PERSPECTIVE

A vaccine to prevent initial loss of cognition and eventual Alzheimer's disease in elderly persons

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Abstract

Prevention is better than cure and prevention of Alzheimer's disease (AD) may be possible. In elderly persons who are cognitively normal, synaptic hypometabolism as shown by reduced cerebral uptake of fluorodeoxyglucose (^{18}F-FDG), provides a premonitory signal of potential, future loss of cognition if those individuals also have present evidence of amyloid deposition seen in the Pittsburgh compound B positron emission tomography (PIB-PET) scan for amyloid. Those are the persons who should be targeted if one aims to prevent AD. The synaptic hypometabolism implies that the brain's availability of adenosine triphosphate (ATP) is inadequate for performance of all required synaptic functions. This review first describes the basis for asserting that reduced cerebral uptake of ^{18}F-FDG accurately reflects synaptic hypometabolism; second, explains the basis for asserting that hypometabolism implies inadequate ATP; third, shows that amyloid beta (Aβ) itself, Aβ modified by pyroglutamate to become a molecule termed pE(3)Aβ, and cyclophilin-D, in concert are the main contributors to inadequate synaptic ATP and that, therefore, reducing all of their levels would neutralize their combined effect and correct the hypometabolism. pE(3)Aβ is more neurotoxic than unmodified Aβ; and cyclophilin D inhibits ATP synthase and reduces ATP formation. Finally, this review describes an mRNA self-replicating vaccine that will raise brain levels of ATP by reducing Aβ, pyroglutamate-modified Aβ, and cyclophilin-D, and thereby—in cognitively normal elderly persons who have synaptic hypometabolism—prevent initiation of the process that terminates in AD.

KEYWORDS
Abeta, ATP-synthase, cyclophilin-D, inadequate ATP level, mRNA vaccine to prevent original cognitive loss, normal cognition, prevent Alzheimer's disease, prevent cognitive loss, pyroglutamate-modified Abeta, synaptic hypometabolism

1 | INTRODUCTION AND BACKGROUND

Almost no medical problem can be either eliminated or neutralized unless the primary cause can be countered before secondary and tertiary events transpire. Diabetes mellitus is best held in check by insulin; complications of hypertension are best prevented if blood pressure is maintained below a threshold level; early treatment of cancers is always the most effective; rheumatoid arthritis benefits from

early use of anti-inflammatory drugs; and it is widely recognized that treatment for human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) should begin immediately when the diagnosis is made. The concept by Hardy and Higgins,[1] and Selkoe[2] that the primary cause of Alzheimer's disease (AD) is cerebral amyloid or its oligomers, was based upon rational analysis of the facts known at the time. The most likely reason why, subsequently, so many trials based upon the amyloid hypothesis have failed either to reverse or halt AD, is because once the causal process is in motion it may be unstoppable due to the known multiplicity of abnormalities of the brain in AD, many of which are also present at the earlier stage of mild cognitive impairment (MCI). On the other hand, it may be possible to prevent AD before it has even commenced, which is the time when there is not yet cognitive loss but when amyloid is detectable by Pittsburgh compound B positron emission tomography (PIB-PET) scan and when synaptic metabolism as shown by fluorodeoxyglucose PET (^{18}F-FDG-PET) scan is reduced.[3] That condition has been termed pre-clinical AD.[4] Because synapses account for a large portion of the brain's energy use, an immediate question is whether preventing this reduction of neuronal adenosine triphosphate (ATP) might prevent the start of cognitive loss in predisposed persons. This article will first describe the basis for asserting the presence of synaptic hypometabolism before cognitive loss has appeared. Second, it will describe the basis for asserting that hypometabolism, as shown by low uptake of ^{18}F-FDG, implies inadequate synaptic ATP. Third, it will show that amyloid beta (Aβ) itself, pyroglutamate-modified Aβ, and cyclophilin-D are, in concert, the main contributors to inadequate synaptic ATP and, therefore, that reducing their input would neutralize their combined effect and correct that hypometabolism. Finally, it will describe a self-replicating, mRNA vaccine that will reduce the levels of Aβ, pyroglutamate-modified Aβ, and cyclophilin-D, thereby preventing the process that terminates in AD.

