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ImmunoPEGliposome-mediated reduction of blood and brain amyloid levels in a mouse model of Alzheimer's disease is restricted to aged animals

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ABSTRACT

The accumulation of extracellular amyloid-beta (Aβ) and intracellular neurofibrillary tangles (hyperphosphorylated Tau) in the brain are two major neuropathological hallmarks of Alzheimer's disease (AD). Active and passive immunotherapy may limit cerebral Aβ deposition and/or accelerate its clearance. With the aid of a newly characterized monoclonal anti-Aβ antibody we constructed immunoPEGliposomes with high avidity for capturing Aβ in the periphery. The functionality of these vesicles in modulating Aβ uptake by both human brain capillary endothelial hCMEC/D3 cells (suppressing uptake) and THP-1 phagocytes (stimulating uptake) was confirmed in vitro. The multivalent immunoliposomes dramatically reduced circulating and brain levels of $A\beta_{1\text{-}40}$, and particularly $A\beta_{1\text{-}42}$, in "aged" (16 month-old), but not "adult" (10 month-old) APP/PS1 transgenic mice on repeated intraperitoneal administration. Furthermore, the immunoPEGliposome-mediated reduction in amyloidosis correlated with lower levels of glial fibrillary acidic protein (GFAP) and reactive glia (GFAP-positive cells). This treatment also lowered the ratio of phosphorylated Tau to total Tau. The therapeutic efficacy of immunoliposome treatment was superior to free monoclonal antibody administration (at an equivalent antibody dose). The potential mechanisms and significance of age-dependent immunoliposome therapy in AD is discussed.

1. Introduction

Dementia deprives people of their memory, functionality and dignity. The Delphi study estimated that there were 24.3 million people with dementia in the world in 2001, with majority suffering from Alzheimer's disease (AD) and vascular dementia, and predicted that this would rise to 42 million in 2020 and 81 million by 2040 [1]. Currently, AD is the fourth-leading cause of death in adults after heart disease, cancer and stroke in the world [1,2]. AD is a chronic neurodegenerative disorder characterized by the accumulation of amyloid (Aβ) plaques and neurofibrillary tangles in specific regions of the brain [3–6]. It is generally believed that an increased deposition of Aβ peptide in senile plagues, presumably arising from amyloidogenic cleavage of membrane bound precursor protein (APP) by β (BACE1)- and γ-secretase, is the main cause

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of neuronal dysfunction and death in AD [7–9]. This leads to the production of Aβ peptides of different length, of which the $Aβ_{1-40}$ is the major species and the $Aβ_{1-42}$ is the most fibrillogenic and predominant component in AD plaques [9]. On the other hand, formation of neurofibrillary tangles containing tau protein is proposed to result from an imbalance between Aβ production and clearance [7–9]. Furthermore, the blood-brain-barrier (BBB) dysfunction plays an important role in the development of AD, and may even precede neuron degeneration in AD brain [10–12].

Current therapeutic options for AD, however, are limited and to date none have provided a cure [1,2]. Accordingly, a number of alternative and innovative therapeutic approaches are being accelerated [13–22]. Among the most promising approaches to date are active and passive immunotherapeutic interventions that limits cerebral Aβ deposition and/or accelerate its clearance, however, their safety profile is still disputed [16,21,23]. Passive immunotherapy refers to direct administration of anti-Aβ monoclonal antibodies for precise targeting of Aβ epitopes [19]. The underlying mechanisms of passive immunotherapy are poorly understood. Antibodies may enter the brain; a process presumably limited by AD stage-dependent changes in function and permeability of the BBB. On brain entry antibodies may bind to amyloid plaques inducing disassembly and/or activate microglial cells for plaque clearance through Fc receptor-mediated processes and lysosomal degradation [23]. Similarly, Megga et al. [24] have suggested that peripherally administered intravenous immunoglobulin (IVIG) may penetrate the BBB and bind to Aβ deposits in mouse brain. The therapeutic effects of IVIG may partly be related to the presence of naturally occurring Aβ antibodies, and such antibodies are abundant in human plasma, and are known to decline with age and advancing AD [25]. On the other hand, antibodies may exert their main effect in the periphery, where they bind to circulating forms of Aβ molecules [19] and restrict Aβ translocation into the brain. This mode of binding may further alter Aβ conformation so that it is less likely to form the fibrillar aggregates associated with AD. In addition, the binding of antibody to Aβ may accelerate clearance and degradation by macrophages of the reticuloendothelial system, and generate a concentration gradient across the BBB, thereby promoting Aβ efflux from the brain into the systemic circulation. This phenomenon is termed as the "peripheral sink" hypothesis [19,21,26,27]. The peripheral sink hypothesis may further explain some of the beneficial effects of active immunization with a non-fibrillogenic Aβ peptide, which elicited an IgM response to Aβ and resulted in both reduction of amyloid burden and cognitive improvement [28]. Due to its large size, IgM is unlikely to translocate into the brain. Unlike IgG, IgM is multivalent. Accordingly, IgM multivalency may allow formation of larger and more stable Aβ immune aggregates in the blood (i.e., high avidity). This process may accelerate removal of excess circulatory soluble Aβ by macrophage compared with IgG, and drawing even more soluble Aβ from the brain.

