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Neuropathology and Amyloid- β Spectrum in a Bapineuzumab Immunotherapy Recipient

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Abstract

The field of Alzheimer's disease (AD) research eagerly awaits the results of a large number of Phase III clinical trials that are underway to investigate the effectiveness of anti-amyloid- β (A β) immunotherapy for AD. In this case report, we review the pertinent clinical history, examine the neuropathology, and characterize the A β profile of an AD patient who received bapineuzumab immunotherapy. The patient received four bapineuzumab infusions over a 39 week period. During the course of this treatment, there was no remarkable change in cognitive impairment as determined by MMSE scores. Forty-eight days after the fourth bapineuzumab infusion was given, MRI revealed that the patient had developed lacunar infarcts and possible vasogenic edema, probably related to immunotherapy, but a subsequent MRI scan 38 days later demonstrated resolution of vasogenic edema. The patient expired due to acute congestive heart failure complicated by progressive AD and cerebrovascular accident 378 days after the first bapineuzumab infusion and 107 days after the end of therapy. Neuropathological and biochemical analysis did not produce evidence of lasting plaque regression or clearance of A β due to immunotherapy. The A β species profile of this case was compared with non-immunized AD cases and non-demented controls and found to be similar to non-immunized AD cases. SELDI-TOF mass spectrometric analysis revealed the presence of full-length A β ₁₋₄₂ and truncated A β peptides demonstrating species with and without bapineuzumab specific epitopes. These results suggest that, in this particular case, bapineuzumab immunotherapy neither resulted in detectable clearance of amyloid plaques nor prevented further cognitive impairment.

Keywords

active immunotherapy; Alzheimer's disease; amyloid- β ; amyloid plaques; bapineuzumab; cerebral amyloid angiopathy; neurofibrillary tangles; passive immunotherapy

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Introduction

Alzheimer's disease (AD) is the most common form of dementia affecting the elderly population worldwide. At present there is no effective treatment to modify the course of this disease. It is widely believed that central to the pathogenesis of AD is the profuse accumulation of amyloid- β (A β) peptides in the gray matter as amyloid plaques and in the walls of cerebral blood vessels. A promising potential therapy to stop the progression of AD is the administration of passive or active immunizations against the A β peptides. In this case report, we examined the clinical history and neuropathology as well as characterized the A β species extant after immunotherapy in a patient who received infusions of bapineuzumab, a humanized antibody (3D6) raised against the N-terminus (residues 1-5) of the A β peptide [1;2]. We compared the bapineuzumab-treated patient with 6 non-immunized AD cases, and 5 non-demented controls (NDC).

Clinical and neuropathological reports and treatment synopsis

The patient had a past medical history notable for depression, hypercholesterolemia, hypertension, coronary atherosclerosis, coronary stenting, asthma, and gastrointestinal reflux. The patient's mother had died of late onset AD. The patient started to experience memory problems in 1999. At that time the patient had a Mini Mental State Examination (MMSE) score of 29 while EEG and cranial CT imaging demonstrated no abnormalities. In 2000, the patient was diagnosed as having mild dementia and Aricept was prescribed then later changed to Exelon in 2001. In 2002, the patient had an MMSE score of 26 and was still capable of playing golf. By 2004 the patient was forgetful, sleeping excessively, with decreased interest in daily activities; the MMSE score was 22. The patient was enrolled in a bapineuzumab double blind study during which the patient received 6 doses of placebo between June 2005 and September 2006. In February 2007, the patient entered the open-label extension study and was placed on active bapineuzumab immunotherapy. A total of 4 doses of bapineuzumab (0.5 mg/kg per dose) were administered over the course of 39 weeks, with the last dose given in the first week of November 2007. Along the course of immunotherapy, four MMSE scores were obtained which fluctuated between 11 and 8. During this time, the patient had an acute myocardial infarction in August 2007. In the last week of December 2007, the patient presented with an ataxic gait. An MRI scan was interpreted as showing an acute lacunar infarct in the left head of the caudate nucleus and putamen, with signs of vasogenic edema in the same region. In early January 2008, the patient was admitted to the hospital with ataxia, gait disturbance, and a decreased level of consciousness. The CSF analysis revealed a protein concentration of 61 mg/dl and 5 mononuclear white blood cells with no signs of infection. Imaging with CT revealed no acute changes. Intravenous Dexamethasone was administered. Three days after the CT scan, an MRI scan was interpreted as showing acute infarction and/or edema in the left frontal periventricular white matter and also involving the basal ganglia. An EEG demonstrated epileptiform waves with severely disorganized rhythm. The patient was started on Keppra. In subsequent days, the patient began to improve in terms of following commands, orientation, and ambulatory and speech capacities. The patient was discharged to a nursing home in mid-January, with noticeable clinical improvement. A follow-up MRI scan a week later reported a lacunar infarct in the left caudate with increased white matter hyperintensities in the right temporal tip and right frontal lobe, with worsening of the mental status, presumably due to an evolving stroke. The patient was hospitalized in a non-ambulatory condition with severe cognitive and verbal impairments and was re-started on Decadron and Keppra. An MRI scan demonstrated apparent progression of the white matter and basal ganglia abnormalities although this was not considered to represent vasogenic edema. The patient expired in the third week of February 2008 at age 79. The direct cause of

