ALZHEIMER'S DISEASE: A HYPOTHESIS ON PATHOGENESIS

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ABSTRACT

Alzheimer's disease (AD) is the major cause of dementia. It is a systemic disorder whose major manifestations are in the brain. AD cases can be categorized into two groups on the basis of the age of onset - before or after about age 60. The majority of cases, 90-95 percent, are in the late onset category. Early onset cases are largely, if not all, familial (FAD). These are caused by mutations in the genes for the amyloid precursor protein (APP), presenilin 1 (PS1), and presenilin 2 (PS2). In contrast late onset cases are mainly sporadic.

The disorder is characterized by intraneuronal fibrillary tangles, plaques, and cell loss. The brain lesions in both early and late-onset AD are the same, and in the same distribution pattern, as those seen in individuals with Down's syndrome (DS) and in smaller numbers in normal older individuals.

Extensive studies of AD have yet to result in a generally accepted hypothesis on the pathogenesis of the disorder. Major emphasis has been placed on the role of amyloid, the neurotoxin formed by the action of free radicals on preamyloid.

The observation that AD lesions are frequently present in normal older individuals prompted the hypothesis that AD is the result of faster than normal aging of the neurons associated with it. This hypothesis provides plausible explanations for FAD and AD.

FAD is associated with mutations in APP, PS1, and PS2. These substances, along with their normal counterparts, undergo proteolytic processing in the endoplasmic reticulum (ER). The mutated compounds, aside from increasing the ratio of βA_{42} to βA_{40} , may down-regulate the calcium buffering activity of the ER in a manner akin to one or more of the many compounds known to do so. Decreases in the ER calcium pool would cause compensatory increases in other calcium pools, particularly in mitochondria. Increases in mitochondrial calcium levels are associated with enhanced formation of superoxide radical formation, and hence of the rate of aging.

SAD may be caused by nuclear and/or mitochondrial DNA mutations beginning early in life that enhance mitochondrial superoxide radical formation in the neurons associated with the disorder.

The above explanations for FAD and AD are suggestive of measures to prevent and for treatment.

INTRODUCTION

Alzheimer's disease (AD) is the major cause of dementia (1). AD cases can be categorized into two groups on the basis of age of onset as shown in Table 1.

Table 1. Alzheimer's disease: Categories

- 1) Late onset, after about age 60, 90-95 percent of cases (2), largely non-familial, i. e., sporadic (SAD)(2); there is a slight increased incidence in near relatives (1,3,4).
- 2) Early onset, before about age 60, 5-10 percent of cases (2), almost all familial (FAD); most are associated with chromosome 14 (2,5) (gene product: presenilin 1 [PS1]), some with chromosome 1 (6) (gene product: presenilin 2 [PS 2]), while a few have mutations of the gene for the amyloid precursor protein (APP) on chromosome 21 (6,7).

AD is a widespread disorder whose major manifestations are in the brain (8,9). The disorder is characterized by intraneuronal fibrillary tangles, plaques, and cell loss (1,8,10-13); major steps in pathogenesis have been summarized (14). The pathologic lesions in both early and late onset AD are the same (1) and have the same distribution pattern. The lesions spread through the brain with time in a predetermined fashion producing a constantly changing pattern (15) of cognitive (1,16,17) and noncognitive symptoms (15). These same lesions are present in the brains of Down's syndrome (DS) individuals after about age 40 (18) and are frequently observed in elderly non-demented persons (1,19,20); the distribution pattern for both groups is the same as for AD.

Atypical clinical presentations occur in about 10 percent of individuals with SAD (1). These include progressive aphasia, visual agnosia, personality disturbances and pure memory loss; such focal features may predominate for up to four years before the typical generalized deficit is apparent (1).

Systemic manifestations include those listed in Table 2 (9,14,21-24).

Table 2. Systemic manifestations of SDAT include:

- 1) Calcium content of cultured skin fibroblasts from patients with Alzheimer's disease is increased while mitochondrial oxidation of glucose and glutamine are depressed (9,14,21).
- 2) There is a decrease in the major membrane phospholipids, phosphatidylcholine and phosphatidylethanolamine, in brain (22) as well as in erythrocytes (23).
- 3) Cytochrome oxidase was depressed in mitochondria from platelets of five out of 6 patients with a diagnosis of Alzheimer's disease (24).

Age is a major risk factor for AD. The prevalence increases exponentially with age (8,25). The age-specific death rate of individuals with AD is 2-4 times that of individuals in the general population (8).

