18-Month study of intravenous immunoglobulin for treatment of mild Alzheimer disease

Norman R. Relkin [a], Paul Szabo [a,b], Basia Adamiak [a], Tuna Burgut [a], Carmen Monthe [a], Richard W. Lent [c], Steven Younkin [d], Linda Younkin [d], Richard Schiff [e], Marc E. Weksler [b,*]

[a] *Department of Neurology, The Weill Cornell Medical College, New York, NY, USA*
[b] *Department of Medicine, The Weill Cornell Medical College, New York, NY, USA*
[c] *Department of Pathology and Laboratory Medicine, The Weill Cornell Medical College, New York, NY, USA*
[d] *The Mayo Clinic, Jacksonville, FL, USA*
[e] *Baxter BioScience, Westlake Village, CA, USA*

Abstract

Intravenous immunoglobulin (IVIg) has been proposed as a potential agent for Alzheimer's disease (AD) immunotherapy because it contains antibodies against beta-amyloid (Aβ). We carried out an open label dose-ranging study in 8 mild AD patients in which IVIg was added to approved AD therapies for 6 months, discontinued, and then resumed for another 9 months. Infusions were generally well-tolerated. Anti-Aβ antibodies in the serum from AD patients increased in proportion to IVIg dose and had a shorter half-life than anti-hepatitis antibodies and total IgG. Plasma Aβ levels increased transiently after each infusion. Cerebrospinal fluid Aβ decreased significantly at 6 months, returned to baseline after washout and decreased again after IVIg was re-administered for an additional 9 months. Mini-mental state scores increased an average of 2.5 points after 6 months, returned to baseline during washout and remained stable during subsequent IVIg treatment. Our findings confirm and extend those obtained by Dodel et al. [Dodel, R.C., Du, Y., Depboylu, C., Hampel, H., Frolich, L., Haag, A., Hemmeter, U., Paulsen, S., Teipel, S.J., Brettschneider, S., Spottke, A., Nolker, C., Moller, H.J., Wei, X., Farlow, M., Sommer, N., Oertel, W.H., 2004. Intravenous immunoglobulins containing antibodies against beta-amyloid for the treatment of Alzheimer's disease. J. Neurol. Neurosurg. Psychiatry 75, 1472–1474] from a 6-month trial of IVIg in 5 AD patients and justify further studies of IVIg for treatment of AD.
© 2008 Elsevier Inc. All rights reserved.

Keywords: Intravenous immunoglobulin; Alzheimer's disease; Amyloid beta peptides; Immunotherapy

1. Introduction

Immunotherapy is one of several treatment strategies now being studied as part of efforts to discover safe and more effective means of treating Alzheimer's disease (AD). Seminal studies by Schenk and his associates demonstrated that active immunization with amyloid beta peptide 1–42 (Aβ42) in complete Freund's adjuvant decreased cerebral amyloid deposits and improved cognition in an APP-transgenic mouse model of early onset, familial AD (Schenk et al., 1999; Morgan et al., 2000). Unfortunately, active immunization of

AD patients with Aβ42 in the adjuvant QS-21 led to a therapeutically meaningful anti-Aβ antibody response in only 20% of the AD patients and sterile encephalitis associated with T cell infiltration in 6% of the AD patients (Orgogozo et al., 2003; Gilman et al., 2005).

Passive immunization with murine polyclonal and some, but not all, murine monoclonal, anti-Aβ antibodies was effective in inhibiting the AD-like neuropathology seen in APP-transgenic murine models of AD (Bard et al., 2000). The presence of natural polyclonal anti-Aβ antibodies in normal human blood and the lower titers of these antibodies in AD patients led to the proposal that intravenous immunoglobulin (IVIg) may be of use for passive immunotherapy in AD (Weksler et al., 2002; Dodel et al., 2002). IVIg is a pool of human immunoglobulins obtained from the blood of

* Corresponding author at: Weill Medical College, 1300 York Avenue, New York, NY, USA. Tel.: +1 212 794 6478; fax: +1 212 746 6382.
E-mail address: Weksler@med.cornell.edu (M.E. Weksler).

thousands of healthy donors that has been approved for more than two decades by the United States Food and Drug Administration for treatment of immune deficiency disorders and other indications but not AD. An initial study of IVIg in patients with neurologic diseases but not AD suggested that IVIg increased blood anti-Aβ antibody levels and fostered Aβ clearance (Dodel et al., 2002).

Dodel and his associates, subsequently, reported encouraging results of treating 5 AD patients with IVIg for 6 months (Dodel et al., 2004). IVIg therapy of these AD patients was associated with a significant increase in serum Aβ40, a significant decrease in cerebrospinal fluid (CSF) Aβ40, as well as with improved cognitive function in the AD patients. We now report the results of an open label, dose ranging trial of IVIg in 8 mildly affected AD patients. The primary goals of this trial was to evaluate the safety of repeated infusions of IVIg in elderly AD patients and to examine anti-Aβ levels in blood and CSF as a function of IVIg dose. Based on the information available at the initiation of this study, cognitive improvement was not expected. As a safety measure, the Folstein Minimental State Examination (MMSE) was administered throughout the trial to identify any unexpected decline in cognitive function (Folstein et al., 1977). The study was not designed nor powered to fully evaluate changes in cognition associated with IVIg treatment.

2. Methods

2.1. Study design

Our study was originally designed as an open label, dose-finding, phase I trial of IVIg in patients with mild AD that included 6 months of active treatment followed by a 3-month washout period. In light of an unexpectedly positive therapeutic response in the first 6 months that diminished with washout of IVIg, all patients were offered and agreed to participate in an open label extension study providing an additional 9 months of IVIg therapy. The original study and its extension were approved by the Weill Cornell Institutional Review Board (IRB) and the Scientific Advisory Committee of the Weill Cornell General Clinical Research Center (GCRC). The trial was conducted in accordance with Good Clinical Research practices. Patients as well as caregivers gave informed consent for their participation.