death was attributed to acute congestive heart failure complicated by progressive AD and stroke.

The brain weighed 1000 grams. The dura was normal and the leptomeninges showed mild fibrosis. There was mild to moderate gyral atrophy. The base of the brain showed severe patchy atherosclerosis of the vessels of the circle of Willis. The mammillary bodies were normal while the uncus showed moderate atrophy without signs of herniation. The brainstem and cerebellum were externally unremarkable. Cerebral slices showed moderate and marked enlargement of the frontal horns and of the body of the lateral ventricles, respectively. The basal ganglia showed a reddish cavity in the head of the left caudate nucleus and putamen that also focally involved the internal capsule, measuring $2.0 \times 2.0 \times 1.2$ cm. Similar cavities but yellowish in color rather than red and smaller, each about 1 cc in total, were present in the right head of the caudate nucleus and in the right posterior putamen at the level of the lentiform nucleus. The thalamus and subthalamic regions were unremarkable. The amygdala showed moderate atrophy and there was moderate atrophy of the head and body of the hippocampi and the parahippocampal gyri. The substantia nigra showed mild to moderate depigmentation. Axial slices of the brainstem were unremarkable, as were parasagittal slices of the cerebellum.

Paraffin-embedded, H & E-stained sections showed moderate to marked gliosis in the upper neocortical layers, with focal full-depth gliosis and frequent hypertrophic astrocytes. Frequent senile plaques were evident. There were no chronic inflammatory cell infiltrates in gray or white matter and no evidence of edema. There was an old cortical microinfarct in the middle frontal gyrus. The amygdala and entorhinal cortex showed very marked gliosis and moderate tissue rarefaction. The hippocampal formation showed marked gliosis and tissue rarefaction in the outer part of the dentate gyrus molecular layer and moderate gliosis in area CA1, with the latter region also containing neurofibrillary tangles, granulovacuolar degeneration and Hirano bodies but showing no obvious neuronal loss. The grossly-described cavitating lesion in the left head of the caudate nucleus and putamen was seen microscopically to be consistent with a resolving, subacute old infarction. The cavity was filled with foamy macrophages and persisting islands of acellular neuropil and was bordered by frequent hypertrophic astrocytes. Uninvolved striatal areas showed rare large neurons with neurofibrillary tangles. There were multiple mineralized blood vessels in the globus pallidus and other blood vessels in the basal ganglia and thalamus showed thickened collagenous walls. There was an old microinfarct in the left dorsal putamen at the level of the full development of the lentiform nucleus. The nucleus basalis of Meynert was depleted of neurons and multiple remaining neurons contained neurofibrillary tangles. The medial thalamus showed marked gliosis and moderate tissue rarefaction. The substantia nigra was mildly depleted of pigmented neurons without evidence of Lewy bodies. The superior vermis and hemispheric cerebellar cortex showed mild patchy depletion of Purkinje cells but otherwise the cerebellum was unremarkable as were sections of the remainder of the brainstem and major levels of the spinal cord. Large, 3×5 cm H & E-stained sections showed marked and extensive white matter rarefaction in the frontal lobe, with mild rarefaction in the temporal and occipital lobes. There were two old microscopic cortical infarctions in the inferior parietal lobule and eight of these in the lateral occipital lobe. There was an old cystic microinfarct in the cerebellar hemispheric cortex, as well as a single microscopic focus of cortical sclerosis. Large, 3×5 cm sections stained with Gallyas, Campbell-Switzer and thioflavine-S methods showed, in neocortical areas, very frequent senile plaques, with frequent diffuse as well as classical (neuritic and cored) plaques. These plaques were evenly distributed in all sections, with no evidence of focal or patchy depletion. There were very frequent neurofibrillary tangles in all cortical areas as well as in the amygdala, hippocampus, entorhinal, and transentorhinal areas. Amyloid angiopathy was present at sparse densities in the frontal and temporal lobes, with moderate to frequent

