Immunoreactivity of Anti-AβP-42 Specific Antibody with Toxic Chemicals and Food Antigens

Aristo Vojdani1,2* and Elroy Vojdani3

1  Immunosciences Lab., Inc., Los Angeles, CA, USA
2 Department of Preventive Medicine, Loma Linda University School of Medicine, Loma Linda, CA, USA
3 Regenera Medical, Los Angeles, CA, USA

Abstract

Objective: The aim of our study was to examine immunoreactivity between AβP-42, toxic chemicals, and food proteins that could be involved in AD.

Methods: We applied monoclonal anti-AβP-42 to a variety of chemicals bound to human serum albumin (HSA) and 208 different food extracts.

Results: We found that anti-AβP-42 reacts from moderately to strongly with mercury-HSA, dinitrophenyl-HSA (DNP-HSA), phthalate-HSA, and aluminum-HSA, but not to many other tested chemicals bound to HSA nor to HSA alone. This antibody also reacted with 19 out of the 208 food antigens used in the assay. One example of a food that reacted strongly with anti-AβP-42 in our study was canned tuna, although raw tuna reacted only moderately.

Conclusion: Based on these results, we hypothesized that reaction between AβP-42 antibody with chemicals bound to HSA and numerous food antigens might play a role in Alzheimer’s disease (AD). These anti-AβP antibodies could be derived from protein misfolding similar to β-amyloid, or from antibodies to various food antigens that cross-react with AβP-42. Removal of toxic chemicals and food items that share a homology with β-amyloid may be recommended at least for patients in the early stages of AD. Therefore, the role of AβP-42 cross-reactive foods and chemicals bound to HSA in neurodegeneration should be investigated further.

Keywords: AβP-42; Amyloidogenesis; Alzheimer’s Disease; Toxins; Dietary Proteins; Immunoreactivity; Neurodegeneration

Abbreviations: OD: Once in a Day; RT: Room Temperature.

Introduction

According to the National Institute on Aging, Alzheimer’s disease (AD) is the sixth leading cause of death in the United States [1]. In most people, the exact causes of AD are not fully understood. In cases of early onset of AD, genetic component plays a significant role. Late-onset Alzheimer’s, the most common form of the disease, is thought to arise from a combination of genetic, environmental and lifestyle factors that may cause a series of brain changes to occur over decades [1]. The genetic component of AD and its association with APOE-ε4 is well established [2]. Apolipoprotein E (ApoE) is a major cholesterol carrier that supports lipid transport and injury repair in the brain; the ApoE-ε4 allele is not effective as the other variants, so much so that it is the largest known genetic risk factor for late-onset sporadic AD [3]. The APOE-ε4 allele is also associated with increased risk for cerebral amyloid angiopathy and age-related cognitive decline during normal ageing [4].

In relation to the role of environmental triggers, many studies have established that specific infectious agents such as Herpes simplex virus type 1 (HSV-1) [5,6], Gram-negative bacteria [7], Chlamydia pneumoniae [5,8], several types of spirochetes including Borrelia burgdorferi [5,9], fungal infections [5,10,11] oral pathogens [12-16], and bacterial toxins [17,18] are implicated in AD. However, with the exception of a few chemicals, the role of environmental toxins and dietary proteins in association with AD has largely been neglected [19]. Toxic metals such as aluminum [20-24], mercury [25-27] and lead [28, 29] are among the few that are known to cause toxicity to the brain and other organs and have been linked to numerous neurodegenerative disorders, including AD.

Exposure to aluminum and such metals has been followed by aggregation of amyloid-β protein (AβP) on neuronal cells [28] as well as AD-like pathologies, which have been shown in animals as well as in human subjects [29]. Aluminum-induced neurotoxicity includes oxidative stress, mitochondrial dysfunction, inflammatory response, and neurofilibrillar degeneration, possibly through amyloid-β oligomerization [20,22]. In vitro studies have shown that aluminum together with other metals is involved in the formation of AβP aggregation, which leads to amyloid fibrils and the formation of amyloid-like plaque structure [24]. As early as 1999 aluminum had already been shown to be neurotoxic, causing abnormal clustering and aggregation that resulted in neuronal death [30]. A review in 1998 [31] showed that such accumulations of amyloid and extracellular tangles act as irritants, resulting in inflammatory reactions that lead to the production of potentially neurotoxic products that contribute to neuronal loss.