All AD patients were given an initial test dose of 0.4 g/kg of IVIg (Gammagard S/D, Baxter) and then randomly assigned to one of four IVIg doses (0.4 g/kg/2 weeks, 0.4 g/kg/week, 1 g/kg/2 weeks and 2 g/kg/4 weeks) for 6 months of treatment. IVIg was discontinued during a 3-month washout period including months 7, 8, and 9. All patients were then treated with 1 g IVIg/kg every 2 weeks starting in month 10 through month 12 and then with 0.4 g/kg/2 weeks starting in month 13 through month 18.

The initial two study infusions and the first post-washout infusion were carried out with overnight stays in the GCRC

for close observation and to permit collection of specimens for pharmacokinetic analyses. All other infusions were performed in the GCRC outpatient facility. Subjects were pre-medicated with 1000 mg of acetaminophen and 50 mg of diphenhydramine approximately 30 min prior to initiating IVIg infusions through a 24 gauge IV catheter placed in arm vein. IVIg was mixed and prepared within 2 h of administration. Certified infusion pumps were employed to regulate rates of IVIg delivery. Total infusion times were 2–6 h per session depending on the patient's assigned dose and body mass. A single lot of IVIg was used throughout the initial 6-month study and another lot was used throughout the 9-month extension of IVIg therapy.

2.2. Clinical assessments

All subjects underwent neurologic examinations including cognitive testing and examinations of blood and CSF at baseline and at 3-month intervals thereafter. Inclusion criteria included ability to give informed consent, a diagnosis of probable AD by NINDS-ADRD criteria, stable doses of at least one approved AD treatment for at least 3 months, ability to comply with the study protocol and possession of a suitable caregiver. Subjects were excluded if they had known allergy to IVIg therapy, IgA deficiency, renal failure, untreated vascular risk factors, and unstable co-morbid medical conditions or were receiving other investigational AD therapies or certain psychoactive medications. Subjects were also screened to exclude the presence of significant depression, psychosis and other neuropsychiatric disorders. Serum viscosity, IgA levels, renal function, thyroid function, rapid plasma reagin, complete blood count, metabolic profile, human immunodeficiency virus status and hepatitis titers were checked at baseline. Subsets of these tests were repeated throughout the study for safety purposes. MMSE testing was performed by the same rater throughout the study. Cognitive testing was carried out between 3 and 6 days from the time of infusions to avoid possible confounding effects of the pre-medication. Treatment emergent adverse events were classified in accordance with the medDRA system.

2.3. Specimen collection

Blood for Aβ assay was drawn into EDTA-containing tubes used to collect plasma samples. The tubes were centrifuged at $600 \times g$, plasma separated, aliquoted into 1 ml volumes, and stored at $-70\,^\circ$C until sent to the laboratory of Drs. S. and L. Younkin for assay of Aβ40 and Aβ42 by an ELISA method that had been previously validated (Scheuner et al., 1996). Samples were obtained before IVIg therapy was begun (baseline), as well as prior to and at multiple times after IVIg infusions and then weekly throughout course of the initial 6 months of study. Additional specimens were obtained over a 24 h period after the first post-washout infusion and before and after infusions during the 9-month extension. Plasma and serum were prepared by centrifuga-

tion and aliquots stored at −80 °C until time of analysis. CSF was obtained by lumbar puncture (LP) at 5 time-points over the 18 months of study (baseline, 1–3 months, 6 months, 9 months and 18 months). All LPs, except those at baseline and post-washout, were performed within 3–6 days of the most recent IVIg infusion. LPs were carried out in the lateral decubitus position using sterile techniques, Quincke needles and gravity drainage, with care taken to avoid exposing CSF to any amyloid-absorbing plastics. CSF protein and cell count was determined to exclude serum contamination and aliquots were flash-frozen to −80 °C until time of analysis.

2.4. Biomarker analyses

Aβ40 and Aβ42 in CSF was measured using a Biosource Aβ kits that have been validated for this purpose. The Biosource Aβ42 kit has a detection limit of 2.2 pM; recovery (spiking) when Aβ42 is added to CSF was 116%; and was specific for Aβ42 with no cross-reactivity at 22 nM of Aβ 1–12; 12–20; 12–28; 22–35; 1–40. The Biosource Aβ40 kit has a detection limit of 1.3 pM; recovery (spiking) when Aβ40 is added to CSF of 87%; and specificity for Aβ40 with no cross-reactivity at 11 nM with Aβ 1–12; 12–20; 12–28; 22–35; 1–42. As these kits have not been validated for plasma, plasma Aβ40 and Aβ42 were determined in the laboratory of Drs. S. and L. Younkin whose method has been validated (Scheuner et al., 1996).

Serum Aβ antibody levels were measured during the initial 6 months of study using a midpoint titer in place of an end-point titer derived from ELISA dilution curves as previously reported (Weksler et al., 2002). Serum anti-hepatitis B surface antibody was measured by ELISA using plates coated with hepatitis B surface antigen to capture the antibody and a horseradish peroxidase-labeled hepatitis B surface antigen to reveal the bound anti-hepatitis B surface antibody. A luminogenic substrate and an electron transfer agent were added to the wells and the horseradish peroxidase in the bound conjugate catalyzed the oxidation of a luminol derivative producing light. The light signals were read by the VITROS ECi/ECiQ system (Ortho-Clinical Diagnostics, Rochester, NY). Total serum IgG level was measured by rate nephelometry of particle formed in a solution of serum and anti-human IgG antibody using the Beckman Immage Instrument (Brea, CA). The half-lives of anti-Aβ antibodies, total IgG, and anti-hepatitis B surface antigen antibody were calculated from blood samples drawn before and at multiple time points after the infusions of 1 g/kg IVIg.

2.5. Statistical analysis

The non-parametric Mann–Whitney rank sum statistic was used to compare the serum anti-Aβ antibody titer before and after infusion of 0.4 g IVIg/kg. The paired Student t-test was used to compare the levels of CSF Aβ40 and Aβ42 at various times during the study compared to their levels before IVIg was first administered. The levels of anti-Aβ and

anti-hepatitis B surface antigen antibodies as well as total IgG were fit to a single dose and multidose plasma curves using a single component exponential model (Kinetica V4.3, Innaphase Corp.).

