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Forum Review

Mitochondrial Cascade Hypothesis of Alzheimer's Disease: Myth or Reality?

MICHELANGELO MANCUSO, FABIO COPPEDÈ, LUIGI MURRI, and GABRIELE SICILIANO

ABSTRACT

Mitochondria are recognized to play a pivotal role in neuronal cell survival or death because they are regulators of both energy metabolism and apoptotic pathways. Morphologic, biochemical, and molecular genetic studies suggest that mitochondria might be a convergence point for neurodegeneration, including Alzheimer's disease (AD). The functions and properties of mitochondria might render subsets of selectively vulnerable neurons intrinsically susceptible to cellular aging and stress. However, the question, "Is mitochondrial dysfunction a necessary step in neurodegeneration?" is still unanswered. This review presents the ways in which malfunctioning mitochondria and oxidative stress might contribute to neuronal death in AD. *Antioxid. Redox Signal.* 9, 1631–1646.

INTRODUCTION

Alzheimer's disease (AD) is devastating, and, despite great strides in recent years, still puzzling in its causes and mechanisms. Although specific mutations in amyloid-β (Aβ) protein precursor (*APP*) and presenilin (*PS*) genes have been associated with the relatively rare forms of familial AD, the causes of the much more frequently occurring sporadic AD (SAD) accounting for 90–95% of AD cases, remain unknown, and the mechanisms leading to neuronal death are still unclear. To date, the amyloid-β cascade hypothesis remains the main pathogenetic model of AD (69). However, although this cascade is potentially viable in familial AD cases with mutation in *APP* and *PS* genes, its role in the SAD is unclear (97).

In the past 15 years, research has been directed at clarifying the involvement of mitochondria and defects in mitochondrial oxidative phosphorylation (OXPHOS) in late-onset neurodegenerative disorders, including AD (5, 62). Although the aetiology of AD remains largely unclear, accumulating evidence suggests that mitochondrial dysfunction and oxidative stress occur in brain and peripheral tissues of AD patients, supporting the idea that mitochondria may trigger the abnormal onset of neuronal degeneration and death in AD.

This article focuses on the role of mitochondria and its metabolism in AD and reviews some of the recent relevant genetic and biochemical data.

BASICS OF MITOCHONDRIAL GENETICS

Mitochondria evolved from a symbiotic relation between aerobic bacteria and primordial eukaryotic cells unable to use oxygen, developed more than a billion years ago. This symbiotic relation became permanent as the bacteria evolved into mitochondria, providing to the host cells the aerobic metabolism, a much more efficient way to produce energy than is anaerobic glycolysis (27). As the site of oxidative phosphorylation, these double-membrane organelles provide a highly efficient route for eukaryotic cells to generate ATP from energy-rich molecules. Electrons from oxidative substrates are transferred to oxygen, *via* a series of redox reactions, to generate water (32). In the process, protons are pumped from the matrix across the mitochondrial inner membrane through the electron transport chain (ETC, which consists of four multimeric complexes—I to IV—plus two small electron carriers, coenzyme Q—or ubiquinone—and cytochrome *c*). When protons return to the

Department of Neuroscience, Neurological Clinic, University of Pisa, Italy.

mitochondrial matrix down their electrochemical gradient, ATP is synthesized *via* complex V (ATP synthase) (75). Mitochondria are the only organelles of the cell besides the nucleus that contain their own DNA (mitochondrial DNA; mtDNA) and their own machinery for synthesizing RNA and proteins (27). Mitochondria have adapted to their new intracellular environment by reducing their genome size to ~16,500 base pairs.

This reduction has increased their replication rate and, thus, ensures the transmission of the mitochondrial genome to two daughter cells. This improved transmission is assumed to be accomplished by the transfer of many essential genes to the nucleus, where the proteins are transcribed into mRNA, translated on cytoplasmic ribosomes, and selectively imported back into the mitochondrion. Human mtDNA (Fig. 1) is a 16,569-kb circular, double-stranded molecule, which contains 37 genes: two rRNA genes, 22 tRNA genes, and 13 structural genes encoding subunits of the mitochondrial respiratory chain, the "gas station" of oxidative phosphorylation, where ATP is generated (27). In addition to its mRNA, rRNA, and tRNAs genes, the mtDNA encompasses a 1-Kb control region.

MITOCHONDRIA, APOPTOSIS, AND FREE RADICAL GENERATION

Despite the critical role of the mitochondrion in maintaining life, it plays an equally important role in mediating cell-death pathways, namely apoptosis. Apoptosis or programmed cell death (PCD) is a physiologic process necessary for organ development, tissue homeostasis, and elimination of defective or potentially dangerous cells without a concomitant inflammatory response in the surrounding tissues. Apoptosis is characterized by cell shrinkage, DNA fragmentation, chromatin condensation, and nuclear fragmentation, and is a mechanism involved in many physiologic and pathologic processes, including cancer and neurodegeneration (55).

Proteins released from mitochondria into the cytosol are important inducers of apoptosis. Indeed, the molecular mechanisms of the biochemical cascades of apoptosis are beginning to be understood and involve several effectors that mediate mitochondrial dysfunction and the subsequent release of proapoptotic proteins, which are able to trigger intracellular pathways, finally resulting in degradation of proteins and DNA. Initiation of apoptosis can be activated either by cell-surface death receptors or by the release of cytochrome *c* from the mitochondria (mitochondrial pathway) into the cytosol; however, both pathways converge and result in the activation of a family of cysteine proteases called caspases, the intracellular executors of apoptosis, ultimately leading to the activation of caspase-dependent nucleases, which result in DNA fragmentation. Death receptors such as Fas receptor and tumor necrosis factor receptor 1 (TNF-R1) contain an intracellular domain termed the death domain, with which they can interact with downstream molecules, leading to the activation of caspases. The mitochondrial pathway is initiated by the withdrawal of growth factors and is regulated by members of the Bcl-2 family proteins such as Bid, Bax, and Bad. A third pathway of apoptosis is initiated by DNA damage and mediated through a p53-dependent mechanism. Together with classic apoptosis, PCD also can occur in an apoptosis-like or caspase-independent mitochondrial manner, in which the apoptosis-inducing factor (AIF) is re-

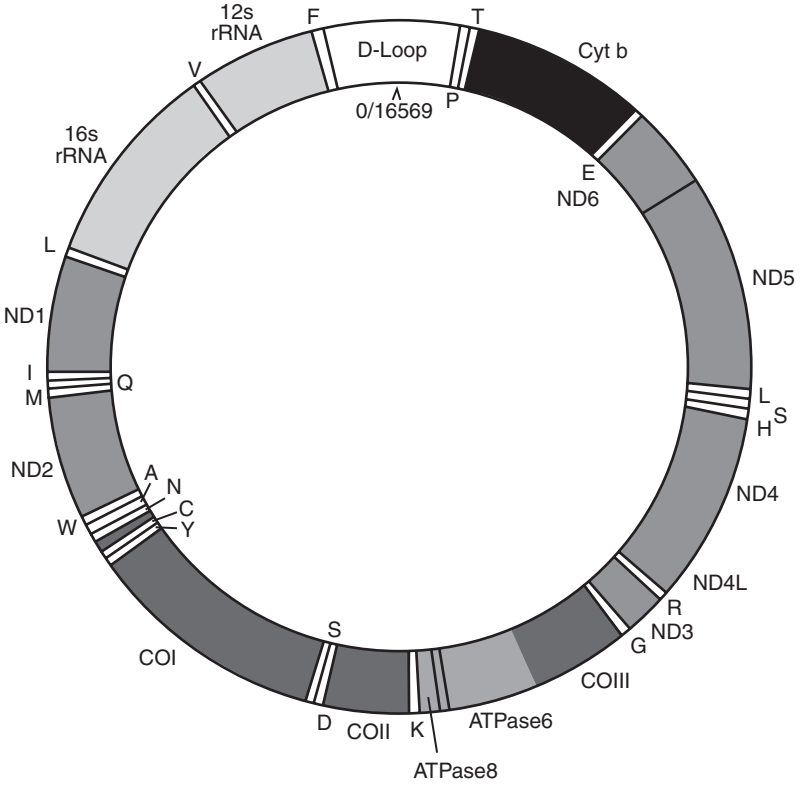

FIG. 1. Mitochondrial DNA (modified from MITOMAP: http://www.mitomap.org).

leased from mitochondria and translocated to the nucleus, with a subsequent recruitment and activation of DNA nucleases (for a review, see ref. 55). The mitochondrial intermembrane space sequesters many proapoptotic factors such as cytochrome *c*, Smac/DIABLO, and endonuclease G. A multiplicity of mitochondrial insults can cause the release of cytochrome *c* into the cytosol, which can then bind to Apaf1 and pro-caspase 9 to form the apoptosome and initiate a downstream caspase cascade, leading to cell death (22). During apoptosis, engagement of the mitochondrial pathway involves the permeabilization of the outer mitochondrial membrane (OMM), which leads to the release of cytochrome *c* and other apoptogenic factors (34). The proteins of the Bcl-2 family, such as Bax or Bak, are key regulators of this program, and their main function is to control mitochondrial permeability and, particularly, the release of apoptogenic proteins from this organelle. Another early characteristic of apoptotic cell death is the disruption of ETC (42). Aβ toxicity in neurons induces apoptosis and a disruption in the ETC (65). Finally, mitochondrial function is disrupted during apoptosis by caspase cleavage of the p75 subunit of complex I of the ETC (89).