Mercury has also been reported as a risk factor for AD due to its well-known neurotoxicity. Mercury ions bind to tubulin, inhibiting guanosine triphosphate (GTP) nucleotide binding capacity and reducing its biological activity, leading to microtubule degeneration [25]. In vitro and animal studies have shown that mercury causes hyperphosphorylation of tau protein and increased formation of AβP aggregation [26].

*Corresponding author: Aristo Vojdani, PhD, MSc, CLS. Immunosciences Lab., Inc, 822 S. Robertson Blvd., Ste. 312 Los Angeles, CA 90035, USA, Tel: (310) 657-1077; Fax: (310) 657-1053; E-mail: drari@msn.com

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Phthalates and bisphenol A (BPA) are used as plasticizers in water bottles, food cans, and many other products. As such, they can leach or migrate into food and water, and hence through estrogenic action or epigenetic modification may affect human health [32,33]. If BPA crosses the blood-brain barrier, it can bind to a target enzyme called protein disulfide isomerase (PDI). PDI is a stress protein found in the endoplasmic reticulum of many cells, including neural tissue, and is involved in protein folding. Normally this enzyme effectively inhibits α-synuclein fibril formation, but the S-nitrosylation of PDI by chemicals leads to a loss of enzymatic activity and the enhancement of protein misfolding and α-synuclein aggregation that are found in AD and Parkinson’s disease [34,35]. It is important to note that BPA has been shown to affect the prefrontal cortex and hippocampus where it interferes with synapse formation, resembling events that occur in AD [36,37].

Prenatal exposure to phthalates was shown to be associated with poor cognition and social impairment mainly in girls, who are more vulnerable to the neurotoxic effects of phthalates than boys [38-40]. Phthalates have also been shown to significantly inhibit the activity of acetylcholinesterase, and upregulate myelin basic protein (MBP) and glial fibrillary acidic protein (GFAP) in a zebrafish model [41]. Furthermore, it was observed that prenatal exposure to phthalates caused cognitive dysfunction and an increase in tau protein phosphorylation in rats [42,43]. Organic solvents containing benzene ring, such as phenol and alkylphenol, are found in the paint, paper, cleansing agent, and textile industries, and have been shown to exert an estrogenic effect and an increase in the expression of amyloid and precursor protein-2 accumulation, which may result in neuronal degeneration in AD brains [19,44]. Since humans are exposed to so many chemicals, their synergistic effect may contribute to immunotoxic, neurotoxic and AD-like pathology [19,45,46]. Autoantibodies to AbP are detected in the elderly and in AD. These antibodies may exert a protective role by inhibiting the activity of toxic peptides. They may also induce an immune attack against AbP, activating inflammatory cascades that kill the neurons in which AbP resides [47,48]. The exact source of these antibodies is not clear. They could be derived as a response to haptenic chemicals bound to AbP or other proteins or peptides, or these antibodies may also arise from a reaction to other antigens, such as pathogens or foods that cross-react with the amyloid peptides [49-52]. A search for food proteins that share similarity with AbP was done by Carter [49], who found that many foods display a large number of tetrapeptide sequences matching those of β-amyloid. This suggests that these and possibly other food proteins could play a rather unexpected role in AD. We resolved then to examine whether this close homology of diverse antigens with AbP and possibly the misfolding of proteins generated by the binding of haptenic chemicals to various proteins constitute an autoimmune component of AD that is triggered by these homologies and neo-antigen formation. To confirm this immunoreactivity or cross-reactivity, we applied monoclonal antibodies made against AbP-42 with 20 haptenic chemicals bound to human serum albumin (HSA) as well as with 208 purified dietary proteins commonly consumed in raw and cooked forms.

