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THE ROLE OF CHOROID PLEXUS IN IVIG-INDUCED BETA-AMYLOID CLEARANCE

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Abstract—We have shown that intravenous immunoglobulin (IVIG) contains anti-Aβ autoantibodies and IVIG could induce beta amyloid (Aβ) efflux from cerebrospinal fluid (CSF) to blood in both Multiple Sclerosis (MS) and Alzheimer disease (AD) patients. However, the molecular mechanism underlying IVIG-induced Aβ efflux remains unclear. In this study, we used amyloid precursor protein (AβPP) transgenic mice to investigate if the IVIG could induce efflux of Aβ from the brain and whether low-density lipoprotein receptor-related protein-1 (LRP1), a hypothetic Aβ transporter in blood–CSF barrier (BCB); could mediate this clearance process. We currently provide strong evidence to demonstrate that IVIG could reduce brain Aβ levels by pulling Aβ into the blood system in AβPP transgenic mice. In the mechanistic study, IVIG could induce Aβ efflux through the *in vitro* BCB membrane formed by cultured BCB epithelial cells. Both receptor-associated protein (RAP; a functional inhibitor of LRP1), and LRP1 siRNA were able to significantly inhibit the Aβ efflux. Should Aβ prove to be the underlying cause of AD, our results strongly suggest that IVIG could be beneficial in the therapy for AD by inducing efflux of Aβ from the brain through the LRP1 in the BCB.

Key words: choroid plexus, IVIG, LRP1, Aβ clearance.

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Abbreviations: Aβ, beta amyloid; AβPP, amyloid precursor protein; A2M, alpha-2-macroglobulin; AD, Alzheimer's disease; apoE, apolipoprotein E; BCB, blood–CSF barrier; CP, choroid plexus; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; IVIG, intravenous immunoglobulin; LRP, lipoprotein receptor-related protein; PBS, phosphate-buffered saline; RAP, receptor-associated protein; RT, room temperature; TEER, transepithelial electrical resistance.

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INTRODUCTION

Alzheimer's disease (AD) is the most common type of dementia in the elderly. Beta-amyloid (Aβ, a 39–42 amino acid proteolytic product of the amyloid precursor protein (AβPP)) accumulation in the brains extracellular space is believed to be one of several contributing factor to AD pathology (Ogomori et al., 1989). Increased levels of Aβ in the brains of AD patients may result from either the overproduction of Aβ or an inadequate metabolism/clearance within the brain. It may be a primary event that leads to amyloid plaque deposition and subsequently to the cascade of other neuropathological changes associated with the disease. Therefore, various therapeutic approaches are aimed to reduce the amount of Aβ-peptide including inhibition of β- and γ-secretase activity, inhibition of toxic Aβ fibrillation/aggregation, and enhancing Aβ clearance (Poduslo et al., 1999; Ghosh et al., 2002).

Recently, it has been shown that immune-mediated clearance pathways may have an important impact on plaque development in the brain (Schenk et al., 1999; Bard et al., 2000). It has been suggested that antibodies against Aβ could prevent amyloid deposition, ameliorate amyloid-mediated behavioral deterioration, and even clear existing plaques (Janus et al., 2000; Morgan et al., 2000). The Aβ peripheral sink hypothesis was also made based on the finding that Aβ can be transported out of brain when antibodies were mainly present in blood (DeMattos et al., 2002). As early as 1980, intravenous immunoglobulin (IVIG), an immune globulin product from human plasma, was used in the treatment of a variety of diseases (Fabian, 1980). A substantial amount of research has reported that there are abundant Aβ autoantibodies in IVIG and these autoantibodies or IVIG may be effective for the treatment of AD and other neurodegenerative disorders (Dodel et al., 2002, 2010; Neff et al., 2008; Relkin et al., 2009). Treatment with IVIG increased both CSF and serum levels of anti-Aβ antibodies and significantly lowered CSF levels of Aβ in AD patients, possibly by facilitating transport of Aβ from the CSF to the serum (Dodel et al., 2002). Most recently, clinical data demonstrated that IVIG treatments may slow down hypometabolic development in AD brains (Dodel et al., 2010). However, the mechanism underlying the IVIG-induced brain Aβ efflux/clearance remains to be determined.