The transport of high-energy electrons through the ETC can also be a source not only of ATP but also of reactive oxygen species (ROS), as the high-energy electrons can react with O_2 to form superoxide (Fig. 2) (44). It has been estimated that up to 2% of the O_2 consumed by healthy mitochondria is converted to superoxide, and this amount is higher in damaged and aged mitochondria. A wide variety of ROS are produced in the course of normal metabolism in biologic systems, and they have several important physiologic functions. However, the accumulation of ROS beyond the needs of the cell can potentially damage several biomolecules, including lipids, proteins, and nucleic acids. The cells possess an intricate network of defense mechanisms to neutralize excessive accumulation of ROS and, under physiologic conditions, are able to cope with the flux of ROS. During the transfer of electrons to molecular oxygen, an estimated 1–5% of electrons in the respiratory chain lose their way, but the remaining majority participate in the formation of O_2^-. When the ETC is inhibited, the electrons accumulate in the early stages of the ETC (complex I and coenzyme Q), where they are donated directly to molecular oxygen to give a superoxide anion (115). The superoxide anion is detoxified by the mitochondrial Mn superoxide dismutase to give hydrogen peroxide (H_2O_2) (see Figs. 2 and 4). Then H_2O_2 is converted to H_2O by glutathione peroxidase. Chronic ROS exposure can result in oxidative damage to mitochondrial and cellular proteins, lipids, and nucleic acids, and acute ROS exposure can inactivate the TCA cycle aconitase and the iron–sulfur centers of ETC complexes I, II, and III, resulting in a shutdown of mitochondrial energy production (115). Oxidative stress describes a condition in which cellular antioxidant defenses are insufficient to keep the levels of ROS below a toxic threshold. Some tissues, especially the brain, are much more vulnerable to oxidative stress because of their elevated consumption of oxygen required to produce energy and the consequent generation of large amounts of ROS. It is well documented that free radical–induced oxidative damage, particularly to neuronal lipids, nucleic acids, and proteins, is extensive in the brain of AD individuals. Peroxidation of cellular membrane lipids in AD brains is marked by increased levels of 4-hydroxynonenal (HNE), malondialdehyde (MDA), lipid hydroperoxides and isoprostanes, and thiobarbituric acid–reactive substances (TBARS). Regarding nucleic acids, increased levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) have been observed in both nuclear and mitochondrial DNA (nDNA and mtDNA) isolated from brain regions of AD subjects. DNA is not the only nucleic acid undergoing oxidative modification in AD, and a growing body of evidence indicates a role for RNA oxidation in the pathophysiology of AD. Concerning protein oxidation in AD brain, oxidative attack on proteins results in the formation of protein carbonyls, often with loss of functionality of the parent protein. Protein carbonyls are a common finding in brain samples from AD individuals. Moreover, in AD pathogenesis, evidence exists for lipid peroxidation–derived protein modifications. (for a recent review on markers of oxidative damage in AD, see ref. 62). Nitric oxide (NO) is a gas synthesized by a family of enzymes denominated nitric oxide synthases (NOS), present in most of the cells of the body. NO is involved in several cellular functions, and, depending on the site of production, it works as a vasorelaxant agent, as a neurotransmitter, and participates in the immune response. NO is thermodynamically unstable, and if produced in excess, it changes from a neuromodulator to a neurotoxic factor. NO became particularly harmful under pathologic conditions involving the production of ROS, when it undergoes oxidative/reductive reactions, producing toxic compounds (Figs. 3 and 4), leading to protein oxidation, nitrosylation, and nitration. It has been largely demonstrated that several brain regions in AD have higher nitrotyrosination levels than those of controls (43). The use of proteomics is a promising tool to identify specifically the oxidatively modified proteins in AD brain, providing insights into potential pathways involved in neurodegeneration. Proteomic studies indicated several such proteins that can be classified as those dealing with energy metabolism, glutamate reuptake, ac-

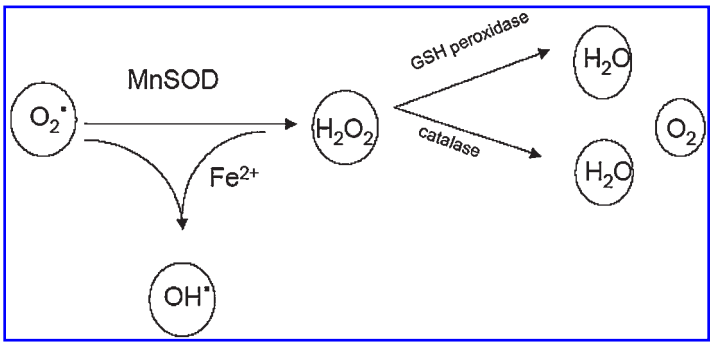

FIG. 2. ROS production in the cell. $O_2^{\cdot}$, superoxide anion; $OH^{\cdot}$, hydroxyl radical; MnSOD, manganese superoxide dismutase; H_2O_2, hydrogen peroxide.

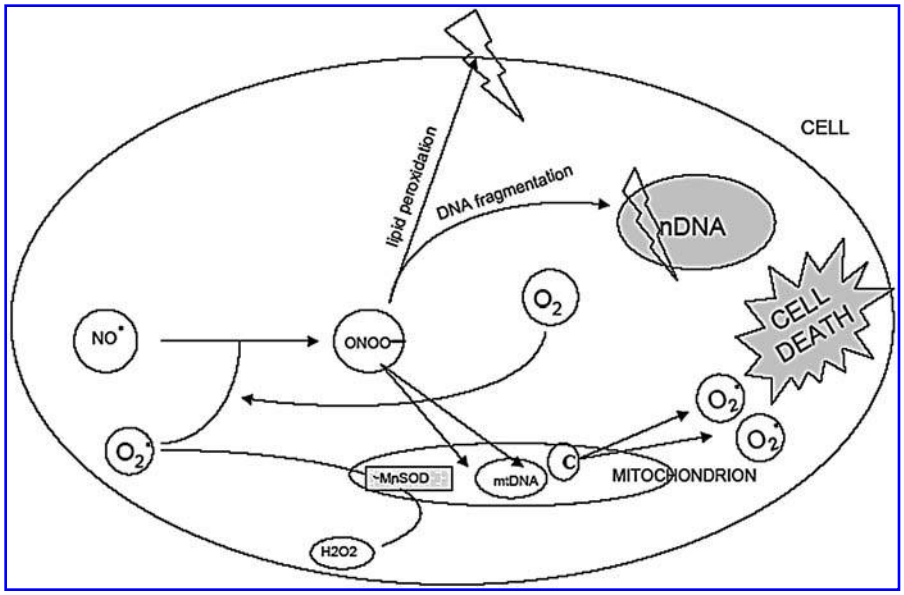

FIG. 3. Nitric oxide metabolism. NO, nitric oxide; $O_2^{\cdot}$, superoxide anion; $ONOO^-$, peroxynitrite; MnSOD, manganese superoxide dismutase; H_2O_2, hydrogen peroxide.

tivity of the proteasome, and others involved in the maintenance of neuronal structure and functions. All these mechanisms are compromised in AD, indicating that the use of proteomics identified oxidatively modified proteins whose altered function is consistent with the disease (9). Markers of oxidative damage have been largely observed not only in AD brain tissues but even in biologic fluids and peripheral tissues of AD individuals, including blood, urines, and cerebrospinal fluid (CSF) (recently revised by our group; ref. 62). Mild cognitive impairment (MCI) is a clinical condition between normal aging and AD, characterized by a memory deficit without loss of general cognitive and functional abilities. Several recent studies on MCI patients suggest that oxidative damage could be one of the earliest events in the neurodegenerative process leading to AD (52, 67). Keller and colleagues (52) measured the amount of protein carbonyls, TBARS, and MDA in brains of MCI and early AD patients and normal controls, observing that the measured markers of oxidative damage to proteins and to lipids were increased in individuals with MCI and early AD compared with normal control subjects (52). Moreover, we performed a study by a comet-assay analysis to evaluate the level of primary and oxidative DNA damage in leukocytes of AD, MCI, and healthy control individuals, observing that the amounts of both primary DNA damage and oxidized bases were significantly higher in AD and MCI patients, compared with controls, giving a further indication that oxidative damage, at least at the DNA level, is an earlier event in AD pathogenesis (67). The comet assay, a recently developed method to assess DNA damage, is a very

