J Neural Transm (2007) [Suppl 72]: 207–215

Printed in Austria

Journal of Neural Transmission

Enhanced apoptosis, oxidative stress and mitochondrial dysfunction in lymphocytes as potential biomarkers for Alzheimer's disease

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Summary Alzheimer's disease (AD) is the most common progressive neurodegenerative disease. Today, AD affects millions of people worldwide and the number of AD cases will increase with increased life expectancy. The AD brain is marked by severe neurodegeneration like the loss of synapses and neurons, atrophy and depletion of neurotransmitter systems in the hippocampus and cerebral cortex. Recent findings suggest that these pathological changes are causally induced by mitochondrial dysfunction, increased oxidative stress and elevated apoptosis. Until now, AD cannot be diagnosed by a valid clinical method or a biomarker before the disease has progressed so far that dementia is present. Furthermore, no valid method is available to determine which patient with mild cognitive impairment (MCI) will progress to AD. Therefore, a correct diagnosis in the early stage of AD is not only of importance considering that early drug treatment is more effective but also that the psychological burden of the patients and relatives could be decreased. In this review, we discuss the potential role of elevated apoptosis, increased oxidative stress and mitochondrial dysfunction as biomarker for AD in a peripheral cell model, the lymphocytes.

Keywords: Lymphocytes, Alzheimer's disease, biomarker

Alzheimer's disease (AD) is the most common neurodegenerative disease affecting more than 25 million people world wide (Wimo et al., 2003). AD manifests as gradual deterioration in memory and cognition, behavior and the ability to perform activities of daily living. The AD brain is marked by severe neurodegeneration like the loss of synapses and neurons, atrophy and depletion of neurotransmitter systems in the hippocampus and cerebral cortex. The majority of AD patients suffer from sporadic AD where ageing itself represents the main risk factor. The minority of AD patients are affected from rare genetic mutations in the amyloid precursor protein (APP) or in the presenilins PS1 and PS2. The clinical progress of these familiar forms is characterized by an early onset of cognitive symptoms. The sporadic and familiar forms share the same pathological hallmarks. They are both characterized by deposition of β-amyloid (Aβ) plaques, accumulation of intracellular neurofibrillary tangles, and pronounced neuronal cell loss. Altered proteolytic processing of APP resulting in the production and aggregation of neurotoxic forms of amyloid beta (Aβ1-40, Aβ1-42) is considered to be central for AD (Selkoe, 2004). Currently, the main hypothesis concerning the origin of AD is based on the neurotoxic effect of Aβ causing increased apoptosis in neurons, elevated oxidative stress, hindered energy metabolism, mitochondrial dysfunction, and consequently synaptic dysfunction (Malaplate-Armand et al., 2006; Kriem et al., 2005).

The diagnosis of AD is still largely based on exclusion criteria of secondary causes and other forms of dementia with similar clinical profile, thus the diagnostic accuracy is only suboptimal. In the United States a diagnostic accurancy of 50–60% and 80–90%, respectively at specialized centers is reached using the common criteria (NINCDS-ADRDA) (Ferris and Yan, 2003; Turner, 2006). Until now, no valid clinical method or biomarker is available to accurately identify AD in the very early phase and to determine which patient with mild cognitive impairment (MCI) will progress to AD (Frisoni et al., 2004; Borroni et al., 2006). This is of special relevance because drug treatment is more effective in the early stage of the disease. Therefore, a valid and easy accessible biomarker for AD or a combination of biomarkers representing the multiplicity of pathophysiological processes taking place in AD would

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simplify the diagnosis, increase the accuracy and enhance the efficacy of drug therapy. At the moment, two different types of biomarkers are discussed: cerebrospinal fluid (CSF) markers like total tau protein or Aβ1-42 and markers in plasma or peripheral cell types like lymphocytes, platelets or fibroblasts (Migliore et al., 2005a). In this review we focus on lymphocytes as a peripheral cell model for AD. Lymphocytes show similar defects like neurons in AD. We and others observed elevated apoptosis, increased oxidative stress and changes in mitochondrial function in lymphocytes. The strong advantage of lymphocytes as a peripheral model compared to CSF is the simple non-invasive, inexpensive and time-saving separation from blood of patients. Repeated samples from patients can be taken as the particular study requires. Therefore, lymphocytes could be an applicable cell model to find a valid and easy detectable biomarker for AD.

Similar effects of AD relevant stressors on mitochondrial dysfunction and apoptosis in human lymphocytes and neuronal cell lines

Lymphocytes show similar reactions to AD relevant stressor like the neuronal like cell line, PC12 cells. We investigated the effects of staurosporine, Aβ1-42, H_2O_2, sodium nitroprusside and complex inhibitors of the mitochondrial respiratory chain on apoptosis and mitochondrial membrane potential (MMP) in human lymphocytes. Staurosporine, which is widely used to induce apoptosis in a variety of cell types, leads to a significant increase in apoptotic cells in human lymphocytes as well as in PC12 cells (Table 1) (Leutz et al., 2002). Additionally, lymphocyte treatment with Aβ1-42 and H_2O_2 results in enhanced apoptosis (Eckert et al., 1998). Treatment of lymphocytes with relevant concentrations of sodium nitroprusside, a NO donor, induces a reduction of MMP in both cell types. Again, the different inhibitors of the respiratory chain initiate a decrease of MMP in human lymphocytes and PC12 cells. Therefore, different AD relevant stressors lead to similar effects like elevated apoptosis or decreased MMP in human lymphocytes and PC12 cells. These results suggest that lymphocytes are a suitable peripheral cell model to study AD relevant pathological changes like apoptosis, oxidative stress or mitochondrial dysfunction.

Table 1 *Comparison of the effects of different stressor in human lymphocytes and PC12 cells on MMP and apoptosis*

	Human lymphocytes	PC12 cells
MMP		
Stressors:		
SNP	↓	↓
Complex inhibitors		
Complex I Rotenone	↓	↓
Complex II Thenoyltrifluoroaceton	↓	↓
Complex III Antimycin	↓	↓
Complex IV Natriumazide	↓	↓
Complex V Oligomycin	↓	↓
Apoptosis:		
Aβ1-42	↑	↑
Staurosporin	↑	↑
H_2O_2	↑	↑

Data published in part (Eckert et al., 1998a; Leutz et al., 2002).