The blood–brain barrier (BBB) and blood–cerebrospinal fluid (CSF) barrier (BCB) are two brain barriers that separate the systemic blood circulation from the brain. The BBB is mainly composed of tightly connected cerebral capillary endothelial cells and prevents substances from leaving the blood and crossing the capillary walls into the brain tissues. Unlike the capillaries that form the BBB, the choroid plexus (CP), located within brain ventricles, tight junctions between the choroidal epithelial cells that seal one epithelium to another, constituting the BCB (Smith, 1991) that was thought to regulate efflux of molecules from CSF into the blood (Brown et al., 2004). It has been reported that Aβ transports across the BBB into the brain from the systemic circulation via the receptor for advanced glycation end-products (RAGE), while out of the brain via the low-density lipoprotein receptor-related protein (LRP)-1 (Deane et al., 2004; Donahue et al., 2006). Additionally, several studies have also shown Aβ transports across the BCB (Sasaki et al., 1997; Monro et al., 2002; Serot et al., 2003; Crossgrove et al., 2005) and presents in the CP of AD patients (Kalaria et al., 1996). Since the CP is in direct continuity with the cerebral interstitial fluid (ISF) and the CSF, Aβ in the brains extracellular space can freely enter into the CSF. Immunoreactive Aβ and its precursor protein AβPP in the CP (Sasaki et al., 1997; Crossgrove et al., 2007) suggests that the CP may be involved in the brains Aβ clearance (Crossgrove et al., 2005; Behl et al., 2009a; Gu et al., 2011). Thus, it is interesting to explore the role of the BCB in the brain Aβ clearance and IVIG-induced efflux of Aβ from brains.

Low-density LRP1 is a member of the LDL receptor family and has various unrelated functions; it participates in cholesterol and lipid transport, and plays a role in the clearance of extracellular proteases and proteinase complexes of apoptotic cells and debris. Aβ binds to alpha-2-macroglobulin (A2M) and apolipoprotein E (apoE). LRP1 serves as a receptor for these two proteins, and LRP1-mediated clearance of A2M and apoE contributes to a reduction in Aβ levels (Herz and Strickland, 2001). LRP1 may play a role in the BBB to transfer Aβ from the brain to the blood (Kounnas et al., 1995; Knauer et al., 1996; Goto and Tanzi, 2002; Moir and Tanzi, 2005; Yamada et al., 2009). Recently, LRP1 was identified in the CP. Reduced LRP1 expression in the CP following lead (Pb) treatments may be associated with Pb-induced accumulation of Aβ in the brain (Behl et al., 2009a; Gu et al., 2011). It was suggested that Aβ elimination from CSF was possibly mediated by LRP1 expressed at the CP (Fujiyoshi et al., 2011). These evidences suggest that LRP1 may be involved in the IVIG-induced Aβ clearance in the peripheral clearance system.

While much work has been done in immunization treatments for Alzheimer's disease and a peripheral sink hypothesis has been proposed extensively, the molecular mechanism underlying antibody-induced efflux of Aβ from brain remains unclear. To address these questions, we used AβPP transgenic mice and an *in vitro* BCB system to investigate whether IVIG could induce efflux of Aβ from the brain through the BCB and if LRP1 mediated this process.

EXPERIMENTAL PROCEDURES

Animals and treatments

An AβPP transgenic mouse model overexpressing human AβPP with a mutation (V717F) that causes an autosomal dominant form of familial AD was utilized (Gu et al., 2011). In this study, 3-month-old mice showed high levels of human Aβ protein in brains without having plaques, which was consistent to previous reports demonstrating these mice had an age-related accumulation of Aβ plaques in brains starting from 6 months of age (Bales et al., 1999). AβPP transgenic mice (AβPP V717F) (on a C57BL/6 genetic background) were bred in the Animal Center at the Indiana University School of Medicine. Mice were housed three to five mice per cage, fed with food and water *ad libitum*, and maintained in a 12-h light/dark cycle facility. A group of 3-month-old AβPP transgenic mice ($n = 6$ per group) were i.v. injected in the lateral tail vein with 500 μg (20 mg/kg) IVIG or IVIG in which anti-Aβ antibodies had been depleted (IVIG w/o anti-Aβ) once a week for 2 weeks. Hippocampus, cortex, CSF and plasma samples were collected after 2 weeks treatment. Plasma was collected immediately after CSF was collected. Fresh samples were immediately tested afterward. Animal protocols pertinent to this study were approved by the Indiana University School of Medicine Laboratory Animal Resource Center.

Depletion of anti-Aβ antibody from IVIG

The protocol was adapted from a previously described method (Du et al., 2001, 2003). The column was packed with NHS-Sepharose 4B (Pharmacia Biotech, Piscataway, NJ, USA) labeled with $A\beta_{1-40}$ (generous gifts from Eli Lilly, Indianapolis, IN, USA 0.6 mg/ml drained Sepharose) and was equilibrated and washed with phosphate-buffered saline (PBS) (pH 7.4). After passing purified human IVIG (Bayer, Pittsburgh, PA, USA) through the column, unbound fraction (IVIG w/o anti-Aβ) was collected.

Culture of choroidal epithelial Z310 cells

The immortalized Z310 rat choroidal epithelial cell line was created by Dr. Zheng (Zheng and Zhao, 2002). Cells were cultured as previously described (Zheng and Zhao, 2002; Shi et al., 2008b). In brief, the cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 10 ng/mL epidermal growth factor (EGF), 100 U/mL of penicillin, 100 mg/mL of streptomycin and 40 mg/mL of gentamycin in a humidified incubator with 95% air and 5% CO_2 at 37 °C and passaged twice a week.