2 | SYNAPTIC HYPOMETABOLISM IS SEEN BEFORE COGNITIVE LOSS HAS APPEARED

Among nine proteins in cerebrospinal fluid (CSF) that participate in core synaptic processes, Lleó et al. found that six were reduced in preclinical AD.[5] Lim et al. caused stress to cholinergic neurotransmission by administering scopolamine to 63 persons aged 55 to 75, who had subjective memory complaints but normal neuropsychological test results.[6] Aβ PET imaging showed that 15 subjects were Aβ +ve and therefore had preclinical AD and 48 were Aβ –ve. At 5 hours post dose, cognition was reassessed; the Aβ +ve group performed significantly worse than the Aβ –ve group, with large magnitudes of effect for three of the cognitive tasks. In the Baltimore Longitudinal Study of Aging (BLSA), O'Brien et al. examined the *post mortem* brains from eight subjects with preclinical AD and eleven age-matched controls.[7] Although neurons were not significantly lost in either CA1 or any other subdivisions of the hippocampus in subjects with preclinical AD, there were significant decrements in the levels of synaptic proteins, Rab3A, synaptobrevin, and synaptotagmin. Price et al. confirmed that neurons themselves are unaffected: they studied four persons with presymptomatic

AD having Clinical Dementia Rating (CDR) scores of 0, and 14 healthy, non-demented controls.[8] They examined sections from the CA1 area of the hippocampus and entorhinal cortex with Nissl and Bielchovsky stains. Neither in the entorhinal cortex nor in the hippocampal CA1 did the number of neurons differ between the healthy controls and those with preclinical AD.

3 | SYNAPTIC HYPOMETABOLISM, SHOWN BY LOW UPTAKE OF ^{18}F-FDG, IMPLIES INADEQUATE SYNAPTIC ATP

Dienel pointed out that evidence for glucose being the obligatory fuel for brain metabolism comes from insulin-induced hypoglycemia, in which the cognitive status progressively changes to lethargy, stupor, and coma as plasma glucose level falls.[9] Administration of glucose rapidly reverses these behavioral consequences and, of other tested compounds, only mannose and maltose are effective, whereas many others, for example, glycerol, ethanol, lactate, pyruvate, glyceraldehyde, fumarate, acetate, β-hydroxybutyrate, and galactose, are ineffective. Although adult brain tissue can metabolize most of those substrates, blood-brain barrier (BBB) transport capacity and their normal blood concentrations are insufficient to deliver enough of these alternative oxidative fuels to the brain to support cognitive activities.

Calculations show that metabolism of $\approx$1 molecule of glucose produces $\approx$28 molecules of ATP by the oxidative pathways per synapse and $\approx$2 molecules of ATP by glycolysis.[9] Synaptic activity accounts for the majority of energy expenditure by the brain, and ATP formation in the brain is primarily from oxidative phosphorylation of glucose,[10] so when cerebral glucose metabolism is reduced there is also reduced ATP available. The cerebral metabolic rate for glucose (CMR_{glc}) is assessed by means of ^{18}F/fluoro-2-deoxy-D-glucose (^{18}F-FDG) PET scanning.[11] ^{18}F-FDG enters the cell by the same membrane transport mechanism as glucose. After penetration of the cellular membrane via glucose transporters, both ^{18}F-FDG and glucose are phosphorylated by hexokinase but unlike glucose-6-phosphate, ^{18}F-FDG-6-phosphate is not a substrate of glucose-6-phosphate isomerase and does not undergo further metabolism in the glucose pathway. The interested reader may refer to illustrations in one of the earliest studies, showing ^{18}F-FDG scans in the occipital cortex when one half of the visual field is stimulated and the other half is not so stimulated: the side contralateral to stimulation is bright and the other side is dark.[11]

4 | ELDERLY PERSONS WITH NORMAL COGNITION ARE PREDISPOSED TO FUTURE AD IF THEY HAVE SYNAPTIC HYPOMETABOLISM AND CEREBRAL DEPOSITION OF AMYLOID