Extensive studies of AD have yet to result in a generally acceptable hypothesis (26-29). Major emphasis has been placed on the role of amyloid (28-33), the neurotoxin formed by action of free radicals on preamyloid (14,34). Although amyloid has a significant role in AD (28-33), it may not be the initiator of the disorder (14,35).

Recent studies of AD are largely concerned with proteolytic processing of APP to form the β A peptides - the major components of the plaques, and of the presenilins in the endoplasmic membrane (36-42). Other areas include calcium homeostasis and free radical formation (43-47), inflammation (48-53), and fibril formation (54,55).

The observation that AD lesions are frequently present in normal older individuals prompted the hypothesis (14) that AD is the result of faster than normal aging of the neurons associated with it. The major purpose of this paper is to advance plausible explanations for the postulated faster neuronal aging. These explanations are suggestive of measures to prevent and to treat.

PATHOGENESIS

To help clarify the association of AD with age (8,25) and oxidative stress (14,27,56-58), aging will be discussed before advancing plausible explanations for the postulated faster than normal aging of neurons associated with AD.

A. Aging.

Aging is the accumulation of diverse deleterious changes in the cells and tissues that increase the risk of disease and death (59,60). Aging changes can be attributed to developmental and genetic defects, the environment, disease processes, and an inherent process, referred to as "the aging process". The chance of death of an individual of a given age in a population - readily available from vital statistics data - serves as a measure of: 1) the average number of adverse changes, i.e., aging changes, accumulated by persons of that age, and 2) physiologic age, i.e., "true age", in contrast to chronological age. The chances for death in a population determine the average life expectancy at birth (ALE-B). ALE-B serves as a rough measure of the span of healthy, productive life, i.e., the functional life span.

Effect of improved living conditions.

Conventional means (CM) of increasing the ALE-B of a population by decreasing the chances for death through improvements in general living conditions, e.g., better nutrition, housing, medical care, are becoming increasingly futile (59,60). This is illustrated in Figure 1 by the curves of the logarithm of the chance of death versus age for Swedish females for various periods from 1751 to 1992 (61,62); a straight line represents exponential increases with age. The chances

Figure 1. Age-specific death rates of Swedish females in various periods from 1751 to 1992.

for death in the developed countries are now near limiting values while ALE-B's approach plateau values of around 76 years for males and 82 years for females.

Thus, as living conditions in a population approach the optimum, and premature deaths a minimum, the logarithmic curve of the chance of death versus age shifts towards a limit determined by the sum of: 1) the irreducible contributions to the chance of death by aging changes that can be prevented to varying degrees by CM, e.g., those due to the environment and disease, and 2) contributions that can be influenced little, if at all, by CM, i.e. those due to the inherent aging process. The now near limiting chances for death rise almost exponentially after about age 28. Only 1-2 per cent of a cohort die before this age.

The aging process.

The inherent aging process is the major risk factor for disease and death in the developed countries after age 28 (59,60). It limits average life expectancy at birth to about 85 years and the maximum life span to around 122 years. This innate process is caused by chemical reactions that arise in the course of normal metabolism which, collectively, produce aging changes that exponentially increase the chance of death with advancing age even under optimal living conditions; this is illustrated in Figure 2 by a plot of the chances for death in 1985 for the entire population of the United States as function of age.

Figure 2. Chance of dying in 1985 as a function of age: Total population of the United States.

Many theories (59,60) have been advanced to account for aging. No theory is generally accepted. The Free Radical Theory of Aging (FRTA) (60,63,64) is a promising hypothesis; the subsequent discussion is based on the assumption that it is correct. It, and the simultaneous discovery of the important, ubiquitous involvement of free radicals in endogenous metabolic reactions, was proposed in 1954 (63,64). The theory arose from a consideration of aging phenomena from the premise that a single common process, modifiable by genetic and environmental factors, was responsible for the aging and death of all living things. The FRTA postulates that the common process is the initiation of free radical reactions. These reactions, however initiated, could be responsible for the progressive deterioration of biological systems over time due to their inherent ability to produce random change. The theory was extended in 1972 (65), and in 1983 (66), with the suggestions that: 1) most free radical reactions were initiated by the mitochondria at an increasing rate with age, and 2) the life span is determined by the rate of free radical damage to the mitochondria; this rate determines the rate of increase of mitochondrial superoxide radical formation with age, and in turn, the age when mitochondrial derived free radical reactions cause death by overwhelming the cellular defenses against such reactions. Collectively, the free radical reactions initiated by the mitochondria constitute the inherent aging process.

