentally with kuru virus. Note numerous curled membranes (arrows). Unpublished work of Liberski et al. Original magnification, ×12,000 (bar = 1 μm). **(B)** Severe astrocytosis of human CJD brain. Cajal gold sublimate. Original magnification, ×1000, bar = 10 μm.

Neuropathology of Transmissible Cerebral Amyloidoses

Introduction

All SSVE are the transmissible cerebral amyloidoses (2,3) and are characterized by two basic neuropathogenetic processes: the accumulation of amyloid deposits and an impairment of the axoplasmic transport (7). Both of these processes may be interconnected. It must be stressed, however, that data to support the role of the axoplasmic transport in the pathogenesis of the SSVE are still mostly circumstantial. In the transmissible brain amyloidoses, this pair is probably responsible for the development of the "classical" neuropathological triad (vacuolation, astrocytosis, neuronal loss; Fig. 1) and only recently recognized pathological phenomena, namely autophagic vacuoles (8,9).

That the amyloid plaque is an important neuropathological finding has been recognized from the time of the seminal kuru research (Fig. 2); the last was even "facetiously" called "the galloping senescence of the juvenile" (10), but this phrase became meaningful only recently when the pivotal role of the amyloid plaque was appreciated.

Except for Gerstmann-Sträussler-Scheinker disease (GSS) and kuru, however, "classical" amyloid plaques occur in the minority of SSVE cases like human Creutzfeldt-Jakob disease (CJD) or natural scrapie (11,12), whereas in all cases, the presence of amyloid can be detected *in situ* by immunohis-

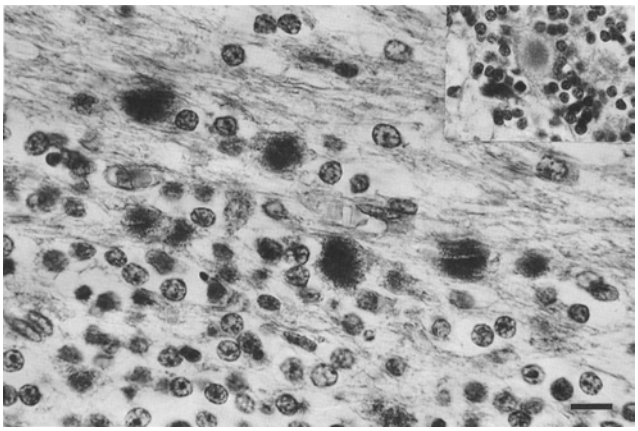


Fig. 2. Typical kuru plaques in human brain affected with kuru. PrP immunohistochemistry. Inset, PAS staining. Courtesy of D. Carlton Gajdusek. Original magnification, ×1000, bar = 10 μm.

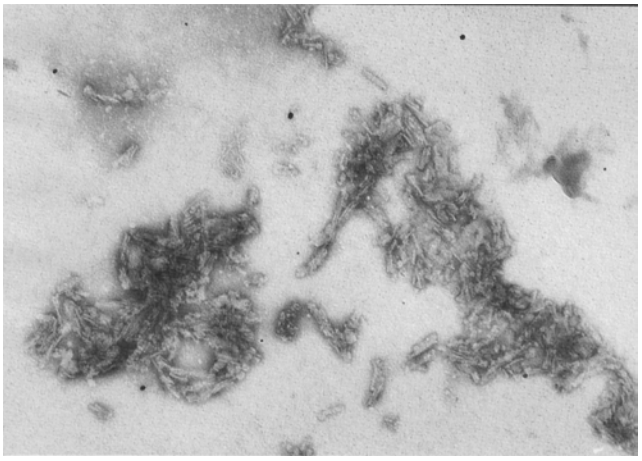


Fig. 3. Scrapie-associated fibrils isolated from hamster brains infected with the 263K strain of scrapie virus. Unpublished work of Liberski et al. Negative staining with phosphotungstic acid. Original magnification, ×20,000.

tochemistry *(13–18)*, or, in brain homogenates, in the form of scrapie-associated fibrils (SAF) *(19)* (Fig. 3) or prion rods *(20)*. The distinction between SAF and prion rods is based on subtle ultrastructural differences between them. Thus, SSVE are "hidden amyloidoses" in the sense that the classical amyloid is hidden from examination of brain tissues of the majority of the SSVE cases by classical neuropathological techniques *(21)*.

