The hunt for dying neurons: Insight into the neuronal loss in Alzheimer's disease

Zilkova M[1,2], Koson P[1,2], Zilka N[1,2]

Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia. **Norbert.Zilka@savba.sk**

Abstract

Neuronal loss is one of the major pathological hallmarks of neurodegenerative disorders including Alzheimer's disease (AD). Using rigorous quantitative methods, the distinct pattern of neuronal loss in pathological conditions such as neurodegeneration and in normal aging was clearly shown. Furthermore, the decrease of total neuronal numbers correlated in a considerable extent with the presence of neurofibrillary degeneration in the same brain regions. However, it appears that neurofibrillary tangles are not the only cause of reduction of neuronal populations, but also alternative triggers could induce neuronal death in this disease. Various inducers, most probably, activate different cell death pathways. Recently, apoptosis has been implicated as a possible mechanism for neuronal death. There is essentially no evidence of apoptosis in AD that would meet all criteria of its classical definition. Therefore it was suggested, that other modes of cell death could contribute to neuronal loss in AD and related disorders *(Tab. 2, Ref. 70)*.

Key words: Alzheimer's disease, neurodegeneration, neuronal loss, stereology, apoptosis, programmed cell death.

How to quantify neuronal loss?

One of the principal issues had to be solved in Alzheimer's disease was the question whether neuronal loss is present as inherent part of this neurodegenerative disorder or not. The description of neuronal loss on histological sections from brain autopsies of AD patients could help to resolve this issue. However, the classical qualitative histopathology often uses terms like "many", "few" and "present" or "absent". These descriptions are definitely helpful and sufficient for describing pathological changes present in the tissue in a great extent. Where the histomorphological changes are more discrete, such terms are very insufficient to test whether there is present statistically significant difference in studied parameters (e.g. cell or synaptic numbers, volume of a brain region, etc.). In this case, there is a need "to attach numbers to the more or less subjective terms used in descriptions" (Schmitz and Hof, 2005). The aims of neuropathologists in the past to determine quantitatively the extent of neuronal loss in AD and to uncover which of the brain regions are the most affected failed very often because of inappropriate methodology. The quantitative neuromorphological approaches, called assumption-based methods (Abercrombie, 1946), used in relatively imminent past, were found to be very biased and led to inconclusive and contradictionary results. Fortunately, the need for unbiased quantitative histomorphological data in neuroanatomy and neuropathology resulted in the past decades into development of new methodological approaches overally named as design-based methods. These methods known also as "stereological" or simply "stereology" encompass broad variety of three-dimensional approaches ("3D probes") that enable to count directly the objects in a defined volume of tissue (Sterio, 1984, Gundersen et al, 1988). The deeper insight into stereology and closer description of such methods would go far beyond the objectives of this review (for comprehensive look on the use of design-based stereology in neuroscience see e.g. Schmitz et Hof,

[1]Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia, and [2]Centre of Excellence for Alzheimer's Disease and Related Disorders, Slovakia

Address for correspondence: N. Zilka, Institute of Neuroimmunology, Slovak Academy of Sciences, Dubravska cesta 9, SK-845 10 Bratislava, Slovakia.

Acknowledgement: This work was supported by research grant VEGA 2/6183/26 and ESF project No. 13120200026.

Tab. 1. Stereological studies on neuronal loss in different brain regions in Alzheimer's disease.

Anatomy – brain region	Studied subjects	Results – Neuronal loss	Reference
Hippocampus	7 AD 14 ND	CA 1 68% subiculum 47% hilus 25%	West, 1994
	13 AD 10 ND	fascia dentata and subiculum 23%	Simic, 1997
	28 cases (neuropathologic Braak staging I-V)	CA 1 33% (stage IV) 51% (stage V) subiculum 22% (stage V)	Rössler, 2002
	14 AD 8 pAD 11 ND	CA 1 48% subiculum 24% hilus 14%	West, 2004
Entorhinal cortex	10 AD 10 ND	48 % (layer II of entorhinal cortex up to 90%)	Gomez-Isla, 1996
Nucleus basalis of Meynert	8 AD 12 ND	42-89%	Cullen, 1997
	9 AD 12 MCI 9 ND	56%	Mufson, 2000
Neocortical regions			
Superior temporal sulcus	34 AD 17 ND	more than 50%	Gomez-Isla, 1997
Prefrontal cortex (Brodmann´s area 9)	19 cases (neuropathologic Braak staging I-V)	up to 90% (selective vulnerability of specific populations of pyramidal neurons)	Bussière, 2003a
Subcortical structures			
Amygdala	9 AD 6 ND	51-56%	Vereecken, 1994
Edinger-Westphal nucleus	7 AD 12 ND	67%	Scinto, 2001
Substantia nigra	11 AD 24 ND	no significant neuronal loss	Kemppainen, 2002

AD – patients with Alzheimer's disease, MCI – patients with Mild Cognitive Impairment, pAD – preclinical AD patients (cognitively intact, but with substantial numbers of neuritic amyloid beta plaques, ND – nondemented patients.

2005). It is noteworthy to mention that introduction of stereological methods in neurosciences played important role in the discovering of specific patterns in neurodegenerative processes during the development of Alzheimer's disease.