3. Results

3.1. Demographics

Eight patients (1 male, 7 female) with mild, probable AD participated in this study. At baseline, their ages ranged from 67 to 86 years (mean 74.3). MMSE scores ranged from 20 to 29 (mean 23.5). They did not show significant signs of depression (mean GDS 4) or other psychopathology (mean NPI 4). All subjects were receiving stable doses of a cholinesterase inhibitor and in some cases memantine for at least 3 months prior to enrolling in the study.

3.2. Serum anti-Aβ antibody levels after IVIg infusion

Serum anti-Aβ antibody levels were measured in all AD patients before and after the infusion of a test dose (0.4 g/kg) of IVIg (Table 1). Before the test dose, 3 of the 8 patients had detectable serum anti-Aβ antibody titers (average titer 43) and 5 patients had no measurable titers (average titer <10). After the test dose of IVIg, all patients had detectable anti-Aβ antibody titers and the increase in titer was significant ($p < 0.005$, Mann–Whitney rank sum test). The average post-infusion titer was higher in the 3 patients with anti-Aβ antibody titers before IVIg infusion (average titer 103) compared to the 5 patients without anti-Aβ antibody titers before IVIg infusion (average titer 47). It should be noted that the lowest dose of IVIg used in this study appeared to double the serum anti-Aβ antibody titer in AD patients.

After the 0.4 g/kg test dose of IVIg, groups of 2 patients were randomly assigned to various doses of IVIg for the following 6 months. The average titer of anti-Aβ antibodies in

Table 1
IVIg infusion increases serum anti-Aβ antibody levels in all AD patients[a]

Anti-Aβ antibody titer	
Before test dose of IVIg	After test dose of IVIg
ND	27
ND	37
ND	44
ND	51
ND	76
37	92
42	103
50	113

[a] Blood was drawn before and immediately after a 0.4 g/kg test dose of IVIg administered to each AD patient. The anti-Aβ antibody mid-point titer of serum was derived from ELISA dilution curves as previously reported (6). A single lot of IVIg, diluted to 10 mg/ml saline, was used to normalize all serum specimen. The lower limit of detection in this assay was a titer of 10. Any titer below this was indicated as not detectable (ND).
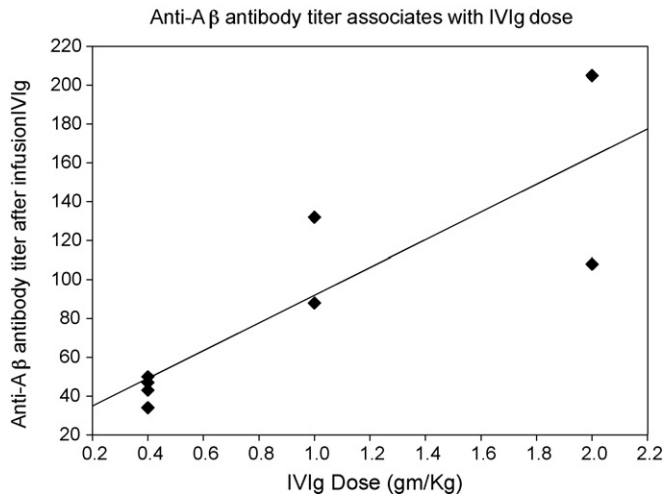
Fig. 1. Anti-Aβ antibody titer correlates with IVIg dose. The anti-Aβ antibody titer immediately after IVIg infusion is plotted against the IVIg dose. Four patients received 0.4 g IVIg/kg, two patients received 1.0 g IVIg/kg, and two patients received 2.0 g/kg. The correlation ($R^2 = 0.72$) was significant ($p < 0.01$).

3.3. Half-life of infused anti-Aβ antibodies, anti-hepatitis B surface antigen antibody, and total IgG in patients with AD

A transient and reproducible increase in serum anti-Aβ antibody titers occurred at all doses of IVIg tested but was most easily appreciated in patients receiving 1 or 2 g IVIg/kg infusions (Fig. 2). The apparent half-life of serum anti-Aβ antibodies, calculated from multiple infusions of 2 g IVIg/kg over the first 4 months of IVIg therapy, averaged 9.3 days. The level of anti-hepatitis B surface antibody and total IgG, also present in IVIg, were also measured in the same serum samples and used to calculate the average serum half-lives of anti-hepatitis B antibody (16.8 days) and total IgG (15.5 days) in the same patients (Table 2). The shorter half-lives of the anti-Aβ antibodies than the half-lives of either anti-hepatitis B surface antibodies or total IgG suggest their more rapid removal from the blood probably due to an *in vivo* interaction of anti-Aβ antibodies with Aβ or other ligands, e.g. anti-idiotypic antibodies, and clearance from the peripheral circulation.

serum 1 h post-infusion, averaged from multiple infusions of 0.4 g/kg, 1 g/kg, or 2 g/kg, respectively, was, 44, 110, and 157. There was a significant ($p < 0.01$) correlation ($R^2 = 0.72$) between the amount of IVIg infused and the titer of serum anti-Aβ antibody level immediately following the infusion (Fig. 1).