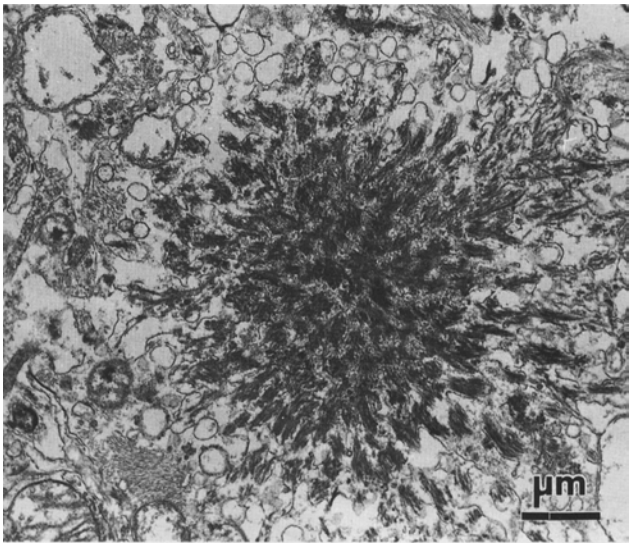


Fig. 4. Typical kuru plaque from a case of Gerstmann-Sträussler-Scheinker (GSS) disease from the original Austrian family. Unpublished work of Liberski and Budka. Original magnification, ×12,000, bar = 1 μm.

PrP Amyloid Plaques

Typically, classical amyloid plaques consist of a core surrounded by dystrophic neurites (DN) *(22)*. Cores are immunostained with antibodies against the scrapie isoform of prion protein (PrP^{SC}) *(13–18)*, but not β(A4), and thus are molecularly, but not morphologically, different from those of AD. The PrP-plaque is enveloped by astrocytic processes (hence, the term "glial plaques" *[23]*). Overall, more than 75% of kuru patients exhibit amyloid plaques *(11)*. In comparison, only a small number of CJD patients of European or American descent show plaques *(11,24,25)*. By contrast, Japanese CJD patients typically show numerous plaques *(15)*. Such a difference may, in part at least, reflect differences between different virus strains isolated from hosts of different genetic background.

Prototypical for all the SSVE is the kuru plaque. Ultrastructurally, kuru plaques consist of fibrils densely interwoven at the center to form a core that is surrounded by a peripheral lighter zone consisting of radially oriented fibrils (Fig. 4) *(26–29)*.

GSS disease deserves a separate comment. It is an extremely rare familial disorder discovered in 1936 by Austrian investigators *(30)*. The position of GSS disease within the spectrum of CJD was

established by transmission to nonhuman primates *(11)*; however, the close similarity to kuru based on a common appearance of amyloid plaques was pointed out by Seitelberger *(31)* even before the slow virus era was initiated by a seminal work of Gajdusek and Gibbs. Plaques in GSS disease are highly pleomorphic *(16,17,30–38)*. In addition to kuru plaques, primitive plaques and senile plaques of the "cocarde" type identified by German investigators (typical for AD) and multicentric plaques ("compound plaques") have been described in GSS disease, and these are regarded as a hallmark for GSS disease (Fig. 5). The exact morphology of amyloid plaque varies in terms of number of amyloid cores and numbers of participating dystrophic neurites. At one end of the spectrum is the kuru plaque, which consists of a stellate core with minimal numbers of dystrophic neurites (or none at all) (Fig. 4). Typical for GSS disease, multicentric plaques consist of several such cores of different sizes and shapes *(16,17,32–38)* (Fig. 5) and numerous dystrophic neurites. The primitive plaque, which consists of several dystrophic neurites without amyloid fibrils, occupies the other end of the spectrum. It seems, however, that the exact morphology of the plaque may not be its inherent property, but rather the characteristic modified by the pre-existing structure of a given brain region. Indeed, the typical "sea-urchin"-like kuru plaque is encountered mostly in the cerebellum, where comparative studies in AD are conspicuously lacking (Liberski and Budka, unpublished observations), whereas the multicentric plaque is commonly encountered in the superficial cortical layers. Finally, noncongophilic (thus, pre- or para-amyloid) PrP-immunoreactive deposits (which are analogous to diffuse plaques of AD) were reported in experimental scrapie *(14,39)* and in human CJD *(40)*.

It was hypothesized that an impairment of axonal transport leads to accumulation of cytoskeleton proteins within dystrophic neurites (DN) *(7,41–43)*. These cytoskeleton proteins, by contrast to AD, are partially phosphorylated neurofilament proteins *(44)*, but the immunoreactivity of DN in experimental scrapie for τ protein was also reported (45). However, in contrast to AD, only a proportion of plaques in experimental scrapie was τ-immunopositive (for example, plaques in the white matter were repeatedly negative). The significance of this phenomenon is unknown at the present time, but it points to a further similarity with AD.