Distinct pattern of neuronal loss in Alzheimer's disease and nondemented cases

In the eighties and nineties of the 20th century there was a controversy about whether normal aging and Alzheimer's disease represent a continuum, where AD exemplifies exaggerated aging rather than a true disease ("If we live long enough, will we all be demented?", Drachman, 1994) or these processes are two different entities from both clinical and pathological perspectives (West et al, 1994; Gomez-Isla et al, 1996). In an attempt to distinguish these two possibilities, many scientific groups applied stereological methods for quantitative assessment of neuronal numbers to determine possible neuronal loss in the brain regions profoundly affected in AD, hippocampus and entorhinal cortex. These brain areas are afflicted both among the first during Alzheimer's disease and simultaneously with the most prominent concomitant neuropathological features of AD (neurofibrillary tangles and senile plaques). For that reason the hippocampus and the entorhinal cortex, as parts of the limbic system, responsible among other functions also for memory processing and learning, were first of the central interest. West et al (1993, 1994) conducted two of the first rigorous quantitative stereological studies on hippocampal formation, where he studied individual hippocampal subdivisions (dentate gyrus, hilus, CA1, CA2–3 and subiculum). In the group of 45 non-demented cases, across the age range of 13 to 85 years, he found substantial loss of neurons in the subiculum (52 %) and in the hilus of the dentate gyrus (31 %), while three remaining hippocampal subdivisions showed no significant change (West et al, 1993). In the study carried out one year later he compared the regional pattern of neuronal loss in individual hippocampal subdivisions of AD patients and

nondemented cases and found striking differences. The most distinctive Alzheimer's disease related neuronal loss was seen in the CA1 region of the hippocampus. In the normal ageing group there was almost no neuron loss in this region, therefore West (1994) suggested that neurodegenerative processes associated with normal ageing and with AD are qualitatively different. Different pattern of neurodegenerative changes and decreased neuronal number in hippocampus during development of Alzheimer's disease was validated by other scientific groups disproving the idea that AD and accelerated aging are the synonymous terms for the same process (Simic et al, 1997; Kril et al, 2002; Rössler et al, 2002). In the subsequent years further quantitative stereological studies in different brain areas brought rising range of evidences for prominent neuronal loss in AD. Evidently to regions accompanied with neuronal loss in AD belongs entorhinal cortex (Gomez-Isla et al, 1996), several neocortical areas (Gomez-Isla et al, 1997; Bussiére et al, 2003 a) and at least some subcortical brain structures (Tab. 1). It has been also clearly demonstrated that the extent of neuronal loss progressed with the severity of the disease (Rössler et al, 2002), whereas in preclinical AD cases in cognitively intact persons with substantial numbers of neuritic beta amyloid (Aß) plaques, was found no significant neuronal loss compared to age-matched nondemented cases (West et al, 2004). Additionally, more detailed stereological outlook based on quantification of immunohistochemically stained pyramidal neurons showed selective vulnerability of specific neuronal populations with their pronounced loss in AD (Bussiére et al, 2003 a).

Relationship of neuronal loss to major neuropathological hallmarks of Alzheimer's disease

The pathognomonic feature of AD on histopathological level is presence of specific pathological structures in the brain tissue. Two types of abnormal protein deposits are characteristic: senile plaques present in extracellular space and neurofibrillary tangles (NFT) placed intraneuronally and later after the death of neuronal cell extraneuronally. Both types of deposits consist of fibers formed from aggregated proteins, beta amyloid in senile plaques and tau protein in case of neurofibrillary tangles. One of the principal questions had to be answered was to determine the relationship of these pathological deposits to neuronal loss and thus to find out their possible causative role in the neurodegeneration in AD. And here again stereology was the method of choice to confirm or to reject these hypotheses. In 1991 Braak published the paper qualitatively describing regionally specific progression of neuropathological changes in Alzheimer's disease. He observed that the distribution pattern of amyloid deposits turned out to be of limited significance for differentiation of neuropathological stages. In contrast to this, the neurofibrillary lesions, exhibited a characteristic distribution pattern permitting the differentiation the severity of the disease into six stages, suggesting a topographical progression of neurodegeneration in Alzheimer's disease in the human brain. According to the Braak model of neuropathological staging of AD, neurofibrillary tangles emerge first in the transentorhinal cortex followed by their appearance in the proper entorhinal cortex and the hippocampal CA 1 region and only later in the isocortical association areas (Braak and Braak, 1991). Strikingly, these regions, as confirmed by above mentioned stereological studies in subsequent years, were affected with severe neuronal loss. However, some of these studies conducted quantifications only of neuronal numbers and not of the load of the neurofibrillary pathology (West et al, 1994; Simic et al, 1997), providing thus just indirect evidence suggesting the possible causative role of NFT pathology. Several stereological studies aimed on quantification of all amyloid plaques, NFTs and neuronal numbers were conducted with the aim to obtain straightforward evidences of mutual relationship of all these for Alzheimer's disease characteristic neuropathological hallmarks. The study carried out by Bobinski in hippocampal formation found strong regional correlations between the relative decreases in the total number of neurons and the relative increase in the total number of neurofibrillary tangles, implicating so "neurofibrillary pathology as a possible etiologic proximate factor in neuronal and volumetric loss in the hippocampal formation of AD patients" (Bobinski et al, 1996). He also confirmed in this work that the decrease in the total number of hippocampal pyramidal neurons plays a causative role in hippocampal formation volumetric loss. In addition, the statistical analysis sugessted that neurofibrillary pathology, not beta amyloid deposition, underlies hippocampal volumetric loss. Comparative study was realized also in entorhinal cortex (Gomez-Isla et al, 1996). The author demonstrated that neuronal number in entorhinal cortex in AD was inversely proportional to NFT formation and neuritic plaques, but was not related significantly to beta amyloid deposition. Very similar observations were found out one year later in superior temporal sulcus by the same author (Gomez-Isla et al, 1997), where again both neuronal loss and neurofibrillary tangles increase in parallel with the duration and severity of illness. We can pressume that in case when the neurofibrillary deposits are possibly toxic for neuronal cell in so substantial extent that could lead to neuronal death, we should definitely observe clearly inverse proportion between reduction of neuronal numbers and increase amount of extracellular NFTs in the ratio 1:1. In other words every extracellular NFT (ghost tangle) would represent "a tombstone" of one died neuron. Nevertheless, in the work by Gomez-Isla was also prominently pointed out that although the inverse proportion of NFT load and neuronal number is present, the amount of neuronal loss excedded by manyfold the amount of neurofibrillary tangles accumulated. Corresponding conclusions for excedding neuronal loss when compared to NFT loads were drawn in later survey in hippocampus (Kril et al, 2002). This indicated also that at least certain proportion of neurons are lost with non-NFT-related mechanisms (Kril et al, 2002; von Gunten et al, 2006) including possibly contribution of oxidative stress and excitotoxic mechanisms inducing neuronal death that could potentially precede NFT formation. These and other latest stereological studies employed more profound look on specific types of neurofibrillary pathology, quantifying not only total amount of NFTs, but discrimi-