3.4. Changes in plasma Aβ40 and Aβ42 levels following infusion of IVIg

The blood level of Aβ peptide in APP-transgenic mice increases after the injection of the monoclonal anti-Aβ antibody m266 and the magnitude of the increase correlated
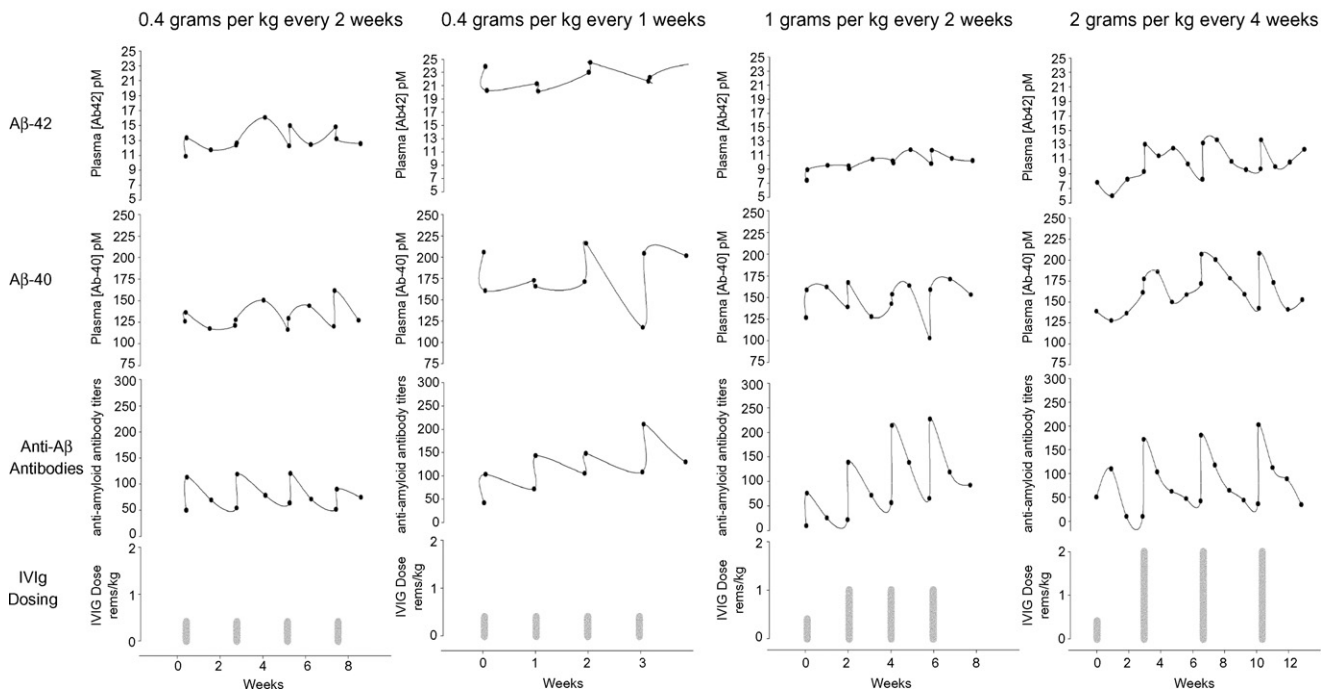

Fig. 2. Increase in plasma Aβ40 and Aβ42 follows injection of IVIg. The increase in Aβ40 and Aβ42 was directly associated with the infusion IVIg. Shown are the levels of plasma Aβ40 (pM), Aβ42 (pM) and anti-Aβ antibody (titer) in one patient from each of the four treatment doses of IVIg.

Table 2
Steady-state half-life of specific antibodies and total IgG in AD patients infused in IVIg[a]

Immunoglobulin	IVIg dose (g/kg)	Half-life (days)
Serum titer anti-Aβ antibody	2	9.8
Serum titer anti-hepatitis B surface antibody	2	16.8
Total serum IgG (mg/ml)	2	15.5

[a] Blood was collected before the infusion of 2 g IVIg/kg into an AD patient and at multiple times after the IVIg infusion. The serum anti-Aβ antibodies, anti-hepatitis B surface antigen titers were measured by ELISA and total serum IgG were described as described in Section 2. The apparent serum half-lives were calculated from serum curves obtained across multiple doses of IVIg during the first 4 months of IVIg therapy from fitting serum values at various times before and after infusion of IVIg using a single dose and multidose curves using a single component exponential model (Kinetica V4.3, Innaphase Corp.).

directly with the cerebral Aβ load (DeMattos et al., 2002). Fig. 2 demonstrates a consistent and repeated increase in plasma Aβ40 and Aβ42 levels in a patient who received 2 g/kg IVIg infusions. It should be noted that the increases in plasma Aβ40 and Aβ42 were lower following the test dose of 0.4 g/kg than following 3 doses of 2 g IVIg/kg. A direct correlation between all IVIg doses and the increase in plasma Aβ40 ($R^2 = 0.77$) was observed. A direct correlation was also observed between IVIg doses of 1 g/kg and 2 g/kg and Aβ42 ($R^2 = 0.26$). However, only the correlation

between Aβ40 and all IVIg doses reached statistical significance ($p < 0.01$).