Regardless of disease and the affected species, DN are ovoid (spheroidal) in shape and usually

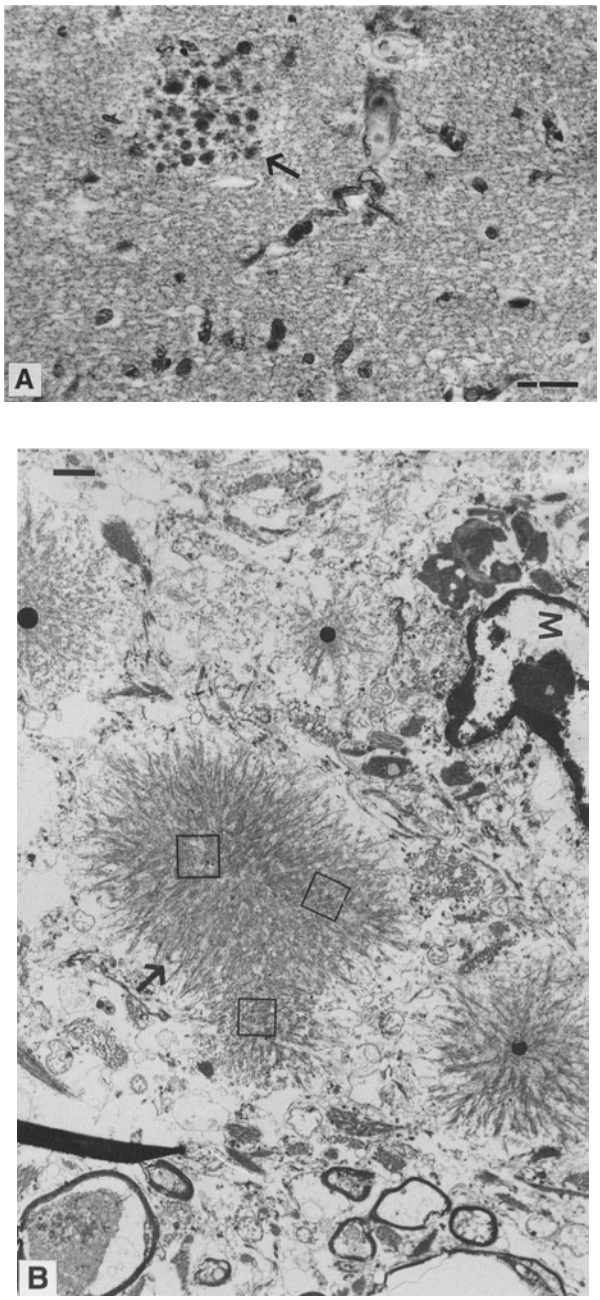
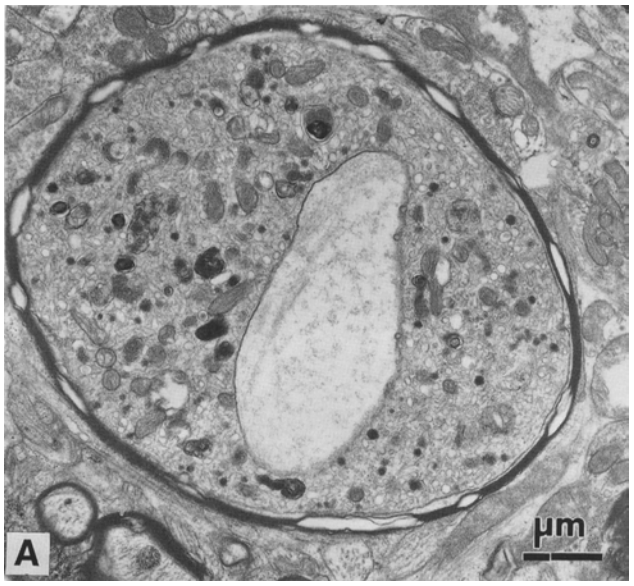


Fig. 5. Amyloid plaques from a case of GSS disease. **(A)** Multicentric plaque (arrow) consisting of several amyloid cores. PAS staining; original magnification, ×1000, bar = 10 μm **(B)**. Kuru (circle) and multicentric plaques. Note three cores (squares) merging to form a multicentric plaque (arrow). Original magnification, ×4400, bar = 1 μm.

observed as isolated structures (Fig. 6A) found against a background of devastated neuropil. Occasionally, DN form clusters that have been interpreted as premature or abortive neuritic plaques



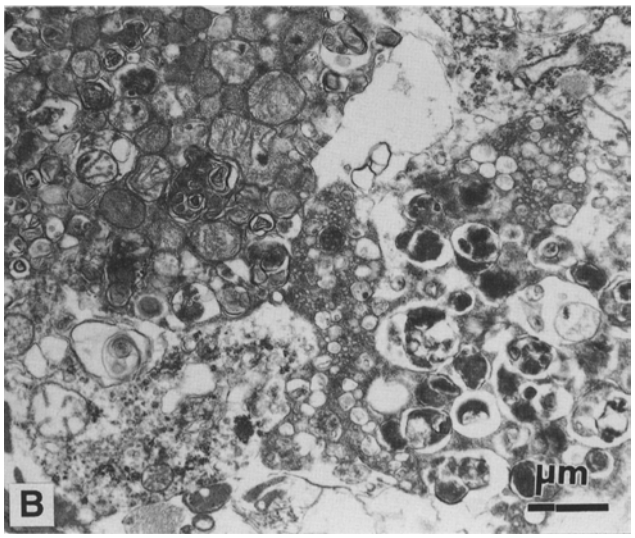


Fig. 6. Dystrophic neurites. **(A)** Isolated dystrophic neurite from hamster brain infected with the 263K strain of scrapie virus. Note mitochondria and abnormal subcellular organelles immersed within neurofilamentous masses. Original magnification, ×12,000, bar = 1 μm **(B)**. Several dystrophic neurites from a case of GSS disease. Original magnification, ×12,000, bar = 1 μm.