Here we have addressed the peripheral-sink hypothesis [19,21,26,27] by a multivalency approach involving conjugation of a recently in-house developed anti-Aβ monolclonal antibody [29,30] (referred to as STAB-MAb) to PEGylated liposomes. We limited our studies to stealth liposomes, since they are stable and offer the necessary attributes for detailed pharmaceutical characterization, reproducibility, up scaling and manufacturing processing. The engineered immunoPEGliposomes, with IgG antibodies attached to the distal end of the reactive PEG chains, were first optimized for Aβ targeting and modulation of Aβ uptake by human brain capillary endothelial cells (suppressing uptake) and macrophage cell lines (stimulating uptake) in the presence of serum/plasma proteins. The beneficial effect of immunoPEGliposome therapy was then confirmed in the double APP/PS1 transgenic mouse model that resembles the amyloid pathology in the brain of AD patients [22,31,32]. The results demonstrated an age-dependent efficacy of immunotherapy as whole.

2. Materials and methods

2.1. Materials

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), cholesterol, L-cysteine, anhydrous dimethyl sulfoxide and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) were obtained from Sigma-Aldrich (Copenhagen, Denmark). 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)$_{2000}$] ammonium salt ($mPEG_{2000}$-DSPE), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[maleimide(polyethylene glycol)$_{2000}$] ammonium salt ($MPB\text{-}PEG_{2000}$-DSPE), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl) ammonium salt (Liss-Rhod-PE) and polycarbonate filters were from Avanti Polar Lipids, Inc. (AL, USA). Phosphocholine assay kit was obtained from MTI diagnostics GmbHP (Idstein, Germany). Novex SilverXpress Silver Staining Kit, Novex 4–12% Bis-Tris Gel 1.0 mm 12 well, Novex SDS Sample Buffer, NuPAGE Reducing Agent, Novex Tricine SDS Running Buffer, CBQCA Protein Quantitation Kit, Qubit Protein Assay Kit, Novex® ECL, chemiluminescent substrate and Hoechst 33342 nuclear dye were purchased from Life Technologies (CA, USA). Boric acid and Amicon Ultra Centrifugal Filters 3 kD and 50 kD were obtained from MERCK KGaA (Darmstadt, Germany). Sepharose CL-4B gel was obtained from GE-Healthcare (Brøndby, Denmark). Lepirudin (Refludan®) was from Hoechst (Frankfurt-am-Main, Germany). Compstatin and the control peptide were a kind gift from Prof. Tom Eirik Mollnes (Oslo University Hospital, Norway).

The Aβ peptides and peptide fragments used in this study were provided by different sources: $Aβ_{1-40}$ (SP-BA40-1), $Aβ_{1-42}$ (SP-BA42-1), $Aβ_{1-43}$ (SP-BA43-5), $Aβ_{29-40}$ (SP-BA2940-1), $Aβ_{29-42}$ (SP-BA2942-1) and $Aβ_{25-35}$ (SP-BA2535-1) were supplied by Innovagen (Lund, Sweden), whereas fragments $Aβ_{1-11}$ (ab120834) and $Aβ_{12-28}$ (ab120838) were supplied by Abcam (Cambridge, UK), and fragment $Aβ_{35-42}$ (RP20145) was obtained from BioNova Cientifica SL (Madrid, Spain). TAMRA-labeled $Aβ_{1-42}$ (Abs/Em = 544/572 nm) was obtained from AnaSpec Inc. (CA, USA).

Antibodies were obtained from different sources: anti-Arc and anti-Tau5 antibodies were from Abcam (Cambridge, UK); anti-synapsin, anti-PSD95, anti-BACE (D10E5), and anti-GAPDH were from Cell Signalling Technology (MA, USA); anti-Aβ (1-16, 6E10) was from Covance (CA, USA); anti-GFAP was from Dako (Glostrup, Denmark); anti-PHF1 was kindly provided by Dr. P. Davies (Albert Einstein College of Medicine, New York, USA).

2.2. Preparation of Aβ peptides for ELISA

Aβ peptides and peptide fragments were diluted to 0.5 mg/mL in 10 mM NH_4OH and incubated at room temperature for 10 min. Samples were sonicated for 10 min in a sonicator bath. The final preparation was lyophilized (Lyophilisation, SpeedVac) and stored at −80 °C. Prior to use, samples were re-suspended in 60 mM NaOH.

2.3. ELISA measurements

96-well ELISA plates (Greiner Bio-One GmbH, Frickenhausen, Germany) were coated with 100 μL of Aβ or Aβ peptide fragments in a carbonate buffer, pH 9.6, at 37 °C for 2 h. For blocking, 100 μL of 3% w/v BSA (in PBS pH 7.4, containing 0.05% v/v Tween 20) was added and incubated for 1 h at 37 °C. Primary antibodies (100 μL) were