densities in the parietal and occipital lobes. Immunohistochemical staining for phosphorylated alpha-synuclein showed frequent Lewy bodies and Lewy-related neurites in the amygdala and superior cervical sympathetic ganglion, with sparse densities of Lewy-related pathology in the olfactory bulb and dorsal medulla. No synuclein-immunoreactive histopathology was present in frontal, temporal or parietal neocortex.

As the neuropathological findings met diagnostic criteria for both AD (NIA-Reagan “high” probability that dementia was due to AD [3]) as well as the Binswanger’s type of vascular dementia (NINDS-AIREN criteria [4]), both diagnoses were assigned. The acute infarct of the left head of the caudate nucleus and putamen described on MR imaging during the final months of life correlated well with the postmortem histological appearance of a resolving, subacute to old infarct in the same region. There was no neuropathological evidence, however, that corresponded to the imaging findings suggestive of a more extensive infarction involving the frontal lobe white matter. Rather, it is likely that this imaging finding corresponded to the extensive chronic white matter rarefaction seen on large sections of the frontal lobe, consistent with leukoariosis. The presence of Lewy bodies restricted to the brainstem and limbic regions was insufficient for the diagnosis of dementia with Lewy bodies (DLB Consortium criteria [5]), and therefore the diagnosis of dementia with Lewy bodies was not assigned. Analysis of DNA isolated from postmortem cerebellum indicated an apolipoprotein E (ApoE) genotype of $\epsilon 4/\epsilon 4$.

In addition to the immunized individual, 5 neuropathologically-confirmed NDC and 6 neuropathologically-confirmed non-immunized AD cases were provided by our own Brain Bank at Banner Sun Health Research Institute (BSHRI) for comparison. The NDC included 2 females and 3 males with a mean age of death of 82 years. The AD group was composed of 1 male and 5 females with mean disease duration of 9 years and average age at the time of death of 82 years. Detailed neuropathological and biochemical data on these NDC and AD individuals has been recently published [6].

Materials and Methods

Neuropathological evaluation

All cases, including the bapineuzumab case presented here as well as the non-immunized AD and NDC individuals, were evaluated for total plaque score, plaque density, total neurofibrillary tangle score, white matter rarefaction (WMR) score, CERAD criteria, neuritic plaque score [7] and Braak stage [8]. These neuropathological assessments are detailed in a previous publication [9]. Histopathological appraisals of the AD lesions were obtained by thioflavine-S staining, Switzer-Campbell silver staining and Gallyas staining [9]. In addition, the leptomeningeal membranes from the bapineuzumab patient from both cerebral hemispheres were separated from the surface of the brain prior to its coronal sectioning, washed 12 times with 4 L each of cold distilled water to remove blood, spread on the surface of 5 inch plastic Petri dishes, allowed to dry in an oven at 60°C overnight, fixed for 2 h with absolute ethanol and stained by thioflavine-S. Based on experience, the degree of vascular leptomeningeal amyloidosis was semi-quantitatively classified as severe.