(Fig. 6B). Large numbers of them form primitive plaques. Different numbers of DN also surround amyloid plaques (Fig. 7). The internal structure of DN is similar regardless of their origin, location, and classification as dendrites or axons (Figs. 6 and 7). DN contain mitochondria, some of which exhibit features of degeneration, and numerous pleomorphic, membrane-bound inclusions. Ultrastructurally, these inclusions can be classified as:

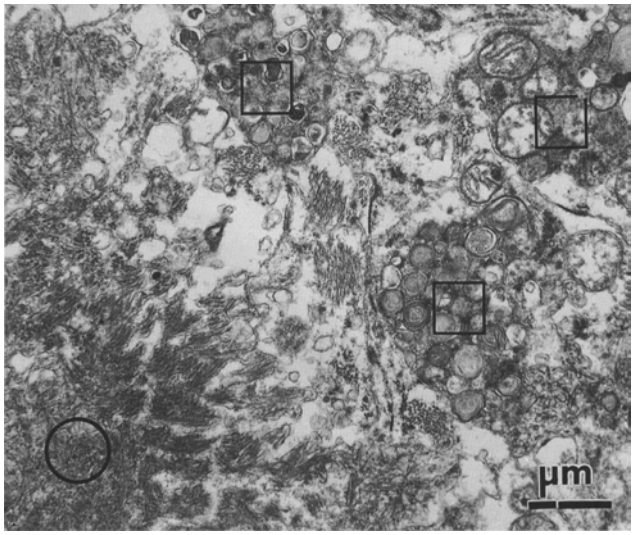


Fig. 7. A margin of amyloid plaque from a case of GSS disease. Note dystrophic neurites (squares) and amyloid plaque core (circle). Unpublished work of Liberski and Budka. Original magnification, ×12,000, bar = 1 μm.

1. Electron-dense homogeneous structures;
2. Multigranular;
3. Multivesicular;
4. Multilamellar; and
5. Electron-lucent cisterns and arranged, frequently tubular profiles of endoplasmic reticulum.

The third part of the amyloid plaque is the glial cells. Although microglial cells were observed as a part of amyloid plaques in experimental scrapie *(46)*, they were detected only recently in plaques of GSS *(47,48)*. Thus, the last putative difference between plaques in both types of amyloidoses has been removed *(49)* (Fig. 8). Barcikowska et al. *(47)* observed that microglial cells were associated with almost all compact kuru or multicentric plaques, but not with diffuse deposits in GSS, which may be an important clue in understanding their role in the morphogenesis of amyloid plaques (*see* section on AD for further discussion).

Amyloid precursor proteins are processed in as yet uncharacterized steps to yield modified amyloid proteins in SSVE and AD. Several recently discovered point mutations and inserts in the cellular genes encoding for the amyloid precursor proteins in SSVE and AD presumably increase the probability of such conformational changes, possibly by

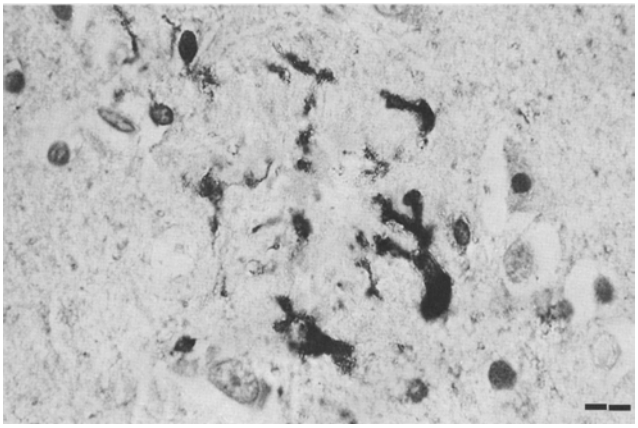


Fig. 8. Numerous ferritin-immunopositive microglial cells within a perimeter of amyloid plaque from a case of GSS disease. Unpublished work of Barcikowska et al. Original magnification, ×1000, bar = 10 μm.

making the amyloid fibril formation thermodynamically preferable *(10)*. Microglial cells may participate in such a process, either as producer/processor cells, phagocytic cells, or both. Astrocytic cells are probably only a reactive element.

Tubulovesicular Structures

TVS (Fig. 9) were first described by David-Ferreira and coworkers in experimental scrapie in mice as "particles and rods ranging in diameter from 320 to 360 A" *(50)*. The reported size of TVS has differed slightly according to different investigators, but factors inherent in electron microscopic techniques (namely swelling or dehydration) can account for this variation.