Elevated apoptosis in lymphocytes of AD patients

Despite the various genetic and environmental factors that may lead to AD, increasing evidence from AD brain tissue, transgenic animals, and cell lines suggest that the underlying neurodegeneration is associated with morphological and biochemical features of apoptosis (Culmsee and Landshamer, 2006; Mattson, 2004). Apoptotic hallmarks are DNA fragmentation, cytoplasmic shrinkage, chromatin condensation and caspase activation (Jellinger, 2006). Two major signaling pathways lead to apoptosis, the TNF-receptor-mediated (extrinsic) and the mitochondria-based (intrinsic) pathway. The extrinsic pathway is activated by the stimulation of death receptors, e.g. cytokine receptors of the TNF family like the Fas receptor (CD 95), whereas the intrinsic pathway is associated with perturbed mitochondrial function including a loss of MMP, increase in reactive oxygen species (ROS) and the release of cytochrome C followed by caspase 9 and caspase 3 activation. Evidence that many neurons undergo apoptosis in AD includes elevated neuronal DNA-fragmentation in AD postmortem brain tissue, and high levels of activated apoptotic proteins such as caspase 3 and BAX in neurons that exhibit neurofibrillary tangle pathology (Mattson, 2004; Eckert et al., 2003). APP and PS mutations are shown to be sufficient to trigger apoptosis in AD animal models (Keil et al., 2004; Marques et al., 2003). Furthermore, recent findings indicate that the expression of mutant PS1 or mutant APP in PC12 cells sensitizes cells to apoptosis (Eckert et al., 2001d; Guo et al., 1997). In addition to genetic evidence that Aβ induces neuron degradation *in vivo*, recent *in vitro* experiments suggest that oligomeric, intracellular Aβ and not aggregated Aβ like previously thought leads to apoptosis (Malaplate-Armand et al., 2006; Kriem et al., 2005; Deshpande et al., 2006).

Studies in lymphocytes from sporadic AD patients have provided evidence for elevated apoptosis in peripheral blood cells (Table 2). Aging itself induces an increase in

Table 2. *Elevated apoptosis in lymphocytes from AD patients and transgenic animals*

Author	Significant changes in sporadic AD patients compared to aged controls	Significant changes in transgenic animals
Eckert et al. (1998a)	– enhanced basal levels of DNA-fragmentation	
Eckert et al. (1998b)	– enhanced basal levels of DNA-fragmentation – enhanced spontaneous apoptosis – increased apoptosis after oxidative stress (d-ribose)	
Eckert et al. (2001b)		PS1 mutations – enhanced basal apoptosis – increased apoptosis after oxidative stress (d-ribose, H_2O_2)
Schindowski et al. (2003)	– enhanced basal apoptosis – enhanced spontaneous apoptosis – increased apoptosis after oxidative stress (d-ribose)	App and PS1 mutations – enhanced basal apoptosis – enhanced spontaneous apoptosis – increased apoptosis after oxidative stress (d-ribose)
Tacconi et al. (2004)	– significant increase in caspase-3, caspase-6, caspase-8 activity	
Lombardi et al. (2004)	– hyperexpression of Fas mRNA and surface Fas receptor	
Frey et al. (2006)	– enhanced basal apoptosis – enhanced spontaneous apoptosis – increased apoptosis after oxidative stress (d-ribose) – increased caspase 3-activity – increase in Fas expression	
Schindowski et al. (2006)	– enhanced basal apoptosis	

vulnerability to apoptosis (Schindowski et al., 2000). This enhanced susceptibility seems to be even more pronounced in lymphocytes from sporadic AD patients (Eckert et al., 2001a, 2003; Schindowski et al., 2006). Elevated DNA fragmentation was seen in freshly prepared AD lymphocytes compared to controls and spontaneous apoptotic cell death after 24 h was significantly elevated. Importantly, elevated basal apoptosis from AD patients correlated significantly with the Mini Mental State Examination (MMSE)

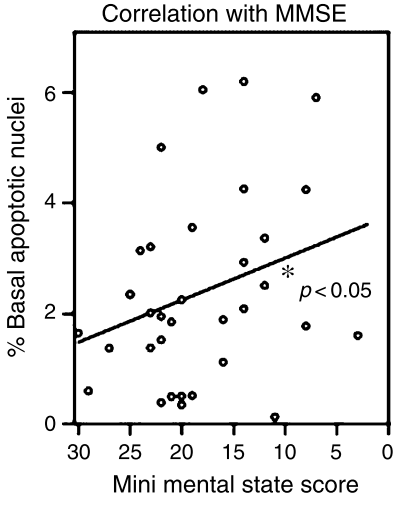

Fig. 1. Correlation of apoptosis in lymphocytes of AD patients with MMSE. Basal levels of apoptotic nuclei in lymphocytes of sporadic AD patients correlate significantly with cognitive decline determined with MMSE ($n = 34$, $^*p < 0.05$) (Schindowski et al., 2006)

of these AD patients (Fig. 1). Furthermore, lymphocytes from AD patients showed an increased vulnerability to pro-apoptotic stimuli like 2-desoxy-ribose (D-ribase) or staurosporine. Analysis of activated lymphocytes gave further evidence for elevated levels of apoptosis in these peripheral blood cells. Significantly elevated levels of DNA-fragmentation were found in activated AD lymphocytes undergoing spontaneous *in vitro* apoptosis or enhanced apoptosis after the treatment with D-ribose. These result point to a faster turnover of apoptotic pathways in AD patients (Eckert et al., 2001a). Importantly, a robust difference in cell death sensitivity between AD patients and patients suffering from vascular dementia was detected.

Moreover, increased CD 95 expression on the surface of T cells from sporadic AD patients and elevated caspase-3, caspase-8 and caspase-9 levels in comparison with non-demented controls refer to an enhanced proneness of AD lymphocytes to cell death (Lombardi et al., 2004; Tacconi et al., 2004; Frey et al., 2006). These findings suggest involvement of the extrinsic and intrinsic apoptotic pathway. CD 95/Fas leads via the extrinsic pathway to apoptotic cell death by the activation of the initiator caspase-8 and the effector caspase-3. Since the activation of the effector caspase-3 is shared by the intrinsic and extrinsic pathway and cytochrome c release from mitochondria is followed by caspase-9 activation, the intrinsic apoptotic pathway could be also important for the increased vulnerability of lymphocytes from AD patients.