Application of the Free Radical Theory of Aging.

The FRTA suggests that improvements in CM which increase ALE-B do so by decreasing the rate of accumulation of aging changes associated with sub-optimal living conditions (Table 3).

Table 3. Free radical theory of aging: How CM increases ALE-B.

Improvements in CM which increase ALE-B do so by decreasing the rate of accumulation of aging changes associated with suboptimal living conditions. For example:

- Better nutrition provides more compounds, e.g., vitamins C and E, and β -carotene, to decrease free radical damage.
- Housing improvements may decrease the amount of food metabolized — and free radical production, needed to maintain body temperature.
- In the case of disease, free radicals are widely involved (67,68) so that measures to improve prevention and treatment would be expected to have beneficial effects on life span.
- Tissue injury causes free radical formation, thus better accident prevention measures should reduce the accumulation of aging changes.

The FRTA further suggests the possibility that measures other than CM to decrease the rates of initiation and/or the chain lengths of free radical reactions can also decrease aging changes, even under optimal living conditions, and in turn the rate of aging and of disease pathogenesis. Many studies now support this possibility (60,67,68).

Dietary antioxidants throughout life.

Most animal studies have used dietary antioxidant supplements to decrease chain lengths; these augment the natural defenses against free radical reaction damage. For example, addition of one percent by weight of 2-mercaptoethylamine hydrochloride (2-MEA) to the diet of male LAF1 mice, started shortly after weaning, increased ALE-B by 30 percent, Figure 3 (69); the maximum life span was increased little, if at all. When 0.5 percent of ethoxyquin was added to the diet of male and female C3H mice, the increase in ALE-B was 20 percent for both groups with no change in the maximum life spans (70).

Thus, antioxidant supplements in mice - living under conditions of good animal care, i.e., production of free radical reaction-induced aging changes secondary to suboptimal living conditions is small - increase the ALE-B. The percentage of older animals in the studies was increased, while the senescence periods (69,70) were decreased as there was little, if any, effect on the maximum life spans. The latter is attributed to the exponentially increasing production of aging changes with age by the innate aging process which progressively nullify the beneficial effects of the antioxidants, keeping increases in maximum life span minimal.

Exposure to antioxidants early in life.

Free radical reactions may also produce significant life-shortening changes during the short period of high mitotic and metabolic rate of early life (71). Consideration of early events in embryogenesis (71) suggest that these reactions, aside from those associated with

Figure 3. Effect of adding 0.5% and 1.0% by weight of 2-mercaptoethylamine hydrochloride (2-MEA) to the diet, starting shortly after weaning, on the life span of male LAF₁ mice.

the environment, may first arise in the developing embryo between the 40-60 cell blastocyst stage, fourth day of gestation, and well after implantation, ninth day of gestation. During this interval of exposure to the elevated O₂ tension of the uterine cavity, female embryos have two functioning X-chromosomes - thus may be better protected against free radical damage, while males have one; discussed further below.

The onset of mitochondrial function, and the associated formation of superoxide radicals in the course of normal metabolism, is on gestational day 14 in the rat (72,73): presumably about the same time in man. There are at least two other sources of free radical reactions in early life: 1) steroid estrogens, elevated in pregnancy (74), can be converted to catechol estrogens that serve as a source of superoxide radicals via the quinone/hydroquinone redox system (75), and 2) sporadic episodes of transient increases in superoxide radicals, secondary to ischemia-reperfusion injury, due to uteroplacental hypoperfusion caused by transient vasoconstriction (73,76,77).

The possibility that free radical reactions in early life may shorten life span was assessed twice (71) because of the unexpected results of the first study; the percentage increase in life span was greater for the male, than for female offspring. In the second

study groups of female Swiss mice were maintained on a semisynthetic diet, with and without an added antioxidant supplement (0.2%w ethoxyquin, 0.25%w 2-MEA, or 1.0%w sodium hypophosphite) from one month before mating until the offspring were weaned. The male and female offspring were separated, placed on a pellet commercial diet (no added antioxidants), and survival curves obtained (Figure 4). Addition of 2-MEA, the most effective of the compounds evaluated, to the maternal diet increased the ALE-B of male offspring by 15 percent and of females by 8 percent (71). The results of both experiments were similar. The second study confirmed the unexpected results of the first one. The antioxidants had no apparent adverse effects on the pregnancies.