Tab. 2. The overview of the modus of cellular death observed in Alzheimer's disease.

DNA damage	Markers for cell death	Brain area	Modus of cell death	Literature
TdT labeling - apoptotic morphology (shrunken, irregular cellular shape, apoptotic bodies)	**c- JUN**	Entorhinal cortex	***Apoptosis***	***Anderson et al., 1996***
TdT labeling - caspase 3 associated with DNA fragmentation - no significant correlation was found between DNA fragmentation and Bcl 2	**CASPASE 1 , CASPASE 3, Bcl 2**	Neocortex	***Apoptosis***	***Masliah et al., 1998***
TdT labeling **Klenow assay** - significant difference (AD vs CTRL) **Apostain assay** - no significant difference		Hippocampus	***Apoptosis***	***Adamec et al., 1999***
	PARP (Poly (ADP- Ribose) Polymerase)	Frontal and temporal cortex, hippocampus	***Apoptosis***	***Love et al., 1999***
	CASPASE 3	Hippocampal formation, frontal cortex	***Apoptosis***	***Su et al., 2001***
	CASPASE 6	Temporal and frontal cortex	***Apoptosis***	***Guo et al., 2004***
	TRAIL (Tumour-necrosis factor related apoptosis inducing ligand)	Cerebral cortex	***Apoptosis***	***Uberti et al., 2004***
	FAP-1 (Fas associated phosphatase-1)	Hippocampal formation	***Apoptosis***	***Savaskan et al., 2005***
	FADD (Fas-associated death domain)	The nucleus basalis of Meynert	***Apoptosis***	***Wu et al., 2005***
	FasL (Fas ligand), CASPASE 8, FODRIN CCP	Hippocampal formation, frontal cortex	***Apoptosis***	***Su et al., 2003***
	TRADD (TNFR-associated death domain), TNFR1 (TNF receptor) and activated JNK (c-Jun N-terminal kinase)	Hippocampal formation	***Apoptosis***	***DelVillar and Miller, 2004***
TdT labeling - non apoptotic morphology (no apoptotic bodies, no shrunken or condensed nuclei)		Occipital cortex, temporal cortex, hippocampus, hypothalamus	***Necrosis***	***Lucassen et al., 1997***
TdT labeling - majority of labeled nuclei did not show the classical morphological features of apoptosis	**APS (v- JUN, apoptosis spesific protein), c- JUN, Bcl 2**	Temporal cortex, hippocampus, entorhinal cortex	***Non apoptotic cell death***	***Stadelmann et al., 1998***
TdT labeling - majority of labeled nuclei did not show the classical morphological features of apoptosis		Parietal cortex, cingulate gyrus, lateral, basal temporal and entorhinal cortex, hippocampus, subiculum	***Necrosis or non apoptotic cell death***	***Lassmann et al., 1995***
TdT labeling - non apoptotic morphology (no chromatine condensation)		Hippocampal formation, entorhinal cortex, inferior temporal cortex	***Necrosis, non apoptotic programmed cell death***	***Troncoso et al., 1996***

nating in details between intracellular (iNFT) and extracellular (eNFT) tangles. Such approach provides additional information about the dynamics of neurodegenerative process on subcellular level in the neuronal cell. It helps to address the answer to one important question, whether the intracellular NFTs are inherently toxic to neurons or whether the neurons can persist with such NFT load in "transitional stage". As mentioned above the proportion of eNFT to neuronal numbers provided information about potential alternative non-NFT-related mechanisms in neuronal death in AD. In contrast, the data from iNFTs counts brought the evidence that substantial proportion of neurons affected by neurofibrillary degeneration may persist for a very long periond of time in late stages of AD and may preserve some function even at stages where dementia is clinically overt. From these results it has been also indicated that the production of eNFTs is a rather slow process that is truly important only in late stages of the disease and that iNFTs may be "far more relevant to the etiopathogenesis of AD" (Bussiére et al, 2003 b; Hof et al, 2003).

