ELECTRON MICROSCOPIST’S VIEW OF THE ALZHEIMER’S PLAQUE

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Alzheimer’s disease (AD) is characterized by extracellular aggregation and deposition of Amyloid-beta peptide in the form of diffuse and fibrillar plaques. More than 50 years ago electron microscopic studies in humans have characterized the structure of the amyloid plaque and neurofibrillary tangles. More recently animal models of AD-type amyloidosis have provided excellent opportunities to study plaque structure during the development and expression of AD-type pathology. Ultrastructural data from a variety of transgenic mice overexpressing mutant amyloid precursor proteins, mutant presenilins, with or without human ApoE knock-in isoforms, are highly comparable to classical electron microscopic findings in AD. This review is an attempt to evaluate, from an electron microscopist’s point of view, the structural identity of AD type pathology, and the mature amyloid plaque in particular. Biomed Rev 2012; 23: 9-17.

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PROLOGUE
In November 1901, Dr Alois Alzheimer admitted Auguste D., a 51 year old patient, to the Frankfurt hospital because of progressive memory loss, delusions and hallucinations. On the 3rd of November 1906, Dr Alzheimer delivered a lecture at the meeting of the Psychiatrists of South West Germany where he presented the clinicopathological data from his long time patient Auguste D. who suffered from “presenile dementia” and died at the age of 55. His presentation would not have been possible without the development of new and special staining techniques by Max Bielschowsky (silver staining) and his friend Franz Nissl (Nissl staining) who introduced him to brain histopathology. Four years later, in 1910 the great psychiatrist and his senior colleague Emil Kraepelin proposed the name Alzheimer’s disease (AD). Regrettfully, as is the case with so many great achievements in science, history and art, what was not properly acknowledged at that time, was the equally important contribution of another man - a young Italian physician, Dr Gaetano Perusini. Together with Dr Alzheimer he studied Auguste D.’s brain. As witnessed by many publications, Dr Alzheimer himself expressed his wish to entrust the investigation of this “new” disease to Dr Perusini. In fact the first 4 cases of Alzheimer’s disease published by
Alzheimer’s laboratory were authored by Perusini. In 1998 an Italian colleague, B. Lucci, described the contribution of his country fellow in the article entitled “The contribution of Gaetano Perusini to the definition of Alzheimer’s disease” published in Italian Journal of Neurological Sciences 19: 49–52. Recently, the silver stained slides of Auguste D’s brain have been rediscovered and their re-examination has confirmed what was described then represents the same diagnostic entity that we recognize now. In the same line of thoughts it would have been very helpful to understand if her case was related to the autosomal dominant form of this disease, but regretfully, no family history for Frau Auguste has been retrieved to this date.

INTRODUCTION

Alzheimer’s disease is characterized by the formation of amyloid plaques, progressive degeneration of neuronal populations in the neocortex and limbic system, appearance of neurofibrillary tangles and by cognitive impairment that is manifested decades after brain tissue pathology has begun. More than 50 years ago Robert Terry and Michael Kidd presented their very detailed ultramorphological studies on the amyloid plaque in postmortem AD brains. They described the characteristic neuritic changes and the structure of neurofibrillary tangles formed by paired helical filaments (PHF) in dystrophic or dying neurons (1-5). AD plaques were described of being composed of extracellular deposits of amyloid-β (Aβ) and other plaque-associated proteins and surrounded by dystrophic neurites and glial cells. A classical plaque had a “central fibrillar core”, “axons and dendrites filled with an excess of neurofibrils”, and “cell processes filled with dense bodies” (4). The earliest sign of plaque formation according to Wisniewski and Terry was the appearance of few abnormal neurites, followed by “wisps” of deposited amyloid (6). In 1989 Yamaguchi et al characterized the structure of the “diffuse plaque” (7). In 1992 they described the deposition of amyloid in AD related angiopathy (8). Scanning electron microscopy has also been applied mainly for the study of tangles and PHF (9). Since 1996 few systemic human electron microscopic (EM) studies have been published (10,11).

THE ERA OF THE TRANSGENIC MOUSE MODEL OF AD

The generation of transgenic (tg) mice as models overexpressing mutant human amyloid precursor protein (APP) and/or presenilin 1 (PS1) and PS2, tg APP/PS1 mice expressing in addition the human apolipoprotein E (ApoE) isoforms, as well as mutant Tau, has made substantial contributions to understanding of the structural changes in the brain during the development of the AD-type pathology (12-17). Importantly, they have replicated major pathological features such as the diffuse and compact amyloid plaque, the massive neuritic dystrophy, and glial activation (11, 18-20). No mouse model has fully recapitulated the entire neuropathological spectrum of AD-like brain lesions, like PHF in dystrophic neurons. Nevertheless tg animals have been an invaluable tool to study AD (21-24). Since no PHF were observed even in the oldest PDAPP mice, many have agreed that other approaches such as accelerated aging or increased longevity of experimental mice might be needed to recapitulate tangle pathology (18). Historically the first animal tg models were based on the overexpression of single or multiple mutant molecules associated with familial AD (FAD) (16). Masliah et al made a detailed EM comparison of the neurodegenerative pathology found in AD and the PDAPP tg mouse line (11, 16). Overproduction of a mutant human APP in mice produced amyloid fibril deposition, diffuse and mature amyloid plaque formation at 8 months of age. Axonal dystrophy, microglial and astroglial involvement have been reported by this and other groups (11, 18, 25). In many human and animal studies the relationship between amyloid deposition/turnover and microglia has been extensively discussed (2, 11, 19, 26-32). To date however, it remains poorly understood. Various reports show that microglia cells may be the driving force in depositing or clearing fibrillar amyloid (31-34). Others like Grathworth et al have shown that the formation and maintenance of the amyloid plaque can occur in almost complete absence of microglia and that neuritic dystrophy may develop independent of microglia (35).