Materials and Methods

Monoclonal antibodies

Commercially available antibodies were purchased from different companies. Rabbit monoclonal anti-amyloid-β1-42 (biolsequence DAEFRHDSGYEVHHQKLYPFAEDVGSNKAGILMLVMGVGYVIA) produced by Abcam’s RAbMab® technology was purchased from Abcam. This antibody reacts strongly to human Aβ 42 monomers, oligomers and fibrils, but not with human muscle fibrils. Additional information about the specificity of this antibody is provided in the Abcam package insert (ab201061) and in an article by Hatami et al. [53]. Affinity-purified mouse anti-amyloid-β1-42 was purchased from BioLegend, San Diego, CA USA. This antibody reacted strongly with formalin-fixed, paraffin-embedded diseased human brain tissue.

The mouse monoclonal antibody (mAb β P2-1) to AbP peptide residues of 104-118 CKTHPHFVIPYRCL was purchased from Thermo Fisher Scientific. Its specificity was confirmed by Western blot, immunoprecipitation, ELISA, and immunohistochemistry. According to the product package insert, this antibody is specific for native, non-denatured amyloid precursor protein (APP) from human and monkey. It does not cross-react with mouse, rat APP or other APP homologs.

Proteins and peptides

Amyloid β -peptide 1-42 at purity greater than 95% was purchased from GenScript Piscataway, NJ, USA. Recombinant tau protein was obtained from rPeptide Watkinsville, GA USA. Wheat globulin, β + β casein, glutamic acid decarboxylase peptide was synthesized by BioSynthesis Lewisville, TX USA.

Chemicals bound to HSA

Dinitrophenyl-HSA (DNPHSA), aflatoxin-HSA, diethyl phthalate, and other chemicals were purchased from Sigma-Aldrich, St. Louis, MO, USA. Protein disulfide isomerase (PDI) was purchased from Creative Biomart.

Binding of phthalate to HAS

Preparation of diethyl phthalate was done according to the method described by Zhou et al. [54]. Briefly, 2 mg of diethyl phthalate was dissolved in methanol and added dropwise to 25 mg of HSA dissolved in 5 mL of 0.14 Tris-HCl buffer. After stirring for 1 hour at room temperature, the conjugates were dialyzed using tubes at a cutoff of 6,000 Dalton against four changes of 0.01 M PBS pH 7.2. After completion of dialysis, the mixture was filtered and kept at -20°C until used.

Binding of mercury to HAS

For this preparation, 100 mg of HSA was dissolved in 9 mL of buffer solution containing potassium chloride and sodium borate 0.05 M, and the pH was adjusted to 9.4 with 0.1 N NaOH. Then, 25 mg of mercury chloride was dissolved in 1 mL of buffer and added dropwise to the HSA solution. The reaction mixture was stirred overnight, dialyzed against 0.1 M PBS using tubing with a cutoff of 6,000 Da kept at -20°C.

Binding of aluminum and glyphosate to HAS

Aluminum hydroxide bound to HSA was prepared according to Lu et al. [55]. Glyphosate or N-phosphomethylglycine was bound to HSA using the method described by Yue et al. [56].

All other chemicals bound to HSA including pyrethroid-HSA and formaldehyde-HSA were done according to the methodology described by Vojdani in 2014 [57].

Preparation of dietary antigens

Food antigens were prepared from products purchased from the supermarket in both raw and cooked forms. For that preparation, 10 g of food product was put in a food processor using 0.1 M of phosphate buffer saline (PBS) at pH 7.4. The mixer was turned on and off for 1 h and then kept on the stirrer overnight at 4°C. After centrifugation
at 20,000 g for 15 min, the top layer, which contained oil bodies, was discarded. The liquid phase was removed and dialyzed against b0.01 M of PBS using dialysis bags, with a cutoff of 6,000 kDa. Dialysis was repeated three times to ensure all small molecules were removed. After dialysis, all samples were filtered through a 0.2 micron filter to remove any debris. Protein concentrations were measured using a kit provided by Bio-Rad (Hercules, CA, USA). Different peptides were purchased from Bio-Synthesis (Lewisville, TX, USA). Lectin and agglutinins including pea lectin and lentil lectin were purchased from Sigma-Aldrich (St. Louis, MO, USA). The complete list of foods used for antigen preparation is shown in [58].