The above illustrated findings cannot be explained by changes in the distribution of lymphocyte subsets. No changes in subset distribution of T, B or NK cells were found in AD patients compared to aged controls (Schindowski et al., 2003, 2006). In contrast, a significant decrease in T lymphocytes was determined in healthy persons >60 years compared to young persons <30 years (Schindowski et al., 2002). Again, no changes in the distribution of T lymphocyte population in AD patients compared to aged controls were found (Schindowski et al., 2003, 2006; Frey et al., 2006), but a significant loss of $CD3^+$, $CD4^+$ and $CD8^+$ occurred during aging. Interestingly, several recent findings indicate that mainly $CD4^+$ cells contribute to the increased apoptotic levels in peripheral lymphocytes of AD patients, whereas no changes in the susceptibility of $CD8^+$ T cells to apoptosis were determined (Fig. 2) (Schindowski et al., 2003, 2006; Frey et al., 2006).

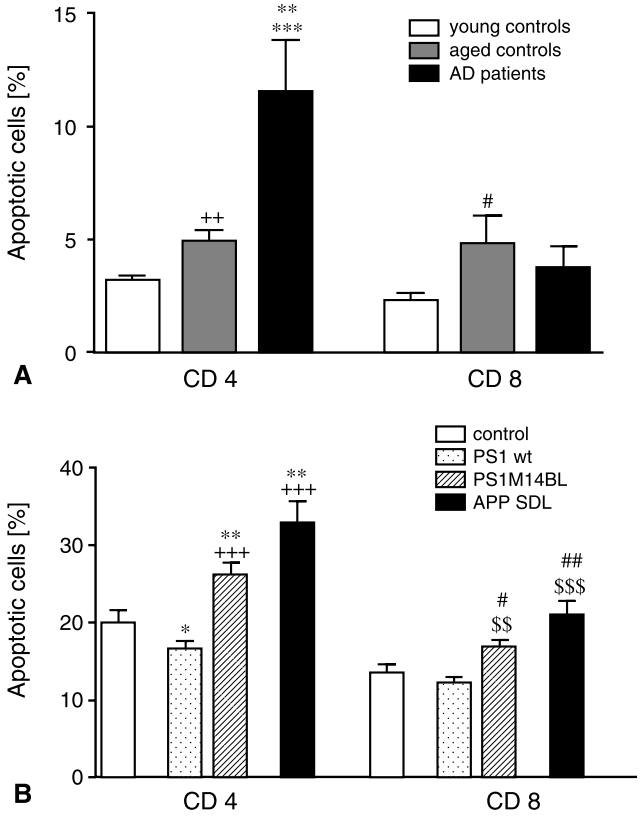

Fig. 2. Increased spontaneous apoptosis in $CD4^+$ lymphocytes compared to $CD8^+$lymphocytes of AD patients and PS1 and APP transgenic mice. **A** Spontaneous *in vitro* apoptosis in $CD4^+$ and $CD8^+$ T cells from young controls ($n = 11$), non-demented aged controls ($n = 12$), and AD patients ($n = 12$) after 24 h incubation, $^{**}p < 0.01$ vs $CD4^+$ from aged controls; $^{***}p < 0.001$ vs $CD4^+$ from young controls; $^{++}p < 0.01$ vs $CD4^+$ from young controls; $^{\#}p < 0.05$ vs $CD8^+$ from young controls. **B** Spontaneous *in vitro* apoptosis in $CD4^+$ and $CD8^+$ T cells from nontransgenic, controls and transgenic animals expressing either mutant human APP (APP695SL) or mutant human PS1 (PS1 M146L) or human wild-type PS1 (PS1 wt) ($n = 6$/group). $^{*}p < 0.05$, $^{**}p < 0.01$ vs $CD4^+$ from control; $^{++}p < 0.01$, $^{++}p < 0.001$ vs $CD4^+$ from PS1wt; $^{\#}p < 0.05$, $^{\#}p < 0.01$ vs $CD8^+$ from controls, $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$ vs $CD8^+$ from PS1wt; modified according to Schindowski et al. (2003)

Besides aging, apolipoprotein E (ApoE) genotype is the most important risk factor for sporadic AD. The three major human isoforms E2, E3 and E4 differ in two amino acids in the positions 112 and 158. The isoform ApoE4 is associated with an increased risk to develop AD. Different effects of ApoE 4 contributing to the pathophysiology of AD like the modulation of the deposition and clearance of Aβ, the impairment of the antioxidative defense system or an increased phosphorylation of Tau are currently discussed (Huang, 2006). Interestingly, lymphocytes from AD patients bearing one or two Apo ε 4 alleles (heterogen ε4/ε3 or homogen ε4/ε4) exhibit a higher rate of apoptotic cell death and caspase 3 activation than Apoε3/ε3 carrier (Frey et al., 2006; Schindowski et al., 2006).

Further, elevated apoptosis was also found in lymphocytes of familiar AD-patients and AD animal models bearing AD relevant APP or PS1 mutations (Fig. 2) (Parshad et al., 1996; Eckert et al., 2001b; Schindowski et al., 2003), supporting the idea that AD specific changes lead to elevated susceptibility of T lymphocytes.

Increased oxidative stress in lymphocytes of AD patients

A large body of evidence suggests that enhanced oxidative stress plays an important role in the dysfunction and apoptotic death of neurons in AD. Studies in post mortem brain tissue of AD patients provided evidence for increased levels of cellular oxidative stress, immunohistochemistry revealed increased protein oxidation, protein nitration, and lipid peroxidation in brain areas with neurofibrillary tangles and Aβ plaques (Perry et al., 2000; Mattson, 2002). Additionally, alteration in levels of antioxidant enzymes such as catalase, Cu/Zn-superoxide-dismutase, and Mn-superoxide-dismutase support the evidence for increased oxidative stress in AD post-mortem tissue and AD animal models (Aksenov et al., 1998; Schuessel et al., 2005, 2006). Membrane lipid oxidation, particularly toxic for neurons, leads to the generation of toxic aldehyds such as 4-Hydroxynonenal (HNE) or malondialdehyde (MDA). The mechanism how oxidative stress accumulates in AD is still unknown but several findings suggest a link between Aβ toxicity and generation of reactive oxygen species (Abdul et al., 2006). Lipid membrane damage is promoted by Aβ aggregates (Murray et al., 2005; Schuessel et al., 2006) and enhanced ROS were found as a consequence of

Table 3. *Increased oxidative stress in lymphocytes from AD patients and transgenic animals*

Author	Significant changes in sporadic AD patients compared to aged controls	Significant changes in transgenic animals
Mecocci et al. (1997)	– elevated basal levels of oxidative DNA damage	
De Leo et al. (1998)	– increased Mn-superoxide-dismutase mRNA levels	
Morocz et al. (2002)	– elevated basal levels of oxidative DNA damage – elevated levels of oxidative DNA damage after oxidative stress (H_2O_2)	
Mecocci et al. (2002)	– elevated basal levels of oxidative DNA damage	
Kadioglu et al. (2004)	– elevated basal levels of oxidative DNA damage	
Migliore et al. (2005)	– elevated basal levels of oxidative DNA damage	
Leutner et. al. (2006)	– enhanced basal ROS levels – elevated ROS levels after staurosporine	
Schüssel et al. (2006)		– elevated ROS levels – increased HNE levels

Aβ mediated mitochondrial dysfunction (Keil et al., 2004; Marques et al., 2003).