TVS have been reported in the natural diseases in ruminants, scrapie in sheep, and BSE in Friesian Holstein cattle *(51–53)*, as well as in nearly all models of scrapie in rodents studied to date *(54–58)*. When hamsters infected with the 263K strain of scrapie were studied, TVS were not initially detected *(54)*. This model of scrapie produces higher infectivity titers than any other, and the negative finding was used to support the notion that TVS was not a significant ultrastructural feature of the disease (much less the infectious agent or an aggregate of it). Subsequently, however, three groups of investigators reported TVS in this model *(55–57)*.

In human CJD, TVS were first reported only in 1992 *(58,59)*. TVS were found consistently in chimpanzees inoculated either with human or chimpanzee CJD and in experimental CJD in mice *(57,58,60)*.

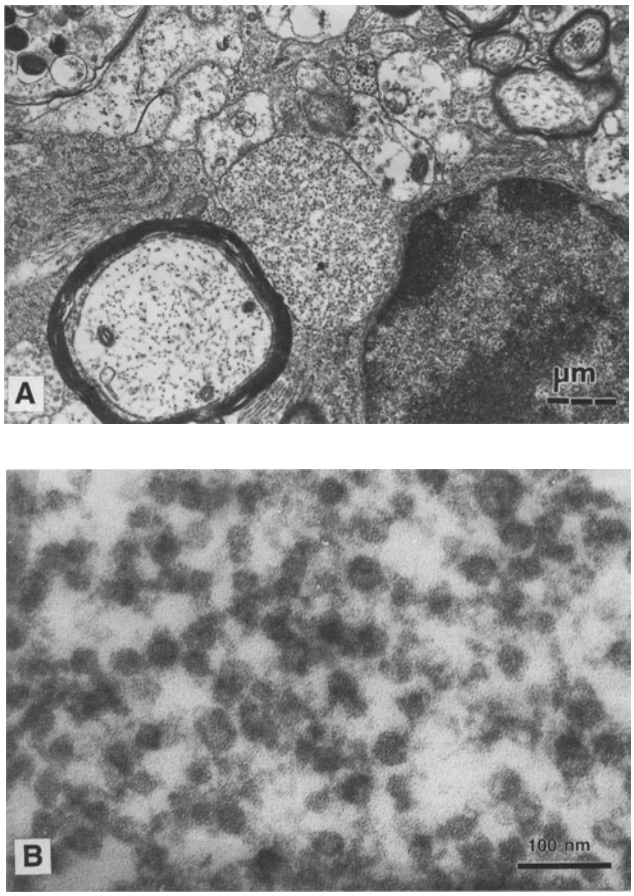


Fig. 9. Low **(A)** and **(B)** high-power electronmicrographs of tubulovesicular structures from scrapie-affected hamster brain. Original magnification (A) ×12,000, bar = 1 μm; (B) ×140,000, bar = 100 nm.

The discovery of TVS in CJD was followed by a similar finding in GSS (Liberski and Budka, manuscript in preparation).

The structure of TVS has been variably described. In most published electron micrographs, TVS appear as spheres measuring between 20 and 40 nm in diameter (Fig. 9). Liberski et al. *(57–59)* demonstrated short tubular forms of TVS. Thus, it is evident that TVS are pleomorphic structures in at least two forms—spheres and short tubules. Circular profiles of TVS may correspond to short tubules cut in transverse or oblique sections. Some investigators believe that this pleomorphism of TVS precludes them from being a part of or aggregate of the infectious scrapie agent. Although I do not prejudge the true nature of TVS, it is noteworthy to remember the pleomorphism of other viruses. Virions of hepatitis delta virus, for example, exist as spheres and short tubules *(61)*.

The nature and significance of TVS are completely obscure. In experimental CJD, TVS appear early in the incubation period, preceding the onset of clinical disease *(57,58)*. Furthermore, in scrapie-infected hamsters, TVS precedes the appearance of other neuropathological changes *(57,58)*. The approximately 1000-fold lower titer of the Fujisaki strain of CJD in murine brain, compared to the 263K strain of scrapie, may account for the delayed appearance of TVS in experimental CJD. TVS-containing processes are more numerous in hamsters infected with the 263K strain of scrapie agent than in those inoculated with the 22C/H strain, which is associated with lower titer in brain than 263K (Liberski, unpublished data). The apparent correlation between the number of neuronal processes containing TVS and the infectivity titer may also explain why their number in natural diseases was so low *(56,58,59)*.

Despite considerable progress in the understanding of the molecular biology of SSVE, the causative agent remains obscure. However, the presence of a virus-like structure, which is consistently observed in all SSVE (which by definition are virus-like disorders), naturally occurring and experimentally induced, and which roughly correlates with the titer of the agent in brain, should attract enough interest to characterize it molecularly and consider it as more than only an interesting ultrastructural finding.