Inhibition of LRP1

Z310 cells were pre-incubated with 100 nM recombinant receptor-associated protein (RAP, EMD Millipore, Billerica, MA, USA) for 1 h before the Aβ was added into the inner chamber. The LRP1 siRNA was obtained commercially from Sigma–Aldrich. The sequences of sense strand: Th-5′-CCUAUCUUUGAGAUCCGAA-3′; antisense strand: Th-5′-UUCGGAUCUCAAAGAUAGG-3′ (Behl et al., 2009a). Cells were seeded at a density of 1×10^5 cells/well in a 6-well plate in cell culture medium. After 24 h, the RNA/transfection system was prepared as described in manufacturer's protocol: 3 μL 10 μM siRNA was diluted in 500 μL opti-MEM I (Invitrogen, Grand

Island, NY, USA). 5 μL lipofectamine RNAiMAX (Invitrogen, Grand Island, NY) was added into the diluted siRNA and incubated for 20 min at room temperature (RT). Cell culture medium was replaced with 500 μL of the above mixture along with 2.5 mL of cell culture medium to obtain a total of 3 mL medium/well. The final concentration of siRNA was 10 nM. The solution containing siRNA was mixed gently by rocking the plate back and forth. Cells were transfected with scrambled siRNA as a negative control. Cells were grown for an additional 24 h and then cultured in the transwell transport device. After 2 days cell culture, Aβ clearance experiment was performed. The knockdown efficiency was then analyzed by Western blot analysis.

Determination of LRP1 and RAP Levels by Western Blot

Mouse CP and Z310 cells were homogenized (1:10, wt/vol) on ice in RIPA buffer (Sigma–Aldrich, St Louis, MO, USA) with protease inhibitor cocktail (Roche, Indianapolis, IN, USA). The protein concentration was determined by using the Bradford method. The protein extract (10 μg of protein) was loaded onto a 4–12% Bis–Tris gel, electrophoresed, and then transferred to a nitrocellulose membrane. The blots were probed with antibody directly against LRP1 (1:20,000, abcam, San Francisco, CA, USA) or RAP (1:1000, abcam, San Francisco, CA), followed by a secondary antibody conjugated with horse-radish peroxidase (1:5000) and visualized by utilizing enhanced chemiluminescence. β-Actin was also assayed as loading controls by using its antibody (1:1000, cell signaling, Danvers, MA, USA). Band intensities were quantified and results were reported as a ratio of LRP1 to β-actin (Gu et al., 2011).

Cultures in Transwell transport device

The Transwell transport device is composed of two chambers which are separated by a porous polyester membrane insert (Costar, Corning, NY, USA). Z310 cells were seeded on this membrane. The compartment in the insert is defined as the inner chamber. The lower compartment is defined as the outer chamber. The cells possess tight barrier characteristics when cells are cultured on a permeable membrane (Shi and Zheng, 2005). Aβ was added into the inner chamber and measured in the outer chamber. Prior to cell seeding on the porous polyester membrane within the inner chamber, the membrane was pre-coated with 0.01% collagen (Sigma, St Louis, MO, USA) for 4–5 h at RT. 200 μL aliquots of cell suspensions containing 5.0×10^4 cells were added to the inner chamber followed by addition of 500 μL of the same culture medium into the outer chamber. A cell monolayer was usually formed 3–5 days after seeding. The media was refreshed every 2 days after seeding. The formation of the cell monolayer was judged by three criteria: (1) the cells formed a confluent monolayer without visible spaces between cells under a light microscope; (2) the height of the culture medium in the inner chamber had to be at least 2 mm higher than that in the outer chamber for at least 24 h; and (3) a constant transepithelial electrical resistance (TEER) value across the cell layer was obtained ($61.3 \pm 2.62\ \Omega\ cm^2$) (Shi and Zheng, 2005). The TEER is a combination of the paracellular and transcellular flux of small inorganic ions (predominantly Na^+ and Cl^-) across the monolayer. The latter contribution is commonly negligible due to the high lipid membrane resistance, but especially for monolayers with high paracellular tightness it may contribute to the measured TEER value (Fanning et al., 1999). The TEER value was measured using an epithelial volt-ohmmeter (EVOM, World Precision Instruments, Sarasota, FL, USA) after culturing in Transwell chambers for at least 2 days. The net value was calculated by subtracting the background value, which was measured on a collagen-coated, cell-free chamber (blank), from the value of cell-seeded chamber. The cultures that reached confluence were used in the transport studies. 1 μM $A\beta_{1\text{-}40}$ was added into the inner chamber and the outer chamber media were collected at 1 and 3 h following 10 mg/ml IVIG and/or 100 nM RAP (EMD Millipore, Billerica, MA) or LRP1 siRNA treatments.