3.5. Change in CSF level of Aβ40 and Aβ42 associated with IVIg therapy

Except for the CSF samples taken after 3 months without IVIg, CSF samples were obtained between 3 and 6 days following the IVIg infusion. Fig. 3 shows the percentage changes from baseline values of CSF Aβ42 and Aβ40 levels in 8 patients during IVIg therapy, in 8 patients after 6 months of IVIg, in 7 patients after 3 months without IVIg therapy, and in 8 patients after 9 months of renewed IVIg. After 6 months of IVIg therapy Aβ40 and Aβ42 levels in CSF were significantly lower ($p < 0.003$ paired Student's t-test) than they had been at baseline. At this time point, the MMSE scores had increased (see below). During the 3 months washout period without IVIg therapy, both CSF Aβ40 and Aβ42 levels increased and were no longer significantly different from their baseline levels. There was also a decline in MMSE score (see below). After the 3 months without IVIg all patients were restarted on IVIg therapy at a dose of 1 g/kg every 2 weeks for 3 months and then at a dose of 0.4 g/kg IVIg every 2 weeks for an additional 6 months. Nine months after IVIg was restarted, the levels of CSF Aβ40 and Aβ42 were significantly lower ($p < 0.003$ paired Student's t-test) than they had been at baseline.
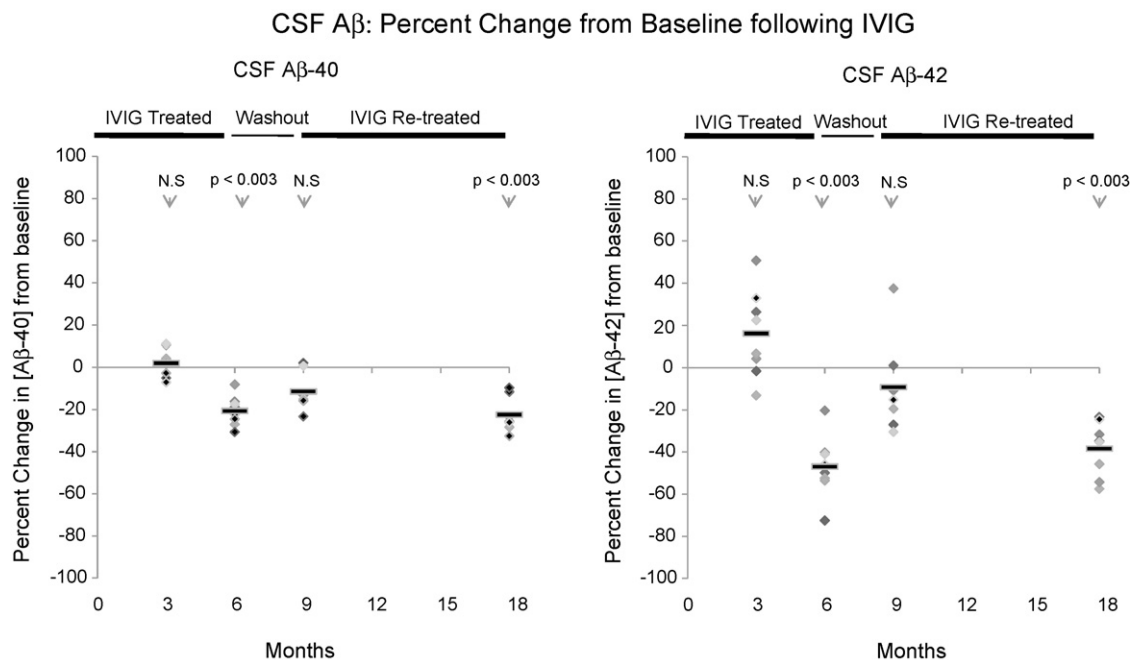

Fig. 3. CSF Aβ40 and Aβ42 were measured during IVIg treatment in all patients before IVIg therapy (baseline), during the course of IVIg therapy (interim), after 6 months of IVIg therapy (6 months), at the end of the 3-month washout period (washout) and at the end of the 9-month period of renewed IVIg therapy (18 months). Individual values and mean values are shown. There was a significant decrease from baseline in CSF Aβ40 and Aβ42 after 6 months of IVIg therapy and after 9 months of renewed IVIg therapy ($p < 0.003$).

Table 3
MMSE in 8 AD patients that received different doses of IVIg[a]

	AD patient number							
	1 (0.4/2 weeks[b])	2 (0.4/2 weeks[b])	3 (0.4/ week[b])	4 (0.4/ week[b])	5 (1.0/2 weeks[b])	6 (1.0/2 weeks[b])	7 (2.0/4 weeks[b])	8 (2.0/4 weeks[b])
MMSE scores								
Before IVIg	24	25	21	21	29	22	26	20
IVIg started								
After 3 months	28	26	23	23	27	24	26	22
After 6 months	29	28	24	25	29	26	27	20
IVIg stopped								
After 9 months	24	24	23	26	27	22	27	18
IVIg restarted								
After 18 months	24	26	26	25	25	23	28	Unable to test

[a] MMSE scores are shown for each patient before IVIg therapy was begun and 3, 6, 9, and 18 months after initial IVIg therapy was begun. Cognitive testing was carried out by the same rater between 3 and 6 days after IVIg infusions to avoid the effects of pre-medication.

[b] IVIg doses.

3.6. MMSE scores in AD patients during 18 months of the study

The MMSE scores were measured in all 8 patients before IVIg therapy and during the 18 months of study (Table 3). Prior to IVIg therapy, the average MMSE was 23.5. After 3 months of IVIg treatment, the mean MMSE score had increased to 24.9 attributable to an increase in MMSE scores in 6 patients, no change in MMSE score from baseline in 1 patient, and a decline in MMSE score from baseline in 1 patient. After 6 months of IVIg treatment, the mean MMSE score increased further to 26.0 with 6 patients showing an increase in MMSE scores above baseline and 2 patients showing no change in their MMSE score from baseline. The IVIg infusions were stopped after 6 months and 3 months later the average MMSE score had fallen to 23.9 with only 3 patients having MMSE scores greater than baseline. Three patients had MMSE scores unchanged from baseline and 2 patients had MMSE scores lower than baseline score. After 9 months of renewed IVIg infusions, the average MMSE scores was 24.0. Five patients had MMSE scores above their baseline values, one patient had a MMSE unchanged from baseline and 2 patients had MMSE scores below their baseline scores. Fig. 4 shows the evolution of the MMSE scores expressed
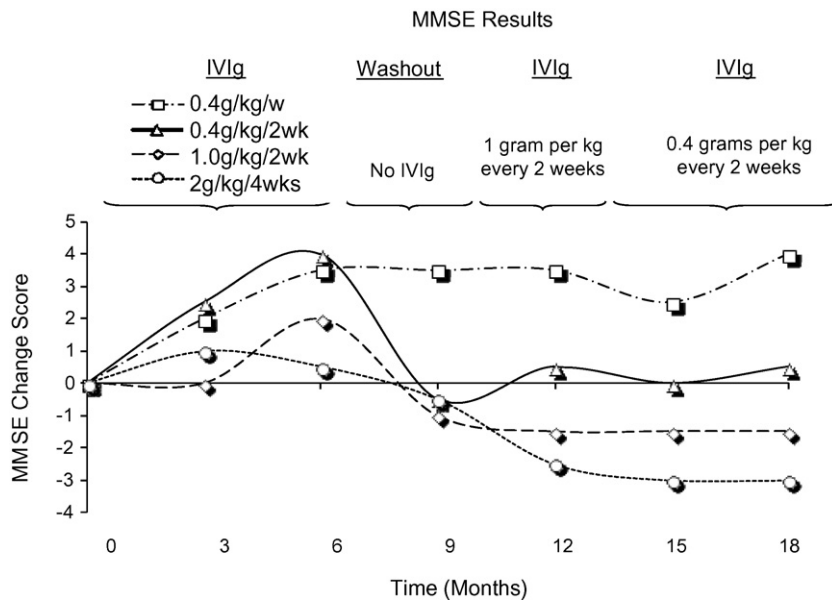