The term "synaptic hypometabolism" refers to a low CMR_{glc} as shown by reduced uptake of the ^{18}F-FDG. The importance of CMR_{glc} before the occurrence of cognitive loss was demonstrated by Mosconi

et al., who followed 77 cognitively normal, 70-year-old subjects in a longitudinal FDG-PET study.[12] During follow-up, 11 developed dementia, 6 of whom had AD, and 19 declined to MCI. Cognitive decline occurred, on average, 8 years after the baseline examination. Those with hypometabolism, that is, a low baseline hippocampal CMR_{glc} (≤ 24 $\mu mol/gm/min$) had the fastest predicted time, 7 years, to cognitive decline; those with an intermediate baseline hippocampal CMR_{glc} (25 to 28 $\mu mol/gm/min$), had a slower predicted time, 9.5 years, to cognitive decline; and those with the highest baseline hippocampal CMR_{glc} (≥ 29 $\mu mol/gm/min$), had the longest predicted time, 12 years, to cognitive decline. Studies by De Santi et al. and De Leon et al. had shown a reduced baseline CMR_{glc} in frontal and temporal lobes with normal aging, and that hypometabolism of glucose in the entorhinal cortex predicted an MCI diagnosis 3 years later with 83% sensitivity and 85% specificity.[13,14] The above studies show cognitive loss as occurring soonest in those individuals with cerebral hypometabolism; note, however, that deliberately raising blood glucose levels to prevent cognitive loss would be inappropriate because many studies have demonstrated that prediabetic levels of blood glucose are themselves associated with either current or future cognitive impairment (see Roriz-Filho et al.[15] for citations).

5 | Aβ, PYROGLUTAMATE-MODIFIED Aβ, AND CYCLOPHILIN-D ARE, IN CONCERT, THE MAIN CONTRIBUTORS TO INADEQUATE SYNAPTIC ATP

5.1 | Aβ and ATP

The importance of Aβ unmodified by pyroglutamate, particularly its soluble oligomers, is widely recognized as an important player in the pathogenesis of AD. As is discussed elsewhere and not expanded upon in this essay, unmodified Aβ is essential but insufficient for that pathogenesis.[16] Both unmodified Aβ and pyroglutamate-modified Aβ participate in causing synaptic hypometabolism in those cognitively normal elderly persons who have potential, future loss of cognition. Du et al. demonstrated accumulation of unmodified Aβ in mitochondria,[17] where Singh et al. showed that it may cause synaptic hypometabolism by preventing the release of ATP into the neuronal cytoplasm.[18] Singh et al. performed multiple computer simulations using programs that judge subcellular locations of proteins as well as the likelihood of protein–protein interactions. Those showed Aβ as forming a very stable complex with adenine nucleotide translocase (ANT). Both unmodified Aβ and pyroglutamate-modified Aβ are abundant in the inner mitochondrial membrane, where Aβ interacts with ANT. The reader is reminded that the mitochondrion has an outer membrane, an inner membrane, the intermembrane space between those two membranes, and a space deep to the inner membrane that is called the matrix. Protrusions from the inner mitochondrial membranes into the matrix form the cristae. The function of ANT is to transport adenosine diphosphate (ADP)/ATP through an exchange-diffusion mechanism. During oxidative phosphorylation, ADP in the neuronal cytosol crosses the external mitochondrial membrane via the voltage-dependent anion channel

and enters the mitochondrial matrix; in exchange, ATP is released from the matrix into the intermembrane space and thence across the outer membrane into the neuronal cytosol. However, because of the ANT–Aβ interaction there is reduced availability of both ANT and ADP, so the availability of ATP is also reduced, which contributes to synaptic hypometabolism.[18,19]

5.2 | Pyroglutamate-modified Aβ

Modification of Aβ by pyroglutamate (pE), produces much-reduced levels of ATP as well as greatly aggravated neurotoxicity. Each of these actions will be explained below but first is an explanation of how pyroglutamate modifies Aβ. The modification occurs because, mediated by the enzyme glutaminyl cyclase, pyroglutamic acid truncates and caps the Aβ oligomeric peptide at position #3 of its N-terminus sequence. That position is occupied by glutamate (E), and pyroglutamate (p) attaches to E, forming a molecule that is written as pE(3)Aβ (sometimes written as pGlu3-Aβ, or AβpGlu3, or $A\beta_{N3(pE)}$; whatever its description in the various cited reports, it will, for uniformity, be written here as pE(3)Aβ). Harigaya et al. showed that pE(3)Aβ in the AD brain comprises $\approx 25\%$ of the total Aβ, but that in senile plaques, pE(3)Aβ forms oligomers at a concentration of 1mcg/ml whereas for $A\beta_{1-42}$ 2.5 mcg/ml was required.[20]