Quantification of Aβ and Aβ antibody by enzyme-linked immunosorbent assay (ELISA)

Levels of Aβ and Aβ antibody were assayed by sandwich ELISA as described previously (Gu et al., 2012) (Hyslop and Bender, 2002). Hippocampus and cortex from mouse brain were extracted with 5 M guanidine buffer. Tissues are homogenized at RT and rotated overnight at 4 °C. Samples were then diluted 1:10 with sample diluted buffer (1% BSA with 0.05% Tween-20). Plasma, CSF samples, and culture media collected from the outer chamber were diluted 1:2 with diluted buffer. Mice samples, media collected from outer chamber, and the immunodepleted IVIG were processed in 96-well ELISA plates that had been coated with antibody 266.2 (generous gifts from Eli Lilly, Indianapolis, IN) to determine Aβ and $A\beta_{1\text{-}40}$ to determine Aβ antibody. After incubation of plates with casein buffer (0.25% casein and 0.05% sodium azide in PBS) for 2 h, samples were loaded overnight at 4 °C. Biotin-3D6 (generous gifts from Eli Lilly, Indianapolis, IN) or biotin-anti-human IgG (Fc specific) (Sigma, St Louis, Mo) was incubated for 1 h at RT, followed by horseradish peroxidase for 1 h. After incubation with tetramethylbenzidine (TMB, Sigma, St Louis, MO) the plates were read for absorbance at 450 nm.

Statistical analysis

Statistical analyses of the differences between groups were carried out by a one-way analysis of variance (ANOVA) with post hoc comparisons by the Dunnett's test. All data are expressed as mean ± SD. Differences between two means were considered significant when p was equal or less than 0.05.

RESULTS

IVIG reduced brain levels but increased blood levels of $A\beta_{total}$ in AβPP transgenic mice

To verify IVIG effect on $A\beta_{total}$ levels in AβPP transgenic mice, we treated a group of 3-month-old AβPP transgenic mice ($n = 6$ per group) by i.v. administration with IVIG or IVIG w/o anti-Aβ for 2 weeks. ELISA analyses showed that CSF concentrations of $A\beta_{total}$ in IVIG-treated mice after 2-week treatments were significantly lower than mice treated with saline (0.16 ± 0.12 ng/ml versus 0.35 ± 0.14 ng/ml, respectively; $p < 0.05$) (Fig. 1A). Additionally, 2-week-treatments of IVIG also significantly reduced $A\beta_{total}$ levels in the hippocampus (0.03 ± 0.01 ng/mg versus 0.06 ± 0.01 ng/mg, $p < 0.001$) and cortex (0.02 ± 0.01 ng/mg versus 0.04 ± 0.01 ng/mg, $p < 0.05$), respectively, as compared to saline-treated mice (Fig. 1C, D). In contrast, plasma levels of $A\beta_{total}$ in 1-week-treated IVIG-treated mice were significantly increased as compared to saline-treated mice (0.15 ± 0.06 ng/ml versus 0.06 ± 0.03 ng/ml, $p < 0.05$) (Fig. 1B). As a control, treatments with IVIG w/o anti-Aβ had little effect on both CSF and plasma levels of $A\beta_{total}$ in transgenic mice as compared to control (Fig. 1). We detected human anti-Aβ antibodies in mice after IVIG treatment. The CSF and plasma levels of human anti-Aβ antibodies were 2.5 ± 3.3 pg/ml and 289.3 ± 41.5 ng/ml. Human anti-Aβ antibodies were not detected in IVIG w/o anti-Aβ-treated mice.

CP Levels of LRP1 and RAP in IVIG treated mice

IVIG reduced $A\beta_{total}$ levels in APP transgenic mice brains may result from IVIG affected LRP1 and/or RAP levels in the CP. LRP1 and RAP in the CP were determined by Western blot. Our results demonstrated that there is no difference between CP levels of LRP1 and RAP in IVIG-treated mice with control mice (Fig. 2).

TEER values of Z310 cells in the *in vitro* BCB model

To specifically examine the role of the CP in the regulation of Aβ efflux from the brain following treatments of IVIG, we used a well-established *in vitro* brain barrier epithelial transport system that had been widely used for BCB studies (Zheng and Zhao, 2002; Shi and Zheng, 2005; Wang et al., 2008; Shi et al., 2008b). Z310 cells were grown in the transwell chamber culture system to form a confluent membrane that possessed tight barrier characteristics as CP (Shi and Zheng, 2005). The tightness of the barrier between two chambers were measured by the TEER after a 2-day culture in Transwell chambers and TEER could reach 63.9 ± 0.85 $\Omega\,cm^2$ at day 3 and day 4 in this study (Fig. 3A), was comparable to the 61.3 ± 2.62 $\Omega\,cm^2$ reported in the previous report