Fig. 4. The MMSE scores over the course of the 18-month study in each treatment group was measured. The choice of 1 g IVIg/kg for the first 3 months of renewed IVIg therapy was chosen before all patients had completed the 6-month trial of IVIg and the intermediate dose 1 g IVIg/kg was chosen. When all data from the 6-month study was available. 0.4 g IVIg/kg given either every week or every 2 weeks was associated with greater increases in MMSE scores between baseline and 6 months of IVIg therapy than when 1 or 2 g IVIg IVIg/kg was given. For this reason, the dose of 1.0 g IVIg/kg was reduced to 0.4 g IVIg/kg every 2 weeks.

as the average of the 2 patients at each IVIg dose. It can be seen that higher doses of IVIg did not lead to higher MMSE scores than lower doses of IVIg.

3.7. Adverse events

IVIg infusions were well tolerated in the majority of cases and no serious treatment emergent adverse events occurred during the 18 months of study. Events that were judged to be treatment related were relatively benign and self-limited and included one episode each of headache, chills and diaphoresis, fever and transient confusion. In all cases, symptoms resolved spontaneously and without sequella. There was no reported episode of agitation associated with the IVIG infusion. There were no adverse events incurred during this study that have not been previously reported in association with IVIg therapy in other patient populations.

4. Discussion

In this study, administration of IVIg to patients with mild AD led to transient, reproducible, and dose-dependent increases in serum anti-Aβ antibody titers and parallel increases in plasma Aβ-40 and Aβ-42 levels. However, the increase in plasma Aβ may underestimate the total amount of Aβ42 mobilized from the CSF into the peripheral circulation as Aβ bound to erythrocytes as well as to plasma proteins (Rogers et al., 2006) would not be detected in the ELISA assay. On the other hand, anti-Aβ antibodies in IVIg may bind plasma Aβ and thereby increasing its half-life and may result in the overestimation of the level Aβ in plasma as measured by ELISA (Levites et al., 2006).

After 6 months of IVIg therapy the CSF Aβ40 and Aβ42 levels decreased. After 3 months without IVIg (washout period) CSF Aβ40 and Aβ42 levels increased to their pretreatment baseline levels. When IVIg therapy was restarted, a decrease in CSF Aβ40 and Aβ42 levels was again observed. These effects are consistent with murine monoclonal anti-Aβ antibody-mediated mobilization of Aβ from the central nervous system into the peripheral circulation of APP-transgenic mice (DeMattos et al., 2002). However, in light of the complex mixture of immunoglobulins in IVIg, other mechanisms could contribute to the alterations in blood and CSF Aβ observed in this study.

Serum half-life of free anti-Aβ antibodies in IVIg-treated AD patients was less than the half-lives of antibodies to hepatitis B surface antigen and total circulating IgG. The more rapid turnover of anti-Aβ antibodies could be the consequence of the binding of free antibodies to their endogenous ligands, including Aβ and/or anti-idiotypic antibodies, and the consequent accelerated clearance of the antibody-ligand complexes. However, certain caveats apply to the interpretation of the calculated half-life. None of our subjects had measurable titers of hepatitis B surface antigen at baseline so that the measured titers of these antibodies can be said

to reflect only IVIg-derived antibodies. In contrast, turnover of anti-Aβ antibodies and total IgG is a function of endogenous immunoglobulin production and clearance as well as the effects of IVIg administration. Thus, the calculated half-life of these antibodies may reflect several factors besides formation of antigen–antibody complexes and their rapid clearance from the blood. For example, it is possible that the more rapid turnover of unbound antibody might occur at higher doses of IVIg because of the saturation of neonatal Fc receptors that normally scavenges IgG and returns it to the circulation. This could, in part, explain why patients receiving the higher doses of IVIg in our study did not have a better outcome than those treated with lower or more frequent doses of IVIg. Thus, when the saturation of neonatal Fc receptors is achieved by high doses of IVIg, the catabolic rate of IgG is increased (Ghetie and Ward, 2000). This may explain, at least in part, why the benefit of IVIg is not directly proportional to its dose.

There is considerable diversity in the isotype and epitope-specificity of anti-Aβ antibodies in blood from healthy humans and IVIg prepared from it. Both IgM and IgG anti-Aβ monomer antibodies can be detected in human serum that react with N-terminal and central region linear epitopes of Aβ monomer. In addition, immunoglobulins in human serum and IVIg reactive with conformational epitopes specific to Aβ oligomers (Relkin et al., 2007) and Aβ fibrils (O'Nuallain et al., 2006) have been found.

Although this study did not include a large enough number of AD patients or testing instruments to establish whether IVIg altered cognitive status, the observed mean improvement of 2.5 points in the group's MMSE scores after 6 months and the decline of these scores towards baseline during the washout period are certainly unexpected and noteworthy. This is a better than expected outcome relative to the 1.5 point decline in MMSE typically seen in AD patients over a 6-month period. Six of the 8 patients treated with IVIg in our study remained at or above their baseline MMSE at the study's completion, which is also a better than expected. Dodel and colleagues also observed improvement in MMSE scores, with comparable improvements in the ADAS-Cog and in tests of visuospatial function over 6 months in 4 of 5 mild to moderate stage AD patients treated with IVIg (Dodel et al., 2004). Passive immunotherapy with anti-amyloid antibodies has also been found to exert positive effects on cognitive impairments in transgenic mice models of AD in some cases as soon as 24 h after treatment (Kotilinek et al., 2002). It is thus possible that IVIg could exert similar benefits for cognition in patients with AD.