Quantification of soluble and insoluble A β by ELISA

Gray matter tissue (100 mg) was homogenized in 6 volumes of 20 mM Tris-HCl, 5 mM EDTA, pH 7.8 with a protease inhibitor cocktail (PIC, Roche Diagnostics, Mannheim, Germany), centrifuged at $435,000 \times g$ for 20 min (Beckman Optima TLA ultracentrifuge, 120.2 rotor; Beckman, Fullerton, CA) and the total protein concentration in the soluble fraction quantified using the Pierce Micro BCA protein assay (Rockford, IL). In addition, 400 mg samples of gray and white matter tissue were homogenized in 3 ml of 90% glass

distilled formic acid (GDFA) and centrifuged at $250,000 \times g$ for 1 h (Beckman LE-80 ultracentrifuge, SW41 rotor). The de-lipidated supernatants were collected and the top fat layer discarded. This procedure solubilized fibrillar, diffuse, membrane-bound, and intra- and extra-cellular oligomeric A β species. Aliquots of the GDFA-soluble fraction were submitted to fast protein liquid chromatography (FPLC) size-exclusion chromatography in 80% GDFA mobile phase (see below). The fractions containing A β peptides were pooled, reduced to 2 ml by vacuum centrifugation (SpeedVac; Savant/Thermo, Waltham, MA) and dialyzed (1000 MW cutoff tubing) against two changes of water, 2 changes of 0.1 M ammonium bicarbonate followed by lyophilization. The samples were dissolved in 500 μ l of 5 M guanidine hydrochloride (GHC), 50 mM Tris-HCl, pH 8.0 and shaken overnight at 4°C. Total protein was determined by the Pierce Micro BCA protein assay. The ELISA kits to quantify A β_{40} and A β_{42} were obtained from Invitrogen (Carlsbad, CA) and Innogenetics (Gent, Belgium), respectively, and performed following the manufacturer's instructions.

Quantification of tumor necrosis factor- α (TNF- α) by ELISA

From each of the specimens, gray matter (100 mg) was homogenized in 20 volumes of 20 mM HEPES, 1.5 mM EDTA, pH 7.4, PIC (Roche) with an Omni TH electric tissue grinder and centrifuged at $3000 \times g$ for 15 min in an IEC Centra CL3R centrifuge (Thermo, Waltham, MA). The supernatants were collected, centrifuged at $40,000 \times g$ for 1 h (Optima LE-80K ultracentrifuge, 50.4 Ti rotor; Beckman). Again, the supernatants were collected and total protein concentrations determined (BCA protein assay, Pierce). Human TNF- α levels were evaluated using an ELISA kit (PromoKine, Heidelberg, Germany) following the manufacturer's directions.

Fast protein liquid chromatography (FPLC)

Cerebral cortex was homogenized in 90% GDFA, and centrifuged and the supernatant was submitted to size-exclusion FPLC using a Superose 12 column (10×300 mm, General Electric, Uppsala, Sweden) as described in detail elsewhere [10]. The fraction containing the A β peptides was reduced by vacuum centrifugation (SpeedVac, Savant Instruments Inc. Farmingdale NY), and stored at -80°C.

High performance liquid chromatography (HPLC)

The FPLC fractions were further purified by HPLC using a reverse-phase C8 column (4.6×250 mm, Zorbax SB, Mac Mod) maintained at 80°C. A linear gradient was developed from 0-60% water/acetonitrile containing 0.1% trifluoroacetic acid (TFA) at a flow rate of 1 ml per min over a period of 120 min and the chromatography monitored at 214 nm. A total of 9 fractions were collected and the volume reduced by vacuum centrifugation (SpeedVac). The fractions were washed 3 times with water (200 μ l each) and the volume reduced by vacuum centrifugation to neutralize the acid. Each fraction was then reconstituted in 2xLDS sample loading buffer (Invitrogen, Carlsbad, CA) containing 50 mM dithiothreitol. Western blots were performed as previously reported [11] using anti-A β_{40} and anti-A β_{42} (Invitrogen) and CT9APP (Millipore, Billerica, MA) as primary antibodies.