The above increases in offspring life spans are attributed to decreases by the 2-MEA in: 1) free radical-induced non-specific aging changes, and 2) life-shortening mutations. The lesser effect in females may be largely due to a normally higher level of protection of female embryos from free radical damage during a short period, about 48 hours in the mouse, just prior to the random inactivation of one of the two functioning X chromosomes in the late blastocyst stage of development - see above. The X chromosome codes for glucose-6-phosphate dehydrogenase (G6PD), a key enzyme in the production of NADPH. The latter serves to maintain glutathione in the reduced state. Glutathione, the major cellular sulphhydryl compound, acts as an antioxidant and as a hydrogen donor for glutathion peroxidase in the reduction of H₂O₂ and hydroperoxides.

Association of disease with age.

The adverse effects of free radical reactions since conception provide a plausible explanation of the association of disease with age (67). The ubiquitous free radical reactions would be expected to produce deleterious changes that accumulate progressively with time. The nature and locations of the changes are influenced by genetic and environmental factors. Those that are more-or-less common to all persons determine the "normal" sequential alterations with age. Superimposed on this common pattern of change are patterns that differ from individual to individual owing to genetic and environmental differences that modulate free radical reaction damage, e.g., defective Cu/Zn SOD in Lou Gehrig disease (78) and probable higher rates of formation of superoxide radicals in the vessel walls in essential hypertension (79). The superimposed patterns of change may become progressively more discernable with time and some may eventually be recognized as diseases, at ages influenced by genetic and environmental risk factors.

In agreement with the above: 1) free radical reactions have been implicated in the pathogenesis of a growing number of disorders - the "free radical diseases" (67,68), some are listed in Table 4, and 2) accumulating recent data demonstrate that many diseases of adulthood that

Figure 4. Effect of adding antioxidants to the maternal diet on the life spans of their offspring: A) Males. B) Females.

shorten life, e.g., breast and prostate cancer, coronary heart disease, hypertension, diabetes mellitus, have their origins in the events of early life (80,81). This is also probably true for Alzheimer's disease.

Table 4. The "Free Radical Diseases" include:

1. Atherosclerosis	7. Fanconi's anemia
2. Cancer	8. Bloom syndrome
3. Alzheimer's disease	9. Amyloidosis
4. Essential hypertension	10. Diabetes mellitus
5. Parkinson's disease	11. Laennec's cirrhosis
6. Cataracts	12. Amyotrophic lateral sclerosis

Decreasing the incidence of "Free Radical Diseases".

Although the exact etiologies of the "free radical diseases" are not known, the probability of developing any one of them should be decreased by lowering levels of the more-or-less random free radical reactions by any means (60,80), e.g., those listed in Table 5.

Table 5. The "Free Radical Diseases": Measures to decrease incidence include:

- 1) During early development enhance antioxidant content of maternal diets, reduce catechol estrogens, and increase efforts to lower episodes of uteroplacental ischemia.
- 2) Caloric restriction.
- 3) Minimize exposure to ionizing radiation.
- 4) Increase antioxidant intake (dietary and/or supplements).
- 5) In the case of a specific disease, lower further by decreasing environmental risk factors – for example, cholesterol in atherosclerosis and carcinogens in cancer.

In some instances it may be necessary to "target" the inhibitor(s) in order to achieve effective inhibitor concentrations. Thus, the blood pressure of spontaneously hypertensive rats is not lowered by intravenously injected Cu/Zn superoxide dismutase (SOD) but it is lowered when the enzyme is coupled with a basic peptide (HB) to form HB-SOD (79). Unlike SOD,

HB-SOD undergoes transendothelial transport and localizes within arterial walls.

B. Hypothesis on pathogenesis of Alzheimer's disease:

Alzheimer's disease is attributed to faster aging, i.e., increased free radical reaction levels, of the brain neurons associated with the disorder, which brings forward in time the normal sequential patterns of neuronal dysfunction and cell loss associated with age. The purpose of this paper is to provide plausible explanations for the faster aging of the neurons involved in the different categories of AD.