Neuropathology of Nontransmissible Cerebral Amyloidoses of AD Type

Introduction

The neuropathology of the nontransmissible cerebral amyloidoses of AD is more florid than that of the transmissible cerebral amyloidoses, but as in the SSVE, it seems that the deposition of amyloid and the impairment of axonal flow linked to the accumulation of abnormal cytoskeleton proteins are the primary events, whereas the other phenomena may be only secondary or, in context of pathogenesis, insignificant.

β(A4) Amyloid Plaques

The morphology of this type of amyloid plaque is basically not very different from that of the PrP-immunoreactive plaque of SSVE *(62,63)*. However, amyloid plaques in AD showed immunoreactivity

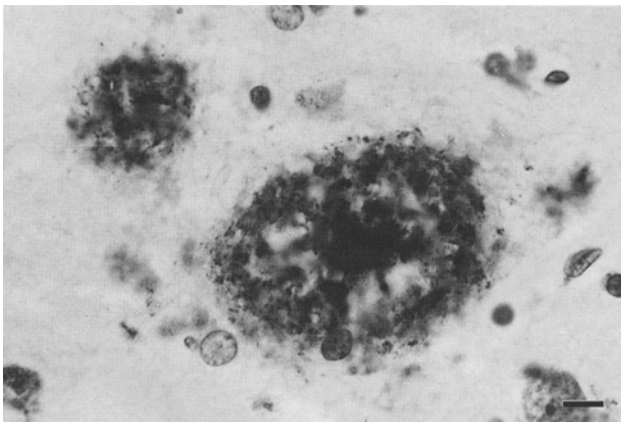


Fig. 10. β(A4)-immunopositive plaques in the brain affected with Alzheimer's disease. Courtesy of Maria Barcikowska. Original magnification, ×1000, bar = 10 μm.

for several complement components, leukocyte cell adhesive molecules, and α1-antichymotrypsin, which are virtually absent from amyloid plaques in experimental scrapie *(62)*. Such immunostaining for molecules involved in many inflammatory reactions may suggest that locally induced inflammatory processes may be associated with the amyloid deposition in the nontransmissible cerebral amyloidoses in contrast to the transmissible brain amyloidoses.

In AD, amyloid plaques are immunoreactive for β(A4) (Fig. 10), and consist of amyloid fibrils, DN, and glial (astrocytes and microglial) cells in different proportions *(64–67)*. According to the still useful classification of Wisniewski and Terry *(66)*, the primitive plaque consists predominantly of DN with little or no amyloid, whereas the classical plaque consists of a β(A4) core surrounded by a corona of DN and glial cells (Fig. 11). Plaques composed of a core, but without a corona, are designated burnt out plaque and are roughly analogous to the kuru plaques of SSVE. Indeed, A4-immunoreactive stellate plaques (morphologically indistinguishable from kuru plaques of the SSVE) were seen in the cerebella of otherwise typical AD (Liberski and Budka, unpublished observation).

The morphogenesis of the plaque is still obscure. The first step is presumably the formation of amorphous plaques (Fig. 12). Amorphous plaques are β(A4) positive, but not congophilic, which suggests that β(A4) or APP in these plaques is not in a β-pleated conformation (pre- or para-amyloid, but not amyloid). There is little or no glial reaction around those plaques. The intermediate step may

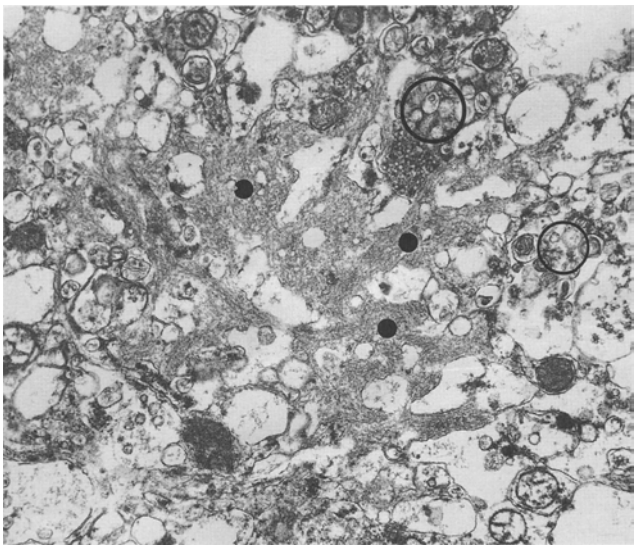


Fig. 11. Classical plaques of Alzheimer's disease. Note amyloid fibers (close circles) and dystrophic neurites (open circles). Original magnification, ×12,000, bar = 1 μm.