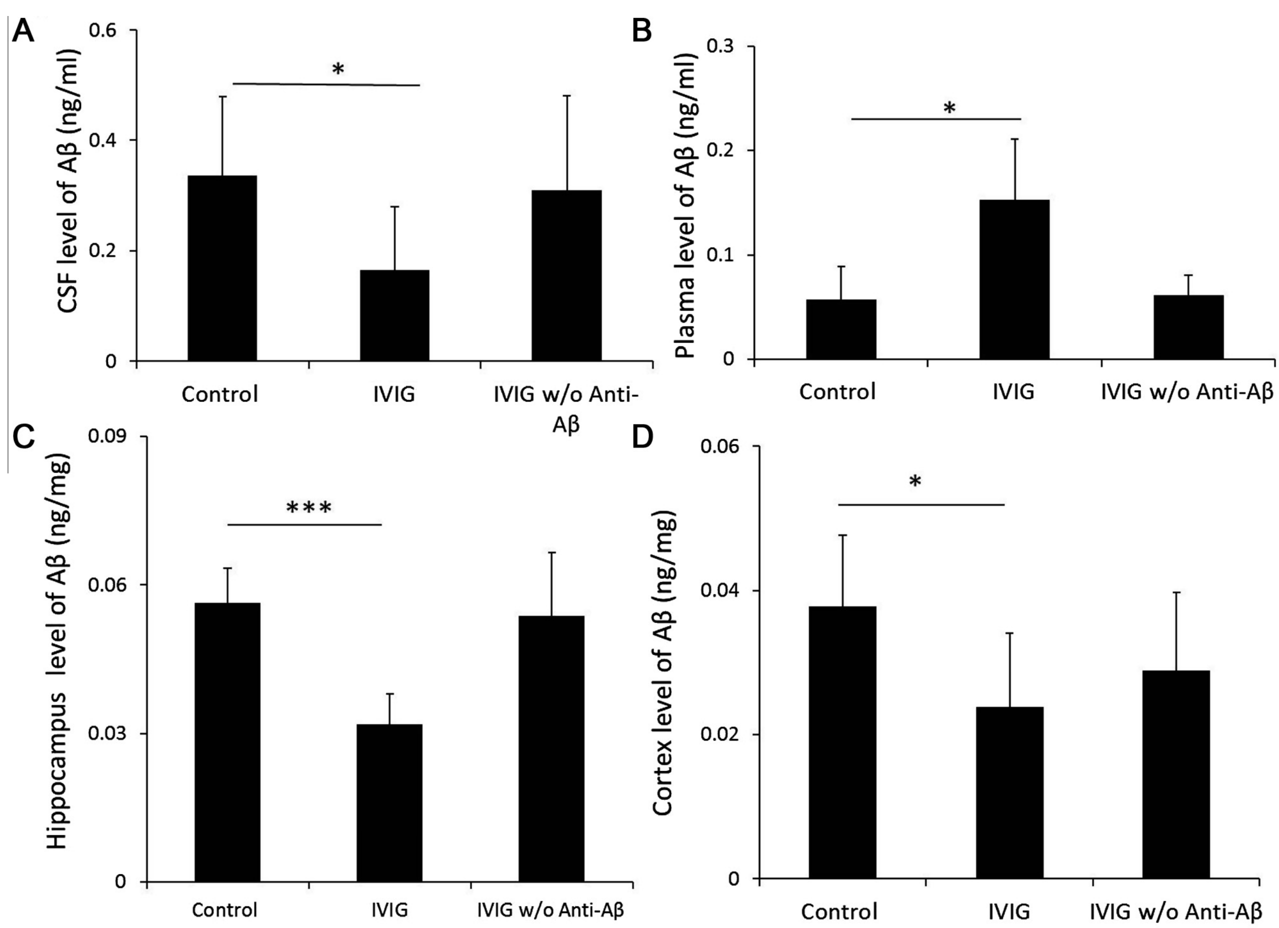

Fig. 1. IVIG treatment decreased brain levels but increased plasma level of $A\beta_{total}$. Three-month-old AβPP transgenic mice received i.v. administration of 0.5 mg/kg as IVIG or IVIG w/o anti-Aβ weekly for 2 weeks. CSF (A), plasma (B), hippocampus (C), and cortex (D), levels of $A\beta_{total}$ in control, IVIG-treated, and IVIG that anti-Aβ autoantibodies has been removed (IVIG w/o anti- Aβ) groups were determined by ELISA. Data represent mean ± SD, $n = 6$. $^{*}p < 0.05$, $^{***}p < 0.001$.