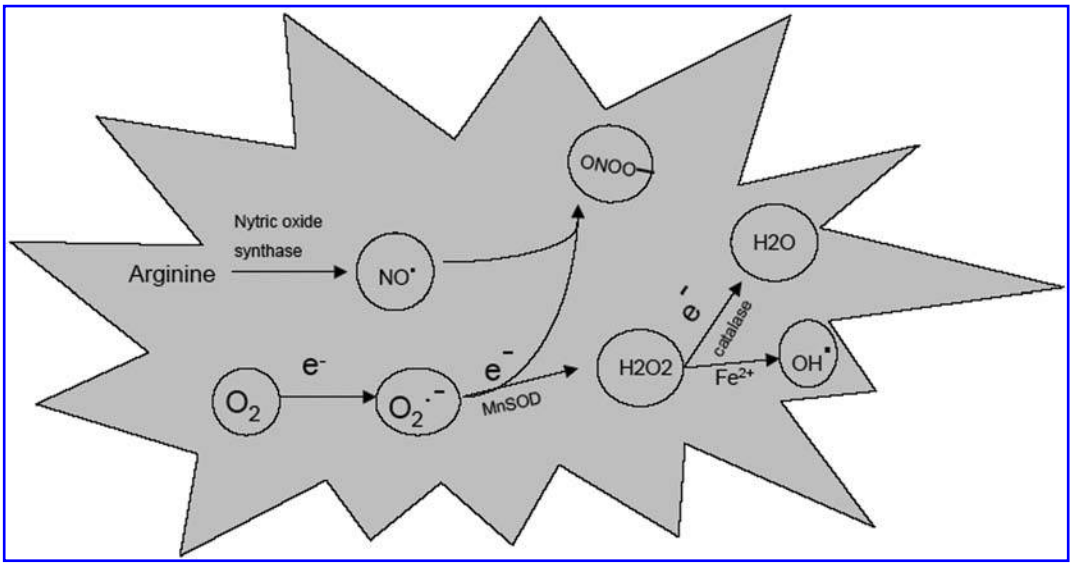

FIG. 4. Mitochondria and oxidative stress. The cartoon represents a "Stressed out" mitochondrion. NO, nitric oxide; $O_2^{\cdot}$, superoxide anion; $ONOO^-$, peroxynitrite; MnSOD, manganese superoxide dismutase; H_2O_2, hydrogen peroxide.

useful test for genotoxicity and represents an invaluable tool for investigating fundamental aspects of DNA damage and resulting cellular responses. This assay is capable of detecting primary DNA damage: DNA single-strand breaks (SSBs), alkali-labile sites, DNA–DNA or DNA–protein crosslinking, and also oxidative DNA adducts and SSBs associated with incomplete excision repair sites. In particular, a recent modification of the standard assay provides the use of two enzymes of the excision-repair system: endonuclease III (endo III) and formamidopyrimidine-DNAglycosylase (fpg), which recognize and cut oxidized pyrimidines and purines, respectively. Enzymes convert these lesions in DNA single-strand breaks, producing fragments that migrate toward the anode during electrophoresis, making up the tail of the comet. Figure 5 shows a nucleus analyzed with this assay in the modified version, which allowed us to show elevated levels of oxidative DNA damage in peripheral leukocytes of MCI and AD patients (67). Under the conditions of oxidative stress, such as those observed in AD and MCI brains, ROS are harmful to lipids, proteins, and DNA, and can trigger apoptosis. The detection of apoptotic cells in postmortem human brain tissues is problematic and has yielded contradictory results. The main problem is caused by the fact that apoptosis occurs within a few hours, whereas the neurodegenerative process leading to dementia and to other neurodegenerative pathologies is a chronic process, making it difficult to detect a substantial number of apoptotic neurons at any given time (90). Even if the question of whether or not the apoptotic process is directly responsible for cellular death in AD brain is still debated, apoptotic cell death has been described in neurons of individuals affected by several neurodegenerative diseases by using *in situ* end-labeling techniques, such as TUNEL. The number of fragmented apoptotic nuclei detected by the TUNEL assay was significantly increased in neurons located in frontal and hippocampal regions of AD patients, in nigral dopaminergic neurons of Parkinson disease (PD) individuals, and in spinal motor neurons of patients affected by amyotrophic lateral sclerosis (ALS), when compared with the respective controls (55). Amyloid peptides and activated caspases accumulate in the frontal cortex and the hippocampus in AD, and recent studies suggest that the two classic hallmarks of AD, amyloid plaques and neurofibrillary tangles, are not independent of each other, but share a common pathway of caspase activation. In this model, Aβ peptides activate caspases, and activated caspases cleave tau, initiating or accelerating the formation of neurofibrillary tangles (21). Moreover, elevated levels of p53 have been observed in postmortem tissues of AD patients, and it has been recently proposed that Aβ peptides can activate apoptosis in a p53-dependent manner (79). The analysis of PCD in neuronal death has led to the identification of several associated phenomena, such as reinitiation of the cell cycle and the key role of oxidative stress, and a causal relation between these events is still largely debated. Based on studies in AD patients and healthy control individuals, evidence has emerged that, under certain circumstances, postmitotic differentiated neurons in the adult human brain are able to reenter the cell-division cycle (72). However, it also seems that in control subjects, the cell cycle does not progress beyond the G_1 phase, and cells are able to redifferentiate into neurons. By contrast, in AD patients, the cell cycle is allowed to progress as far as the G_2 phase, without any evidence of DNA replication. Cell-cycle arrest at this stage does not permit redifferentiation, but is a prelude to irreversible cellular damage or apoptosis or both (72). Moreover, an increased expression of cyclins and cyclin-dependent kinases involved in both the G_1–S and the G_2–M transitions of the cell cycle has been observed in degenerative neurons of AD brains; however, these inductions are not indicative of neuronal cell division, but they are likely the prelude to a PCD (117). The cell-cycle reentry in neurons was observed also in healthy individuals and could be a physiologic mechanism required for repairing oxidative DNA damage in neurons (55).

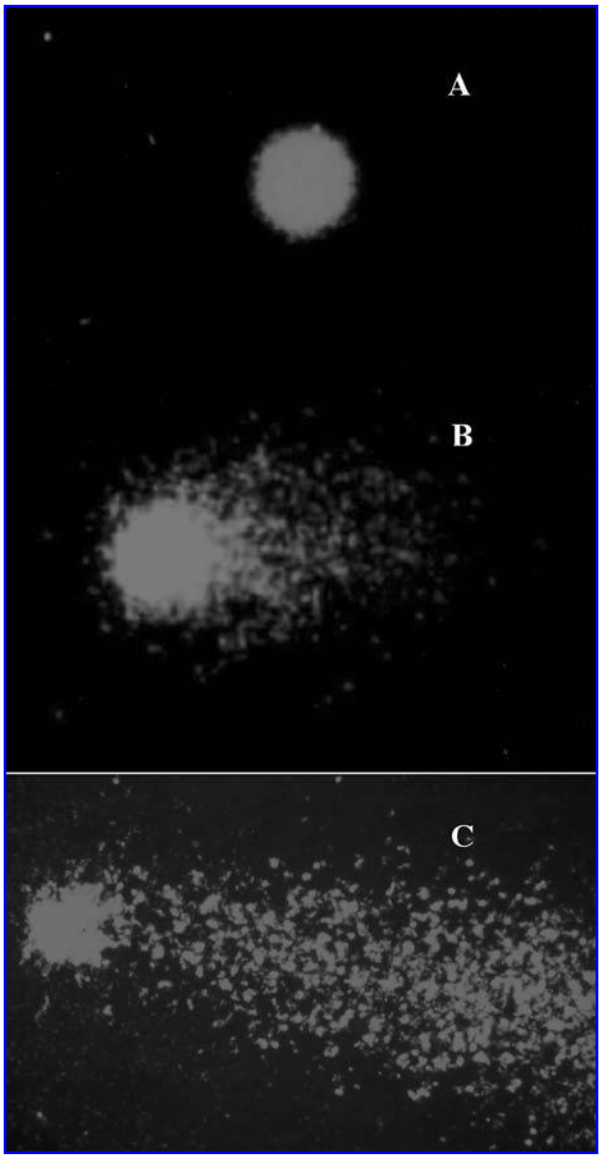

FIG. 5. Comet assay analysis of DNA oxidative damage by the comet assay in leucocytes of control (A) and AD (B, C). (**A**) Nucleus without DNA oxidative damage. (**B**) Nucleus with a moderate level of DNA oxidative damage. (**C**) Nucleus with high levels of DNA oxidative damage.