Elevated oxidative stress is again not only found in neurons of AD patients but also in peripheral cells like lymphocytes and fibroblasts (Table 3) (Drouet et al., 1999; Schindowski et al., 2003; Huang et al., 2005). The leakage of reactive oxygen species (ROS) from mitochondria, e.g. the superoxide anion radical is converted to H_2O_2 which can take part in the Fenton reaction resulting in the production of the reactive hydroxyl radical cumulating in DNA-oxidation. Here, a major product is 8-hydroxy-2-deoxyguanosine (8-OHdG). Our group showed that lymphocytes from AD patients handle oxidative stress differently than lymphocytes of aged-matched controls. Firstly, lymphocytes of AD patients have increased basal ROS levels and secondly they react differently to oxidative stressors like staurosporine. They show increased levels of ROS after the treatment with staurosporine (Leutner et al., 2005). Our findings are supported by results of altered levels and activities of antioxidant enzymes. De Leo et al. provided evidence that the activity of the Cu/Zn superoxide-dismutase in red blood cells is significantly elevated and mRNA levels of Mn-superoxide dismutase are significantly increased in lymphocytes, supporting the hypothesis of an increased level of ROS in AD (De Leo et al., 1998). These results are supported by different groups (Mecocci et al., 1997, 2002; Cecchi et al., 2002; Morocz et al., 2002; Kadioglu et al., 2004; Migliore et al., 2005b). They all found significantly higher concentrations of 8-OHdG in different peripheral cell models. Supporting the hypothesis of elevated peripheral oxidative stress in AD, significantly lower plasma levels of antioxidants were detected in blood from sporadic AD patients compared to aged controls (Mecocci et al., 2002; Straface et al., 2005). Another group reported significantly elevated levels of oxidative DNA damage at basal levels in lymphocytes of sporadic AD and after additional oxidative stress induced by H_2O_2 (Morocz et al., 2002). In addition, DNA-oxidation altered activity and expression of antioxidant enzymes were found in peripheral blood cells of sporadic AD patients (De Leo et al., 1998).

In lymphoblasts and fibroblasts from familial AD patients with PS and APP mutations a clear increase in lipid-peroxidation products, MDA and HNE was found (Cecchi et al., 2002). Furthermore, the anti-oxidant capacity in lymphoblasts from peripheral blood of familial AD patients was reduced (Cecchi et al., 1999). These results are confirmed by findings in transgenic animals. Elevated ROS levels were found in lymphocytes of PS1 mutant mice (Eckert et al., 2001b; Schuessel et al., 2006).

Mitochondrial dysfunction in lymphocytes as a potential biomarker for AD

The increased ROS levels and enhanced apoptosis found in AD brain and periphery can be explained by mitochondrial dysfunction taken place in AD. Mitochondria are essential for the maintenance of cell function and viability. Mitochondria are the major source of ROS. They are exposed to high concentrations of ROS and may therefore be particularly susceptible to oxidative stress. Analyses of AD brains provide substantial evidence for disturbed mitochondrial energy metabolism (Beal, 2000) and for decreased glucose metabolism (Hoyer, 2000; Blass et al., 2002). These metabolic changes are due to the dysfunction of the mitochondrial electron transport enzymes. The most consistent finding in AD is a deficiency in complex I (cytochrome C oxidase) of the respiratory chain (Parker Jr, 1991; Parker Jr et al., 1994; Maurer et al., 2000; Butterfield et al., 2001). Additionally, a reduction of the activities of pyruvate dehydrogenase, isocitrate dehydrogenase and α-ketoglutarate were found in AD brains (Bubber et al., 2005). Fur-

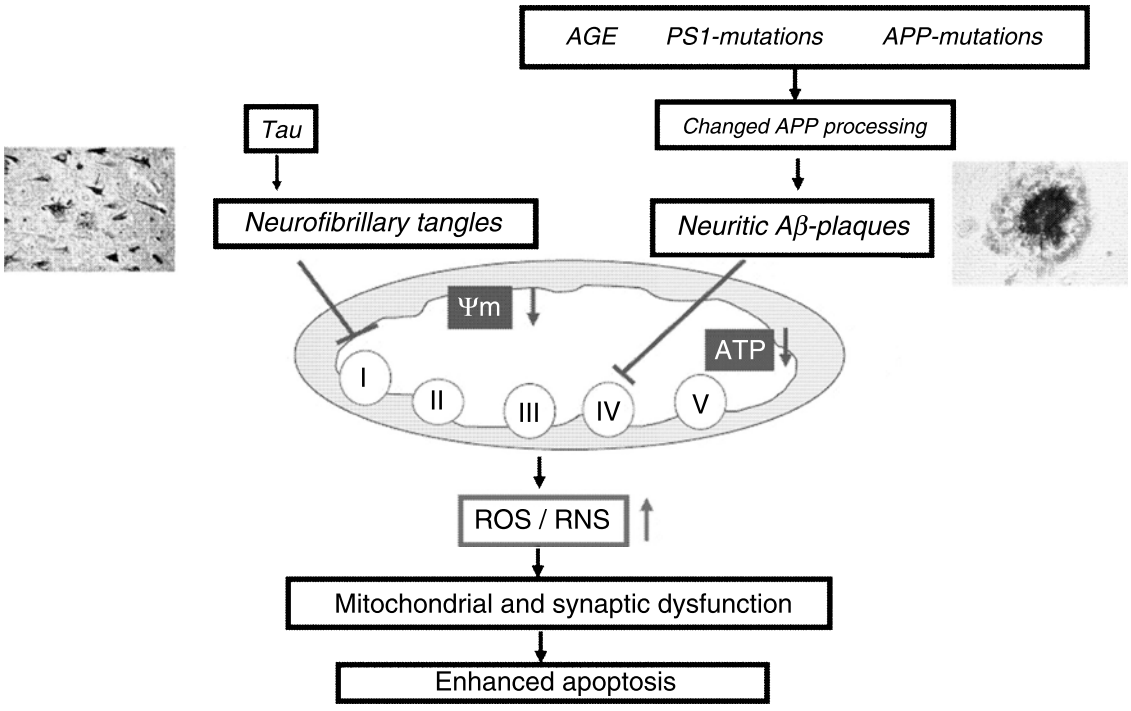

Fig. 3. Mitochondrial dysfunction as an early event in sporadic and familiar AD. Mitochondrial dysfunction as an early common pathway of aging, tau pathology and other unknown risk factors of sporadic AD as well as APP and PS1 mutations, modified according to Hauptmann et al., 2006