Data demonstrate that pE(3)Aβ is highly enriched in the AD brain, particularly in the subcortical regions that are affected in AD. Morawski et al. examined brain tissue from 13 non-demented controls and 11 AD cases, using immunohistochemistry, quantitative real-time polymerase chain reaction (RT-PCR), and enzymatic activity assays for the expression level of glutaminyl cyclase in temporal and entorhinal cortex; they also measured cortical pE(3)Aβ concentrations.[21] In AD brains, glutaminyl cyclase mRNA expression was increased and frequently associated with pE(3)Aβ deposits. The peptide was shown by Güntert et al. as being far more strongly accumulated during progression of AD than unmodified Aβ, which actually decreased, demonstrating the disease-driving role of pE(3)Aβ.[22] They did this by using a lysyl endopeptidase to digest cored plaques from five individuals with AD and analyzed the resultant peptides with various, very sensitive laboratory techniques including time of flight mass spectrometry. They examined levels of peptides having similar lengths but that were derived from either unmodified Aβ or pE(3)Aβ. Following enzymatic digestion, $A\beta_{1-16}$ was the predominant unmodified type and they examined levels of that in contrast to those of pE(3)Aβ3-16; the two molecules have almost identical ionization properties so their levels are comparable. Their table 2 shows that for temporal cortex, frontal cortex, and hippocampus, there was an average 47% increase in the total of pE(3)Aβ3-16 between Braak stages 1V and V1 but a 30% decrease in $A\beta_{1-16}$. A specific anti-pE(3)Aβ antibody created by Hettmann et al. had a binding strength that did not change significantly when that was tested against various mixtures of pE(3)Aβ plus unmodified $A\beta_{1-42}$ oligomers and fibrils and pure pE(3)Aβ aggregates, showing that there is little overlap between the neuropathology caused by the two molecules.[23] All of the above findings, together with the data in the next paragraph,

demonstrate the specificity of pE(3)Aβ and support that a reduction of pE(3)Aβ would have therapeutic benefit.

It is important that pE(3)Aβ adds to the neurotoxicity of Aβ. Russo et al. used cultured hippocampal neurons to show that pE(3)Aβ compared to Aβ_{1-42}, reduced cell viability by 40% (see figure 1 in Russo et al.[24]). He et al. showed that the pE(3)Aβ has a greater propensity to form beta sheets and greater aggregability than Aβ_{1-42}.[25] Grochowska et al. saw more neurotoxicity from mixed aggregates of pE(3)Aβ and Aβ_{1-42} than from Aβ_{1-42} alone, showing that each acts separately, apparently driven by a different receptor interaction pattern of these two oligomeric species.[26] In a mouse model for AD, Wirths et al. showed that intraneuronal accumulation of pE(3)Aβ peptides induce a severe neuronal loss with an associated clinical, neurological phenotype.[27] Wildburger et al. analyzed intact Aβ in the brains of six patients with advanced AD and found 26 unique proteoforms among which canonical Aβ accounted for only 15.3%, but N- and C-terminal truncations accounted for 73%.[28] N-terminal truncations were mostly in the insoluble material, and C-terminal ones were in the soluble material, which is believed to be the most toxic form of Aβ. Because unmodified Aβ comprised a minority of the identified proteoforms, it seems that reducing the level of pE(3)Aβ in addition to that of unmodified Aβ is a therapeutic necessity. Finally, Morawski et al. found that elevated pE(3)Aβ load correlated better with the decline in MMSE than did an elevated concentration of unmodified Aβ.[21] Parenthetically, in contemplating why many trials of vaccines against Aβ have failed, one of the reasons might be because lowering the level of Aβ while leaving pE(3)Aβ unattended may be inadequate.

In brief, the reduction of pE(3)Aβ levels would be beneficial for elderly individuals who are at risk of future loss of cognition.