Neuronal cell death in Alzheimer's disease.

Quantitative studies showing an extensive neuronal loss during the progression of Alzheimer's disease led to the principal question regarding the modus of neuronal death that is characteristic for AD. Although there is a growing evidence of apoptosis as a major mechanism involved in the neuronal death in AD, the

contribution of apoptosis to overall neurodegeneration and dementia remains unclear (Anderson et al, 2000).

Cell death represents a mechanism that regulate balance between proliferation and differentiation during neuronal system development (Martin, 2001), but also during the optimization of adult cells and tissue functions (Krantic et al, 2005). In adult nervous system the neuronal cells represent the population of highly differentiated postmitotic cells, with upregulated protection from programmed cell death. Under pathological conditions, such as acute and chronic cytotoxic insults, "pathological" reinitiation of cell cycle could occur just before cell death (Ankarcrona and Winblad, 2005; Krantic et al, 2005).

Classification of cell death

Cell death has been generally classified into two distinct types, apoptosis and necrosis, which differ structurally and biochemically. The present studies confirmed that morphological descriptions of apoptosis and necrosis do not characterize all variants of cell deaths (Sperandio et al, 2000; Martin, 2001). Neuronal death is not always strictly apoptosis or necrosis, as show basic classification, but it may occur as apoptosis-necrosis continuum or hybrid form of cell death with coexisting characteristics of both extremes (Martin, 2001).

Classical apoptosis is one of best-known phenotypic expression of physiological programmed cell death (PCD), that is mediated by active mechanisms with specific molecules (Schwartz, 1993; Amin, 2000). It is characterized by the presence of DNA condensation in the nuclei, DNA fragmentation at the nucleosome linkage regions, cell shrinkage and partition of cytoplasm and nucleus into membrane bound-vesicles (apoptotic bodies) which contain ribosomes, morphologically intact mitochondria and nuclear material (Majno et al, 1995; Wyllie et al, 1997; Martin, 2001). These apoptotic bodies expressing specific markers on their surface are rapidly recognized and phagocytized by other macrophages or adjacent epithelial cells. Due to this efficient mechanism for the removal of apoptotic cells in vivo no inflammatory response was induced. The mechanism of apoptosis is widely used by the organism during development and in other conditions, where the elimination of unwanted cells is required (Savill et al, 1989).

The molecular basis of classical apoptosis is characterized by two different caspase cascades. Both pathways consist two main phases: initiation and execution, which lead to the activation of cystein-dependent, aspartate-directed proteases called caspases (Krantic et al, 2005). The first pathway represents "mitochondrial pathway" with cytochrome C release from mitochondria and activation of caspase-9 through Apaf-1 and then sequentially activation of caspase-3, -6 and -7 (Li et al, 97).

The second one involves activation of "death-domain containing receptors" such as tumor necrosis factor α receptor 1 TNFαR1 and FAS. The cell surface death receptors recruit adapter proteins, which contain death domains such as FAS associated death-domain protein (FADD) and TNFαR1-associated death-domain protein (TRADD). The TRADD-FADD complex or receptor-associated FADD induce the autolytic activation of the associated caspase 8, which is the critical event that transmits the death signal and then activates caspase 3 due to the proteolysis (Wang, 2000; Takuma et al, 2005).

Apoptotisis-like PCD. Despite difficulty with cell death classification some literature mentioned term apoptosis-like PCD, which represented "mitochondrial pathway caspase independent". In this pathway important role plays apoptosis-inducing factor (AIF) that releases after mitochondrial membrane permeabilization and translocates to the nucleus, where it is associated with large scale DNA fragmentation (Susin et al, 1999).

Non-apoptotic PCD. The main feature of this non-classical form of PCD is the internucleosomal fragmentation of DNA. The cells undergo autophagic death characterized by degradation of cytoplasmic organelles that precedes the nuclear destruction (Martin, 2001; Bursch and Ellinger, 2005). This type of cell death can occur in the nervous system after exposure to neurotoxine or after hypoxia-ischemia and also in various neurodegenerative diseases (Martin, 2001).

Necrosis-like PCD. This kind of programmed cell death involves different cellular organelles than mitochondria and by other proteases than caspases, such as cathepsins and calpains that are triggered from lysosomes and endoplasmic reticulum (Lockshin and Zakeri, 2004). This molecular mechanism represents alternative pathway when caspases are inhibited by oxidative stress or energy depletion (Krantic et al, 2005).