Recent evidence is indicative of a role for DNA oxidative damage in triggering apoptois-like neuronal PCD: oxidative

damage to the DNA has been recently demonstrated to induce the activity of the poly-ADP-ribose polymerase-1 (PARP-1), with a subsequent PARP-1–dependent translocation of AIF from mitochondria to the nucleus, resulting in caspase-independent programmed cell death (109). Further studies are required to prove this hypothesis, which could provide more insight into our understanding of the relation between oxidative DNA damage and neuronal cell death.

MtDNA OXIDATIVE DAMAGE, DNA REPAIR, AND ALZHEIMER'S DISEASE

One of the leading etiologic hypotheses regarding AD is the involvement of free radical–induced oxidative damage in neuronal degeneration. Chronic exposure to ROS is believed to be one of the major contributors to the aging process and to several age-related pathologies including AD. ROS, which are produced mainly in mitochondria during the normal cellular metabolism, can then cause oxidative damage to lipids, proteins, and nucleic acids. The accumulation of nDNA and mtDNA damage is thought to be highly deleterious in postmitotic cells such as neurons, which cannot be replaced through a cellular-division mechanism. Indeed, oxidative base modifications to nDNA and particularly to mtDNA could potentially result in bioenergetic dysfunctions, resulting in neuronal death (113).

In the previous paragraph, we discussed several recent reports on MCI individuals suggesting that oxidative damage occurs early during the progression from a normal aging to dementia (52, 67). Particularly, concerning DNA oxidative damage, an increased amount of DNA oxidative damage was recently confirmed by our group in leukocytes of both MCI and AD patients (67). Moreover, increased levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) have been observed in both nDNA and mtDNA isolated from brain regions of AD subjects compared with controls (66), and a recent study demonstrated that mtDNA had approximately 10-fold higher levels of oxidized bases than nDNA, that guanine is the most vulnerable base to DNA damage, and that multiple oxidized bases are significantly higher in AD brain specimens in comparison with controls (111). Subsequently Wang and colleagues (110) provided further evidence of DNA oxidative damage as an earlier event in the neurodegenerative process leading to AD. Authors quantified multiple oxidized bases in nDNA and mtDNA in several postmortem brain regions isolated from subjects with MCI and age-matched healthy individuals, finding increased levels of 8-hydroxiguanine and 4,6-diamino-5-formamidopyrimidine in both nDNA and mtDNA of MCI subjects, compared with age-matched controls. Increased levels of 8-hydroxyadenine were also observed to be elevated in nDNA from MCI subjects (110).

Thus, the type of DNA damage that is most likely to occur in neuronal cells is oxidative damage, which is primarily removed by the DNA base-excision repair (BER) process, and compelling evidence suggests that BER plays a pivotal role in the development and the maintenance of the central nervous system, and that possible imbalances in BER activities could contribute to neuronal loss (113).

BER is the main pathway to repair DNA base modifications caused by oxidation, alkylation, and deamination. Cells contain several DNA glycosylases, each of them exhibiting a specific substrate spectrum. DNA glycosylases catalyze the first step in the BER process by cleaving the *N*-glycosylic bond between the damaged base and the sugar moiety; after the cleavage, the damaged base is released, resulting in the formation of an abasic (AP) site, which is then cleaved by an AP lyase or AP endonuclease. Repair can then proceed through short- or long-patch BER. In short-patch BER, a single nucleotide is incorporated into the gap and ligated by a DNA ligase. In long-patch BER, several nucleotides (two to seven to eight) are incorporated, followed by cleavage of the resulting 5′ flap structure and ligation (37, 113). In nuclei, both pathways are initiated by DNA polymerase β (Pol β), which adds the first nucleotide into the gap. In short-patch BER, the same polymerase also removes the 5′-sugar phosphate, and the DNA ligase III/XRCC1 complex seals the DNA ends. However, if the 5′-sugar phosphate is reduced or oxidized, and therefore resistant to the action of Pol β, then an additional synthesis of DNA is required to displace the modified 5′-sugar phosphate as part of a flap, which is then removed by flap endonuclease (FEN1). It has been suggested that after Pol β adds the first nucleotide into the gap, it is substituted by Pol δ/ϵ, which continue long-patch BER. DNA ligase I completes the long-patch pathway (26).

The BER takes place either in nuclei or mitochondria, and mitochondria possess independent BER machinery encoded by nuclear genes. Indeed, several BER enzymes have been identified that have both nuclear and mitochondrial forms. For example, among DNA glycosylases, nuclear and mitochondrial adenine-DNA-glycosilases are generated by alternative spliced forms of the *MYH* gene (78). Similarly, nuclear and mitochondrial uracil DNA glycosilases are encoded by alternative splicing and transcription of the *UNG* gene (74). Two isoforms of the human oxoguanine DNA glycosilase (hOGG1) operate in human cells, the alpha and the beta forms, arising as alternative splice products from the *hOGG1* gene (48). Recent findings suggest that the beta form operates in mitochondria, whereas the alpha form operates in the nucleus as well as in mitochondria; however, it seems that the alpha form is responsible for the base-excision activity also in mitochondria, whereas the function of the beta form remains unclear (48, 96).

After the removal of the damaged base, the AP site generated is incised by AP-endonucleases/lyases, such as AP endonuclease 1 (APE1), which comprises more than 95% of cellular AP-site incision activity in human cells and has been localized to the nucleus, the cytoplasm, and the mitochondria in mammalian cells (26, 113). The gaps generated by the action of AP endonucleases/lyases are filled in by either Pol β or Pol δ/ϵ in the nucleus and by Pol γ in the mitochondria. Pol γ is the only DNA polymerase identified in mitochondria and can catalyze the removal of the 5′-sugar phosphate necessary for short-patch BER. To date, no evidence has been found of long-patch BER in mitochondria (113).

The final step in the BER process is the ligation of the nick left by the action of the DNA polymerases. Whereas two different DNA ligases, ligase I and ligase III, operate in the nucleus, only ligase III is operative in mitochondria, where it participates in short-patch mitochondrial BER (56).

Results published by several authors have suggested that an impaired DNA damage could be critical to the etiology of AD (23, 51, 59, 60). Particularly, in 1999, Lowell and co-workers suggested that the brain in AD might be subjected to the double insult of increased oxidative stress and deficiencies in repair mechanisms responsible for the removal of oxidized bases (59). Moreover, the same authors demonstrated a decrease in BER activity in postmortem brain regions of AD individuals, especially in the activity of hOGG1 (60). Others observed a deficiency of the double-strand breaks-repair pathway in AD brains (23, 51). These studies support the concept that, in addition to increased oxidative damage in AD brain regions, a decreased repair of oxidative damage also leads to the accumulation of DNA errors, which could be critical in the pathogenesis of AD (60). MtDNA is believed to be particularly sensitive to oxidative damage because of its proximity to the inner mitochondrial membrane, where oxidants are formed. Because of several of the mtDNA genes encode for subunits of the mitochondrial respiratory chain, oxidative mtDNA damage, if not correctly repaired, could result in mutations and deletions disrupting the function of genes involved in the production of ATP, ultimately leading to mitochondrial dysfunctions, increased production of ROS, and cellular death. Therefore, a decline in one or more BER enzymes may result in the accumulation of nDNA and mtDNA mutations, ultimately leading to the production of altered proteins and possible neurodegeneration (114).

Polymorphisms in DNA-repair genes account for differences in DNA repair between individuals. They have been associated with the risk of several age-related disorders including various types of cancer and could be critical to the etiology of AD (17, 29). HOGG1 removes 8-OHdG and nicks the DNA leaving an AP site, which is then incised and completely repaired by the action of APE1 (50). Studies in humans and other animals suggest that OGG1 is involved in the repair of oxidative damage in neurons, and OGG1 activity was observed either in mitochondria or nuclei of neuronal cells (38, 58, 106). After the observation that the bulk of neuronal DNA damage in AD is acquired by oxidative damage and ROS, we recently decided to study whether or not the common polymorphism Ser326Cys in the *hOGG1* gene could be a possible risk factor for sporadic AD (SAD) (17). We have chosen to investigate the hOGG1 Ser326Cys polymorphism as a candidate risk factor for SAD because, among DNA bases, guanine is the most vulnerable to ROS attack in AD, with 8-OHdG being the major damage (66, 111). Moreover, Vodicka and colleagues (107) recently observed that the capacity to repair oxidative DNA damage was significantly decreased in healthy human subjects bearing the *hOGG1* Cys326Cys homozygous variant genotype, compared with those bearing the wild-type genotype. Multiple studies have shown that an impaired DNA-repair activity gives a higher risk of developing cancer, and the Ser326Cys polymorphism has been associated with the risk of lung, esophageal, and prostate cancers (112). We analyzed a sample of 178 patients with sAD and 146 matched controls, and we failed to find any association between allele or genotype frequencies of the *hOGG1* Ser326Cys variant and the risk of SAD (17). However, we recently observed a strong association between the *hOGG1* Cys326 variant and the risk of ALS in males, and a trend between the presence of this variant and increased levels of markers of oxidative stress in blood cells of ALS subjects, suggesting a possible contribution of an impaired DNA BER in the process leading to motoneuronal degeneration (16).