Surface enhanced laser desorption/ionization-time of flight mass spectrometry (SELDI-TOF) mass spectrometry (MS). A $\beta_{40/42}$ Method

Following HPLC fractionation samples were analyzed using Western blots and the amyloid-containing peaks subjected to SELDI-TOF mass spectrometry as described in a previous publication [10]. Polyclonal anti-A β_{40} and anti-A β_{42} antibodies (Invitrogen) were used at a concentration of 0.38 mg/ml on PS20 chips as capture antibodies (Bio-Rad, Hercules, CA). The molecular mass assignments resulted from 100 averaged shots in a Bio-Rad SELDI

Protein Biology System II with external calibration acquired with the ProteinChip Peptide Mass Calibration Kit (Bio-Rad).

6E10 Method ProteinChip β -Amyloid MPD Kit

The HPLC fractions were pooled to yield one combined sample per case. All experimental steps were carried out at room temperature. The 6E10 protein chips, calibration standards and experimental instructions were provided by the manufacturer (Bio-Rad). Fifty μ l/ml of the internal standard ($A\beta$ Cys1-24 $M_r = 2,979.3$) was added to each calibration standard ($A\beta_{1-16}$, $1-38$, $1-40$ and $1-42$) as well as to each of the specimens (~ 200 – 400 μ l) under investigation. The ProteinChip arrays were equilibrated with 5 μ l of PBS per spot for 5 min. The arrays were then loaded with 5 μ l of sample or calibrant, placed in a humidified chamber and incubated for 1 h. Following removal of the sample, each array was placed in a 15 ml Falcon tube and rinsed 3 times (5 min each) with wash buffer (PBS, 0.5% Triton X-100) and 3 times (5 min each) with PBS. Finally, to desalt the arrays, 10 ml of 0.1 M HEPES was added to each tube, incubated for 5 min and air dried. Acetonitrile (200 μ l) and 1.0% TFA (200 μ l) were added to 5 mg of α -cyano-4-hydroxycinnamic acid (CHCA) and the mix vortexed for 2 min. After centrifugation at $1000 \times g$ for ~ 1 min to remove particles, 200 μ l of the CHCA solution was combined with 400 μ l acetonitrile and 400 μ l 1.0% TFA and vortexed 1 min. Finally, 1 μ l of the prepared CHCA solution was applied to each spot and air dried. The molecular mass assignments resulted from 100 averaged shots in a Bio-Rad SELDI Protein Biology System II with external calibration attained using the ProteinChip Peptide Mass Calibration Kit (Bio-Rad).

Results

Neuropathological examination of the bapineuzumab immunized patient revealed brain atrophy (1000 g), total plaque score of 15 (range 0-15), plaque density as frequent, total neurofibrillary tangles score of 3 (range 0-15), Braak stage of VI (range I-VI), total cerebral cortex amyloid angiopathy of 7 (range 0-12) and total WMR score of 5 (range 0-12). The CERAD NP score classified this brain as definite AD. Figures 1A and B show illustrations of the amyloid core plaques and diffuse amyloid densities and Figure 1C demonstrates neurofibrillary tangle pathology. In general, there were no areas of apparent amyloid plaque clearance (Figure 1). In addition, this patient exhibited severe leptomeningeal cerebral amyloid angiopathy (Figure 1D) and moderate areas of WMR (demyelination).

Immunoassay analysis of the bapineuzumab patient demonstrated high levels of GDFA/GHCl-soluble $A\beta$ in both the gray and white matter, similar to mean levels present in the non-immunized AD group (Figure 2A and 2B). The total gray matter Tris-soluble $A\beta$ level was similar to the mean of the NDC and non-immunized AD groups (Figure 2C). The scatter plots (Figure 2) of the $A\beta$ ELISA show the wide individual variation among the AD group. In the bapineuzumab case, the level of gray matter TNF- α was higher than the mean observed in the non-immunized AD and NDC individuals (Figure 2D). Interestingly, there were no significant differences between AD and NDC TNF- α levels. The levels of TNF- α are in agreement with a previous study using the same technique on control and demented individuals [12].