The age of onset of clinical symptoms in AD is a continuum largely determined by the rate of increase of free radical levels with age in the neurons associated with it:

β A peptides are present in the brain in two fibrillar forms. Most are in a form not recognized by the usual Congo red and thioflavin methods, i.e., preamyloid (14,82) - also named diffuse or amorphous plaques. The minor fraction is amyloid, i.e., recognized by Congo red. The major peptide form in the deposits is βA_{42} . *In vitro* studies demonstrate that conversion of preamyloid to amyloid is induced by free radical reactions (34). This may be also so *in vivo*, owing to the presence in the tissues of H_2O_2 formed in the course of normal metabolism. The intercellular concentration of H_2O_2 would be expected to vary from place to place owing to differences in the cellular environment, and to be gradually increased with age (83,84) by the innate aging process. At some age and in some locations, determined largely by the level of free radical reactions in AD neurons, intercellular H_2O_2 concentrations could begin to exceed the threshold for the activation of microglia cells in a manner akin to the up-regulation of the synthesis of cytokines IL-8 and TNF- α by dendritic cells (85). Then, free radicals formed by activated microglia would serve to: 1) cata-

lyze the oxidative conversion of preamyloid to amyloid, and 2) simultaneously initiate the inflammation responsible for clinical expression of AD. The foregoing suggests that brain tissue H_2O_2 concentrations in individuals without a clinical history of dementia, yet whose brain pathology was compatible with a diagnosis of Alzheimer's disease (86), had not significantly exceeded the threshold level for activation of microglia; possibly because of higher than normal brain antioxidant levels (87).

Thus, slower increases with time of AD neuronal free radical levels are associated with later onset AD. Hence, AD onset ages gradually decline from those associated with old age to blend with those of SAD - and further lowered by the action of apolipoprotein E, followed by FAD, and finally by Down's syndrome (2,18,32).

How apolipoprotein E4 lowers AD onset age is not known. The O_2 mediated interaction between apoE4 (88) and βA_4 suggests a possible explanation: Reaction of tissue O_2 with ApoE4 may serve as a catalyst for the slow oxidative compaction of preamyloid, a process which results eventually in formation of amyloid. Thus, when H_2O_2 -induced microglia activation occurs, less time is required to complete the compaction of preamyloid to amyloid. Hence, the age of onset of clinical symptoms of AD is decreased, more so the longer the period of ApoE4-associated compaction.

Sporadic and familial AD, along with DS, will be discussed below followed by a brief consideration of two related disorders, Dementia pugilistica and Parkinson's disease.

Sporadic AD.

Initiation of the greater than normal increase in oxidative stress with age in SAD is attributed (14,58) to accumulating mutation(s) in one or more of the approximately 1000 mtDNA and nucDNA genes involved in mitochondrial biogenesis and function (89). The mutation(s): 1) probably first occur in a progenitor cell(s) prior to the three-germ cell stage of development - since cells involved in the disorder are of both ectodermal and mesodermal (e.g., fibroblasts) origin, and 2) for the most part have adverse effects on mitochondrial function. The latter augments superoxide radical formation and decreases ATP production - thereby enhancing oxidative stress. In view of the above it is not surprising that efforts to find the gene(s) responsible for sporadic AD have been inconclusive (90,91). This disorder could be initiated by one or more of a large number of combinations of mutations in progenitor cells of the genes associated with mitochondrial function, e.g., those influencing cytochrome oxidase (92). Identification of the initiating mutation(s) would be expected to be made progressively more difficult with time by the continuing accumulation of mutations throughout life.

Clonal expansion during development of a progenitor cell(s) for the neurons associated with SAD may

cause a systemic disorder if some daughter cells are diverted to serve as progenitor cells for the mesoderm and/or endoderm.

If the mutation(s) in the original progenitor cell was in the nucleus the daughter cells are all equally defective. However, if the mitochondrial DNA is involved a spectrum of cells arise in which some have many copies of the mutation(s) while others may have none, since during cell division mitochondria are allotted to daughter cells at random (93). Thus, cells with mutated mitochondrial DNA age at a normal rate or faster.

Neuronal descendants in the brain of a progenitor cell that had a mutated mtDNA are likely to have mitochondria with different numbers of impaired mtDNA's and therefore to be aging at different rates. If the progenitor cell also had nucDNA mutations having adverse effects on mitochondria, the rate of aging of the daughter cells would be further increased.

As daughters of a progenitor cell with impaired mitochondria, present in the brain as neurons, age to become dysfunctional and die - owing to increasing cellular free radical levels (14), they would be expected to contribute to variable degrees (depending on number, extent of mitochondrial impairment, and distribution) to the early appearance of patterns of progressive, constantly changing cognitive and noncognitive symptoms, i.e., Alzheimer's disease.

Further, the distribution of some descendant neuronal cells, responsible for the early atypical focal clinical presentations that occur in about 10 percent of patients (1,17), may be a reflection of differences in the potential progenitor cells.