Enzyme-linked immunosorbent assay (ELISA)

For demonstration of anti-AβP-42 antibody binding to chemicals bound to HSA and food antigens. Chemicals bound to HSA, food antigens, PDI enzyme, and AβP-42 were dissolved in PBS or methanol at a concentration of 1.0 mg/mL and then diluted 1:100 in 0.1 M carbonate-bicarbonate buffer at a pH of 9.5, and 100 µL was added to each well of the polystyrene flat-bottom ELISA plate. Plates were incubated overnight at 4°C and then washed three times with 200 µL 0.01 M PBS containing 0.05% Tween 20 at a pH of 7.4. The non-specific binding of immunoglobulins was prevented by adding 2% bovine serum albumin (BSA) into the PBS and then incubating overnight at 4°C.

Plates were washed, and then, rabbit monoclonal antibodies diluted at an optimal dilution of 1:500 were added to quadraplicate wells coated with each antigen. Plates were incubated for an additional 1 h at room temperature. The plates were then washed five times with Tris-buffered saline (TBS)-TWEEN. Alkaline phosphatase-labeled secondary antibody at a dilution of 1:600 was then added to all wells and incubated again for 1 hour at room temperature. The enzyme reaction was started by adding 100 µL of para-nitrophenylphosphate at a concentration of 1 mg/mL in diethanolamine buffer containing 1 mM MgCl2 and sodium azide at a pH of 9.8. The reaction was stopped 45 min later with 50 µL of 1 N NaOH, and the samples were read by an ELISA reader; the optical densities were recorded.

To determine the specificity of affinity-purified rabbit anti-AβP-42 binding to the chemicals bound to HSA and food antigens, the rabbit anti-AβP-42 was replaced with the same dilution of non-immunized rabbit antibody. After the addition of other ELISA reagents to these 12 control wells, the ODs were measured and their mean was subtracted from the mean OD of all other reactions.

Demonstration of specificity of anti-AβP-42 binding to chemicals-HSA and food antigens

To determine the specificity of this antigen-antibody reaction, serial dilutions of serum as well as inhibition studies were conducted. Anti-AβP-42 was serially diluted from 1:400-1:12,800 in PBS containing 2% BSA, and was then applied to different wells of ELISA plates coated with DNP-HSA, phthalate-HSA, egg yolk and cooked tuna antigens. After the completion of all other ELISA steps, the recorded ODs were converted to different graphs.

Inhibition study

Monoclonal rabbit anti-AβP-42 in the presence or absence of chemicals-HSA or food antigens was used in the inhibition study. In different test tubes, 1 mL of 1:400 diluted rabbit anti-AβP-42 was pre-incubated with 100 µL of diluent containing 100 µg of BSA, HSA, or 1.5-100 µg each of DNP-HSA, phthalate-HSA, egg yolk antigen or cooked tuna antigen. After mixing, the tubes were kept for 1 hour in a 37°C water bath, followed by 4 hours of incubation at 4°C, and then centrifugation at 3,000 g for 10 min. The supernatant was used for measuring the degree of anti-AβP-42 binding to the chemical-HSA or food antigen- coated plates before and after absorption with the specific antigens. After completion of the ELISA procedure, the ODs were converted into graphs.

Measurement of AβP-42 concentrations by Sandwich ELISA

For measurement of AβP-42 concentrations, 100 µL of affinity-purified mouse anti-AβP-42 at a concentration of 500 µg/mL and a dilution of 1:200 in 0.1 M carbonate buffer pH 9.6 were applied to the surfaces of the wells of microtiter ELISA plates. After incubation, addition of 2% BSA, and washing, AβP-42 in standard concentrations of 40, 80, 160, 320, 640, 960, and 1280 pg/mL was prepared, and 100 µL of each preparation were added to the first row of the microtiter plate coated with the antibody. Also, known amounts of AβP-42 in concentrations of 31.25, 62.5, 125, 250, 500 and 1000 pg/mL each were added to duplicate wells. Furthermore, tau protein, α-β casein, and glutamic acid decarboxylase peptides in concentrations of 31.2 to 1000 pg/mL were added to the additional duplicate wells coated with anti-AβP-42 antibody. After 60 min incubation at RT and washing, 100 µL of rabbit monoclonal anti-AβP-42 antibody were added to all wells.