thermore, Hirai et al. found an increase in mitochondrial DNA in neurons of AD patients and ultrastructural changes of the mitochondria (Hirai et al., 2001; Rodriguez-Santiago and Nunes, 2005). These results are confirmed in AD animal and cell models (Anandatheerthavarada et al., 2003; Blanchard et al., 2003; Marques et al., 2003; Keil et al., 2004). Our group found decreased mitochondrial membrane potential and diminished enzymatic activity of respiratory chain complexes III and IV in 3 months old APP transgenic mice, which show no Aβ plaques at this age. We suggest that oligomeric Aβ induces mitochondrial dysfunction in these mice (Hauptmann et al., 2006). Therefore, we suggest that mitochondrial dysfunction is an early event in AD leading to several pathological features of this disease. In addition, we determined reduced complex I activity, impaired mitochondrial respiration and ATP synthesis in P301L tau transgenic mice (David et al., 2005). We propose the following hypothetical sequence of events linking to AD (see Fig. 3). Aβ as well as Tau pathology lead to mitochondrial dysfunction before Aβ plaques or Tau tangles can be detected. Consequently, ATP levels are reduced and ROS production is increased. We suggest that when the inhibition of mitochondrial function has reached a threshold and severe energy deprivation appears, mitochondrial and synaptic dysfunction can appear. Therefore, mitochondrial dysfunction could be an early marker for AD. Furthermore, the detection of mitochondrial dysfunction could become a tool to distinguish between MCI patients who develop AD or not.

Again, mitochondrial dysfunction was not only observed in brains of AD patients, but also in peripheral tissues such as platelets. Several studies showed a decreased cytochrome c activity in human platelets from AD patients (Bosetti et al., 2002; Mancuso et al., 2003; Cardoso et al., 2004). In accordance with these findings, platelets of AD patients show decreased ATP levels and increased levels of ROS (Cardoso et al., 2004).

In lymphocytes of sporadic AD patients only few studies were conducted referring to mitochondrial dysfunction. Our group investigated a protein factor that act upstream of mitochondrial dysfunction, Bcl2 (Schindowski et al., 2006). The antiapoptotic Bcl2 can form heteromers with the proapoptotic Bax and can therefore prevent its apoptogenic activity (Culmsee and Landshamer, 2006). We found a tendency of elevated Bcl2 in T cells of sporadic AD patients compared to aged controls. Again, $CD4^+$ cells were more sensitive to AD related changes. Bcl2 levels were significantly elevated in $CD4^+$ cells compared to $CD8^+$ cells. Interestingly, when splitting up AD-patients into mild (MMSE >20) and severe (MMSE <20) AD, a dual regulation was observed. Bcl2 is up-regulated in mild AD while further progression of the disease the Bcl2 content decreases with cognitive loss. We

Table 4. *Mitochondrial function in a preliminary set of patients with AD or MCI compared to aged controls*

	Mitochondrial membrane potential		ATP levels	
	AD	MCI	AD	MCI
Complex I (Rotenone)	$\downarrow^{*}$	ns	ns	ns
Complex II Thenoyltrifluoroaceton	ns	ns	$\downarrow^{**}$	ns
Complex III Antimycine	$\downarrow^{*}$	$\downarrow^{*}$	ns	ns
Complex IV Natriumazide	$\downarrow^{*}$	$\downarrow^{*}$	ns	ns
Complex V Oligomycine	$\downarrow^{*}$	$\downarrow^{*}$	ns	ns

Mitochondrial dysfunction was investigated in 8–12 AD patients, MCIs and aged controls. MMSE aged controls 29.88 ± 0.35, MCI 27.33 ± 3.04, AD patients 20.12 ± 5.78

ns not significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $\downarrow$ reduction relating to control

suggest that in the early stage of AD, Bcl2 is up-regulated to protect cells against apoptosis. Furthermore, we found in a preliminary set of patients (Table 4) increased sensitivity of complex I–V in lymphocytes of AD patients compared to aged controls (Table 4). The mitochondrial membrane potential was significantly reduced after stressing lymphocytes of AD patients with complex I, III, IV, and V inhibitors of the respiratory chain. Importantly, here we found a graduation of susceptibility to complex inhibitors between AD patients, MCIs and aged controls. Additionally, there was a significant decrease in ATP-levels graduated from AD patients to MCI and aged controls after stimulation with the complex II inhibitor.

However, other groups investigated the basal activities of the complexes of the respiratory chain in lymphocytes of sporadic AD patients. They found no significant differences between aged controls and AD patients (Molina et al., 1997; Casademont et al., 2003).

Conclusion

In several studies, lymphocytes were shown to be a suitable cell model studying pathological changes in AD. This cell type shows similar vulnerability to AD relevant stressors like Aβ1-42 or nitrosative or oxidative stress *in vitro*. Increased basal apoptosis, elevated ROS levels, altered levels of antioxidant enzymes, elevated hydroxyl radical induced DNA-oxidation and increased mitochondrial susceptibility were found in AD patients compared to controls.

According to the proposal of a consensus group on molecular and biochemical markers for AD (Consensus report of the Working group on molecular and Biochemical Markers of Alzheimer Disease, 1998), an ideal biomarker should detect the essential feature of neuropathology of AD. Its sensitivity for detecting AD and its specificity for distinguishing other dementias should be more than 80%. Also, the biomarker should be reliable, reproducible, non-invasive, simple to perform and inexpensive. Keeping these requirements in mind, lymphocytes are an adequate biomarker model. Lymphocytes can be easily obtained from blood samples. Their separation is inexpensive and time-saving. Repeated samples from patients can be taken as the particular study requires.