After sequential FPLC size exclusion and HPLC reverse-phase chromatographic separations (Figure 3), Western blot analyses demonstrated that the bapineuzumab-treated individual harbored monomers, dimers and larger oligomeric $A\beta$ forms (Figure 3). A broad array of amyloid- β protein precursor ($A\beta$ PP) C-terminal fragments were observed comprising the CT99/83 (~ 13 kDa) and some C-terminal peptides with lower molecular weight as well as a longer $A\beta$ PP C-terminal fragment at ~ 40 kDa, which are currently under investigation.

SELDI-TOF mass spectrometry, using anti-A β ₄₀, anti-A β ₄₂ and 6E10 capture antibodies, revealed a heterogeneous mixture of monomeric A β -related peptides starting at positions: 1, 3, 4, 11, and 17 with C-termini at positions 40, 42, 43, 46, 49, and 50. Mass spectrometry also demonstrated elongated A β related peptides with C-termini at positions: 68, 73, 96, and 99 (sequence numbering relative to the A β peptide starting at position 1 Asp). Dimeric forms of A β peptides ending at positions 40, 42, 43, and 46 as well as peptides with N-termini at position 3 with glutamyl modified to pyroglutamyl were also detected.

Discussion

Despite administration of 4 doses of bapineuzumab immunotherapy, thorough examination of large coronal sections covering the 4 cerebral lobes and the hippocampus in this patient revealed extensive numbers of plaques throughout the cerebral cortex. In addition, the finding of severe cerebral amyloid angiopathy in this case generally coincided with increased neuropathology and clinical evolution of AD as previously reported for ApoE4 carriers [13;14]. This subject died with dementia after disease duration of 5 years.

In a recent study, investigators at the University of Pittsburgh reported the neuropathological findings observed in an AD individual who received four 1 mg/kg doses of bapineuzumab. This individual did not complete the clinical trial because of progression of clinical symptoms and died 11 months after the last infusion of bapineuzumab [15].

Immunochemical and histochemical observations demonstrated frequent amyloid deposits in neocortex mainly composed of neuritic plaques with rare diffuse deposits, and scattered diffuse A β accumulation in the neostriatum. A comparison between the magnitude of neuropathological lesions and a ¹¹C-PiB PET study taken 36 months prior to the patient's death, suggested a 'mismatch' between pretreatment radiological and postmortem pathology especially in the neostriatum. In this case, however, it cannot be ruled out that the lack of a significantly noticeable plaque removal may be a consequence of the time interval between the last immunization (11 months) and the time of death. During this period, while amyloid concentrations remain elevated, antibody levels inevitably declined, perhaps diminishing to levels inadequate to prevent plaque reconstitution. Furthermore, a group comparison between 7 placebo and 19 bapineuzumab-treated patients evaluated with ¹¹C-PiB PET showed an estimated decreased mean dye retention of ~25%, interpreted as revealing a reduction in fibrillar amyloid over 78 weeks relative to baseline, while an increased retention was observed in the placebo control group [16]. However, these observations would have been more informative if the differences between baseline and the last available ¹¹C-PiB PET were individually defined and their respective *ApoE* genotypes specified.

In the present investigation, the bapineuzumab-treated patient exhibited no apparent areas of amyloid plaque clearance in contrast to those observed in the actively immunized AN1792 individuals [17-20]. Moreover, in our bapineuzumab-treated patient, the total gray matter and white matter GDF α /GHCI-soluble and the tris-soluble A β levels were similar to the mean observed in the non-immunized AD cases and higher than the NDC.