Other groups hypothesized that the Arg194Trp polymorphism of the BER X-ray repair cross complementing group 1 (*XRCC1*) gene could contribute to genetic susceptibility for sAD. Authors investigated the Arg194Trp polymorphism of the *XRCC1* gene in the DNA samples of 98 patients with AD and 95 healthy subjects and observed a borderline association ($p = 0.056$) between the 194Trp allele and the risk of SAD (28).

The question of whether or not AD is associated with alterations in the BER pathway is still a matter of great interest, and further studies are ongoing, the better to address this question (113). Initial studies on neuronal DNA repair have focused on the nucleotide excision repair (NER) pathway, given the neurologic defects observed in patients with xeroderma pigmentosum and Cockayne syndrome, disorders characterized by neuronal cell death due to the lack of ability to repair DNA damage, and associated with impaired NER activity (36). The NER pathway is required for the removal of a wide variety of forms of DNA damage, including UV-induced photoproducts and other bulky lesions. NER involves at least 25 proteins or complexes of proteins and is divided into global genome repair (GGR) and transcription-coupled repair (TCR). The two pathways differ mainly in the initial steps that recognize the DNA lesion, and different initial recognition factors are involved. After a correct assembly of the NER complex, a fragment of 24 to 32 nucleotides is incised and removed from the damaged strand. Repair is completed by new DNA synthesis using the undamaged strand as a template and ligation. The global repair pathway removes damage overall in the genome, irrespective of genome location and point in the cell cycle, whereas TCR is required for the specific repair of bulky lesions in the transcribed strand of active genes (37). Mitochondria have been shown to lack NER, which operates in the nuclei, removing the majority of DNA lesions. In patients with deficits within the genes encoding for products involved in the NER pathway, progressive neurodegeneration develops. Xeroderma pigmentosum is the typical inherited disorder caused by a defect in NER and due to a deficit in one of seven XP complementation groups (XPA to XPG), leading to an increased development of skin cancers after sunlight or UV exposure, and neurologic complications. Cockayne syndrome and trichothiodystrophy are due to impaired TCR and are associated with skeletal abnormalities and neurologic degeneration (26). Therefore, defects in the NER pathway clearly lead to neuronal dysfunction and neurodegeneration, so that polymorphisms in two genes of the NER pathway (*XPD* and *XPF*) have been recently studied for their possible association with late-onset SAD. The *XPD* gene encodes an ATP-dependent DNA helicase that is required for the opening of the damaged DNA after the damage-recognition step. The *XPF* gene encodes a polypeptide that is part of a complex required for the incision at the 5′ end of the recognized DNA damage. Two common polymorphisms of the *XPD* gene and a silent mutation in exon 7 of the *XPF* gene have been studied in 97 patients with late-onset SAD and in 101 age- and sex-matched controls, but none of them has been found to be associated with the AD risk (29).

MITOCHONDRIA AND AGING

Aging is a predictable, universal, and detrimental process in humans, yet exactly how and why it occurs remains poorly understood. Even if the loss of neurons is part of the normal physiologic process of aging and has been observed in several regions of the senescent human brain, it is also closely associated with functional impairments such as dementia and motor neuron disability in neurodegenerative conditions. Moreover, increasing age is a well-known risk factor for the development of neurodegenerative diseases, including AD.

The initial free radical theory of aging suggested that ROS, which are constantly generated through normal metabolism, cause aging, as well as associated degenerative diseases, by introducing irreversible damage to membranes, proteins, and DNA (46). Because mitochondrial ETC is the major site of ROS production in the cell, and one of the targets of the oxidative damage, it has been postulated that mitochondria may play a role in the aging process. (47). The mitochondrial theory of aging, a correlate of the free radical theory, is also based around the idea of a vicious cycle, in which somatic mutations of mtDNA provoke respiratory chain dysfunction, leading to enhanced ROS production and, in turn, to the accumulation of further mtDNA mutations (47). Over the last decade, evidence has mounted in support of Harman's postulation. Mitochondrial dysfunction and mtDNA mutations [both point mutations and deletion(s)] have been shown to amplify during the course of aging (101). MtDNA shows increased damage with age; levels of 8-hydroxy-2-deoxyguanosine, a product of free radical damage, increases exponentially, and this correlates with the rate of accumulation of mtDNA with deletion (49). In human skeletal muscle, muscle fibers that lack cytochrome *c* oxidase (COX) activity also appear and accumulate in an age-related manner (70).

Recently, a homozygous knockin mice (mtDNA mutator mice) expressing a proofreading-deficient form of the nuclear-encoded mitochondrial DNA polymerase (Polg) was created (100). The mtDNA-mutator mice are born in normal mendelian proportion and have a normal appearance until the age of 25 weeks, at which age the animals developed a wide range of premature aging phenotypes (100). MtDNA mutator mice have a widespread tissue distribution of a linear, deleted mtDNA molecule and random accumulation of mtDNA point mutations. At first glance, these remarkable phenotypes would seem to support the hypothesis for the mitochondrial basis of aging. However, one of the key features of this hypothesis is that mtDNA mutations lead to increased ROS production, which leads to further mtDNA and cellular damage. Surprisingly, increased levels of mtDNA mutations were not associated with increased ROS production or increased oxidative stress in mtDNA mutator mice. Therefore, the premature aging phenotypes in mtDNA mutator mice are not generated by a vicious cycle of massively increased oxidative stress accompanied by exponential accumulation of mtDNA mutations.

Thus, whereas no doubt exists that mitochondrial DNA mutations and COX-deficient fibers accumulate during aging, the extent to which these abnormalities are detrimental to tissue function is uncertain.

Given the increasing incidence of dementia with age, it was supposed that dementia is inevitable for those who survive >100 years; however, several recent studies on nonagenarians and centenarians suggest that dementia and neurodegeneration are not a mere consequence of physiologic aging, and ~20% of centenarians are functionally cognitively intact (84). Healthy aging is presumably the result of complex interactions between several genes, the environment, and stochastic factors. Further studies on nonagenarians and centenarians and their relatives will help us to understand better whether persons who achieve extreme healthy old age are individuals with genetic variants predisposing to long life and resulting in decreased susceptibility to age-related degenerative diseases, or if the hypothesis that they lack many of the variants predisposing to various illness is the most appropriate (84).

MITOCHONDRIA AND ALZHEIMER'S DISEASE

Abnormal mitochondrial morphology in AD

Morphologic alterations in neuronal mitochondria in AD have been reported. Recently, Baloyannis (3) reviewed the morphologic alterations of the mitochondria in 22 brains of AD patients. Morphologic alterations of mitochondria in the Purkinje cells and the climbing and mossy fibers in the cerebellar cortex and in the neurons of the cortex of the brain hemispheres, the thalamus, the globus pallidus, the red nucleus, the vestibular nuclei, as well as the locus coeruleus, are consistently present in AD brains. The majority of the mitochondria, small, round, or elongated, presented disruption of the cristae or osmiophilic inclusions. Morphometric studies of the mitochondria in AD revealed a significant reduction in mitochondria density in endothelial cells as well as in fibroblasts obtained from patients with AD (95). Furthermore, apparently normal dendrites from the frontal cortex of seven patients with AD showed mitochondria with increased-density matrices and paracrystalline inclusions in the intercristal space (59). Last, ultrastructural examination showed that AD cybrid cells contained a significantly increased percentage of enlarged or swollen mitochondria that had a pale matrix and few remaining cristae (102). Other pathologic features such as crystal-like intramitochondrial inclusions and cytoplasmic inclusion bodies were also found in AD cybrids (102).

