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BIOL6301 Project Proposal
Alzheimer's disease

Antibodies Targeting Amyloid β as Treatment for Alzheimer's disease

Alzheimer disease (AD) is the most common neurodegenerative disease. It is estimated that about five million people in the United States are affected by Alzheimer's disease. In the U.S. the total financial cost of the disease exceeds $100 billion dollars (Findeis, 2007). However, the greatest risk factor of acquiring Alzheimer's is age. As the life expectancy of the population is increasing, many people are to be diagnosed with Alzheimer's disease. Thus, it is imperative to understand the molecular mechanism of Alzheimer's, the treatment, and even preventive care of the disease.

A key observation of AD is the aggregation amyloid β peptide deposits in the brain of the patients affected with the disease. Amyloid β peptides are about 39-43 residues long and are the products of the proteolytic processing of its precursor, β-APP (or simply APP), resulting from sequential cleavage by proteases named β- and γ-secretases (Findeis, 2007). Also, genetic mutation in the genes that encode APP, PS-1 and PS-2 greatly affect the levels of amyloid β in the brain.

Unfortunately, the function of amyloid β peptides in the brain is not well understood, although research has been done to show some insight. It is suspected that amyloid β modulates potassium ion channel in neurons (Findeis, 2007). Monomeric form of amyloid β is not toxic to the brain, but its oligomeric form is very toxic to the brain and leads to neuronal cell death. Also, a healthy person possesses mainly two types of amyloid β peptides which are amyloid β 40 and amyloid β 42. Normally, amyloid β 40 is the most prevalent form in a healthy person. However, amyloid β 42 is

usually observed in patients with AD. These amyloid β 42 plaques usually form between nerve cells and affect the communication between them, disrupting normal cognitive function such as memory and decision making which are key symptoms of Alzheimer's.

There is another protein called tau that is found in the plaques along with the amyloid β peptides. Tau is found in neural axons and keep microtubules together. Mutations of tau are mostly clustered in or near the repeat domain (e.g., G262V, V337M, P301L/S/T, ΔK280), they tend to enhance the aggregation propensity of tau and reduce its microtubule affinity (Zempel, 2014) Tau can be phosphorylated at various sites. For instance, it is estimated that tau protein has about 85 phosphorylation potential sites. However, in patients with AD, tau is found to have hyper phosphorylated Ser/Thr-Pro motifs which will dissociate from the microtubules in the axons and lead to their death. Neurofibrillary tangles (agents of tau protein) and senile plaques (agents formed by amyloid β peptide) are two detrimental features in Alzheimer's disease. It was once thought that these two byproducts were thought of as independent mechanisms. However, experimental evidence suggests that both agents are intrinsically related. Understanding amyloid β and tau toxicity as part of a common pathophysiological mechanism may uncover molecular targets to prevent as well as treat the disease (Lloret, Fuchsberger, Giraldo, Vina, 2015).

In this research proposal, we would like to study amyloid β 42 which is the most prevalent cause of AD. We propose the hypothesis that if we can target amyloid β 42 by antibodies it would lead to a decrease in their aggregation levels and suspend its toxic agents in the bodies of patients with Alzheimer's. Also, amyloid β 40 would be measured as a ratio with amyloid β 42 in diagnosing and treating Alzheimer's. The

design of the experiment will be simple yet sophisticated in order to test this hypothesis on several parameters; targeting amyloid β 42 with antibodies as well as measuring the ratio between amyloid β 42 and amyloid β 40 throughout the regions of the brain.

The experiment will consist of creating antibodies to target amyloid β 42 such that it will decrease amyloid β 42 levels in the brain, therefore relieving Alzheimer symptoms. This approach tests the role of the long form of amyloid β 42 in AD and its potential as a target for developing a disease modifying drug. The significance of amyloid β ratios may account for differences in early data that showed variability in levels of amyloid β in individual subjects with and without AD. Elevated levels of amyloid β from one individual to another do not appear to provide a clear picture of the risk of acquiring or having AD. However, the ratio of amyloid β 42 to amyloid β 40 appears to give a clearer correlation of risk and diagnosis (Findeis, 2007).

Using amyloid β ratios in combination with other emerging diagnostic methods has the potential to shift presymptom diagnostic methods from an evaluation risk into a clearer state of successfully identifying individuals who should begin therapy. Furthermore, the importance of the ratio of amyloid β 42 to amyloid β 40 has recently been demonstrated in animals. In transgenic mice in which amyloid β 40 production has been achieved using a viral vector, the increase in amyloid β 40 inhibited amyloid β 42 linked amyloidosis and death. The results showed that even in the presence of increased amyloid β, a shift in amyloid β length toward shorter lengths reduces amyloidosis and toxicity. Altogether, the data from the transgenic mice show that selective reduction of amyloid β 42 will provide an improvement to slow the onset and progression of AD. With this knowledge there is potential to create antibodies that

selectively lower levels of amyloid β 42 relative to amyloid β 40 and as well as targeting longer lengths of amyloid βs (Findeis, 2007).