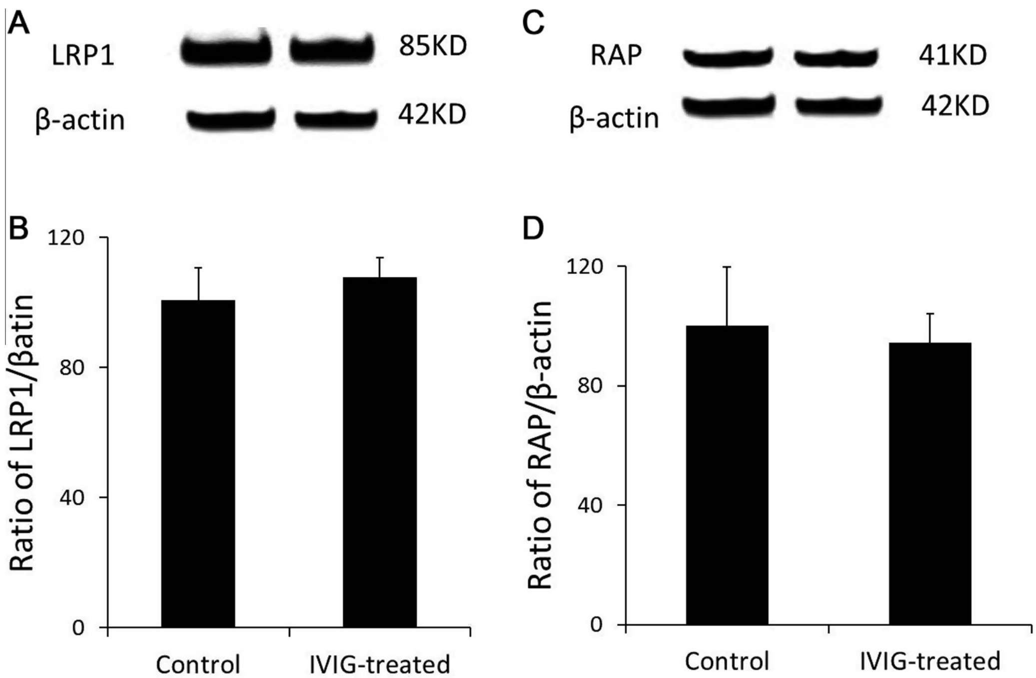

Fig. 2. IVIG treatment did not affect CP levels of LRP1 and RAP. Three-month-old AβPP transgenic mice received i.v. administration of 0.5 mg/kg as IVIG or IVIG w/o anti-Aβ. After 24 h, mouse CPs were collected and CP levels of LRP1 and RAP were measured by Western blot analysis. Data represent mean ± SD, $n = 3$.

(Shi and Zheng, 2005). To test whether IVIG and Aβ affect the rigidity of the cell monolayer, TEER was measured before and after IVIG and Aβ treatments. There was little change between treated and untreated samples (Fig. 3B).

IVIG induced Aβ efflux from inner chamber

1 μM $A\beta_{1-40}$ was added into the inner chamber and the outer chamber media were collected at 1 and 3 h following treatments. This dose regimen was shown to produce a significant accumulation of Aβ in Z310 cells in our previous studies (Behl et al., 2009a,b) and *in vitro* toxicity studies (Shabala et al., 2010). Aβ concentrations in outer chamber were gradually increased from 0 into 0.28 ± 0.06 ng/ml at 1 h and 4.2 ± 0.96 ng/ml at 3 h following treatments. Furthermore, addition of IVIG into the outer chamber markedly increased amounts of Aβ efflux from inner chambers from 0.28 ± 0.06 ng/ml to 2.52 ± 0.22 ng/ml at 1 h ($p < 0.001$) and 4.2 ± 0.96 ng/ml

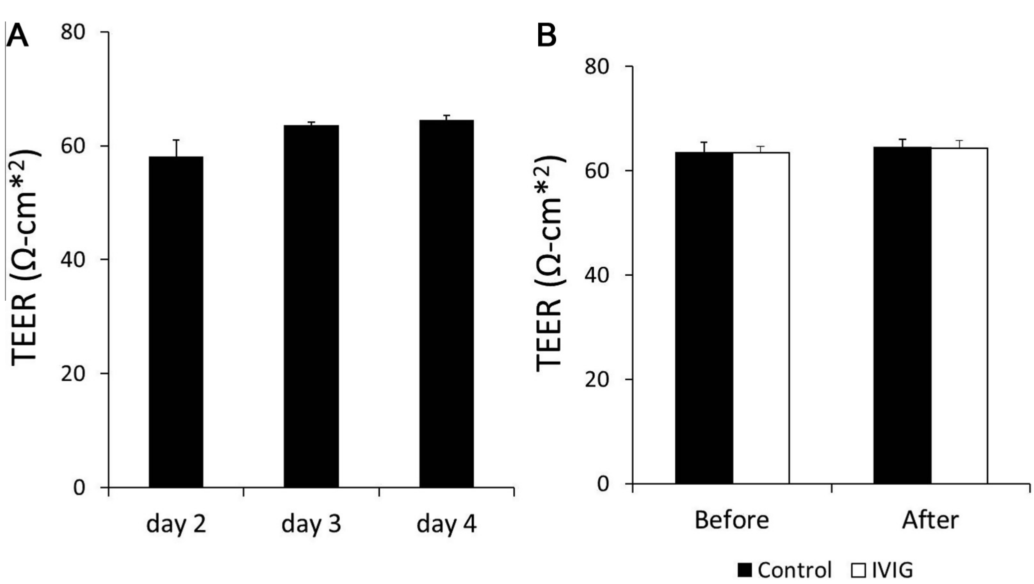

Fig. 3. Transepithelial electrical resistance (TEER) values of Z310 cells in the *in vitro* BCB model. TEER values across the transwell chamber culture system were determined from day 2 to day 4. TEER values reached 63.9 ± 0.85 Ω cm^2 on day 3 (A). Before and after a 3-h incubation with Aβ, TEER values were measured and no difference detected (B). Data represent mean ± SD of three independent experiments, $n = 3$.