5.3 | ATP and the role of ATP synthase

Because synaptic hypometabolism reflects either an inadequate amount or impaired function of ATP, the details of its synthesis and function, and how Aβ may impact them, are all relevant. ATP synthase, located within the inner mitochondrial membrane, has two "fractions" or regions: one binds to oligomycin (which inhibits the ATP synthase) and is labeled F_o, and the other is labeled F_1. Acting together, they are responsible for synthesis of ATP. Beck et al. observed selective loss of the F_o subunit in neuronal mitochondria of AD-model mice, with consequences that included reduced ATP production, elevated oxidative stress, and activation of the mitochondrial permeability transition pore.[29] They also noted that it was the synaptic mitochondria in neurons of AD-model mice that had a 67% reduction of F_o-ATP synthase in comparison to controls, whereas the non-synaptic mitochondria had only a 30% reduction. Using AD-model mice that overexpress mutant human amyloid precursor protein, Gillardon et al. microdissected cortical and hippocampal tissue at the onset of cognitive impairment but before the appearance of amyloid plaque, and separated synaptosomal from non-synaptic mitochondria by density gradient ultracentrifugation.[30] Respiration by mitochondria was initiated

and measured in synaptosomal mitochondria; stage 3 (electron transport) that requires ATP, was reduced to 43.1%, and specific antibody showed amyloid oligomers in these mitochondria. Rhein et al. used human neuroblastoma cells that were either transfected or not transfected with human wild-type amyloid precursor protein (APP).[31] In the transfected cells, production of ATP was reduced by 23% ($P < .05$). As already noted, Singh et al. made analyses using various computer programs, which suggested that Aβ forms a very stable complex with ANT; because both members of this pair are located in the inner mitochondrial membrane, the likelihood is high that Aβ interferes with the function of ANT, which is to exchange ATP from the mitochondrion with ADP in the cytoplasm. That interference would result in a diminished availability of ATP to neuronal synapses.[18] Although there is no information about whether pE(3)Aβ also interacts with ANT, nevertheless the following data show that pE(3)Aβ is also responsible for lowered ATP levels. Gunn et al. measured lipid peroxidation by mouse cortical neurons after exposure to Cu^{2+} and ascorbate, in the presence or absence of Aβ or pE(3)Aβ.[32] They found that both peptides caused lipid peroxidation; and Lopez et al. reported that lipid peroxidation was accompanied by a dramatic diminution of ATP.[33] Increased reactive oxygen species (ROS) in AD, which would produce lipid peroxidation, has been known for many years.[34] Thus, via several mechanisms, both Aβ and pE(3)Aβ lower ATP levels, preventing which would prevent synaptic hypometabolism. Another benefit of raising ATP levels is that heat-shock proteins 70 and 40 inhibit the self-assembly of pE(3)Aβ into fibrils in a reaction that requires ATP.[35,36]

In brief, both Aβ and pE(3)Aβ lower ATP levels, and increasing them would be beneficial for elderly individuals who are at risk of future loss of cognition because of synaptic hypometabolism caused by reduced levels of ATP.

5.4 | Cyclophilin D

Cyclophilin D in the mitochondrial matrix translocates to the inner mitochondrial membrane to collaborate with ATP synthase subunit 9 to open the mitochondrial permeability transition pore that results (see next section) in uncoupling of oxidative phosphorylation and reduced formation of ATP. Baines et al. generated mice overexpressing cyclophilin D, which caused them to have mitochondrial swelling and cell death.[37] Several reports have demonstrated that reducing the level of cyclophilin D enhances production of ATP, which clearly would be advantageous for persons with synaptic hypometabolism. Cyclophilin D is a product of the Pfif gene, and Baines et al. also created Pfif null mice, the homozygotes of which produced no cyclophilin D and whose heterozygotes produced half-normal levels; those mice had mitochondria resistant to both swelling and opening of the mitochondrial permeability transition pore (MPTP) caused by Ca^{2+}.[37] Thus, reducing cyclophilin D negated its deleterious consequence of excessively raising Ca^{2+}. Cyclophilin D also binds ANT, which is another way whereby cyclophilin D reduces ATP.[38] Giorgio et al. showed that binding to the F_0F_1-ATP synthase and impairing its enzymatic activity is yet another