The binding of pure Aβ-42 peptide and other proteins to the Aβ-42 first antibody and its sandwich with the second antibody was measured by the addition of alkaline-phosphatase labeled anti-rabbit, followed by substrate and color development, then reading of the ODs at 405 nM. After generation of a standard curve, the obtained values of AβP-42 concentration were compared to the true values.

Results

Using rabbit monoclonal antibody against AβP-42, we measured immune reactivity with various chemicals bound to HSA and compared them with the level of this immune reaction with its target antigen AβP-42 as a positive control, and HSA alone as a negative control.

Compared to the mean OD of control wells coated with HSA alone or an OD of 0.17, the anti- AβP-42 specific antibody reacted with a phthalate-HSA OD of 1.9, aluminum-HSA OD of 1.2, DNP-HSA OD of 1.1, and reacted to a lower degree with a mercury-HSA OD of 0.75. Compared to 3SD above the mean of the ODs of the control wells, the p values for phthalate-HSA, aluminum-HSA, DNP-HSA, and mercury-HSA were p<0.00001. Reactions of the same antibody with aflatoxin-HSA, glyphosate-HSA, and pyrethroid-HSA were similar to the control wells (Figure 1). Furthermore, the reaction of this antibody with formaldehyde-HSA, isocyanate-HSA, bisphenol A, tetrabromobisphenol A, tetrachloroethylene, and parabens was also comparable to the wells coated with HSA alone (data not shown). Since PDI is a stress protein that is involved in the inhibition of protein misfolding and in the aggregation of α- synuclein, the reactivity of AβP-42 antibody with PDI was examined. The reaction of anti-AβP-42 antibody with PDI resulted in an OD of 1.0 (23%), which is considered moderate (Figure 1). Similarly, by testing the anti-AβP-42 antibody for its possible immunoreactivity with 220 different antigens, we found that in comparison to AβP-42 antibody binding with AβP-42 peptide with an OD of 3.8, numerous foods reacted from moderately to strongly with this antibody. The OD for wells coated
with BSA alone was 0.15, for spinach 0.32, and for egg white 0.45, which were slightly greater than 3SD above the mean of control wells. This immunoreactivity was significantly higher (p<0.00001) with seaweed and scallop with an OD of 0.8, wheat extract and pea lectin with an OD of 0.85, pea protein with an OD of 1.4, egg yolk with an OD of 1.55, cow’s milk with an OD of 1.8, α+β casein with an OD of 2.4, and for α-gliadin 33-mer and other wheat peptides such as β-amylase, CXCR3-binding gliadin peptide, and wheat globulin with ODs from 2.4 to 3.2 (62-83% of anti-AβP binding to AβP-42). In addition to milk and wheat antigens, the strongest reactions of AβP-42 antibody was observed with canned tuna extract (OD of 2.8) but not raw tuna (only a moderate OD of 0.7), lentil lectin (OD of 2.1), squid (OD of 2.1), and hazelnut (OD of 2.0) (Figure 2). The reactivity of this antibody with the additional 189 food extracts used in this study was comparable to the wells coated with BSA alone (Figure 1).
Determination of the specificity of anti-AβP-42 antibody binding to chemicals-HSA and food antigens

First, we used unimmunized rabbit serum and examined its reaction with chemicals bound to HSA and with all 208 food antigens, obtaining no OD above 0.25. We also used mouse monoclonal antibody made against AβP 104-118 AA, and reacted them with all chemicals bound to HSA or the 208 foods. None of the ODs were higher than 0.3. These results support the specificity of AβP-42 binding to some of the chemicals bound to HSA or food antigens listed in (Figures 1 and 2). Further experiments such as dilutions of anti-AβP-42 antibody and inhibition of the antibody-antigen reaction were performed (Figure 2).