to 7.61 ± 0.91 ng/ml at 3 h ($p < 0.001$) of treatments (Fig. 4A).

Low density LRP (RAP) partially inhibited efflux of Aβ from inner chamber

We next examined whether LRP1 was involved in Aβ efflux with or without IVIG induction. Hundred nanomolar RAP, an inhibitor of LRP1 or saline, was added into the inner chamber for 1 h followed by the addition of 1 μM Aβ. Without IVIG treatments, Aβ concentrations in the outer chamber were dramatically reduced by RAP from 0.28 ± 0.06 to 0.02 ± 0.01 ng/ml 1 h after Aβ treatments and 4.2 ± 0.96 to 1.12 ± 0.51 ng/ml 3 h after Aβ treatments ($p < 0.001$). Furthermore, RAP treatments also significantly blocked IVIG-induced Aβ efflux from inner chambers 1 h (from 2.52 ± 0.22 to 1.77 ± 0.1 ng/ml, $p < 0.001$) and 3 h (from 7.61 ± 0.91 to 5.41 ± 0.92 ng/ml, $p < 0.001$) (Fig. 4B).

LRP1 knockdown by siRNA partially inhibited efflux of Aβ from inner chamber

To test whether Aβ efflux following IVIG exposure was indeed mediated by LRP1 in the BCB, we assessed Aβ efflux *in vitro* BCB model following LRP1 knockdown by siRNA. The knockdown efficiency was analyzed by Western blot analysis. As shown in data presented in Fig. 5, introducing LRP1 siRNA to the cells caused a significant reduction of LRP1 at protein (84.7%) expression levels as compared to the scrambled siRNA controls (Fig. 5A, B). Following LRP1 knockdown by siRNA, the Z310 cells were then divided into four groups (Fig. 5C): scrambled siRNA control without IVIG treatment, LRP1 knockdown control without IVIG treatment, scrambled siRNA control with IVIG treatment, and LRP1 knockdown cells with IVIG exposure. One micromolar Aβ was added into the inner chamber. Aβ concentrations in outer chamber were quantified as before using ELISA. Without IVIG treatments, Aβ concentrations in outer chamber were reduced by LRP1 knockdown from 0.28 ± 0.08 to 0.02 ± 0.004 ng/ml 1 h after Aβ added and 4.0 ± 0.31 to 1.17 ± 0.38 ng/ml 3 h after Aβ added ($p < 0.05$). Furthermore, after LRP1 was knocked down and followed by IVIG treatment, IVIG-induced Aβ efflux from inner chambers were reduced from 2.39 ± 0.51 to 1.1 ± 0.28 ng/ml after 1 h Aβ added ($p < 0.001$) and 7.26 ± 1.14 to 2.86 ± 0.86 ng/ml after 3 h Aβ added ($p < 0.001$) (Fig. 5C). These results support the hypothesis that increased efflux of Aβ may be mediated at least in part by IVIG effect on LRP1.

DISCUSSION

IVIG has been clinically used to treat a variety of diseases (Fabian, 1980) and currently, at least two clinical trials are performed to examine whether IVIG could be used to treat AD. (Dodel et al., 2002, 2010; Neff et al., 2008; Relkin et al., 2009). We have identified anti-Aβ autoantibodies (IgG) in human serum and CSF that may act in an immune-mediated Aβ clearance pathway (Du et al., 2001; Dodel et al., 2002). Our early report demonstrated that treatments with IVIG dramatically increased serum levels of Aβ and significantly lowered CSF levels of Aβ in AD and Multiple Sclerosis (MS) patients, possibly by facilitating the transport of Aβ from the CSF to the serum by IVIG contained anti-Aβ autoantibodies (Dodel et al., 2002). However, the molecular mechanism underlying IVIG-induced efflux of Aβ from brain remains unknown. The studies presented in this report took the advantage of the AβPP transgenic mice, which specifically