Kurt et al (2001) provided the first detailed ultrastructural study on tg mice co-expressing mutant APP and PS1 (19). They showed robust amyloid deposition months earlier than APP tg mice. A number of other studies using different APP tg models have all reported mature amyloid plaques (36-42). Based on these and other studies, overwhelming evidence has been accumulated to support the idea that FAD-like mouse models produce extensive amyloid plaque, microglial cell activation and axonal dystrophy (11, 26, 38). Our ultrastructural studies in three (Fig. 1) and seven months old APP/PS1 transgenic mice have shown that the structure of the plaque and its immediate surroundings also strikingly resemble classical finding in humans (1-5). In these tg mice the mature amyloid plaque also presented with a dense fibrillar core surrounded
by a great number of elaborate microglial cell processes forming characteristic invaginations (or bays) where parallel amyloid fibrils accumulated (Fig. 1). Importantly, microglial processes showed morphological signs of phagocytic activity with neuritic debris and/or aggregation of lysosomes. This also appeared to be true for microglia that surrounds the plaque periphery. Studies have shown that in tg AD-like mouse models microglial response around plaques occurs often and relatively soon (43-44).

The ε4 allele of ApoE is known so far to be the only firmly established risk factor for the more common sporadic forms of AD (39-41). Our extensive ultrastructural examination of the mature plaque structure and neuritic pathology in seven month old APP/PS1 tg mice each expressing one of the three human ApoE isoforms - ApoE2, ApoE3 and ApoE4 (45) have shown that all the signature signs of the mature neuritic AD-like plaque are also observed at this time point. Structurally, the plaque and axonal degeneration morphologies were also similar to that of tg APP/PS1 mice with the mouse ApoE (Fig. 1-3; see also Sanchez-Varo et al. 2012). Even at 3 months of age APP/PS1 mice exhibit a number of very dystrophic axonal terminals with synaptic figures that seem to have preserved their pre- and

Figure 1. Low power electron micrograph of a mature amyloid plaque seen in the cortex of APP/PS1 tg animal. The amyloid core is represented by a star-shaped epicenter (*), surrounded by many processes (black arrow) of microglial cells. One of these processes at the lower right shows autophagic vacuoles (white arrow). The amyloid core is surrounded by corona of dystrophic spheroids (**) filled with degenerating mitochondria, autophagic vacuoles and lysosome-like structures.
Figure 2. Electron micrograph showing higher magnification of two moderately degenerated axons with normal mitochondria (*) and autophagic vacuoles (**).

Figure 3. Electron micrograph showing axonal profiles at advanced stage of degeneration. At the center of this micrograph, a grossly degenerated terminal with preserved pre- and post- synaptic density and condensed axoplasmic matrix is also observed.
post synaptic density (Fig. 3); interestingly, the postsynaptic dendritic shafts and spines did not show evidence of dystrophy. Grossly dilated and dystrophic presynaptic terminals have been discussed in aged APP751 (Swedish double mutation) tg mice (38), in 4 month old PS1M146L/APP751SL tg mice with human ApoE (46). The latter findings were discussed as the initial degenerative process underlying functional disturbance prior to neuronal loss. We have found that a corona of degenerating neuritic processes is represented by profiles at different stages of swelling and dystrophy and organelle compaction (Fig. 1-3). We also observed that in APP/PS1 tg mice and also tg APP/PS1 with human ApoE isoform knock-in tg mice (45) morphological changes in myelinated axons distant from the plaque and the swollen and dystrophic profiles within the plaque are strikingly similar. We have been also consistently encountered transitions from myelinated to unmyelinated segments along a single axon and these segments contained similar dystrophic changes. Based on such observations we can speculate with high degree of confidence that the vast majority of “dystrophic neuritic profiles” are indeed demyelinated segments of axons. To date almost all ultrastructural studies agree with this concept (25, 37, 46). Electron microscopic studies in postmortem AD also support the predominant axonal origin of the plaque’s dystrophic neurites (12, 47-49). Many years ago, in their classical ultrastructural studies, Tery and Wisniewski (1-2) have suggested that the earliest precursors of the senile plaque is the abnormally swollen and degenerating neurite filled with numerous mitochondria, lamellar and dense bodies. They even proposed that plaques develop from small clusters of these dystrophic neurites. In good agreement with APP-immunoreactive spheroids in an early onset AD patients, motor deficits have been reported (47, 50-51). Likewise in 10 and 14 months old APP/PS1 models, in mice transgenic for human four-repeat tau, or human ApoE4, evidence for motor deficits caused by substantial disturbance of axonal transport and spinal cord axonal degeneration has been reported (52). It has been suggested that axonal swellings such as these found in and around the plaque, precede and participate in plaque formation, probably due to focally increased Aβ secretion or lysis of Aβ rich swellings (53). Plaques with identical morphology have been observed by us and by others (19, 45) in the white matter as well. Since white matter is devoid of neuronal cell bodies, dendritic processes and axonal terminals, it has been speculated that amyloid deposition is likely to be extraneuronal (19). The evolution of dystrophic axonal changes included in addition diverse axoskeletal abnormalities.