Necrosis. Accidental or pathological cell death initiated by rapid and severe failure to homeostasis is called necrosis. This cell death occurs when cells are injured by extreme physical stress or chemical changes. The process is characterized by the presence of massive ion influx, mitochondrial swelling, nonspecific DNA breakage in the nuclei and ultimately plasma-membrane rupture (Majno et al, 1995; Martin, 2001). The nuclear pyknosis occurs as condensation of chromatin into irregularly shaped clumps in contrast with apoptotic regularly shaped aggregates. Due to the ultimate breakdown of the plasma membrane, the cytoplasmic contents including lysosomal enzymes are related into the extracellular fluid. Therefore, in vivo, necrotic cell death is often associated with extensive tissue damage resulting in an intensive inflammatory response (Van Furth and Zwet, 1988). It is believed that cellular necrosis is passive result rather than specific molecular program, however some specific pathways may cause necrosis (Martin, 2001). The differences between classical apoptosis and necrosis are easy distinguishable, but there are also intermediate variants between these two extremes.

Neuronal cell death in Alzheimer's disease

Predominant mechanism of pathologic death in CNS injury is necrosis while physiological cell death occurring during brain development is considered to be apoptosis (Takuma et al, 2005). Despite of this facts, the neurobiological mechanisms by which neuron are dying in Alzheimer's disease have not been fully elucidated. Many studies suggest that in AD-related neurodegeneration an apoptotic cell death mechanism is involved (Ander-

son et al, 1996), while others argue against it (Vitek et al, 1994; Lucassen et al, 1997; Stadelmann et al, 1998). Enthusiasm for apoptosis goes from a character of this type of cell death. It is thought that neuronal death in degenerative disease is a selective at the individual cell level and not associated with inflammation (Dickson, 2004). On the other hand, detailed studies provided evidence of abnormal oxidative stress, advanced glycation-end product (AGE), as underlying causes of necrotic cell death (Vitek et al, 1994). Recent research into mechanisms of cell death in AD has led to the understanding of how apoptosis and necrosis contribute to neurodegeneration. The key results of the neuronal death studies are shown in the Table 2. The dates are highly condensed and details need to be sought in the original paper. All of the selected studies demonstrate the spectrum of current opinion of the neuronal death in AD.

Apoptosis is an active mechanism of cell death that may occur in Alzheimer's disease (Anderson et al, 1996; Lassmann et al, 1995). Various markers for apoptosis have been described in AD brains, but majority of them was not upregulated compared with control brains. Apoptosis specific protein (ASP), useful marker strongly associated with cardinal features of apoptosis such a chromatin and cytoplasmic condensation, was not increased in AD brains. On the other hand activated caspase 3 was elevated in AD and exhibited a high degree of colocalization with NFT and senile plaques (Stadelmann et al, 1998; Su et al, 2001). It has been also reported an upregulation of the anti-apoptotic protein Bcl 2 in DNA fragmented cells and down-regulation in tangle bearing neurons (Su et al, 1996; Satou et al, 1995). Some investigators demostrated high level of the DNA repair protein PARP and its end-product poly(ADP-ribose) in AD (Love et al, 1999).

DNA damage belongs to the more studied death stimuli, which in many cells leads to apoptotic death via a pathway dependent on p53. Biochemical correlates of these morphological features have emerged during the subsequent years of study of this phenomenon. The first and most dramatic is DNA fragmentation, that was described by Wyllie in 1980. However, in some apoptotic systems (e.g., Fas killing of tumor cells) artificially enucleated cells lacking a nucleus still die, showing that the nucleus is not always necessary for apoptotic cell death. Independence of DNA breaks are critical, because techniques that detect double-stranded DNA breaks, such as TUNEL, are not specific for apoptosis. Indeed, necrotic cells in three models of necrosis were brightly stained by TUNEL (Collins et al, 1992). Several investigators have reported increased number of DNA strand breaks in AD brain tissue compared with age-matched control brains (Lassmann et al, 1995; Stadelmann et al, 1998; Cotman, 1998). Despite the large number of neurons with DNA fragmentation identified in the hippocampus of AD brain, only exceptional of them displays the morphological characteristics of apoptosis including cell shrinkage, chromatin condensation, nuclear fragmentation and cytoplasmatic condensation (Bancher et al, 1997; Lucassen et al, 1997; Stadelmann et al, 1998). Moreover the majority of degenerating cells was not located within amyloid deposits and did not contain neurofibrillary tangles (Lassmann et al, 1995; Cotman, 1998). Except blunt-ended double stranded DNA breaks or breaks with protruding 3 termini labeled by TUNEL technique it has been demonstrated in AD brains also increasing number of single and double stranded DNA breaks with 5 termini detected by Klenow assay and single stranded DNA detected by Apostain assay (Adamec et al, 1999).

The last studies accumulate the evidence which support essential role of apoptosis in AD pathogenesis (Ankarcrona and Winblad, 2005), with mitochondrial dysfunction and endoplasmic reticulum stress in a main role (Takuma et al, 2005). Direct evidence for mitochondrial dysfunction in AD comes from reports about reduction of cytochrome c oxidase activity in dentate gyrus and hippocampus of AD patients (Ojaimi and Byrne, 2001; Takuma et al, 2005). Next observations suggest that also alteration in mitochondrial DNA (mtDNA) or beta amyloid (Aß) may cause mitochondrial dysfunction (Eckert et al, 2003) and resulting energy deficit may trigger the onset of neuronal apoptosis in AD.

