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Dystrophic neurites accumulating autophagic vacuoles show early stages of neuritic destruction

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Abstract

We re-examined the database of some 20,000 electron micrographs from the Echigo-1, the 263K-strain or the 22C-H of scrapie-infected hamsters to look for the cytoplasmic clearance. We reevaluated the largest database in the world of photographed dystrophic neurites for the presence of cytoplasmic clearance as shown in transgenic fruit flies transfected with Aβ-42. In several neurites, we found electron-lucent areas not bound by any membranes or only partially bound; thus, they were not autophagic vacuoles as the latter are membrane-bound and contain cargo. Those changes were not observed in every examined neurite and no correlation with any other changes were noticed. In some neurites, which could be traced over several sections, the electron-lucent areas were evident to change size, i.e. to expand.

Key words: *autophagy, prion disease, clearance.*

Introduction

Creutzfeldt-Jakob disease (CJD) and Gerstmann-Sträussler-Scheinker disease or syndrome (GSS) are prototypic human prion diseases [1]; for which several experimental models in hamsters, mice or bank voles are currently available. The best known, not transgenic, models are mice infected with the K. Fu (Fujisaki) strain of GSS and the Echigo-1 strain of CJD [12,13,24,27]. The ultrastructural pathology of rodent models of human prion diseases are characterized by a spongiform change, dystrophic neurites containing abundant autophagic vacuoles and lysosomal dense bodies and the presence of tubulovesicular structures (TVS), disease-specific particles of unknown significance [10,15]. We [12-14,24,25,27] and then others [5] pioneered the research on autophagy in prion diseases; however, the exact role of autophagy has never been clearly elucidated [2,7].

In diseases of protein misfolding, including prion diseases, autophagy seems to play a protective role by removal of toxic protein aggregates [19,22,29] and the name "a guardian against neurodegeneration" was coined for this function [3]. However, the role of autophagy in neurodegeneration was also considered.

Recently, a role for autophagy in *Drosophila* flies transfected with a construct encoding Aβ-42, a major amyloidogenic peptide in Alzheimer disease

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(AD) was published [18] and demonstrated the presence of dystrophic neurites, not unlike those seen in AD [6,16,30], filled with autophagic vacuoles and lysosomal electron-dense bodies, and showing areas of the cytoplasmic clearance. The latter finding suggests the leakage of lysosomal enzymes form autolysosomes that initiate degeneration and, probably, the loss of neurites.

The latter publication prompted us to re-examine our database of some 20,000 electron micrographs from Echigo-1 CJD-infected, 263K-strain and 22C-H strain of scrapie-infected hamsters to investigate evidence of cytoplasmic clearance as found in the *Drosophila* model.

Material and methods

Creutzfeldt-Jakob disease strain, animals, incubation period of illness

Outbred 6-week-old golden Syrian hamsters (Medical University of Lodz, Department of Oncology, Lodz, Poland) were inoculated intracerebrally with 0.05 ml of a 10% (w/v) centrifugation-clarified hamster brain suspension containing the Echigo-1 strain of the CJD agent [23]. Control animals were sham inoculated intracerebrally with the same volume of saline. We used five hamsters for each experiment; the control group consisted of 2 hamsters. In the first passage in our laboratory (7^{th} serial passage after initial isolation), the incubation period was approximately six months. The experiment was repeated twice with the similar results.

Scrapie strains

Hamsters were inoculated with the 263 K or 22C-H strains of scrapie [9,17]. These strains are widely used experimental tools primarily because of the relatively short incubation periods which, for mice, ranged from 16 to 18 weeks and for hamsters from 9 to 10 weeks for the 263K strain and 24-26 weeks for the 22C-H strain. Appropriate control animals were sham inoculated with saline.

Electron microscopy

Hamsters in the terminal stage of CJD and control sham-inoculated hamsters at the same interval after inoculation were anaesthetized with ketamine. They were perfused by an intracardiac injection with saline followed by 150 ml of 1.25% glutaraldehyde and 1% paraformaldehyde prepared in cacodylate buffer (pH 7.4) and then by 50 ml of 5% glutaraldehyde and 4% paraformaldehyde.

Perfused animal carcasses were held at 4°C for at least 2 hours, after which brains were removed and several 1-mm^3 samples were dissected under a binocular microscope from parietal cortex, corpus callosum, CA2 region of the hippocampus, thalamus, cerebellum and the brain stem. Those samples were postfixed in 1% osmium tetroxide for 1-2 hours, dehydrated through a series of graded ethanols and propylene oxide, and then embedded in Epon resin (Serva). Semi-thin sections were stained with toluidine blue, blocks trimmed, and ultrathin sections stained with lead citrate and uranyl acetate. Specimens were examined using a JEM 100 C transmission electron microscope.

Results

The ultrastructural picture of the Echigo-1, 263-K and 22C-H infected hamsters were described elsewhere [13,14,24]. Briefly, spongiform change, astrocytosis, TVS and dystrophic neurites were readily found.

We reevaluated the largest database in the world of photographed dystrophic neurites for the presence of cytoplasmic clearance as shown in transgenic fruit flies transfected with Aβ-42 [18]. In several neurites, we found electron-lucent areas not bound by any membranes or only partially bound; thus, they were not autophagic vacuoles as the latter are membrane-bound and contain cargo (Figs. 1-4). Those changes were not observed in every examined neurite and no correlation with any other changes was noticed. In some neurites, which could be traced over several sections, the electron-lucent areas were evident to change size, i.e. to expand.