Currently approved drugs for the treatment of AD target the metabolic deficits observed in the disease associated with reduced brain function include two classes of drugs, the acetylcholinesterase inhibitors (AChEI) tacrine, donepezil, rivastigmine, galantamine and the N-methyl-D-aspartate (NMDA) receptor antagonist memantine. These drugs appear to provide short-term benefits to patients based on enhancement of remaining cognitive function. However, they do not appear to provide significant delay of disease progression. Because of the limited benefit of these drugs, the main focus of treatment of AD patients is therefore monitoring of the progress of the disease and providing an appropriate level of supportive care (Findeis, 2007). In 2012, the first national plan to address AD in the U.S. was released, the National Alzheimer's Project Act legislation. There has been increases in U.S. federal funding for AD, particularly in the areas of biomarker discovery, genetic link and related biological underpinnings, and prevention studies for Alzheimer's. (Synder, Hendrix, Bain, Carrillo, 2015).

As research continues to explore the onset and progression of AD, an expanding range of potential mechanisms of intervention has emerged with the goal of developing disease-modifying therapies that will slow, if not reverse, the progression of disease. Experimental therapies based on modulation of amyloid β pathways can be broadly described as being based on three types of approaches: prevention of production of amyloid β, prevention of the formation of toxic forms of amyloid β, and prevention of toxic effects of amyloid β. That’s why we believe producing antibodies can be added as an effective approach to stop the progression of AD (Findeis, 2007).

The most advanced of these approaches is that of immunotherapy, creating antibodies, based on vaccination with amyloid β. The potential importance of immunotherapy approaches to AD was first demonstrated by Solomon in 1997, in experiments that demonstrated that antibodies raised against amyloid β 28 bound to and disaggregated premade amyloid β fibrils. Antibodies were also shown to block the toxic effects of fibrillary amyloid β on cells. Subsequent experiments with active vaccination for amyloid β produced extraordinary positive results in transgenic mice. That is why in our hypothesis for treating AD, antibodies would be produced to combat the progression of amyloid β 42 in comparison with amyloid β 40 (Findeis, 2007).

Selective lowering amyloid β 42 has emerged as a disease-specific strategy for reducing the amyloid forming potential of all forms of amyloid β. Slowing or stopping the formation of new deposits of amyloid β, inhibiting the formation of soluble toxic oligomers of amyloid β, and thereby slowing or halting the progression of neurodegeneration. Drugs that selectively lower amyloid β 42 levels effectively will demonstrate biochemical efficacy promptly in early clinical trials. Once effective dose levels are attained, plasma and cerebrospinal fluid levels of amyloid β 42 and amyloid β 40 can be measured by established ELISA methods. In this way, dose levels that maintain reduction of amyloid β 42 can be identified for use in future trials based on empirical reduction of amyloid β 42 by the use of antibodies (Findeis, 2007).

Certainly use of anti-amyloid β antibodies could be a promising path for treating Alzheimer's and it supports the hypothesis which describes that amyloid β peptides are the main culprits of the disease. However translating these results effectively and without causing harm in humans poses a great challenge. The side effects like

meningoencephalitis or microhemorrhages have been reported due to excessive T helper (T1) mediated inflammation. The number of strategies to modify vaccines to avoid the unwanted T cell response is being considered. The use of second generation vaccines like amyloid β peptides linked to carrier proteins including viral structures or other independent T cell epitopes and DNA vaccines expressing amyloid β fragments holds promise as a modifying therapy for Alzheimer's disease (Klafki, Wolfgang, Staufenbiel, Kornhuber, Wiltfang, 2006).

Other consideration that can't be ignored is that the transport of antibodies from the blood to the brain is known to be limited because of blood brain barrier (BBB). In other words, the blood brain barrier is so effective that even therapeutic drugs can't cross it and thus are unable to repair the diseased brain. The major challenge for the delivery of antibodies following either active or passive immunization is the transport of large proteins to the central nervous system (Spencer, Masliah, 2014).

Previous studies with monoclonal antibodies for treatment of Alzheimer's have shown approximately 0.1% of injected antibodies across the BBB with rest either metabolized in the liver or excreted through kidneys (Spencer, Masliah, 2014). By targeting the receptors in the BBB for active transport of proteins to the central nervous system from blood can come as a promising approach that will most likely be a challenging task for creating an ultimately successful immunotherapy combatting the molecular mechanisms of Alzheimer's.

Also, a key issue which needs to be addressed is the simultaneous targeting of both amyloid β and tau related pathology. Studies have demonstrated a strong interplay between soluble amyloid β peptides and tau in Alzheimer's disease pathocascade

(Nisbet, Polanco, Ittner, Gotz, 2014). Furthermore, the complexity of Alzheimer's disease asks for the use of combination therapy for best results. This immunization approach can also be used for the development of monoclonal antibodies or related immunotherapeutics to be used alone or in combination with other agents to treat different stages of the disease. As we continue to learn more about the pathology of Alzheimer's disease at molecular level, we will find more plausible approaches for future immunotherapy of Alzheimer's disease.

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