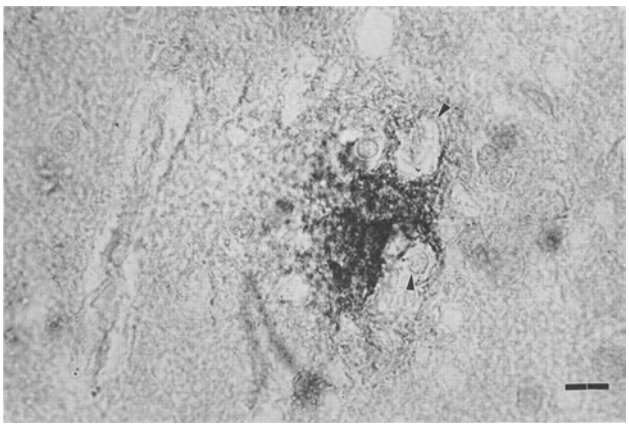


Fig. 12. Amorphous β(A4)-immunopositive amorphous plaque. Courtesy of Maria Barcikowska. Note unstained nuclei. Original magnification, ×1000, bar = 10 μm.

be the so-called A plaque, consisting of preamyloid, but with an admixture of microglial cell(s) in the center *(68)*. In AD, the microglial cell is an unequivocal component of amyloid plaque *(49,69–77)*. This was further demonstrated by ultrastructural studies showing that microglial cells are associated with every compact plaque if sectioned serially *(78)*. Whether the microglia are secreting cells (amyloid producer/processor cell) or phagocytosing cells is still open to debate. The ultrastructural observations that amyloid fibrils are intimately associated with the endoplasmic reticulum, which is involved with protein synthesis, favors a processing rather than a phagocytosing function of these cells *(49)*. On the other hand, the fact that abundant microglial cells are not associated with diffuse plaques may support the opposite view. The alternative possibility is, however, that microglial cells invade diffuse plaques at later stages of their morphogenesis and participate in organization ("crystallization") of diffuse plaque to yield the compact plaque. Analogous to AD, amyloid fibers in experimental scrapie and CJD were demonstrated in endoplasmic reticulum of microglial cells *(49,79)*.

Neurofibrillary Tangles

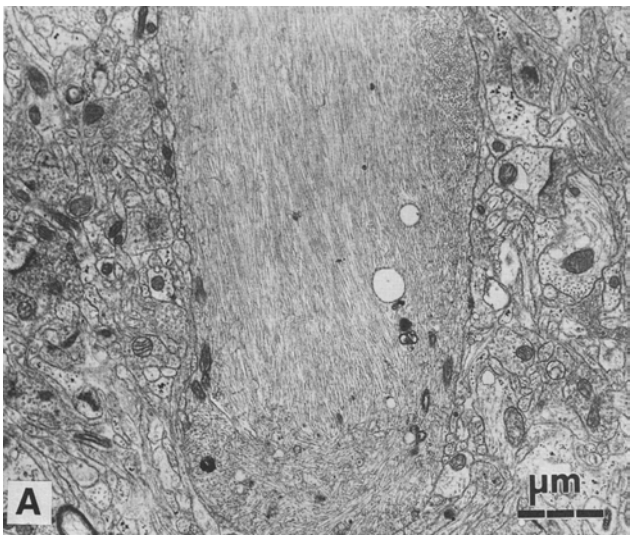
As in the SSVE, an impairment of axonal transport seems to be linked to accumulation of abnormal cytoskeleton proteins *(42,80,81)*. In AD, such an accumulation is designated neurofibrillary tangles (NFT). Flame-shaped NFT occur intracellularly, or extracellularly as "ghost" tangles *(82,83)*. The tinctorial properties of NFT, particularly birefringence following Congo red staining, classify NFT as being amyloid. (It must be emphasized here that the term amyloid denotes only the β-pleated conformation of any protein that causes its congophilia.) Ultrastructurally, NFT consist of paired helical filaments (PHF; *84,85)* (Fig. 13). Immunohistochemically, mainly abnormally phosphorylated τ protein *(82,83)* is present but also β-amyloid *(86,87)*, neurofilament proteins, MAP2, actin, and ubiquitin are detected within NFT. Thus, in AD, two biochemically distinct amyloids are observed.