Finally, it is very difficult to assess the relative contribution of apoptosis to neuronal death in AD, because of chronic nature of the disease process. Some neurons exhibit morphological features of apoptosis, but many degenerating neurons do not show any evidence of this type of cell death. It has been suggested that apoptosis might not be the only mechanism of cell death in Alzheimer's disease (Su et al, 1994; Troncoso et al, 1996; Yuan and Yanker, 2000).

Conclusion

Neuronal loss is widely accepted as a hallmark of Alzheimer's disease. Recent neuropathological research confirmed the distinct pattern of neuronal loss in AD and in normal aging. It has been shown that neuronal loss in AD affects specific brain areas, that are not afflicted in normal elderly, showing progressive reduction in neuronal numbers correlating with the severity of the disease. The quantitative histopathological studies demonstrated also the relationship of neurofibrillary tangles to neuronal loss, suggesting neurofibrillary degeneration as one of important upstream factors leading to neuronal cell death. Furthermore, large body of evidence is showing that apoptosis is important process underlying neurodegeneration. There is also mounting evidence that other events may contribute to neuronal loss in AD as well. Therefore, it is being slowly accepted that various neuronal cell death pathways could be involved in neuronal loss in AD.

References

Abercrombie M. Estimation of nuclear population from microtome sections. Anat Rec 1946; 94: 239—247

Adamec E, Vonsatel JP, Nixon RA. DNA strand beaks in Alzheimer's disease. Brain Res 1999; 849: 67—77

Amin F, Bowen ID, Szegedi Z, Mihalik R and Szende B. Apoptotic and non-apoptotic models of programmed cell death in MCF-7 human breast carcinoma cells. Cell Biol Int 2000; 24: 253—260.

Anderson AJ , Su JH, Cotman CW. DNA damage and apoptosis in Alzheimer's disease: colocalization with c-Jun immunoreactivity, relationship to brain area and effect of postmortem delay, J Neurosci 1996; 16: 1710—1719.

Anderson A, Stoltzner S, Lai F, Nixon R. Evidence for DNA damage and apoptosis in Down syndrome and Alzheimer disease. Neurobiol Aging 2000; 24: 511—524.

Ankarcrona M, Winblad B. Biomarkers for apoptosis in Alzheimer's disease. Int J Geriat Psychiat 2005; 20: 101—105.

Bancher C, Lassmann H, Jellinger KA. Mechanisms of cell death in Alzheimer's disease. J Neural Transm 1997; 50: 141—152.

Bobinski M, Wegiel J, Wisniewski HM, Tarnawski M, Bobinski M, Reisberg B, De Leon MJ, Miller DC. Neurofibrillary pathology — correlation with hippocampal formation atrophy in Alzheimer disease. Neurobiol Aging 1996; 17: 909—919.

Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol (Berl) 1991; 82 (4): 239—259.

Bursch W, Ellinger A. Autophagy a basic mechanism and potential role for neurodegeneration. Folia Neuropathol 2005; 43 (4): 297—310.

Bussiére T, Giannakopoulos P, Bouras C, Perl DP, Morrison JH, Hof PR. Progressive degeneration of nonphosphorylated neurofilament protein-enriched pyramidal neurons predicts cognitive impairment in Alzheimer's disease: stereologic analysis of prefrontal cortex area 9. J Comp Neurol 2003 a; 463 (3): 281—302.

Bussière T, Gold G, Kovari E, Giannakopoulos P, Bouras C, Perl DP. Stereologic analysis of neurofibrillary tangle formation in prefrontal cortex area 9 in aging and Alzheimer's disease. Neuroscience 2003 b; 117 (3): 577—592.

Collins RJ, Harmon BV, Gobe GC, Kerr JFR. Internucleososmal DNA cleavage should not be the sole criterion for identifying apoptosis. Int J Rad Biol 1992; 61: 451—453.

Cotman CW. Apoptosis Decision Cascades and Neuronal Degeneration in Alzheimer's Disease. Neurobiol Aging 1998; 19: 29—32.

Cullen KM, Halliday GM, Double KL, Brooks WS, Creasey H, Broe GA. Cell loss in the nucleus basalis is related to regional cortical atrophy in Alzheimer's disease. Neuroscience 1997; 78 (3): 641—52.

Del Villar K, Miller CA. Down-regulation of DENN/MADD, a TNF receptor binding protein, correlates with neuronal cell death in Alzheimer's disease brain and hippocampal neurons. Proc Natl Acad Sci USA 2004; 23: 124210—4215.

Dickson DW. Apoptotic mechanisms in Alzheimer neurofibrillary degeneration: cause or effect? J Clin Invest 2004; 114: 23—27.

Drachman DA. If we live long enough, will we all be demented? Neurology 1994; 44 (9): 1563—1565.

Eckert A, Keil U, Marques CA, Bonert A, Frey C, Schussel K Mitochondrial dysfunction, apoptotic cell death, and Alzheimer's disease. Biochem Pharmacol 2003; 66: 1627—1634.

Giannakopoulos P, Herrmann FR, Bussiere T, Bouras C, Kovari E, Perl DP, Morrison JH, Gold G, Hof PR. Tangle and neuron numbers, but not amyloid load, predict cognitive status in Alzheimer's disease. Neurology 2003; 60 (9): 1495—500.