Impaired energy metabolism in AD

Impaired energy metabolism and abnormalities of mitochondrial respiration are autopsy features of brain tissue affected by neurodegeneration, but also of peripheral cells (platelets and fibroblasts) of AD patients. The reduction in brain energy metabolism appears to relate to the clinical disabilities of AD patients and can also precede the clinical symptoms by decades. A number of positron emission tomography investigations have shown that energy metabolism in AD brain is reduced (2, 41). Further, reduced cerebral metabolism has been shown in the temporoparietal cortices of AD patients, and these changes precede both the neuropsychologic impairment and the cortical atrophy typically found with the conventional neuroimaging (6). Functional magnetic resonance spectroscopy has also shown a decreased ratio of phosphocreatine to inorganic

phosphate in the AD brain (86). In agreement with these studies, a decreased glucose utilization in skin fibroblasts of AD patients has been observed (108). Because mitochondria are the powerhouse of all cells, damage to mitochondria will inevitably impair energy metabolism. The main pathway for oxidation of glucose in the brain is the tricarboxylic acid (TCA) cycle (the Krebs cycle), which takes place in the mitochondria. The oxidative decarboxylation of pyruvate, the product of glycolysis, by the pyruvate dehydrogenase complex (PDHC) provides acetyl CoA to initiate the TCA cycle, which includes eight different enzymes. Deficiency in the two key enzymes of the rate-limiting step of the TCA cycle, PDHC and α-ketoglutarate dehydrogenase complex, (KGDHC) has been documented in AD cases by multiple groups, suggesting defects in glucose metabolism in the AD brains (40, 94). Further, Aβ can inhibit both COX and KGDHC in isolated brain mitochondria (13).

Recently, Bubber and co-workers (8) performed a complete study of the TCA-cycle enzymes in AD brains. The study confirmed not only the decreased activity of PDHC and KGDHC enzymes, but also of the isocitrate dehydrogenase, whereas the activities of succinate dehydrogenase and malate dehydrogenase were increased. Further, all the changes observed in TCA-cycle activities correlated with clinical state, suggesting that their imbalance in AD could lead to diminished brain metabolism, resulting in the decline in brain function.

Impairment of mitochondrial respiration

Defects in ETC inhibit production of ATP and increase production of ROS. Deficient COX activity has been reported by several authors in different brain regions (7, 71, 93, 116), as well as in platelets (83) and fibroblasts (99) of sAD patients. Involvement of other mitochondrial OXPHOS complexes is less documented and more controversial. Cardoso and collaborators (11), studying mitochondria isolated from AD platelets, did not find differences between AD and controls in NADH-ubiquinone oxidoreductase (complex I) or succinate dehydrogenase-cytochrome *c* reductase (complex II/III) acitivities, whereas the ATP levels were decreased. Moreover, we have reported that COX but not F1F0-ATPase activity was decreased in hippocampus and platelets of sAD cases (7, 63), and that the impaired COX activity could have functional consequences on energy metabolism (63).

The "cybrid model" of AD

Thus, the inhibition of mitochondrial OXPHOS observed in sAD, particularly the COX deficiency, has an etiologic impact. The question then arises as to the origin of the COX deficiency in SAD. It could result from an environmental toxin or an acquired or inherited mtDNA mutation(s). To address the relevance and potential causes of COX defect in AD, the cytoplasmic hybrid ("cybrid") technique, first described by King and Attardi (54), has been applied. In this technique, mitochondria/mtDNA from human AD and control platelets is transferred to culturable cells depleted of endogenous mtDNA(ρ0 cells). The resulting AD cybrids showed a transferred COX defect and revealed a number of downstream consequences that recapitulate the pathology observed in AD brain (53, 98, 103). Associated with this COX deficiency is increased oxidative stress, manifested by elevated oxidative stress enzymes and protein adducts from 4-hydroxynonenal. Remarkably, AD cybrid cells have elevated intracellular and extracellular Aβ peptide levels (53). If cultured densely with concentrated media, AD cybrids will lay down Congo red–positive deposits reminiscent of early amyloid plaques (53). AD cybrids also show elevated spontaneous death with apoptotic nuclear morphology (81), increased cytoplasmic cytochrome *c* levels and caspase-3 activity (53), and elevated cleavage of caspase substrate (81) not observed in non-AD cybrids. Again, the spontaneous alterations in cell death and cell-death pathways observed spontaneously in AD cybrids can be reproduced by exposing non-AD cybrids to exogenous aggregated beta amyloid or oxidative stress (81, 82). Whereas mitochondria are normally transported down neuronal processes by independent motor protein systems, in differentiated AD cybrids, this mitochondrial transport is impaired (104), suggesting that delivery of mitochondria to the synapses might be impaired *in vivo*.

Recent studies (10) documented that the alterations in AD cybrid oxidative status renders these cells vulnerable to Aβ 1–40 cell death because these cells manifest an excessive decrease in the mitochondrial membrane potential, excessive mitochondrial release of cytochrome *c*, and caspase enzyme activation.

All these and other studies demonstrate that SAD cybrid cell lines express the morphologic and biochemical phenotype observed *in vivo* in SAD, reinforcing the concept that primary mtDNA changes could be responsible for the "mitochondrial features" of SAD and could be the origin of the increased oxidative stress and Aβ deposition found in SAD brain.

MtDNA mutations and AD

However, even though cybrid technology strongly supports the role of the mtDNA in the disease, no causative mutations in the mtDNA have been reported. Currently, several groups are trying to define the specific mtDNA mutation(s) responsible for the mitochondrial phenotype observed in SAD cybrids. An increase of somatic mtDNA rearrangements has been observed in AD brains. The mtDNA "common deletion," for instance, has been observed to be elevated about 15-fold in AD brains (19). Further, the A-to-G transition at nucleotide position 4336 in the mtDNA was observed more frequently in AD patients compared with controls (92). MtDNA control region (CR) mutations have been recently reported as more common in AD brains compared with controls (20). These mutations preferentially affect important functional domains and are also present in a high proportion of the mtDNA of the brains. In particular, two heteroplasmic changes were specific to AD brains (T414C, present in 65% of AD brains, and the T477C), whereas others were predominantly expressed in AD. Further, cloning and sequencing of the mtDNA CR from patient and control brains showed that AD brains had an average 63% increase in heteroplasmic mtDNA CR mutations, and in AD subjects older than 80 years, a 130% increase in these mutations was found. The AD brains showed also an average 50% reduction in mtDNA content and in the ND6 complex I transcript, which can likely reduce the mitochondrial oxidative phosphorylation. However, a previous study involving a larger number of tissue samples did not identify the same T414C mutation in brains of AD patients (15). Furthermore, Elson and co-workers (31) sequenced the complete cod-

ing regions of 145 autopsy AD brain samples and 128 normal controls. They observed that for both synonymous and nonsilent changes, the overall numbers of nucleotide substitutions were the same for the AD and control sequences.

It has been speculated that mtDNA mutations that accumulate with age might lead to impaired energy generation and to increased amounts of ROS, both resulting in cell damage. Polymorphisms in mtDNA may cause subtle differences in the encoded proteins and, thus, minimal changes in OXPHOS activity and free radical overproduction. This could predispose an individual, or a population sharing the same mtDNA genotype, to an earlier onset of apoptotic processes, such as accumulation of somatic mtDNA mutations and OXPHOS impairment. The opposite could be true for different polymorphism(s), which could be beneficial, increasing OXPHOS activity and/or reducing ROS production. Common mtDNA polymorphisms determine classes of continent-specific genotypes, defined as "haplogroups," which can be detected by RFLP analysis. In Europe, nine different mitochondrial haplogroups have been identified (H, I, J, K, T, U, V, W, X). Specific mitochondrial haplogroups have been linked to longevity (73, 119). Thus, if they can be associated with longevity, the same or other haplogroups could be involved in the other side of the spectrum of the life cycle, the neurodegeneration and, thus, death. Results of previous studies on the role of European mtDNA haplogroups in AD are controversial. The most relevant have been carried out independently by diverse research groups in Europe and the United States. It has been suggested that inherited mitochondrial haplogroups K and U may influence AD risk in whites. By studying an Italian sample of subjects, Carrieri *et al*. (12) hypothesized that K and U may act by neutralizing the effect of the major AD risk factor apolipoprotein E (*APOE*) ϵ4 allele, as they are present at a lower frequency in ϵ4 carriers than in noncarrier AD patients, whereas in controls, independence between ϵ4 allele and mtDNA haplogroups is seen. Furthermore, the same authors detected a lowering of the ϵ4 allele odds ratio from statistically significant to nonsignificant values in patients with haplogroups K and U (12). Another recent report regarding a possible link between AD and mtDNA genotypes showed that males classified as haplogroup U had a significant increase in risk of AD, whereas females demonstrated a significant decrease in risk with the same U haplogroup, independent of *APOE* genotype (105). Conversely, two studies indicated that mtDNA haplogroups were not associated with AD, either individually or by grouping together closely related haplogroups (15, 31). These studies are strong, as they included only neuropathologically proven cases of AD of European descent. We recently genotyped predefined European mtDNA haplogroups in 209 patients with AD and 191 matched controls (64). To minimize the risk of a "genetic contamination," which could lead to false associations between gene markers and disease, we were careful to enroll in the study only patients and controls of clear Tuscan origin (with at least three generations of Tuscan-born relatives). Tuscany is an area of Central Italy that was the core of the Etruscan colonization. The Etruscan language is not of Indo-European origin, and it was spoken in the area up to the first century, when it was replaced by Latin, a language of the Indo-European family. Roman penetration in Etruria was mainly political and cultural. Even though some colonies of veterans were established in the territory, no massive replacement of population is known from historical sources. Sharp genetic change associated with historical linguistic differentiation in central Italy was also noted by Barbujani and Sokal (4). For these reasons, Tuscany has been selected for our study. We reported that the frequency of haplogroups H, I, J, K, T, U, V, W, and X was not significantly different between the AD patients and control groups (64). Further, no significant differences were noted between genders in mtDNA haplogroups distribution in both AD patients and control groups. In light of the findings by Carrieri *et al*. (12), we evaluated whether an interaction between *APOE* polymorphism and mtDNA haplogroups was present in patients. The *APOEϵ4* allele was confirmed as a risk factor for AD, as it was found at a significant higher frequency in patients than in controls (23.3% *vs.* 8.11%, respectively), but no association between *APOE* alleles and mtDNA haplogroups was observed, taking into account whether patients were carriers of the *APOEϵ4* allele. Our data also excluded any association between mtDNA haplogroups, age at onset, and mean survival with the disease (64). In those studies involving a large group of patients, a correct population selection to minimize the risk of a genetic contamination is essential. Further studies will be required to define the contribution of mtDNA haplogroups, if any, to the pathogenesis of AD.