Considering the applicability of the above discussed parameters, elevated apoptosis, increased oxidative stress and mitochondrial dysfunction are essential for the neuropathology of AD. Therefore, they meet one crucial criteria of the consensus group. Regarding the specificity, we detected robust differences in cell death susceptibility between AD and vascular dementia. For oxidative stress and mitochondrial dysfunction, studies comparing different forms of dementia need to be conducted. Furthermore considering the reliability, elevated apoptosis and increased oxidative stress were found in many studies. The measurement of oxidative stress as a biomarker has one disadvantage. Oxidative stress is also found in other neurodegenerative disease e.g. Parkinson disease (PD). Increased levels of MDA in serum, plasma and CSF for example were observed in PD patients (Ilic et al., 1999). Furthermore, elevated oxidative DNA-damage could be detected in lymphocytes of PD patients (Petrozzi et al., 2002) as well as significantly increased levels of 8-OHdG in serum and CSF of PD patients (Kikuchi et al., 2002).

From our point of view, mitochondrial dysfunction could be a promising concept as a biomarker for AD. Although in AD, like in PD no basal changes of complex activities of the respiratory chain in lymphocytes could be detected (Martin et al., 1996), we found enhanced susceptibility of complex I–V of the respiratory chain in a small sample of AD patients. Importantly, only here a graduation of susceptibility between AD patients, MCIs and aged controls could be detected. These results need to be confirmed in larger sample of patients.

Taken together, lymphocytes are a promising cell model for establishing biomarkers for AD, but further studies need to be conducted to evaluate which is the most adequate biomarker.

References

Abdul HM, Sultana R, Keller JN, St Clair DK, Markesbery WR, Butterfield DA (2006) Mutations in amyloid precursor protein and presenilin-1 genes increase the basal oxidative stress in murine neuronal cells and lead to increased sensitivity to oxidative stress mediated by amyloid beta-peptide (1–42), H_2O_2 and kainic acid: implications for Alzheimer's disease. J Neurochem 96: 1322–1335

Aksenov MY, Tucker HM, Nair P, Aksenova MV, Butterfield DA, Estus S, Markesbery WR (1998) The expression of key oxidative stress-han-

dling genes in different brain regions in Alzheimer's disease. J Mol Neurosci 11: 151–164

Anandatheerthavarada HK, Biswas G, Robin MA, Avadhani NG (2003) Mitochondrial targeting and a novel transmembrane arrest of Alzheimer's amyloid precursor protein impairs mitochondrial function in neuronal cells. J Cell Biol 161: 41–54

Beal MF (2000) Energetics in the pathogenesis of neurodegenerative diseases. Trends Neurosci 23: 298–304

Blanchard V, Moussaoui S, Czech C, Touchet N, Bonici B, Planche M, Canton T, Jedidi I, Gohin M, Wirths O, Bayer TA, Langui D, Duyckaerts C, Tremp G, Pradier L (2003) Time sequence of maturation of dystrophic neurites associated with A beta deposits in APP/PS1 transgenic mice. Exp Neurol 184: 247–263

Blass JP, Gibson GE, Hoyer S (2002) The role of the metabolic lesion in Alzheimer's disease. J Alzheimers Dis 4: 225–232

Borroni B, Di Luca M, Padovani A (2006) Predicting Alzheimer dementia in mild cognitive impairment patients. Are biomarkers useful? Eur J Pharmacol 545: 73–80

Bosetti F, Brizzi F, Barogi S, Mancuso M, Siciliano G, Tendi EA, Murri L, Rapoport SI, Solaini G (2002) Cytochrome c oxidase and mitochondrial F1F0-ATPase (ATP synthase) activities in platelets and brain from patients with Alzheimer's disease. Neurobiol Aging 23: 371–376

Bubber P, Haroutunian V, Fisch G, Blass JP, Gibson GE (2005) Mitochondrial abnormalities in Alzheimer brain: mechanistic implications. Ann Neurol 57: 695–703

Butterfield DA, Aksenov M, Markesbery WR (2001) Altered expression of cytochrome c oxidase and NADH dehydrogenase in Alzheimer's disease brain: implications for oxidative stress and neurodegeneration. J Neurochem 77: 16

Cardoso SM, Proenca MT, Santos S, Santana I, Oliveira CR (2004) Cytochrome c oxidase is decreased in Alzheimer's disease platelets. Neurobiol Aging 25: 105–110

Casademont J, Miro O, Rodriguez-Santiago B, Viedma P, Blesa R, Cardellach F (2003) Cholinesterase inhibitor rivastigmine enhance the mitochondrial electron transport chain in lymphocytes of patients with Alzheimer's disease. J Neurol Sci 206: 23–26

Cecchi C, Fiorillo C, Sorbi S, Latorraca S, Nacmias B, Bagnoli S, Nassi P, Liguri G (2002) Oxidative stress and reduced antioxidant defenses in peripheral cells from familial Alzheimer's patients. Free Radic Biol Med 33: 1372–1379

Cecchi C, Latorraca S, Sorbi S, Iantomasi T, Favilli F, Vincenzini MT, Liguri G (1999) Gluthatione level is altered in lymphoblasts from patients with familial Alzheimer's disease. Neurosci Lett 275: 152–154

Culmsee C, Landshamer S (2006) Molecular insights into mechanisms of the cell death program: role in the progression of neurodegenerative disorders. Curr Alzheimer Res 3: 269–283

David DC, Hauptmann S, Scherping I, Schuessel K, Keil U, Rizzu P, Ravid R, Drose S, Brandt U, Muller WE, Eckert A, Gotz J (2005) Proteomic and functional analyses reveal a mitochondrial dysfunction in P301L Tau transgenic mice. J Biol Chem 280: 23802–23814

De Leo ME, Borrello S, Passantino M, Palazzotti B, Mordente A, Daniele A, Filippini V, Galeotti T, Masullo C (1998) Oxidative stress and overexpression of manganese superoxide dismutase in patients with Alzheimer's disease. Neurosci Lett 250: 173–176

Deshpande A, Mina E, Glabe C, Busciglio J (2006) Different conformations of amyloid beta induce neurotoxicity by distinct mechanisms in human cortical neurons. J Neurosci 26: 6011–6018

Drouet M, Lauthier F, Charmes JP, Sauvage P, Ratinaud MH (1999) Age-associated changes in mitochondrial parameters on peripheral human lymphocytes. Exp Gerontol 34: 843–852

Eckert A, Cotman CW, Zerfass R, Hennerici M, Muller WE (1998) Lymphocytes as cell model to study apoptosis in Alzheimer's disease: vulnerability to programmed cell death appears to be altered. J Neural Transm 259–267