mechanism by which cyclophilin D decreases the synthesis of ATP.[39] ATP synthase is organized into supramolecular units called synthasomes that increase the efficiency of ATP production, and Beutner et al. showed that deletion of cyclophilin D was advantageous, by increasing synthasome assembly in various tissues including the brain.[40] In an AD mouse model, Du et al. showed that abrogation of cyclophilin D resulted in persistent, life-long protection against Aβ toxicity thereby suggesting that blockade of cyclophilin D may be of benefit for AD treatment.[41] The same group of investigators showed that in mice with streptozotocin-induced diabetes, blockade of the F_1F_0 ATP synthase–cyclophilin D interaction by cyclophilin D ablation, protected against decrease of ATP synthesis; and, further, that the absence of cyclophilin D alleviated deficits in synaptic plasticity, learning, and memory in the diabetic mice.[42]

In brief, reduction of cyclophilin D would lead to increase in ATP formation and benefit persons with synaptic hypometabolism.

6 | CALCIUM AND MAGNESIUM INVOLVEMENT IN ATP FORMATION; THE CRITICAL IMPORTANCE OF THE PROTON MOTIVE FORCE ($\Delta\Psi$); HOW $\Delta\Psi$ MAY CAUSE HIGH MITOCHONDRIAL CA^{2+} LEVELS AND LOWER ATP FORMATION

The ultimate cause of synaptic hypometabolism is reduced availability of ATP, largely due to its reduced production by mitochondria, and its regulation involves both Ca^{2+} and Mg^{2+}. For more extensive details than provided in this synopsis, in which oversimplification of the many complexities is intended to assist understandability, the interested reader is referred to recent reviews.[43–45] This section will briefly discuss the synthesis of ATP and its role as a store of energy; the concept behind the motive force of protons; the uptake and removal from mitochondria of Ca^{2+}; the mitochondrial permeability transition (MPT); the MPTP; and the crucial role of cyclophilin D. Within the intermembrane space, addition of a third phosphate group to ADP adds the energy that is stored as ATP. This addition requires complex V (ATP synthase) and complexes I, II, and IV (cytochrome c oxidase) of the electron transport chain (ETC), that transport protons in the form of H^+ across the inner mitochondrial membrane (which is almost impermeable but has a variety of ion channels and transporters). In the matrix, the oxidation of NADH produces the H^+ (the proton), and the ETC transports the H^+ into the intermembrane space, which now becomes electrically positive; and the matrix, having lost its H^+ is now electrically negative. That translocation of protons by complexes 1, 111, and 1V, from the mitochondrial matrix into the intermembrane space, creates the force to drive ATP synthesis by ATP synthase. The energy of this concentration gradient is the so-called proton motive force ($\Delta\Psi$).[42,46] $\Delta\Psi$ is regulated by the Ca^{2+} concentration in the mitochondrial matrix, where the net level of Ca^{2+} is the difference between Ca^{2+} uptake and its extrusion: uptake is via the mitochondrial Ca^{2+} uniporter (MCU) transporting Ca^{2+} into the mitochondrial matrix: the main extrusion pathway is the Na^+/Ca^{2+} exchanger pumping Ca^{2+} from the matrix, back into the intermembrane space.

Wescott et al. described experiments on isolated mitochondria that elucidated the main mechanisms involved in ATP formation, for which the three critical components are ADP (the substrate), Ca^{2+} (the regulator), and $\Delta\Psi$ (the energy source).[45] By using isolated mitochondria and controlling the levels of Ca^{2+}, Wescott et al. showed that Ca^{2+} regulates mitochondrial use of carbohydrates and amino acids in the tricarboxylic acid cycle, and through this regulation, increased Ca^{2+} powered by $\Delta\Psi$, augments ATP production. If, however, the Ca^{2+} concentration in the mitochondrial matrix becomes excessive, Ca^{2+} may then cause the MPTP to swell and open; when that happens, the inner mitochondrial membrane can no longer maintain a barrier to protons, leading to dissipation of the proton motive force, that is, $\Delta\Psi_m$, and now there is uncoupling of oxidative phosphorylation and reduced formation of ATP.[47,48] Further, extrusion of excessive Ca^{2+} requires ATP and causes its further depletion.[49] Those high levels of Ca^{2+} may be prevented by dantrolene, discussed in detail in Fessel.[3] Finally, and of note, Mattiason et al. showed that mitochondria isolated from the hippocampal CA1 region are more susceptible to an excessive Ca^{2+} load than are mitochondria isolated from the CA3 region,[50] which is relevant in the present context because CA1 is more important than is CA3 for memory and cognition.