As shown in (Figure 3), in proportion to the dilutions of anti-AβP-42 antibody, the ODs of the antibody binding to DNP-HSA, phthalate-HSA, egg yolk antigen or tuna antigen declined significantly. For example, the reaction of anti-AβP-42 at a dilution of 1:400 with tuna antigen gave an OD of 2.4, a dilution of 1:3200 gave an OD of 0.95, and a dilution of 1:12800 gave an OD of 0.33, which is almost equivalent to the ELISA background (Figure 3).

To further demonstrate the specificity of this antibody-antigen reaction, different amounts of chemicals bound to HSA or food proteins (inhibitors) in concentrations ranging from 3-100 µg were added to the antibody before performing the ELISA on the plates coated with the optimal concentrations of the same antigens. The addition of the anti-AβP-42 antibody in the presence of higher concentrations of specific antigens in the liquid phase resulted in significant inhibition of the anti-AβP-42 antibody binding to the chemicals-HSA or food antigens. This inhibition of antigen-antibody reaction was insignificant when HSA was added to the liquid phase or when the antigen concentration in the liquid phase was below 6 µg (Figure 4).

For the proper quantification and demonstration of anti-AβP-42 antibody binding to AβP-42 peptide, known concentrations of AβP-42 peptide were used in Sandwich ELISA to generate a standard curve, showing an increase in proportion to the concentration of AβP-42, with a resulting correlation coefficient of 0.998 (Figure 5A). Furthermore, AβP-42, tau protein, α+β casein, and GAD-65 were diluted in serum diluent buffer to obtain final concentrations of 31.25, 62.5, 125, 500 and 1000 pg/mL which were then measured in the assay against the standard...
The exact source of these anti-AβP antibodies is not clear, but they could be derived from immune response to aggregated forms of β-amyloid, from protein misfolding, or from antibodies to completely different antigens that cross-react with AβP-42. Aluminum, phthalate and dinitrophenyl are three chemicals bound to HSA that reacted significantly with anti-AβP-42. Because aluminum compounds are added to so many commercially-prepared products, from colored candies, cheese, coffee whiteners, to even drinking water, aluminum uptake into the bloodstream begins in utero and continues throughout life [19-24]. Although most absorbed aluminum is excreted, some of it manages to bind to different tissue proteins, particularly in the intestinal mucosa and in the brain [61-65]. Moreover, aluminum accumulation in the brain affects the memory and cognition, alters synaptic activity, activates microglia, and promotes β-amyloid and neurofilament aggregation, all of which are hallmarks of neurodegenerative disorders [66]. Aluminum is one of the factors that accelerate AβP-42 monomer aggregation by cross-linking anionic amino acids contained in the AβP-42 sequence to form AβP-42 aggregates [67]. This was reviewed very elegantly by Kawahara [22]. This may be one explanation as to why high levels of antibodies to AβP-42 and other amyloid proteins are detected in patients with AD [45,46]. Similar mechanisms of action may be applied to the aluminum binding to human albumin, where the aluminum may affect the functional properties of albumin or other proteins, leading to the formation first of dipoles and then of clusters that may mimic AβP-42 oligomerization or protein misfolding similarities [68]. This explanation is supported by the findings that numerous age-related disorders are now recognized to be related to the accumulation of different misfolded proteins that result in the production of autoantibodies called anti-oligomer antibodies [69].

Additional studies are needed to determine whether or not the addition of aluminum to albumin indeed results in protein misfolding, and if its injection into animal models results in the production of anti-oligomer antibodies that will react with AβP-42 and other associated misfolded proteins and peptides.

A similar interpretation may be applied to DNP-HSA or the plasticizer diethylphthalate-HSA, the neo-antigens to which anti-AβP-42 has reacted strongly (Figure 1). Using molecular modeling, the binding of phthalate plasticizers to HSA was examined in vitro. Fluorescence quenching data revealed that interaction of phthalate with HSA resulted in alterations in the conformational and secondary
structures of HSA. Thermodynamic analysis also showed that hydrophobic sources were the main interaction for phthalates with HSA-protein [54].