A possible link between the "mitochondrial cascade" and the "amyloid cascade" hypothesis

As reported earlier, the "mitochondrial cascade hypothesis" could explain many of the biochemical, genetic, and pathologic features of SAD (Fig. 6). Accumulation of somatic mtDNA mutations accelerates normal aging, leads to oxidative damage, and causes energy failure, increased production of ROS, and accumulation of Aβ, which in a vicious manner reinforces the mtDNA damage, the impairment of the mitochondrial respiration, and the oxidative stress. The result is the activation of the mitochondrial permeability transition pore, the release of cytochrome *c,* and the induction of caspase-mediated apoptosis.

A similar hypothesis has a strong link with the amyloid cascade hypothesis, as it suggests that the adverse consequences of a primary mitochondrial respiratory chain defect and Aβ peptide can be synergistic.

Although the amyloid plaques characteristic of AD consist of extracellular aggregates of the toxic Aβ peptide, researchers are increasingly recognizing that amyloid species may exert toxicity from within the cell. Before plaques are observed, intracellular aggregates of Aβ form early in mice overexpressing APP and strongly correlate with cognitive impairment (77). How intracellular Aβ might cause cellular dysfunction remains unclear, although some sites of action have been identified. In particular, Anandatheerthavarada and collaborators (1) linked amyloid to the mitochondrion, which at that time was not yet widely recognized as a site of amyloid accumulation or toxicity. In their laboratories, these authors showed, for the first time, that APP is targeted to neuronal mitochondria, under some physiologic and pathologic conditions, by virtue of its chimeric NH_2-terminal signal. APP is also targeted to mitochondria of cortical neuronal cells and selected regions of the brain of a transgenic mouse model for AD. Chemical cross-linking, together with immunoelectron microscopy, showed that the mi-

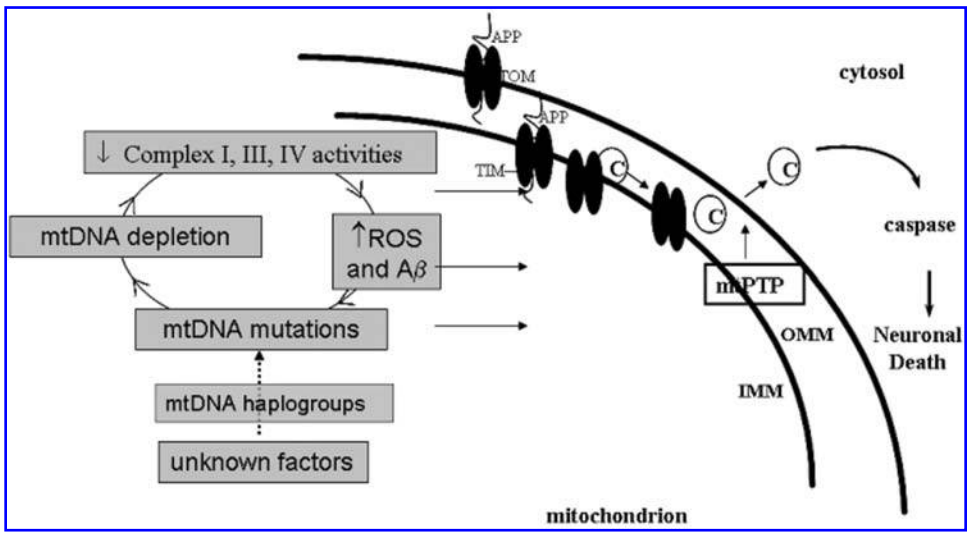

FIG. 6. A proposed mechanism of mitochondria-induced cell death in AD. Aβ, amyloid-β; ROS, reactive oxygen species; mtPTP, mitochondrial permeability transition pore; C, cytochrome *c*; IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane; APP, amyloid precursor protein; TOM and TIM, protein importation translocases of the mitochondrial outer and inner membranes.

tochondrial APP exists in NH_2-terminal inside transmembrane orientation and in contact with mitochondrial translocase proteins. Mutational studies show that the acidic domain, which spans sequence 220–290 of APP, causes the transmembrane arrest with the amino terminus inside and the COOH-terminal 73-kD portion of the protein facing the cytoplasmic side. Accumulation of this transmembrane-arrested full-length APP was associated with reduced COX activity, decreased ATP synthesis, and loss of the mitochondrial membrane potential. When the acidic APP220–290 domain was deleted, transmembrane arrest did not occur, and no mitochondrial dysfunction was therefore observed.

These data were obtained in transgenic mouse model for AD. Recently, experiments from the same group (24) were conducted in fresh brain hemispheres of both genders from humans clinically diagnosed with AD and nondementia, obtained at autopsy. They found that nonglycosylated full-length and C-terminally–truncated APP was associated with mitochondria in samples from the brains of individuals with AD, but not with mitochondria in samples from nondemented subjects. Moreover, within AD brain samples, levels of mitochondrial APP were higher in more affected brain areas (frontal cortex, hippocampus, and amygdala) and in subjects with more-advanced disease. Further, they confirmed that APP forms complexes with mitochondrial inner and outer membrane translocases TOM40 and TIM23 in AD, and that mitochondrial accumulation of APP is associated with decreased COX activity, increased levels of H_2O_2 in mitochondria, and block of the transport of nuclear encoded proteins. Last, they observed that mitochondrial accumulation of APP directly correlates with mitochondrial dysfunction in various brain regions in AD. They concluded that the abnormal accumulation of APP across mitochondrial import channels, causing mitochondrial dysfunction, is a hallmark of human AD pathology.

These data on mitochondrial APP complement a growing literature that mitochondria may interact with factors involved in Aβ metabolism, and that mitochondria, APP, and Aβ metabolism might be interconnected in the cascade, leading to neurodegeneration and dementia. Mitochondrial dysfunction is a hallmark of Aβ-induced neuronal toxicity in AD. Lustbader and co-workers (61) recently demonstrated that Aβ-binding alcohol dehydrogenase (ABAD), which is localized to the mitochondrial matrix, may be a direct molecular link from Aβ and mitochondrial toxicity. Their data demonstrate that ABAD and Aβ directly interact in mitochondria in AD, and that this interaction promotes leakage of ROS, mitochondrial dysfunction, and cell death, potentially underlying the mechanism of Aβ-induced mitochondrial toxicity. Again, evidence also indicates that γ-secretase, which is essential to cleave APP and create Aβ, is present in mitochondria (45). Insulin-degrading enzyme (IDE), which is important for Aβ removal, can be targeted to mitochondria by alternative translation initiation (57). The presequence peptidase PreP, which is localized to the mitochondrial matrix and is responsible for degrading presequences and short peptides, also can degrade Aβ (35).

Conversely, mitochondrial dysfunction and oxidative stress may alter APP processing, leading to intracellular accumulation of Aβ (39, 80). Further, oxidative stress increases the activity of the β-secretase, the enzyme responsible for N-terminal cleavage of Aβ from the APP (30).