In brief: in the presence of high mitochondrial Ca^{2+} levels, there is both decreased formation and increased use of ATP. The decrease of ATP, particularly in CA1 synapses, would lessen if cyclophilin D were reduced by, for example, a vaccine.

Mg^+ participates in several ways in the regulation of ATP production by the mitochondria: first, because the mitochondria are major pools of Mg^+; second, because both Mg^+ and Ca^{2+} levels in the mitochondrial matrix affect mitochondrial dehydrogenases and, therefore, mitochondrial respiration rate; and third, because an increase in Mg^+ antagonizes opening of the MPTP, this counters the effect of excessive mitochondrial Ca^2. For these reasons, in clinical practice, Mg^+ levels should be kept at the upper levels of the normal range, by use of oral supplementation.

7 | A MULTIVALENT COLLABORATION AMONG Aβ, PE(3)Aβ, ATP SYNTHASE, AND CYCLOPHILIN D

A constellation of unmodified Aβ, pE(3)Aβ, and cyclophilin D participates in a multivalent collaboration that lessens the effect of ATP synthase and eventuates in reduced formation of ATP. Because it is not unmodified Aβ alone but a collaboration with its pE(3)Aβ variant that participates in the multivalent collaboration, it seems possible that this explains the failure of so many attempts, including by various vaccines, to demonstrate benefit to AD from reducing the amount of Aβ. A vaccine that not only reduces Aβ but also reduces pE(3)Aβ and cyclophilin D, would result in increased neuronal ATP and decrease the synaptic impairment caused by inadequate ATP. Such a vaccine should be beneficial for cognitively normal elderly persons who have synaptic hypometabolism (and potentially, also, for MCI/AD patients). The concept may be tested in animal trials and if those were successful, then ultimately in clinical trials in humans.

8 | A VACCINE USING MRNA METHODOLOGY. THE BENEFIT OF A SELF-AMPLIFYING MRNA VACCINE AGAINST Aβ, PE(3)Aβ, AND CYCLOPHILIN D

Only the briefest synopsis of the possible methodology is provided here: for further details the reader is referred to Pardi et al.[51] and Zhang et al.[52] mRNA has the capacity for self-replication, which means that susceptible persons may be vaccinated just once and the effect should be long-lasting because a self-replicating vaccine induces long-term antibody production, which is an important asset in the present context. An additional reason to use an mRNA-based vaccine is that a single vaccine can incorporate several antigens and thereby minimize the number of oral drugs that might be required to achieve the same therapeutic objective. The basic principle of mRNA vaccines is to use the host cell's transcription system to produce target antigens that stimulate adaptive immunity. Traditional mRNA-based vaccines contain only the target antigen gene and cannot replicate themselves. Self-amplifying mRNA vaccines encode the engineered genome of an RNA virus; one commonly used for this purpose is a modified vaccinia virus Ankara, which is an attenuated poxviral vector that is widely used to develop vaccines because of (1) its absence of harmful effects in humans, (2) its excellent safety record, (3) its capacity to express multiple antigens, and (4) its ability to express antigen-specific humoral and cellular immunity.[53] The vaccine contains the virus's non-structural protein gene, and its structural protein gene is replaced by the mRNA(s) of interest. The resulting proteins are antigens expressed at a high level due to the amplification effect of the RNA template, and these replicons cannot produce infectious virions because the gene for the structural protein of the virus has been deleted. Cells replicate, including neurons and glia, and the daughter cells maintain the parental molecular biology. A self-amplifying mRNA vaccine would produce a long-lasting effect and should protect the individual for many years from synaptic dysfunction, whereas a traditional vaccine would produce an effect of far shorter duration. Another major advantage of mRNA vaccines is that by targeting multiple antigens they minimize the number of oral drugs needed.

9 | VALIDITY TRIALS

The initial trials should be in AD-model rodents. The vaccine should be produced with the collaboration of a vaccinologist. Only after an animal trial has shown that the vaccine produces a significant rise in available ATP would a trial in humans be contemplated. The primary goal of a human trial would be to demonstrate that the vaccine raises ATP levels and prevents cognitive loss in elderly persons who have not yet lost cognition. Accrual of subjects would require that they are age 70 to 75 years, and have health adequate to allow follow-up of >5 years; uncontrolled diabetes and hypertension would be among many exclusions, so accrual to the trial would be slow.