In general, a remarkable range of variations in A β values existed among the individuals in the NDC and AD groups. Similar disparities were also observed in a previous comparison of two AN1742 immunized cases studied in our laboratory [20]. In a more recent study of 7 AN1792 immunized AD individuals the range of A β peptide levels among these cases as measured by ELISA was extensive which supports the contention of a wide spectrum of human responses to amyloid removal [6]. The wide variability of amyloid plaque removal by AN1792 in individual patients has also been recently reviewed by Boche et al. [21].

In bapineuzumab-treated individuals, vasogenic edema in the presence of the *ApoE4* allele may be complicated by the abundant vascular amyloidosis that probably exacerbated the inflammatory response. Both the classical and alternative complement cascades are preferentially activated by fibrillar A β ₄₂ which is more plentiful in AD *ApoE4* individuals [22-24]. As part of the humoral branch of the immune system, complement responses promote inflammation, opsonization and cytolysis. These inflammatory reactions are compounded by the activation of microglia [25;26], and the secretion of proinflammatory cytokines and chemokines that result in the breaching of the blood-brain barrier and promotes diapedesis of vascular monocytes/macrophages, as observed in an *in vitro* model [27]. Interestingly, the bapineuzumab-treated individual had a level of TNF- α about twice the average observed in AD and NDC, suggesting an ongoing inflammatory reaction.

The interaction of antibodies with vascular A β may also be hampered by the tight enmeshing of the amyloid fibrils with the extracellular matrix molecules. In support of this assumption, it is necessary to employ collagenase to release the A β filaments from the extracellular vascular matrix during the amyloid purification procedures [28]. However in Tg mice, the 3D6 antibody appears to remove or prevent the deposition of vascular amyloid [29].

The antigen-antibody recognition sites for the N-terminal sequence of A β have been investigated using X-ray crystallography for 3 potentially therapeutic monoclonal antibodies 12A11, 10D5, and 12B4 [30]. These antibodies are capable of recognizing A β in amyloid plaques as well as soluble forms and therefore should, in principle, be efficacious anti-amyloid therapeutic interventions. The bapineuzumab antibody was very efficient in removing plaque amyloid in A β PP transgenic mice [31]. This efficacy may reflect the fact that the N-terminal degradation, interfering modifications and ancillary molecular steric hindrance prevalent in humans are absent or present in negligible quantities in transgenic mice [32;33]. We have observed that the A β peptides in humans with sporadic AD and familial AD have substantially degraded N-termini with A β molecules starting at positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 14, 15, 16, 17, and 19 [20;34-36]. N-terminal truncated A β peptides starting at positions 3, 4, 5, 8, and 9 have also been detected in CSF of living individuals with MCI [37], and in amyloid deposits of NDC individuals [38]. In human AD cases, the A β in amyloid plaque cores and capillaries contains a high proportion of isoaspartyl modifications at position 1 and 7 [34] that drastically changes the progression of the peptide bonds and secondary structure of A β . In addition, there are significant amounts of A β N-terminally degraded peptides at positions 3 and 11 with modified glutamyl to pyroglutamyl residues [39-41] that strongly alter the secondary and tertiary structures of these molecules. Therefore, key antigenic determinants in human amyloid plaques that the bapineuzumab antibody specifically target could either be chemically modified to yield alterations in N-terminal primary and secondary structures or masked due to binding to ancillary molecules or absent entirely. SELDI-TOF mass spectrometry of the bapineuzumab patient revealed the persistence of full-length A β _{40/42} as well as a spectrum of N-terminally truncated forms, at least two of which (starting at position A β ₁₁ and at position A β ₁₇) have been recognized as potentially toxic [42;43] as well as several oligomeric species.

Further complications for hopes to intervene against amyloid deposition pathology arise from the fact that in humans a large amount of A β deposits are of the diffuse type which are mainly composed of P3 peptides corresponding to the sequence of A β ₁₇₋₄₂ [44], although diffuse amyloid deposits also contain a variable amount of A β ₁₋₄₂. P3 is a highly insoluble and potentially toxic A β -related species that entirely lacks the N-terminal region epitopes which the bapineuzumab IgG therapy specifically target. It will be of interest to see whether or not Lilly's solanezumab or Pfizer's ponezumab immunization protocols elicit the severe

inflammatory response which has been interpreted as the result of T-cell A β epitopes in the central and C-terminal domains of the A β molecule.