Mercury is another chemical which extensive epidemiological and demographical studies have reported to have a strong association with AD [25]. In fact, according to one review article, some autopsy studies found increased levels of mercury in the brain tissues of AD patients but not in the blood, urine, hair or cerebrospinal fluid [25]. Furthermore, in in vitro and animal studies it was demonstrated that mercury causes tau protein phosphorylation, and the increased formation of amyloid-β protein [25]. Similar structural changes to HSA molecules due to mercury binding could be a plausible explanation for the detection of immune reactivity between AβP-42 antibody and mercury-HSA reported in our study. This is supported by the findings that, the same antibody did not react with HSA alone, formaldehyde-HSA, aflatoxin- HSA, and many additional chemicals bound to HSA that were used in our study. Further studies are needed to examine the differences between these two groups of chemicals, one group to which the AβP-42 antibody reacted strongly, and the second group to which the AβP-42 antibody did not (Figure 1).

However, it is much easier to explain the reaction of anti-AβP-42 antibody to PDI, an enzyme that is known to be the target for endocrine disruptors such as BPA and phthalates [34,35]. This binding of anti-AβP-42 with PDI may result in the loss of the latter’s functionality, and contribute to the α-synuclein aggregation and the production of the antibodies that are detected in AD [70] and Parkinson’s disease [71]. In relation to food association with AD, almost all the published articles are about proper diets that could cut the risk of AD, such as the MIND diet, which stands for “Mediterranean-dash Intervention for Neurodegenerative Delay” [72]. Since, in our recent studies [58,73], we demonstrated that polyclonal and monoclonal antibodies made against some food antigen antibodies to cross-react with Aβ-42 in such a way so as to demonstrate a role in the amyloidogenesis that is characteristic of AD. To some extent, this is because HSA binds with 95% of Aβ in the blood and inhibits plaque formation in peripheral tissue. HSA also binds with endogenous fatty acids, especially hydroxynonenal, which can penetrate the BBB and then reach the brain, where, through the induction of oxidative stress, it may contribute to senile plaque formation, especially in ApoE4 individuals [84]. Furthermore, HSA is known for its ability to bind with additional molecules such as pharmaceuticals, aluminum and heavy metals, as shown in our studies. Similar to fatty acids binding to HSA, the binding of toxic chemicals to HSA can result in BBB penetration and the deposition of these molecules’ antibodies and immune complexes into the amyloid plaque. This binding of aluminum, mercury or other haptenic molecules to HSA may compete with Aβ binding to HSA, resulting in a decrease in the concentration of active HSA on one hand, but the availability of a higher level of Aβ on the other hand. Based on this mechanism of action of HSA in the prevention of amyloid plaque formation, plasma exchange for increasing the level of HSA is used in clinical trials to reduce the levels of Aβ in the blood, hence reducing Aβ penetration into the brain, and inhibiting amyloid plaque formation [85,86]. For now the results of our research provide a brief list of chemicals that can change human protein (HSA) so as to be recognized by the anti-AβP-42 antibody, as well as a menu of dietary proteins the antibodies of which may immunologically impact amyloid-β protein oligomerization; removal of these food items plus those shown by Carter [49] which share a homology with β-amyloid may be recommended at least in patients in the early stages of AD. Furthermore, as has been recommended in earlier studies [24-26,32], the most effective public health preventive measure for neurodegenerative disorders would be the elimination of mercury, aluminum, plasticizers and other toxic chemicals from human contact.