10 | CONCERNS

There are several concerns about administering a vaccine to healthy persons in the present context.

1. pE(3)Aβ-42 may not be the only variant of Aβ1-42 that affects synapses (note that 1 to 42 indicates a length of 42 amino acids; thus, 3 to 42 has a length of 40 amino acids, 3 to 38 a length of 36 amino acids, etc.). Schlenzig et al. showed that variants of the pE(3) with different lengths had different abilities to impair synaptic function.[54] Therefore, although using pE(3)Aβ-42 and intact Aβ may omit several relevant antigens (see #2 and #5 below), it may not be practical to incorporate them.

2. As noted above, Wildburger et al. analyzed intact Aβ in the brains of patients with advanced AD and found that unmodified Aβ comprised only 15.3% of the 26 identified proteoforms;[28] it is possible that other Aβ molecules of different lengths would not be covered by the proposed vaccine.

3. Cytokine release is of major concern because the report of a "cytokine storm" that developed in six healthy human volunteers administered an anti-CD28 super-agonistic monoclonal antibody, during a phase I clinical trial.[55] There are premonitory signs of a cytokine storm and preventative action is possible.

4. Next, there is a risk that a vaccine might provoke auto-immune diseases. "Non-self" molecules such as pE(3)Aβ can induce the production of antibodies that subsequently react with epitopes of "self" origin and cause autoimmune disease. If the proposed vaccine were subjected to validation by clinical trial, a family history or personal history of autoimmune disease should be an exclusionary criterion.

5. Another risk is antibody-dependent cellular cytotoxicity (ADCC), an immune mechanism whereby an effector cell of the immune system lyses a target cell, whose membrane-surface antigens have been bound by specific antibodies that were induced by the vaccine. This issue as well as antibody-dependent enhancement (ADE), is reviewed in detail by Arvin et al.[56] Almost always it occurs when an intact virus is part of the vaccine, because antibodies present at the time of an infection may increase the severity of an illness: this is not the case in the present context and with the proposed vaccine. Arvin et al. also warned that the effector function of antibodies is species specific, so that results in animals may not apply to humans; and further, that protective and non-protective antibodies may be elicited by different forms of the same protein, which reminds us of the various lengths of Aβ that may be present in AD.

6. Infectious complications such as the occurrence of progressive multifocal leukoencephalopathy, which is caused by the JC virus, was seen in three multiple sclerosis patients treated with natalizumab, are risks that are related to ADE and ADCC and to the presence of immune suppression.[57] Because prior exposure to the JC virus is very frequent in the general population, this potential possibility cannot be discounted but it was rare, even in patients with immune suppression due to infection by HIV: a history of infection by HIV

should be an exclusionary criterion for potential recipients of a vaccine to prevent ATP reduction.

7. It is also worth pointing to the age-related attenuation of the immune system, because the subjects exposed to the vaccine will usually be over age 70 years.

In brief: although the proposed vaccine might theoretically minimize ATP depletion and benefit synaptic function, risks must be acknowledged and, if possible, minimized. The various risks of vaccination should be considered if animal experiments suggest contemplating human trials. None of these risks to human subjects will actualize unless trials occur in human subjects.

11 | CONCLUSIONS

Synaptic hypometabolism in cognitively normal elderly persons provides a premonitory signal of potential, future loss of cognition. The synaptic hypometabolism, shown by reduced cerebral uptake of ^{18}F-FDG, is due to inadequate availability of ATP caused by the combined actions of unmodified Aβ, of Aβ that is modified by pyroglutamate to become a molecule termed pE(3)Aβ, and of excessive cyclophilin D. The combination of all of the above molecules produces reduced ATP synthase levels and lowered availability of ATP. An mRNA self-replicating vaccine could reduce levels of Aβ, pE(3)Aβ, and cyclophilin D, thereby correcting the deficiency of ATP. That would prevent initiation of the loss of cognition and future AD, in elderly persons who have not yet lost cognition.

CONFLICT OF INTEREST
There are no conflicts of interest.

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