Eckert A, Oster M, Zerfass R, Hennerici M, Muller WE (2001a) Elevated levels of fragmented DNA nucleosomes in native and activated lymphocytes indicate an enhanced sensitivity to apoptosis in sporadic Alzheimer's disease – Specific differences to vascular dementia. Dement Geriatr Cogn 12: 98–105

Eckert A, Schindowski K, Leutner S, Luckhaus C, Touchet N, Czech C, Muller WE (2001b) Alzheimer's disease-like alterations in peripheral cells from presenilin-1 transgenic mice. Neurobiol Dis 8: 331–342

Eckert A, Steiner B, Marques C, Leutz S, Romig H, Haass C, Muller WE (2001d) Elevated vulnerability to oxidative stress-induced cell death and activation of caspase-3 by the Swedish amyloid precursor protein mutation. J Neurosci Res 64: 183–192

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

Ferris SH, Yan B (2003) Differential diagnosis and clinical assessment of patients with severe Alzheimer disease. Alz Dis Assoc Dis 17 Suppl 3: S92–S95

Frey C, Bonert A, Kratzsch T, Rexroth G, Rosch W, Muller-Spahn F, Maurer K, Muller WE, Eckert A (2006) Apolipoprotein E epsilon 4 is associated with an increased vulnerability to cell death in Alzheimer's disease. J Neural Transm 113: 1753–1761

Frisoni GB, Padovani A, Wahlund LO (2004) The predementia diagnosis of Alzheimer disease. Alz Dis Assoc Dis 18: 51–53

Guo Q, Sopher BL, Furukawa K, Pham DG, Robinson N, Martin GM, Mattson MP (1997) Alzheimer's presenilin mutation sensitizes neural cells to apoptosis induced by trophic factor withdrawal and amyloid beta-peptide: Involvement of calcium and oxyradicals. J Neurosci 17: 4212–4222

Hauptmann S, Keil U, Scherping I, Bonert A, Eckert A, Muller WE (2006) Mitochondrial dysfunction in sporadic and genetic Alzheimer's disease. Exp Gerontol 41: 668–673

Hirai K, Aliev G, Nunomura A, Fujioka H, Russell RL, Atwood CS, Johnson AB, Kress Y, Vinters HV, Tabaton M, Shimohama S, Cash AD, Siedlak SL, Harris PLR, Jones PK, Petersen RB, Perry G, Smith MA (2001) Mitochondrial abnormalities in Alzheimer's disease. J Neurosci 21: 3017–3023

Hoyer S (2000) Brain glucose and energy metabolism abnormalities in sporadic Alzheimer disease. Causes and consequences: an update. Exp Gerontol 35: 1363–1372

Huang HM, Fowler C, Xu H, Zhang H, Gibson GE (2005) Mitochondrial function in fibroblasts with aging in culture and/or Alzheimer's disease. Neurobiol Aging 26: 839–848

Huang YD (2006) Apolipoprotein E and Alzheimer disease. Neurology 66: S79–S85

Ilic TV, Jovanovic M, Jovicic A, Tomovic M (1999) Oxidative stress indicators are elevated in de novo Parkinson's disease patients. Funct Neurol 14: 141–147

Jellinger KA (2006) Challenges in neuronal apoptosis. Curr Alzheimer Res 3: 377–391

Kadioglu E, Sardas S, Aslan S, Isik E, Esat KA (2004) Detection of oxidative DNA damage in lymphocytes of patients with Alzheimer's disease. Biomarkers 9: 203–209

Keil U, Bonert A, Marques CA, Scherping I, Weyermann J, Strosznajder JB, Muller-Spahn F, Haass C, Czech C, Pradier L, Muller WE, Eckert A (2004) Amyloid beta-induced changes in nitric oxide production and mitochondrial activity lead to apoptosis. J Biol Chem 279: 50310–50320

Kikuchi A, Takeda A, Onodera H, Kimpara T, Hisanaga K, Sato N, Nunomura A, Castellani RJ, Perry G, Smith MA, Itoyama Y (2002) Systemic increase of oxidative nucleic acid damage in Parkinson's disease and multiple system atrophy. Neurobiol Dis 9: 244–248

Kriem B, Sponne I, Fifre A, Malaplate-Armand C, Lozac'h-Pillot K, Koziel V, Yen-Potin FT, Bihain B, Oster T, Olivier JL, Pillot T (2005) Cytosolic

phospholipase A2 mediates neuronal apoptosis induced by soluble oligomers of the amyloid-beta peptide. FASEB J 19: 85–87

Leutner S, Schindowski K, Frolich L, Maurer K, Kratzsch T, Eckert A, Muller WE (2005) Enhanced ROS-generation in lymphocytes from Alzheimer's patients. Pharmacopsychiatry 38: 312–315

Leutz S, Steiner B, Marques CA, Haass C, Muller WE, Eckert A (2002) Reduction of trophic support enhances apoptosis in PC12 cells expressing Alzheimer's APP mutation and sensitizes cells to staurosporine-induced cell death. J Mol Neurosci 18: 189–201

Lombardi VR, Fernandez-Novoa L, Etcheverria I, Seoane S, Cacabelos R (2004) Association between APOE epsilon4 allele and increased expression of CD95 on T cells from patients with Alzheimer's disease. Method Find Exp Clin 26: 523–529

Malaplate-Armand C, Florent-Bechard S, Youssef I, Koziel V, Sponne I, Kriem B, Leininger-Muller B, Olivier JL, Oster T, Pillot T (2006) Soluble oligomers of amyloid-beta peptide induce neuronal apoptosis by activating a cPLA2-dependent sphingomyelinase-ceramide pathway. Neurobiol Dis 23: 178–189

Mancuso M, Filosto M, Bosetti F, Ceravolo R, Rocchi A, Tognoni G, Manca ML, Solaini G, Siciliano G, Murri L (2003) Decreased platelet cytochrome c oxidase activity is accompanied by increased blood lactate concentration during exercise in patients with Alzheimer disease. Exp Neurol 182: 421–426

Marques CA, Keil U, Bonert A, Steiner B, Haass C, Muller WE, Eckert A (2003) Neurotoxic mechanisms caused by the Alzheimer's disease-linked Swedish amyloid precursor protein mutation – Oxidative stress, caspases, and the JNK pathway. J Biol Chem 278: 28294–28302