The relatively late, and variable, age of onset of clinical expression of SAD suggests that the accumulating mutation(s) in the genes affecting mitochondrial function in the progenitor/daughter cells have only mild, slowly progressing, depressing effect(s). The prevalence of sporadic AD suggests that the mutation(s) postulated to cause it occur at a much higher rate than those of the rare, well-known mitochondrial disorders (93-96) such as the Kearns-Sayre syndrome (96); the latter is attributed to mutation of a mtDNA molecule in a somatic cell during development and is characterized by onset before 20 years of age of ophthalmoplegia, atypical retinitis pigmentosa, mitochondrial myopathy, and one of the following: cardiac conduction defect, cerebellar dysfunction, or elevated cerebrospinal fluid protein.

Early onset AD.

- 1) Familial forms of AD (FAD). Mutations in APP and the presenilins (PS1 and PS2) cause FAD by increasing (28) the: 1) fraction of βA_{42} formed from APP, and 2) rate of aging.

The mutants are ubiquitously expressed transmembrane proteins; APP has a single transmembrane domain while PS1 and PS2 have 6-9 (97). The turnover time of normal APP is short (20-30 minutes)

(98). Turnover times of PS1 and PS2 are also short, about 15 minutes (99); that of the complex formed from the N- and C-terminal fragments is apparently greater than 12 hours (100). Mutations in the APP and the presenilins should further shorten turnover times (101).

APP, PS1, and PS2, as well as their mutated forms, undergo proteolytic processing in the endoplasmic reticulum (ER) membrane (28). Cleavage of APP by α - or β -secretase produces, respectively, membrane-bound 83- and 99-residue C-terminal fragments. These fragments are cleaved by γ -secretase, apparently formed (42) from the N- and C-terminal fragments of either PS1 or PS2; the products of the C99 cleavage include the βA_{40} and βA_{42} peptides. Mutants of the presenilins increase the fraction of βA_{42} (28), and hence increase it further with mutated APP (28).

The increased aging rate of individuals with FAD may be largely secondary to down-regulation of the ER calcium buffering activity by mutations in APP or the presenilins. This activity of the ER is modulated by the ATPase calcium pumps (102-104), located in the ER membrane (105,106), and by the associated Ca^{2+} release channels (107,108). Many compounds influence ER calcium content, including those listed in Table 6. It seems very likely that mutations in APP or the presenilins decrease ER calcium buffering, in a manner akin to that of one or more of the substances known to influence it, resulting in compensatory increases in other calcium pools, particularly in mitochondria. The foregoing is strongly supported by studies with cultured neurons (47,116,117). Increases in mitochondrial calcium content enhances superoxide radical formation, and in turn, the rate of aging (118).

Table 6. Endoplasmic reticulum: Calcium buffering capacity is modulated by many compounds, including:

- 1) phospholamban (109-111)
- 2) the ganglioside GM1 and Gm3 (112,113)
- 3) 6-gingerol (114) and ellagic acid (114)
- 4) procaine (105), caffeine (105), thapsigargin (105), and dantrolene (105)
- 5) the erythrocyte isoenzyme of acylphosphatase (115) – this enzyme inhibits the Ca^{2+} pump by hydrolysing the phosphointermediate formed during the catalytic cycle of the calcium ATPase.

Clinical manifestations of FAD, and death, occur at significantly lower ages than for those with SAD. This can be attributed to a combination of one or more of the contributing causes listed in Table 7.

- 2) Down's syndrome (DS). Individuals with this disorder have three copies of chromosome 21 rather than the normal two. Chromosome 21 codes for both SOD and APP; these gene products are increased in the cells of individuals with DS (119). Persons with this disorder display premature aging (119,120); manifestations include rapid aging of the skeletal system and skin,

Table 7. Clinical manifestations of FAD and death.

These occur at significantly lower ages than for those with SAD. This can be attributed to a combination of one or more of the following:

- 1) Mitochondrial superoxide radical formation is increased secondary to the increased O_2 consumption associated with increased turnover of the mutated forms of APP and of the presenilins.
- 2) Increased formation of the more amyloidogenic forms of $A\beta$, i.e. $A\beta_{42}$.
- 3) Down-regulation of the calcium buffering activity of the ER leading to increased mitochondrial superoxide radical formation.
- 4) Amyloid-associated inflammatory responses (28).

early menopause, short life span, and AD-like pathology (121) after about age 40. The first indication of the latter is generally apathy (119) and recent memory loss (122). In view of the discussion above, FAD cases associated with chromosomes 1 and 14 may also display accelerated aging; published reports focus only on central nervous system manifestations. The premature aging of Down syndrome patients is probably caused by a higher than normal level of oxidative stress in the cells (Table 8).