Similar observations have been reported in APP tg mice, APP/PS1 tg mice, tg mice with human four-repeat tau proteins and in mice expressing mutant human tau (11, 19, 38, 46, 54, 55-57). Axonal swelling, the cytoskeletal pathology, accumulation of dystrophic organelles, such as mitochondria and autophagic vacuoles, while indicative of progressive axonal degeneration, may also indicate disturbed organelle (anterograde/retrograde) transport (54, 57). Interestingly we have often observed instances of only normal mitochondrial accumulation in dilated segments along the neurite. Changes in the axoplasmic cytoskeleton in association with organelle compaction have been consistently observed in experimental traumatic brain injury and these features have been classically interpreted as axonal transport impairment (58-59). Impaired axonal transport appears to be a major pathological alteration in later stages of the AD progression and the axonal pathology described by us and others recapitulates the cardinal features of axonal impairment and possible chronic deficits not only in motor performance.

Currently it is still unclear whether dementia and neurodegeneration are equal to loss of neurons, loss of functional synapses or disturbances of synaptic transmission. Although not directly observed within the plaque, neuronal cell death of non-apoptotic and/or apoptotic origin is an important part of the pathogenesis of the disease and reflects the cause/consequence paradigm of AD. Mitochondrial dysfunction and oxidative stress in degenerating neurites as causative factors for the progressive cell death occurring in tg animals has also been discussed (60). In general, however cell death has emerged as a controversial topic. The exact mechanism of cell death in neurons that develop tangles in AD is not known. In our single time-point studies there was no ultrastructural indication of autophagic or apoptotic cell death although many axonal processes exhibited pathology consistent with the process of autophagy. Our data are similar to data from APP/PS1tg mice where the lysosomal pathology was prevailing in degenerating neurons of older animals (19, 61). It is also difficult to argue that axonal pathology and neuronal cell death are directly related since we have found much less neuronal cell body degeneration compared to the massive axonal dystrophy throughout the brain. Nonetheless, non-apoptotic type of cell death can be substantial given the protracted nature of the disease observed in these animals. Neuronal apoptosis on the other hand has been implicated in aging APP/PS mice (60, 62) based on a number of activated caspase 3 immunoreactive cells (marker of apoptotic cell death) and ultrastructural

morphology. However the involvement of this cell death type in tg animals remains rather uncertain (63). The involvement of apoptotic cell death is also unclear in human AD cases (64). Implicating the potential role of autophagic neuronal cell death, Yang et al have presented evidence for a cross talk between apoptosis and autophagy by showing robust activated caspase 3 staining in autophagic vacuoles in dystrophic neurites of APP/PS1 tg mice (62). In AD brains certain hippocampal neurons have shown features of granulovacular degeneration with the expression of activated caspase 3 (65). Although such findings reinforce the idea of possible link between apoptotic and autophagic neuronal cell death in some experimental models, and also in AD, further studies are needed to clarify the exact nature of cell degeneration.

CONCLUDING REMARKS

After reviewing published EM data from AD, tg APP/PS1 mice with mutant tau, our own data from single tg APP mice, double tg APP/PS1 mice, and tg APP/PS1 mice expressing human ApoE isoforms, we conclude that at ultramicroscopic level they all share the cardinal features of the classic senile plaque. Clinically, despite variable and divergent genetic causes, the outcome in all AD patients is similar as they present with unique postmortem pathology (66). Therefore these tg animals, in addition to being extremely useful for the study of the pathogenesis of AD, they also offer models for the development of treatment strategies. Studies of FAD have contributed to the identification of important molecular processes. Studies on sporadic and late-onset AD cases have shown the relevance of ApoE variants and the population of patients at risk. They have also prompted the search for additional genetic candidates that may produce more complete transgenic animal models that harbor different combination of AD - related human genes. There are, of course many unanswered questions. It is still debated whether dominantly inherited AD and “sporadic” forms express identical biomarker signatures. Sporadic AD is currently conceptualized as a complex, nonlinear, dynamic, and chronically progressive disease and it will be interesting to know if EM can be of any help in charting possible morphological differences. Animal models will help in unraveling the precise mechanism of amyloid secretion, cell and axon degeneration and of synapse dystrophy and elimination. While this search is on, the EM evaluation of the morphological changes will likely still remain a “golden standard” in the complex approach in resolving the various aspects of this dehumanizing disease.

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