Gomez-Isla T, Price JL, McKeel DW Jr, Morris JC, Growdon JH, Hyman BT. Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. J Neurosci 1996; 16 (14): 4491—500.

Gomez-Isla T, Hollister R, West H, Mui S, Growdon JH, Petersen RC, Parisi JE, Hyman BT. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. Ann Neurol 1997; 41 (1): 17—24.

Gundersen HJ, Bagger P, Bendtsen TF, Evans SM, Korbo L, Marcussen N, Moller A, Nielsen K, Nyengaard JR, Pakkenberg B, et al. The new stereological tools: disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. Apmis 1998; 96 (10): 857—881.

Guo H, Albrecht S, Bourdeau M, Petzke T, Bergeron C, LeBlanc AC. Active Caspase-6 and Caspase-6-Cleaved Tau in Neuropil Threads, Neuritic Plaques, and Neurofibrillary Tangles of Alzheimer's Disease. Amet J Path 2004; 165: 523—531.

Hof PR, Bussiere T, Gold G, Kovari E, Giannakopoulos P, Bouras C, Perl DP, Morrison JH. Stereologic evidence for persistence of viable neurons in layer II of the entorhinal cortex and the CA1 field in Alzheimer disease. J Neuropathol Exp Neurol 2003; 62 (1): 55—67.

Kemppainen N, Roytta M, Collan Y, Ma SY, Hinkka S, Rinne JO. Unbiased morphological measurements show no neuronal loss in the substantia nigra in Alzheimer's disease. Acta Neuropathol (Berl) 2002; 103 (1): 43—47.

Krantic S, Mechawar N, Reix S and Quiron R. Molecular basis of programmed cell death involved in neurodegeneration. Trends Neurosci 2005; 12: 670—676.

Kril JJ, Patel S, Harding AJ, Halliday GM. Neuron loss from the hippocampus of Alzheimer's disease exceeds extracellular neurofibrillary tangle formation. Acta Neuropathol (Berl) 2002; 103 (4): 370—376.

Lassmann H, Bancher C, Wisniewski HM. Cell death in Alzheimer's disease evaluated by DNA fragmentation in situ. Acta Neuropathol 1995; 89: 35—41.

Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES and Wahn X. Cytochrome c and dATP-dependent formation of Apaf-1/caspase 9 complex initiated an apoptotic protease cascade. Cell 1997; 91: 479—489.

Lockshin R and Zakeri Z. Caspase-independent cell death? Oncogene 2004; 23: 2766—2773.

Love S, **Barber R, Wilcock GK.** Increased poly(ADP-ribosyl)ation of nuclear proteins in Alzheimer's disease. Brain 1999; 122: 247—253.

Lucassen PJ, Chung WC, Swaab DF DNA damage distribution in the human brain as shown by in situ end labeling; area-specific differences in aging and Alzheimer disease in the absence of apoptotic morphology. J Neuropathol Exp Neurol 1997; 56: 887—900.

Majno G, Joris I. Apoptosis, oncosis and necrosis. An overview of cell death. Amer J Pathol 1995; 146: 3—15.

Martin LJ. Neuronal cell death in nervous system development, disease, and injury. Int J Mol Med 2001; 7: 455—478.

Masliah E, Mallory M, Hansen L A. Caspase dependent DNA fragmentation might be associated with excitotoxicity in Alzheimer disease. J Neuropathol Exp Neurol 1998; 57: 1041—1052.

Mufson EJ, Ma SY, Cochran EJ, Bennett DA, Beckett LA, Jaffar S, Saragovi HU, Kordower JH. Loss of nucleus basalis neurons containing trkA immunoreactivity in individuals with mild cognitive impairment and early Alzheimer's disease. J Comp Neurol 2000; 427 (1): 19—30.

Ojaimi J and Byrne E. Mitochondrial function and Alzheimer's disease. Biol Signals Recept 2001; 10: 254—262.

Rössler M, Zarski R, Bohl J, Ohm TG. Stage-dependent and sector-specific neuronal loss in hippocampus during Alzheimer's disease. Acta Neuropathol (Berl) 2002; 103 (4): 363—369.

Satou T, Cummings BJ, Cotman CW. Immunoreactivity for Bcl 2 protein within neurons in Alzheimer's disease brain increases with disease severity. Brain Res 1995; 697: 35—43.

Savaskan E, Ravid R, Meier F, Muller-Spahn F, Jockers R. Immunohistochemical localization of Fas-associated phosphatase-1 (FAP-1) in Alzheimer's disease hippocampus. Appl Immunohistochem mol Morphol 2005; 13 (2): 190—193.

Savill JS, Fadok V, Henson P, Haslett C. Phagogocyte recognition of cells undergoing apoptosis. Immunol Today 1989; 14: 126.

Schmitz C, Hof PR. Design-based stereology in neuroscience. Neuroscience 2005; 130 (4): 813—831.

Schwartz LM, Smith SW, Jones MEE, Osborne BA. Do all programmed cell death occur via apoptosis? Proc Natl Acad Sci USA 1993; 90: 980—984.