Table 8. Down's syndrome. Elevated oxidative stress indicated by:

- 1) Increased activities of glucose-6-phosphate dehydrogenase (123) in erythrocytes.
- 2) Enhanced activity of glutathione peroxidase (123) in erythrocytes and in cultured fibroblasts.
- 3) DS neurons exhibit a 3-4 fold increase in intracellular reactive oxygen species and elevated levels of lipid peroxidation (124).

Thus, the early appearance of neuronal dysfunction and neuronal loss in the brains of individuals with Down's syndrome may be caused by a higher than normal rate of cellular aging in the brain – as well as throughout the body, owing to increased formation of both superoxide dismutase and APP (with enhanced formation of βA peptides) by their cells.

Dementia pugilistica (DP).

A blow(s) to the head is a risk factor for Alzheimer's disease (125). Repeated blows to the head are associated with DP – the punch drunk syndrome (126,127). The severity of the syndrome correlates with the length of the boxing career and total number of bouts, and has an overall incidence of about 17 percent in professional boxers which rises with age (126,127).

There are numerous neurofibrillary tangles in the cerebral cortex, particularly in the temporal lobe (126,127). In contrast to AD, neuritic plaques are few while diffuse preamyloid plaques (126) are abundant; the diffuse plaques can form within days after head injury (128). Most cases show evidence of past brain hemorrhage (129).

The initiating steps in pathogenesis of DP may be similar to those of sporadic AD. Extracellular iron, e.g., in the form of heme, may disrupt calcium homeostasis

by increasing the influx of calcium through the neuronal membrane by: 1) catalyzing oxidative alterations in the membrane, and 2) increasing intracellular H_2O_2 concentrations (by catalyzing the aggregation rate of extracellular βA peptides to form amyloid, this in turn perturbs the neuronal membrane so as to activate an NADPH oxidase [130]). Increasing neuronal calcium concentration in the foregoing manner should result in neurofibrillary tangle formation (14).

The relative paucity of neuritic plaques is probably because the rate of the oxidative conversion of diffuse plaques to amyloid is about that of a normal individual, slow compared to a person with AD.

Parkinson's disease

The pathogenesis of idiopathic Parkinson's disease may be similar to that of sporadic AD; a defect has been found in Complex I of mitochondria from platelets (131,132) as well as in the substantia nigra (133) of patients with Parkinson's disease. Defective mitochondria have also been found in the skeletal muscle of such patients (134,135): a similar study apparently has not been conducted with Alzheimer's disease patients.

The frequent overlap in clinical manifestations between the two disorders (136-138) may be largely due to overlap in the tissue distribution of daughters of the progenitor cells.

PREVENTION AND TREATMENT

The above discussion suggests measures to prevent and treat AD. The suggested treatments include some that are currently being used (139), e.g., antioxidants and anti-inflammatory compounds.

Alzheimer's Disease

The age of onset of all categories of AD should be delayed by measures to delay conversion of preamyloid to amyloid; see treatment-2, below under Sporadic AD.

Sporadic AD

Prevention. The incidence may be decreased by efforts to minimize free radical reactions involved in the initiation of this disorder. In particular, women who wish to become pregnant should increase their daily intake of antioxidants by consuming: 1) a diet rich in high antioxidant-containing fruits and vegetables, and 2) antioxidant supplements at intake levels deemed "safe" during pregnancy.

Treatment. Presented in Table 9.

Familial AD

Chromosomes 1 and 14

Prevention. 1) Possibly in the future with gene therapy.
2) Measures, e.g., procaine, dantrolene(104), that may help to maintain normal calcium buffering activity of the ER during pregnancy.

Treatment. Same as (2) above plus measures employed for sporadic SDAT.