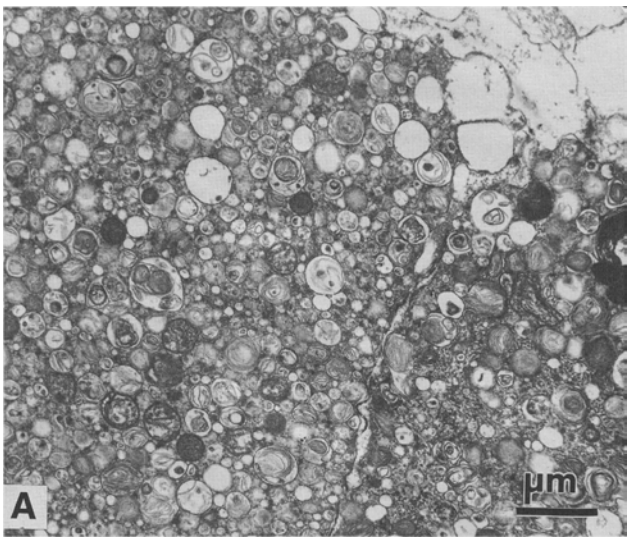
Dystrophic Neurites

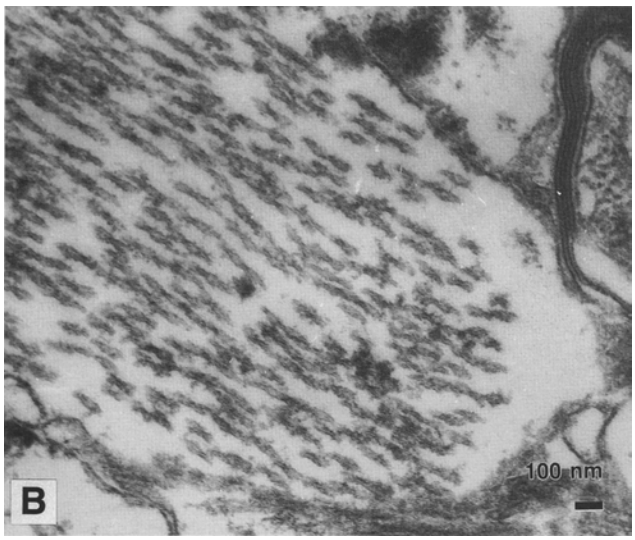
Except for PHF, the morphology of dystrophic neurites and of the recently discovered neurpil threads of AD is the same as in SSVE *(42,60,88,89)*. Ultrastructurally, DN contain degenerating mitochondria and electron-dense, multivesicular, and multilamellar bodies (Fig. 14). In AD, DN are immunopositive for τ (Fig. 15) and β(A4) proteins *(90)*. DN may occur as a part of amyloid plaques or as independent structures.

Conclusions

In conclusion, the basic pattern of neuropathological changes is similar in the transmissible and nontransmissible cerebral amyloidoses, and consists of accumulation of molecularly different amy-







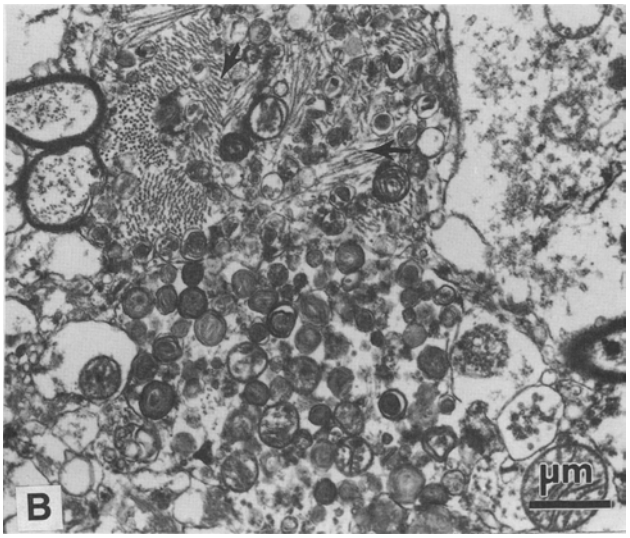
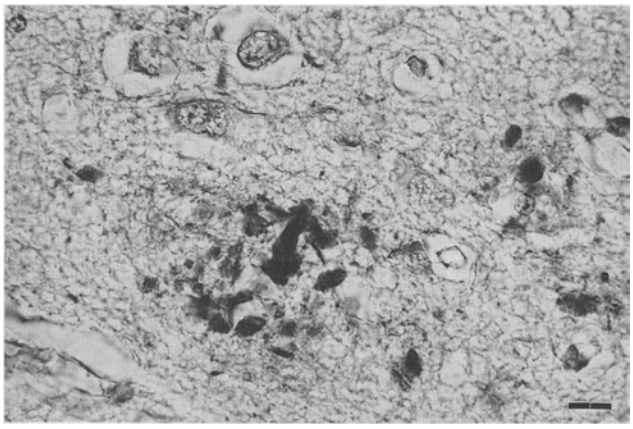


Fig. 13. Low **(A)** and high **(B)** electron micrographs of neuronal processes containing paired helical filaments. Original magnifications (A) ×7000; (B) 50,000 (A) bar = 1 μm (B) = 100 nm.

Fig. 14. *(right column, above)* Dystrophic neurites without **(A)** and with **(B)** paired helical filaments (PHF). PHF marked with arrows at B. Original magnifications, ×12,000, bar = 1 μm.

Fig. 15. *(right column, below)* Numerous τ-positive dystrophic neurites surrounding amyloid plaques in a brain affected with Alzheimer's disease. Original magnification, ×1000, bar = 10 μm.



loids and an impairment of the axoplasmic flow, which is linked to the accumulation of molecularly diverse cytoskeleton proteins.

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