As neither the present study nor that of Dodel and colleagues was double-blind nor placebo-controlled it is not possible to conclude with certainty that IVIg therapy results in changes in Aβ levels or cognition in AD patients. However, the reversal of both the decrease in CSF Aβ40 and Aβ42 levels and cognitive benefits after discontinuation of IVIg for 3 months coupled with decreases in CSF Aβ40 and Aβ42 levels as well as cognitive stabilization following resumption of IVIg treatments supports the hypothesis that IVIg

holds promise for the immunotherapy of AD. The precise mechanisms responsible for the observed cognitive benefits of IVIg remain to be elucidated. In our group of AD patients, their cognitive improvement paralleled their decreased CSF Aβ levels. However, the magnitude of changes in CSF Aβ was not predictive of cognitive outcomes in individual cases. Similarly, neither baseline Aβ antibody titers nor anti-Aβ antibody responses to IVIg correlated with cognitive improvement. Since the number of subjects was small and the indices by which cognitive change was measured were relatively crude, these relationships need to be re-examined in a larger and more detailed study.

It should be remembered that IVIg was first proposed as a treatment for patients with AD because of natural anti-Aβ antibodies in IVIg (Dodel et al., 2002). However, it remains unclear whether anti-Aβ antibodies in IVIg are fully responsible for the observed effects on blood or CSF Aβ levels or the changes in cognition observed in our study participants. However, a recent report indicated that affinity-purified anti-Aβ antibodies from IVIg increased Aβ levels in blood and decreased Aβ levels in CSF from APP-transgenic mice (Wei et al., 2006). Furthermore, IVIg depleted of anti-Aβ antibodies had considerably less effect on Aβ levels in the blood or CSF from these animals. It remains to be determined whether the results with IVIg in APP-transgenic mice reflect the mechanisms active in AD patients. Finally, it is also possible that other activities of IVIg, unrelated to its content of anti-Aβ antibodies, such as the modulation of inflammatory and immune reactions, may complement the effects of anti-Aβ antibodies on cognitive function in AD patients (Nimmerjahn and Ravetch, 2007; Negi et al., 2007).

It is fair to ask why two small studies of IVIg in patients with AD have been associated with encouraging effects on cognitive function not reported in studies of monoclonal anti-Aβ antibodies. This appears paradoxical as monoclonal anti-Aβ antibodies might be expected to increase serum anti-Aβ levels to a greater extent than those achieved with the infusion of IVIg. It is possible that the greater diversity of the polyclonal anti-Aβ antibodies in IVIg might play an important role. For example, the humanized murine monoclonal anti-Aβ antibody being used is specific for the linear N-terminal epitope of the Aβ peptide. In contrast, the polyclonal anti-Aβ antibodies in IVIg include antibodies that react not only with the linear Aβ N-terminal epitope but also the central region epitopes of the Aβ peptide as well as the conformational epitopes expressed on Aβ oligomers (Relkin et al., 2007) and Aβ fibrils (O'Nuallain et al., 2006). Several groups have stressed the advantage of a polyclonal antibody preparations as compared to a monoclonal antibodies to recognize multiple epitopes on a complex antigen (Rodkey, 1995; Haurum, 2006; Klitgaard et al., 2006).

The findings of this study should not be taken as sufficient justification to use IVIg to treat AD patients at the present time. Further studies are needed to establish efficacy, to determine the optimal dosing regimen and to confirm the safety of this agent in the general population of AD patients. Finally,

IVIg is relatively expensive and is in limited supply. There are, however, ways to increase the potency and availability of IVIg. It may be possible to produce IVIg preparations with a higher content of anti-Aβ antibodies, for example, by preparing IVIg from donors with a higher titers of anti-Aβ antibodies or by dissociating anti-Aβ antibodies from bound ligands such as Aβ or anti-idiotypic antibodies that occur *in vivo* and mask the activity of anti-Aβ antibody. A third, and perhaps most intriguing would be to immunize transchromosomic cows whose endogenous bovine immunoglobulin genes are inactivated and replaced by a transchromosome expressing human immunoglobulin genes (Kuroiwa et al., 2002). However, before exploring these avenues in patients with AD, further testing of IVIg must be carried out in larger numbers of patients with AD to establish its safety and efficacy. A phase II trial is currently being carried out for this purpose.

References

Bard, F., Cannon, C., Barbour, R., Burke, R.L., Games, D., Grajeda, H., Guido, T., Hu, K., Huang, J., Johnson-Wood, K., Khan, K., Kholodenko, D., Lee, M., Lieberburg, I., Motter, R., Nguyen, M., Soriano, F., Vasquez, N., Weiss, K., Welch, B., Seubert, P., Schenk, D., Yednock, T., 2000. Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. Nat. Med. 6, 916–919.

DeMattos, R.B., Bales, K.R., Cummins, D.J., Paul, S.M., Holtzman, D.M., 2002. Brain to plasma amyloid-beta efflux: a measure of brain amyloid burden in a mouse model of Alzheimer's disease. Science 295, 2264–2267.

Dodel, R., Hampel, H., Depboylu, C., Lin, S., Gao, F., Schock, S., Jackel, S., Wei, X., Buerger, K., Hoft, C., Hemmer, B., Moller, H.J., Farlow, M., Oertel, W.H., Sommer, N., Du, Y., 2002. Human antibodies against amyloid beta peptide: a potential treatment for Alzheimer's disease. Ann. Neurol. 52, 253–256.

Dodel, R.C., Du, Y., Depboylu, C., Hampel, H., Frolich, L., Haag, A., Hemmeter, U., Paulsen, S., Teipel, S.J., Brettschneider, S., Spottke, A., Nolker, C., Moller, H.J., Wei, X., Farlow, M., Sommer, N., Oertel, W.H., 2004. Intravenous immunoglobulins containing antibodies against beta-

amyloid for the treatment of Alzheimer's disease. J. Neurol. Neurosurg. Psychiatry 75, 1472–1474.