Martin MA, Molina JA, Jimenez-Jimenez FJ, Benito-Leon J, Orti-Pareja M, Campos Y, Arenas J (1996) Respiratory-chain enzyme activities in isolated mitochondria of lymphocytes from untreated Parkinson's disease patients. Grupo-Centro de Trastornos del Movimiento. Neurology 46: 1343–1346

Mattson MP (2002) Oxidative stress, perturbed calcium homeostasis, and immune dysfunction in Alzheimer's disease. J Neurovirol 8: 539–550

Mattson MP (2004) Pathways towards and away from Alzheimer's disease. Nature 430: 631–639

Maurer I, Zierz S, Moller HJ (2000) A selective defect of cytochrome c oxidase is present in brain of Alzheimer disease patients. Neurobiol Aging 21: 455–462

Mecocci P, Cherubini A, Senin U (1997) Increased oxidative damage in lymphocytes of Alzheimer's disease patients. J Am Geriatr Soc 45: 1536–1537

Mecocci P, Polidori MC, Cherubini A, Ingegni T, Mattioli P, Catani M, Rinaldi P, Cecchetti R, Stahl W, Senin U, Beal MF (2002) Lymphocyte oxidative DNA damage and plasma antioxidants in Alzheimer disease. Arch Neurol 59: 794–798

Migliore L, Fontana I, Colognato R, Coppede F, Siciliano G, Murri L (2005a) Searching for the role and the most suitable biomarkers of oxidative stress in Alzheimer's disease and in other neurodegenerative diseases. Neurobiol Aging 26: 587–595

Migliore L, Fontana I, Trippi F, Colognato R, Coppede F, Tognoni G, Nucciarone B, Siciliano G (2005b) Oxidative DNA damage in peripheral leukocytes of mild cognitive impairment and AD patients. Neurobiol Aging 26: 567–573

Molina JA, deBustos F, JimenezJimenez FJ, BenitoLeon J, Gasalla T, OrtiPareja M, Vela L, Bermejo F, Martin MA, Campos Y, Arenas J (1997) Respiratory chain enzyme activities in isolated mitochondria of lymphocytes from patients with Alzheimer's disease. Neurology 48: 636–638

Morocz M, Kalman J, Juhasz A, Sinko I, McGlynn AP, Downes CS, Janka Z, Rasko I (2002) Elevated levels of oxidative DNA damage in lymphocytes from patients with Alzheimer's disease. Neurobiol Aging 23: 47–53

Murray IVJ, Sindoni ME, Axelsen PH (2005) Promotion of oxidative lipid membrane damage by amyloid beta proteins. Biochemistry 44: 12606–12613

Parker WD Jr (1991) Cytochrome oxidase deficiency in Alzheimer's disease. Ann NY Acad Sci 640: 59–64

Parker WD Jr, Parks J, Filley CM, Kleinschmidt-DeMasters BK (1994) Electron transport chain defects in Alzheimer's disease brain. Neurology 44: 1090–1096

Parshad R, Sanford KK, Price FM, Melnick LK, Nee LE, Schapiro MB, Tarone RE, Robbins JH (1996) Fluorescent light-induced chromatid breaks distinguish Alzheimer disease cells from normal cells in tissue culture. Proc Nat Acad Sci USA 93: 5146–5150

Perry G, Nunomura A, Hirai K, Takeda A, Aliev G, Smith MA (2000) Oxidative damage in Alzheimer's disease: the metabolic dimension. Int J Devel Neurosci 18: 417–421

Petrozzi L, Lucetti C, Scarpato R, Gambaccini G, Trippi F, Bernardini S, Del Dotto P, Migliore L, Bonuccelli U (2002) Cytogenetic alterations in lymphocytes of Alzheimer's disease and Parkinson's disease patients. Neurol Sci 23 Suppl 2: S97–S98

Rodriguez-Santiago B, Nunes V (2005) Expression of mitochondrial genes and transcription estimation in different brain areas in Alzheimer's disease patients. Neurobiol Dis 18: 296–304

Schindowski K, Leutner S, Muller WE, Eckert A (2000) Age-related changes of apoptotic cell death in human lymphocytes. Neurobiol Aging 21: 661–670

Schindowski K, Frohlich L, Maurer K, Muller WE, Eckert A (2002) Age-related impairment of human T lymphocytes' activation: specific differences between CD4(+) and CD8(+) subsets. Mech Ageing Dev 123: 375–390

Schindowski K, Kratzsch T, Peters J, Steiner B, Leutner S, Touchet N, Maurer K, Czech C, Pradier L, Frolich L, Muller WE, Eckert A (2003) Impact of aging: sporadic, and genetic risk factors on vulnerability to apoptosis in Alzheimer's disease. Neuromol Med 4: 161–178

Schindowski K, Peters J, Gorriz C, Schramm U, Weinandi T, Leutner S, Maurer K, Frolich L, Muller WE, Eckert A (2006) Apoptosis of CD4+ T and natural killer cells in Alzheimer's disease. Pharmacopsychiatry 39: 220–228

Schuessel K, Schafer S, Bayer TA, Czech C, Pradier L, Muller-Spahn F, Muller WE, Eckert A (2005) Impaired Cu/Zn-SOD activity contributes to increased oxidative damage in APP transgenic mice. Neurobiol Dis 18: 89–99

Schuessel K, Frey C, Jourdan C, Keil U, Weber CC, Muller-Spahn F, Muller WE, Eckert A (2006) Aging sensitizes toward ROS formation and lipid peroxidation in PS1M146L transgenic mice. Free Radic Biol Med 40: 850–862

Selkoe DJ (2004) Alzheimer disease: Mechanistic understanding predicts novel therapies. Ann Int Med 140: 627–638

Straface E, Matarrese P, Gambardella L, Vona R, Sgadari A, Silveri MC, Malorni W (2005) Oxidative imbalance and cathepsin D changes as peripheral blood biomarkers of Alzheimer disease: A pilot study. FEBS Lett 579: 2759–2766

Tacconi S, Perri R, Balestrieri E, Grelli S, Bernardini S, Annichiarico R, Mastino A, Caltagirone C, Macchi B (2004) Increased caspase activation in peripheral blood mononuclear cells of patients with Alzheimer's disease. Exp Nephrol 190: 254–262

Turner RS (2006) Alzheimer's disease. Semin Neurol 26: 499–506

Wimo A, Winblad B, Aguero-Torres H, von Strauss E (2003) The magnitude of dementia occurrence in the world. Alz Dis Assoc Dis 17: 63–67