It is possible, based on the results of our study and the existing accumulated literature and knowledge, to hypothesize a mechanism that takes into account the effects of toxic chemicals such as aluminum, mercury, and phthalate plasticizers, and integrate then with the ability of some food antigen antibodies to cross-react with AβP-42 in such a way so as to demonstrate a role in the amyloidogenesis that is characteristic of AD. To do this it is necessary to consider the blood-brain barrier (BBB).
The BBB is the regulated interface between the peripheral circulation and the central nervous system (CNS). The anatomical substrate of the BBB is the cerebral microvascular endothelium, which, together with astrocytes, pericytes, neurons, and the extracellular matrix, constitute a “neurovascular unit” that is essential for the health and function of the CNS. Tight junctions (TJ) between endothelial cells of the BBB restrict paracellular diffusion of water-soluble substances from blood to brain. The TJ is an intricate complex of transmembrane (junctional adhesion molecule-1, occludin, and claudins) and cytoplasmic (zonula occludens-1 and -2, cingulin, AF-6, and 7H6) proteins linked to the actin cytoskeleton [83,84,87,88]. It has been demonstrated that a number of different types of brain-reactive autoantibodies are not only numerous but ubiquitous in human serum in both healthy individuals and patients suffering from AD, although the levels are higher in subjects with AD [85,89]. While these autoantibodies can normally stay safely in the serum of a healthy person with an intact BBB, under certain conditions the BBB that normally protects the body by keeping out toxic environmental chemicals and unwanted molecules may fail or become permeable. The compromised BBB may then allow these brain-reactive autoantibodies, toxic chemicals, and cross-reactive food antibodies to gain access to the neurons within the brain tissue [85,86,89,90]. The brain-reactive autoantibodies may react with AβP-42 and other neural antigens, increasing intraneuronal amyloid-β deposition [87,91]. The toxic chemicals may have a direct effect on the brain, or the antibodies generated against them may bind to tissue proteins and form neo-antigens. Direct binding of the chemical to an Aβ-42 monomer results in the misfolding of the Aβ-42 peptide, inducing amyloid fibril formation. Additionally, the food antibodies that have penetrated the BBB cross-react with AβP-42, and may also bind to an Aβ-42 monomer, again resulting in the misfolding of the Aβ-42 peptide, further contributing to amyloid fibril aggregation. In conjunction with the formation of immune complexes, release of cytokines, complement cascade activation, and other inflammatory factors, further promote amyloid plaque aggregation, which contributes to AD neuropathology.

We believe that the results of our study show enough indications for immunoreactivity between AβP-42 autoantibodies, food proteins, and toxic chemicals in conjunction with a compromised BBB as having a role in the pathogenesis of Alzheimer’s disease. Effective prevention and early reversal requires knowing the status of each factor whether you have been exposed to toxic chemicals, pathogens, or cross-reactive foods that are involved in amyloidogenesis. We need to get to the cause of cognitive decline and fix any imbalances before the situation becomes irreversible. This means that in order to reverse cognitive decline in a neurodegenerative disease, it is necessary to identify and remove the triggering factors that are causing the brain’s defenses to misfire and produce a harmful instead of protective amyloid response.

The necessary processes can be summarized into these 3 steps:
1. Remove the triggers.
2. Remove the amyloid clusters.
3. Rebuild the synapses destroyed by the disease.

This idea of identifying the cause of the amyloid production, removing that, and then removing the amyloid (using monoclonal antibody) has not been tested, according to the book by Bredesen [88,92].

The linchpin of this idea, of course, is correctly identifying which factors are triggering the patient's condition. Cross-reaction between AβP-42 and various infectious agents demonstrated in our earlier study [60] and with different food antigens shown in the present study indicate that circulating AβP-42 cross-reactive factors may interfere with the accurate measurements of AβP-42 levels in the blood. This, plus the presence of microbial-generated amyloids [88,93] in the blood of healthy subjects and patients with AD, may be a reason for reports of inconsistent results in the measurement of AβP-42 levels in serum [89,90,94,95]. It is also well known that the pathogenesis of typical Alzheimer's disease is quite variable, depending on the age, genetic makeup, environmental exposure and the patient's demographic. Due to these variables, it is possible that a certain percentage of the elderly may develop Alzheimer's dementia even in the presence of a lower amyloid burden that is below the detectable threshold of amyloid-β plaque captured via PET imaging [96]. Due to the lower levels of amyloid-β peptide presented to cell-mediated immunity, these individuals may also exhibit lower levels of antibody to Aβ-42. Therefore, the presence of these other antigens in the blood can interfere with accurate measurements of AβP-42 peptide and antibody levels.

That is why more research should be conducted to find reliable biomarkers for testing in the serum or cerebrospinal fluid of patients with Alzheimer's disease.

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