GENE-ENVIRONMENT INTERPLAY IN AD: ANTIOXIDANTS IN AD THERAPY AND PREVENTION

AD causative and susceptibility genes and AD risk factors

We recently reviewed several genetic and environmental AD risk factors and the importance of their interplay in disease on-

set and progression (18). Mutations in the *APP* and in the *PS-1* and *PS-2* genes are rare, fully penetrant, and responsible for only the early-onset familial forms of the disease. However, the majority of AD cases (90–95%) are age-related sporadic forms whose onset is likely due to the contribution of several susceptibility genes at multiple loci and interaction between them and environmental or stochastic factors. Advanced age is the most reliable environmental risk factor for the sporadic forms of AD, whereas the most highly replicated genetic risk factor is the *APOE-ε4* allele of the A*POE* gene, which imposes a 2.3- to threefold increased risk of AD for copy number carried by an individual, compared with the normal *APOE-ε3* allele. Thus, the effect of the *APOE-ε4* variant is dose related, with a lower estimated risk in heterozygous carriers, compared with homozygous *APOE-ε4* individuals. The *APOE-ε4* variant is associated with high plasma cholesterol levels and is supposed to enhance the deposition of Aβ-peptides and the formation of neuritic plaques. On the contrary, the *APOE-ε2* variant, which is associated with low levels of plasma cholesterol, decreases the individual risk of AD developing. The effect of the *APOE-ε4* allele on AD risk decreases with increasing human age, and both advanced age and the possession of one or two copies of the *APOE-ε4* allele are associated with oxidative stress. The *APOE-ε4* allele is not an AD-causative gene, meaning that its presence is neither necessary nor sufficient to develop the disease, and suggesting the existence of several other AD-susceptibility loci. Many other AD risk factors, including hyperhomocysteinemia and hypercholesteloremia, stroke, severe brain injuries, exposure to metals such as aluminium, and diabetes mellitus, are all directly or indirectly associated with increased oxidative stress. Similarly, polymorphisms in folate and homocysteine-metabolizing genes (particularly in the *MTHFR* gene), in genes whose products participate in inflammation [mainly in members of the interleukin (IL) family, such as *IL-1α*], in the gene encoding for the vascular endothelial growth factor (*VEGF*), and in genes involved in the transport and the metabolism of cholesterol (*CYP46A1, ABCA1, LRP1*) and in that of metals (such as the genes encoding for transferrin and for the hemochromatosis factor, *TF* and *HFE,* respectively), have been associated with AD susceptibility by several authors (for a recent review, see ref. 18). Dietary habits, lack of exercise, and lack of intellectual brain stimulation have been associated with increased AD risk. Conversely, the consumption of unsaturated fatty acids and omega-3 polynsaturated fatty acids, intellectual activity, exercise, and the stimulation of the brain by molecules acquired through smoking and drinking coffee, seem to exert a neuroprotective effect and to reduce the risk of developing neurodegenerative disorders, including AD (18, 87).

Antioxidants in AD treatment and prevention

The evidence that many of the risk factors for AD are also associated with a condition of increased oxidative stress, and that markers of oxidative damage to proteins, lipids, and nucleic acids have been extensively found in brains, peripheral tissues, and biologic fluids of AD individuals, have strongly suggested that the use of molecules exerting antioxidant activity (including vitamins, carotenoids, polyphenols, and flavonoids) could be helpful in AD treatment and prevention (25). Moreover, recent evidence of the presence of an increased oxidative damage in MCI individuals (52, 67) has strengthened the opinion that oxidative damage might be one of the earliest events in the process leading from a normal brain to AD, opening a new field of investigation on the potential effect of antioxidants in delaying the onset and the progression of dementia in MCI individuals. Clinical trials were carried out in either AD and MCI patients to test the effects of antioxidants in these pathologic and prepathologic conditions (33, 85). Results from clinical trials investigating the effects of antioxidant vitamins, such as vitamin E and vitamin C, on cognitive function and dementia are conflicting and inconsistent and seem to point out that the use of a single vitamin is not able to slow the cognitive decline in both MCI and AD patients (85). In contrast, a recent prospective study showed a reduced prevalence and incidence of AD in individuals taking vitamins E and C in combination. However, no significant reduction in risk was found from taking vitamin E or vitamin C alone (118). This area of research is very controversial, and more effort is necessary in the future to address the contribution of the diet to the risk of dementia, and particularly to answer the question of whether a mixture of antioxidant compounds could be helpful in reducing the risk of the disease. Several studies suggest that molecules or nutrients exerting antioxidant properties reduce the incidence of AD. Several agents or nutrients with antioxidant properties, including estrogens, omega-3 polyunsaturated fatty acids, and flavonoids have been proven to reduce the incidence of AD (for a recent review, see ref. 76). In parallel, the contribution of the neurotoxic Aβ peptide and that of hyperphosporylated tau proteins to oxidative damage in AD brains has been revised. Evidence indicates that in the initial phase of AD development, both Aβ deposition and the formation of neurofibrillary tangles of hyperphosphorylated tau protein are consequences of oxidative stress and may function as "shields" to protect the neurons against oxidative injuries; meaning that both Aβ peptide and neurofibrillary tangles might be cellular compensations to increased oxidative stress rather than the main putative source of ROS, as it was previously supposed (68). However, during the progression of the disease, the antioxidant activity of both Aβ and tangles evolves to prooxidant. Several molecular and cellular studies indicate that Aβ peptides enter mitochondria and promote the generation of free radicals, ultimately leading to mitochondrial disruption and cellular death (88). So increasing evidence indicates that genetic and environmental factors associated with an increased risk of AD are also associated with increased oxidative stress, and oxidative stress is one of the earliest detectable events is individuals, such as MCI subjects, at increased risk of developing AD (18, 67). Moreover, dietary manipulation with antioxidants seems to reduce the incidence of AD (25, 76). Therefore, a low intake of calories and the consumption of foods containing a mixture of vitamins and other antioxidants, together with physical exercise and intellectual stimulation of the brain, seem to compose one of the best lifestyles to slow the risk of developing AD.

CONCLUSIONS

AD is associated with multiple changes, including reduced ATP synthesis and altered mitochondrial structure and function in the brain. Although until 10 years ago, it was not clear whether the AD-associated mitochondrial defects were con-

tributing factors in the progression of the disease, now compelling evidence indicates that AD brains are bioenergetically impaired, that this metabolic deficiency appears early in the clinical evolution of AD, that it worsens with clinical deterioration, and that it is associated with mitochondrial enzymatic impairments in the TCA cycle and ETC.

AD cybrid studies over the past few years have provided compelling circumstantial evidence for systemic mtDNA contributions to AD pathogenesis in the brain. However, all the recent genetic studies fail to confirm abnormalities in AD mtDNA, and the exact role and relevance of the reported mtDNA point mutations or haplogroups remain to be clarified.

It is tempting to hypothesize that an interaction between mitochondrial dysfunction and oxidative damage could trigger a vicious cycle, leading to neuronal degeneration and, thus, disease. Indeed, it is clear that a strong relation exists between the mitochondrial cascade hypothesis and the Aβ cascade hypothesis. In addition to forming extracellular aggregates, Aβ, or its precursor APP, has complicated intracellular effects involving a variety of subcellular organelles, including mitochondria. At the same time, mitochondria and their dysfunction may alter APP processing and cleavage to intracellular and intramitochondrial Aβ.

Mitochondria are now at center stage in human neurodegenerative diseases and aging. Their role in those processes is much less "mythic" and much more "realistic" than believed in past years, and we should look forward to exciting developments in this field during the coming years. It will be important to develop a better understanding of the role of mitochondrial energy metabolism in AD and its link with the amyloid hypothesis in aging and AD, because it may lead to the development of effective treatment strategies.

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ABBREVIATIONS

AD, Alzheimer's disease; AIF, apoptosis-inducing factor; APP, amyloid-protein precursor; Aβ, amyloid-β; BER, base excision repair; COX, cytochrome c oxidase; ETC, electron-transport chain; hOGG1, human oxoguanine DNA glycosilase; MCI, mild cognitive impairment; mtDNA, mitochondrial DNA; nDNA, nuclear DNA; NER, nucleotide excision repair; NO, nitric oxide; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; OXPHOS, mitochondrial oxidative phosphorylation; PCD, programmed cell death; ROS, reactive oxygen species; SAD, sporadic Alzheimer disease.

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Address reprint requests to:
Michelangelo Mancuso, M.D., Ph.D.
Department of Neuroscience
Neurological Clinic
University of Pisa
Via Roma 67
56126 Pisa, Italy

E-mail: mmancuso@inwind.it

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