Scinto LF, Frosch M, Wu CK, Daffner KR, Gedi N, Geula C. Selective cell loss in Edinger-Westphal in asymptomatic elders and Alzheimer's patients. Neurobiol Aging 2001; 22 (5): 729—736.

Simic G, Kostovic I, Winblad B, Bogdanovic N. Volume and number of neurons of the human hippocampal formation in normal aging and Alzheimer's disease. J Comp Neurol 1997; 379 (4): 482—494.

Sperandio S, de Belle I, Bredesen DE. An alternative, nonapoptotic form programmed cell death. Proc Nat1 Acad Sci USA 2000; 26: 14376—14381.

Stadelmann C, Bruck W, Bancher C, Jellinger K, Lassmann H. Alzheimer disease: DNA fragmentation indicates increased neuronal vulnerability, but not apoptosis. J Neurophatol Exp Neurol 1998; 57: 456—464.

Stark AK. Pelvig DP, Jorgensen AM, Andersen BB, Pakkenberg B. Measuring morphological and cellular changes in Alzheimer's dementia: a review emphasizing stereology. Curr Alzheimer Res 2005; 2 (4): 449—481.

Sterio DC. The unbiased estimation of number and sizes of arbitrary particles using the disector. J Microsc 1984; 134 (Pt 2): 127—136.

Su JH, Anderson AJ, Cotman CW Immunohistochemical evidence for apoptosis in Alzheimer's disease. Neuroreport 1994; 5: 2529—2533.

Su JH, Satou T, Anderson AJ, Cotman CW. Upregulation of Bcl 2 is associated with neuronal DNA damage in Alzheimer's disease. Neuroreport 1996; 7: 437—440.

Su JH, Zhao M, Cotman CW. Activated caspase-3 expression in Alzheimer's and aged control brain: correlation wit Alzheimer pathology. Brain Res 2001; 898: 350—357.

Su JH, Anderson AJ, Cribbs DH, Tu Ch, Tong L, Kesslack P, Cotman CW. Fas and Fas Ligand are associated with neuritic degeneration in the AD brain and participate in ß-amyloid-induced neuronal death. Neurobiol Dis 2003; 12: 182—193.

Susin SA. et al Molecular characterization of mitochondrial apoptosis inducing factor. Nature 1999; 397: 441—446.

Takuma K, Yan SS, Stern DM, Yamada K. Mitochondrial dysfunction, endoplasmic reticulum stress, and apoptosis in Alzheimer's disease. J Pharmacol Sci 2005; 97: 312—316.

Terry RD, Masliah E, Katzman R. Physical basis of cognitive alterations in Alzheimer•s disease: synapse loss is the major correlate of cognitive impairment. Ann Neurol 1991; 30: 572—580.

Troncoso JC, Sukhov RR, Koliatsos VE. In situ labeling of dying cortical neurons in normal aging and in Alheimer's disease: correlations wit senile plaques and disease progression. J Neuropathol Exp Med 1996; 55: 1134—1142.

Uberti D, Cantarella G, Faccheti F, Cafici A, Grasso G, Bernardini R, Memo M. TRAIL is expressed in the brain cells of Alzheimer's disease patients. Neuroreport 2004; 579—581.

Van Furth R, Van Zwet TL. Immunocytochemical detection of 5-bromo-2-deoxyuridine incorporation in individual cells. J Immunol Methods 1988; 108: 45.

Vereecken TH, Vogels OJ, Nieuwenhuys R. Neuron loss and shrinkage in the amygdala in Alzheimer's disease. Neurobiol Aging 1994; 15 (1): 45—54.

Vitek MP, Bhattacharya K, Glendening JM, Stopa E, Vlassara H, Bucala R et al. Advanced glycation end products contribute to amyloidosis in Alzheimer disease. Proc Natl Acad Sci USA 1994; 91: 4766—4770.

von Gunten A, Kovari E, Bussiere T, Rivara CB, Gold G, Bouras C, Hof PR, Giannakopoulos P. Cognitive impact of neuronal pathology in the entorhinal cortex and CA1 field in Alzheimer's disease. Neurobiol Aging 2006; 27 (2): 270--277.

Wang KKW. Calpain and caspase: can you tell the difference? Trends Neurosci 2000; 23: 20--26.

West MJ. Regionally specific loss of neurons in the aging human hippocampus. Neurobiol Aging 1993; 14 (4): 287--293.

West MJ, Coleman PD, Flood DG, Troncoso JC. Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. Lancet 1994; 344 (8925): 769—772.

West MJ, Kawas CH, Stewart WF, Rudow GL, Troncoso JC. Hippocampal neurons in pre-clinical Alzheimer's disease. Neurobiol Aging 2004; 25 (9): 1205—1212.

Wu CK, Thal L, Pizzo D, Hansen L, Masliah E, Geulac C. Apoptotic signals within the basal forebrain cholinergic neurons in Alzheimer's disease. Exp Neurol 2005; 195: 484—496.

Wyllie AH, Kerr JFR, Currie AR. Cell death: The signigicance of apoptosis. Int Rev Cytol 1980; 68: 251—306.

Wyllie AH. Apoptosis: an overview. British Med Bull 1997; 53: 451—465.

Yuan J, Yanker BA. Apoptosis in the nervous system. Nature 2000; 407: 802—809.

Received June 29, 2006.
Accepted September 2, 2006.