Table 9. Treatment of sporadic AD: The rate of progression may be slowed by a combination of the following measures (60,80 ,140):

- 1) decrease free radical initiation rates
 - a) lower caloric intake to an acceptable level.
 - b) add compounds to the diet that can compete with O_2 for electrons from the mitochondrial respiratory chain (140); spin-traps, nitroxides, and hydroxylamines can apparently do so.
- 2) supplement the diet with antioxidants such as vitamin E and selegiline (141). Specifically, increase the brain concentration of substances that may be able to minimize the expected rise of tissue H_2O_2 in the brain – possibly, lipoic acid, ascorbic acid (142), propofol hemisuccinate (143), and measures to increase metallothionein-III (144,145) (Could this be the unknown [144] normal function of the readily oxidized metallothionein-III?).
- 3) dietary supplementation with compounds employed to improve mitochondrial function; e.g., riboflavin (146), menadione (147), and in particular, Coenzyme Q_{10} (148).
- 4) compounds to depress inflammatory reactions associated with senile plaques (149-151), e.g., indomethacin (149).
- 5) estrogen treatment in postmenopausal women (152).
- 6) possibly by: a) immunization with amyloid- β (153), b) selective inhibition of Alzheimer's γ -secretase activity (154), and c) peptides which bind to $A\beta$ s and prevent β -sheet formation (155).

Chromosome 21

Prevention and treatment. Same as for chromosomes 1 and 14.

Down's syndrome.

Prevention. This disorder is caused by the failure of the two homologs of chromosome 21 to separate during the nuclear division of the maturing oocyte in meiosis I. This failure increases with age which suggest that free radical reactions may be involved. If so, the measures suggested above for the prevention of sporadic AD may be beneficial.

Treatment. If detected during pregnancy, the disorder may be ameliorated by decreasing the level of more-or-less random free radical reactions by measures discussed in the section on aging. After birth, treatment similar to that for sporadic AT should be beneficial.

Parkinson's Disease

Prevention and treatment. As with sporadic AD.

Dementia Pugilistica

Prevention. Measures to prevent injuries to the head.

Treatment. As with sporadic AD. Use of iron chelators.

TESTING THE HYPOTHESIS

Alzheimer's Disease

Sporadic AD.

- 1) Check the pregnancy records of the mothers of AD patients, e.g., in Sweden. They may show the presence of conditions that could predispose to mutations

in the developing embryo, e.g., obesity, smoking, alcohol consumption, antioxidant-poor diet.

Early onset AD.

1) Familial AD.

- a. The life span of affected individuals, as well as of transgenic mice expressing mutant APP or PS genes, may be increased by:
 1. antioxidants capable of passing the blood-brain barrier, to lower brain intracellular concentrations of H_2O_2 , thus delaying the conversion of preamyloid to amyloid.
 2. efforts to maintain normal cellular calcium levels, e.g., administration of procaine to slow release of calcium from the ER.

2) Down's syndrome.

- a. Onset of AD should be delayed by slowing the increase with age of the intracellular concentration of H_2O_2 in the brain, see above.
- b. Check the pregnancy records of mothers with Down's syndrome children. They may suggest that the embryo was under increased oxidative stress.
- c. Supplement the diet of Down's syndrome female mice (Jackson Laboratories, Bar Harbour, Maine: designated B6E1C38, stock number 001924) with antioxidants. This treatment may decrease the incidence of Down's syndrome.

COMMENT

The brain lesions of SAD, FAD, those seen after about age 40 in patients with Down's syndrome, and in older "normal" individuals, are the same and have the same distribution, but differ in the age of onset and the degree of involvement. The foregoing suggests that these disorders have different modes of initiation and a final common pathway. As discussed above, the probable causes of these disorders are listed in Table 10.

Table 10. Probable causes of SAD, FAD, and the AD lesions in Down's syndrome.

SAD. Mutation(s) before or during the 3-germ cell stage of development in a neuronal progenitor cell(s) that impair mitochondrial function.

FAD. Mutations in APP or the presenilins that down-regulate ER calcium buffering capacity.

DS. Increased oxidative stress secondary to higher gene dosage for SOD and APP.

DP. Increased neuronal aging secondary to brain injury.

The age of onset of clinical symptoms - related to the conversion of preamyloid to amyloid, is determined by the free radical level in the AD neurons. Conversion should be slowed by substances that can slow the rate of the normal rise of intercellular H_2O_2 concentration in the brain with age.

The innate aging process serves as the final common pathway for AD.

ABBREVIATIONS

AD, Alzheimer's disease; ALE-B, average life expectancy at birth; APP, amyloid precursor protein; CM, conventional measures (means); DP, dementia pugilistica; DS, Down's syndrome; ER, endoplasmic reticulum; FAD, familial Alzheimer's disease; FRTA, free radical theory of aging; 2-MEA, 2-mercaptoethylamine; SAD, sporadic Alzheimer's disease.

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