Folstein, M.F., Folstein, S.E., McHugh, P.R., 1977. Cognitive defect in medical illness. Ann. Intern. Med. 86, 827–828.

Ghetie, V., Ward, E.S., 2000. Multiple roles for the major histocompatibility complex class I-related receptor FcRn. Ann. Rev. Immunol. 18, 739–766.

Gilman, S., Koller, M., Black, R.S., Jenkins, L., Griffith, S.G., Fox, N.C., Eisner, L., Kirby, L., Rovira, M.B., Forette, F., Orgogozo, J.M., AN1792(QS-21)-201 Study Team, 2005. Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. Neurology 64, 1553–1562.

Haurum, J.S., 2006. Recombinant polyclonal antibodies: the next generation of antibody therapeutics? Drug Discov. Today 11, 655–660.

Klitgaard, J.L., Coljee, V.W., Andersen, P.S., Rasmussen, L.K., Nielsen, L.S., Haurum, J.S., Bregenholt, S., 2006. Reduced susceptibility of recombinant polyclonal antibodies to inhibitory anti-variable domain antibody responses. J. Immunol. 177, 3782–3790.

Kotilinek, L.A., Bacskai, B., Westerman, M., Kawarabayashi, T., Younkin, L., Hyman, B.T., Younkin, S., Ashe, K.H., 2002. Reversible memory loss in a mouse transgenic model of Alzheimer's disease. J. Neurosci. 22, 6331–6335.

Kuroiwa, Y., Kasinathan, P., Choi, Y.J., Naeem, R., Tomizuka, K., Sullivan, E.J., Knott, J.G., Duteau, A., Goldsby, R.A., Osborne, B.A., Ishida, I., Robl, J.M., 2002. Cloned transchromosomic calves producing human immunoglobulin. Nat. Biotechnol. 20, 889–894.

Levites, Y., Smithson, L.A., Price, R.W., Dakin, R.S., Yuan, B., Sierks, M.R., Kim, J., McGowan, E., Reed, D.K., Rosenberry, T.L., Das, P., Golde, T.E., 2006. Insights into the mechanisms of action of anti-beta antibodies in Alzheimer's disease mouse models. FASEB J. 20, 2576–2578.

Morgan, D., Diamond, D.M., Gottschall, P.E., Ugen, K.E., Dickey, C., Hardy, J., Duff, K., Jantzen, P., DiCarlo, G., Wilcock, D., Connor, K., Hatcher, J., Hope, C., Gordon, M., Arendash, G.W., 2000. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. Nature 408, 982–985.

Negi, V.S., Elluru, S., Siberil, S., Graff-Dubois, S., Mouthon, L., Kazatchkine, M.D., Lacroix-Desmazes, S., Bayry, J., Kaveri, S.V., 2007. Intravenous immunoglobulin: an update on the clinical use and mechanisms of action. J. Clin. Immunol. 27, 233–245.

Nimmerjahn, F., Ravetch, J.V., 2007. The antiinflammatory activity of IgG: the intravenous IgG paradox. J. Exp. Med. 204, 11–15.

O'Nuallain, B., Hrncic, R., Wall, J.S., Weiss, D.T., Solomon, A., 2006. Diagnostic and therapeutic potential of amyloid-reactive IgG antibodies contained in human sera. J. Immunol. 176, 7071–7078.

Orgogozo, J.M., Gilman, S., Dartigues, J.F., Laurent, B., Puel, M., Kirby, L.C., Jouanny, P., Dubois, B., Eisner, L., Flitman, S., Michel, B.F., Boada, M., Frank, A., Hock, C., 2003. Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. Neurology 61, 46–54.

Relkin, N.R., Mujalli, D., Shennoy, S.A., Adamiak, B., Weksler, M.E., Kayed, R., Glabe, C., Szabo, P., 2007. IVIg contains antibodies against oligomers and fibrils of beta amyloid. Alzheimer's Dementia 3, S196.

Rodkey, L.S., 1995. Comparative properties of polyclonal and monoclonal antibodies. Methods Mol. Biol. 51, 139–150.

Rogers, J., Li, R., Mastroeni, D., Grover, A., Leonard, B., Ahern, G., Cao, P., Kolody, H., Vedders, L., Kolb, W.P., Sabbagh, M., 2006. Peripheral clearance of amyloid beta peptide by complement C3-dependent adherence to erythrocytes. Neurobiol. Aging 27, 1733–1739.

Schenk, D., Barbour, R., Dunn, W., Gordon, G., Grajeda, H., Guido, T., Hu, K., Huang, J., Johnson-Wood, K., Khan, K., Kholodenko, D., Lee, M., Liao, Z., Lieberburg, I., Motter, R., Mutter, L., Soriano, F., Shopp, G., Vasquez, N., Vandevert, C., Walker, S., Wogulis, M., Yednock, T., Games, D., Seubert, P., 1999. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 400, 173–177.

Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T.D., Hardy, J., Hutton, M., Kukull, W., Larson, E., Levy-Lahad, E., Viitanen, M., Peskind, E., Poorkaj, P., Schellenberg, G., Tanzi, R., Wasco, W., Lannfelt, L., Selkoe, D., Younkin, S., 1996. Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat. Med. 2, 864–870.

Wei, X., Zhao, Z., Ma, R., Dodel, R., Hampel, M., Farlow, M., Du, Y., 2006. Aβ-antibodies in serum and CSF as potential markers of biological activity in AD. In: Proceedings of the 10th International Conference on Alzheimer's Disease and Related Disorders, pp. 103–108.

Weksler, M.E., Relkin, N., Turkenich, R., LaRusse, S., Zhou, L., Szabo, P., 2002. Patients with Alzheimer disease have lower levels of serum anti-amyloid peptide antibodies than healthy elderly individuals. Exp. Gerontol. 37, 943–948.