Although senile plaques have been substantially disrupted by bapineuzumab immunotherapy [16], in a Phase II clinical trial, beneficial effects on cognition were not apparent [2], although this trial was primarily designed as a safety and pharmacokinetic study and not powered for clinical endpoints. An additional explanation for this observation is that subsequent neuropathological examinations have revealed that clinical accuracy for AD diagnosis is about 54-97% [45]. Furthermore, about two-thirds of AD patients show mixed pathologies [45] that are also likely to complicate the effectiveness of therapeutic interventions. It is also important to bear in mind that although senile plaques have been disrupted, the data unequivocally reveal that all potentially toxic amyloid species are not eliminated completely or exported from the brain tissue [6;20;21]. This may explain why in spite of amyloid plaque removal, the immunized patients still died of complications related to AD. Moreover, morphological examinations have consistently revealed that disruption therapy leaves a legacy of plaque skeletal remnants [17;18;20;21] of unknown toxicity in the parenchyma that may harbor a residuum of deleterious amyloid species as well as other molecules recalcitrant to disruption by immunotherapy. Thus far, even for cases in which substantial clearance of plaque amyloid was evident, immunotherapy apparently did not commensurately alter the course of AD, although the much greater statistical power of incoming studies will help decide this issue unequivocally. Regardless of the ultimate capacity of A β immunotherapy to ameliorate soluble A β toxicity and amyloid deposition, this intervention may prove to be a component of wider and more comprehensive strategies designed to mitigate a broader range of molecular pathologies and cognitive dysfunctions.

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Figure 1.

Frontal cortex and leptomeningeal blood vessels histopathology from the Bapineuzumab patient. Amyloid plaques stained by: A) thioflavin-S and B) Campbell-Switzer. C) Gallyas staining demonstrating numerous neurofibrillary tangles. D) Blood vessels stained by thioflavin-S showing severe cerebrovascular amyloidosis. Scale bars for: A and B = 100 μm ; C = 100 μm (inset = 25 μm); D = 250 μm .

Figure 2.

Scatter plots of ELISA data derived from Aβ peptides isolated from Superose 12 size-exclusion FPLC fraction 3 (MW range 2-10 kDa), tris-buffer homogenates and HEPES-buffer homogenates. A) Total gray matter GDFA/GHCl-soluble Aβ peptides in ng per mg of total protein. B) Total white matter GDFA/GHCl-soluble Aβ peptides in pg per mg of total protein. C) Total gray matter tris-soluble Aβ peptides in pg per mg of total protein. D) Total gray matter TNF-α in pg per mg of total protein. NDC, non-demented controls; AD, Alzheimer's disease; Bapi, bapineuzumab immunized AD patient; GM, gray matter; WM, white matter; GDFA, glass distilled formic acid; GHCl, 5 M guanidine hydrochloride

(GHC1), 50 mM Tris-HCl, pH 8.0 buffer; tris, 20 mM Tris-HCl, 5 mM EDTA, pH 7.8 buffer; TNF- α , tumor necrosis factor-alpha.

Figure 3.

Reverse-phase HPLC and related Western blots derived from FPLC fraction 3 from the Bapineuzumab patient. The Western blots were developed with antibodies against A β ₄₀, A β ₄₂ and CT9APP. Lanes # 10 contain the corresponding A β ₄₀ or A β ₄₂ peptides standards. Notice that in both A β cases there were a preponderance of A β dimers over monomers as well as the presence of multiple oligomeric forms. Aside from revealing the presence of the CT99 and CT83 A β PP C-terminal peptides at ~ 13 kDa, which are related to the β - and α -secretase A β PP cleavages, the CT9A β PP antibody also demonstrated the presence of ~40 kDa band, suggesting a longer A β PP C-terminal peptide.