Discussion

We report here that dystrophic neurites in prion diseases in hamsters showed accumulation of lysosomes and autolysosomes and autophagic vacuoles. This phenomenon, albeit neglected in studies of prion diseases, is reminiscent of another protein misfolding disease, AD. This was shown in a classical paper by Lampert [6] and later elucidated in several reports including ours [8,26]. Recently numerous transgenic models of AD have been developed and enabled more detailed studies of the development of autophagic vacuoles. For instance, in APP single

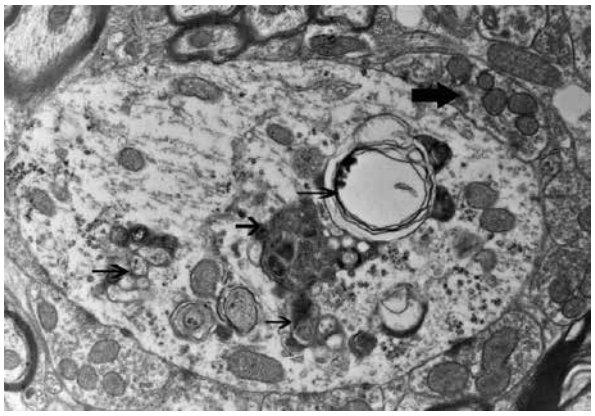

Fig. 1. A dendrite with a connected synaptic terminal (large arrow) and different types of autophagic vacuoles (arrows). The 263K strain of scrapie. Original magnification, 20 000×.

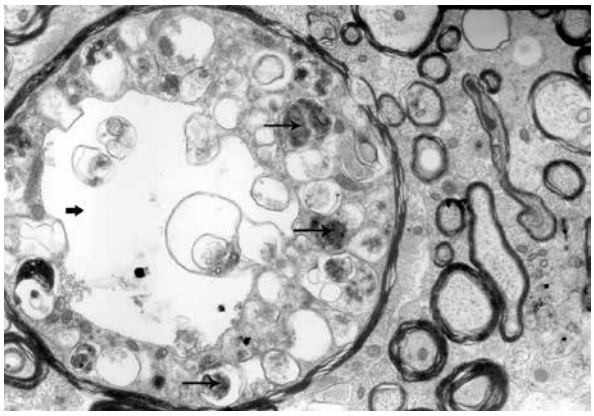

Fig. 2. A myelinated neurite with a large clear space (arrow) surrounded by numerous autophagic vacuoles (arrows). The Echigo-1 strain of CJD. Original magnification, 20 000×.

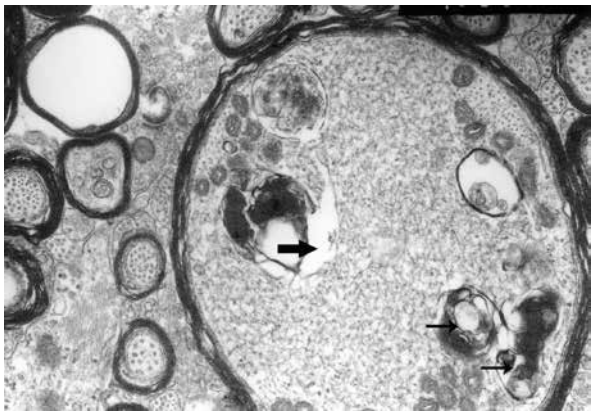

Fig. 3. A myelinated neurite with a narrow clear space (arrow) surrounded by numerous autophagic vacuoles (arrows). The Echigo-1 strain of CJD. Original magnification, 20 000×.

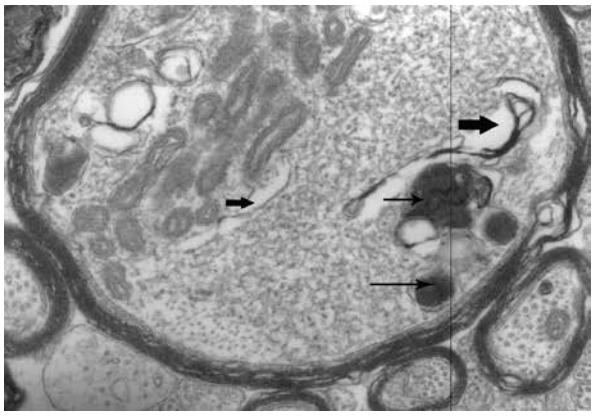

Fig. 4. A myelinated neurite with a large clear space (arrows) surrounded by numerous autophagic vacuoles (arrows). The Echigo-1 strain of CJD. Original magnification, 20 000×.

transgenic and APP co-expressing knocked-in mutant PS1 mice [28], severe neuroaxonal dystrophy was reported. Numerous dystrophic neurites were seen in the vicinity of plaques and in the regions distant from plaques and were stained by anti-phosphorylated APP and anti-phosphorylated 200 kDA neurofilament antibodies. This is analogous to our study on expression of phosphorylated neurofilaments in CJD [11,26]. Massive accumulations of lysosomes and autophagosomes were observed within dystrophic neuritis around plaques in transgenic APP/PS1 mouse models of AD [4]. Axonal lysosomes appeared early in the developing of the disease in a transgenic AD model and it is not the end-stage of pathology. Those authors suggested that dystrophic neuritis accumulating autophagic vacuoles resulted from merging of endosome and autophagosome pathways and it seems that they travel along the axons toward the somata of neurons [20,21]. Interestingly, in variant CJD we also observed abundant dystrophic neurites filled with lysosomes and autophagic vacuoles similar to those seen in neuritic plaques in human brains with AD. The mechanism of dystrophic neurite clearance is unknown but such a process may lead to their removal and it may contribute to deficits associated with neuroaxonal dystrophy.

Disclosure

The authors declare no conflict of interest.

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