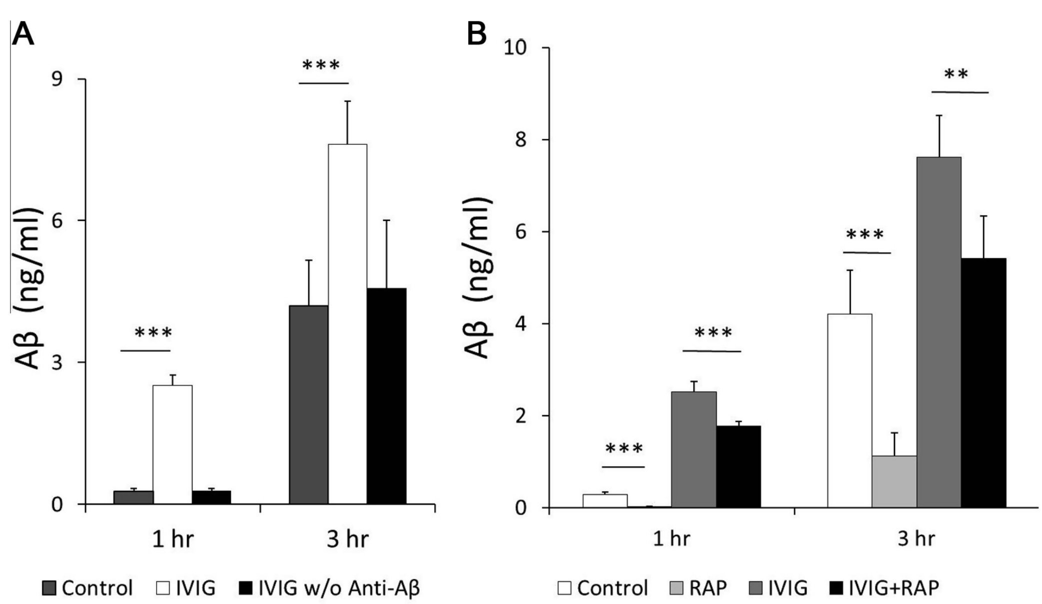

Fig. 4. Media in outer chambers were added with or without 10 mg/ml IVIG or IVIG w/o anti- Aβ for 1 to 3 h after addition of 1uM Aβ into inner chambers. IVIG increases the Aβ efflux from inner chambers (A). Treatment of inner chambers with RAP (the LRP1 inhibitor, 100 nM) significantly inhibited Aβ efflux and IVIG-stimulated Aβ efflux through the formed confluent layer of Z310 cells (B). Aβ in outer chamber media was quantified by ELISA method. Data represent mean ± SD of three independent experiments, $n = 5$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

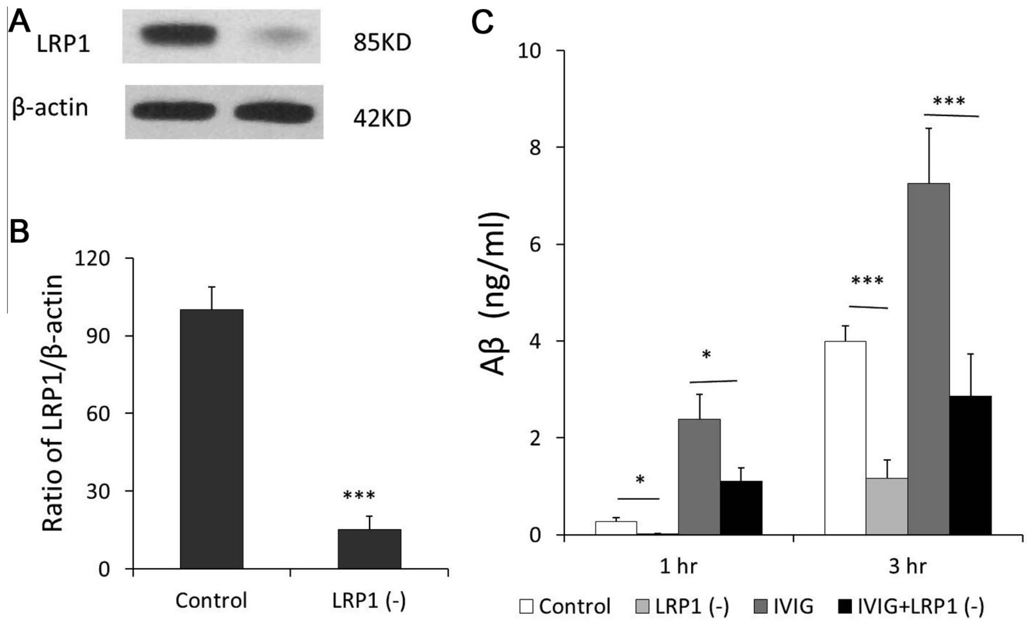

Fig. 5. LRP1 knocked down in Z310 cells by using siRNA. The knockdown efficiency was analyzed by Western blot analysis. Introducing LRP1 siRNA to the cells caused a significant reduction of LRP1 at protein (84.7%) expression levels as compared to the scrambled siRNA controls (A, B). Media in outer chambers were added with or without 10 mg/ml IVIG for 1–3 h after addition of 1 μM Aβ into inner chambers. LRP1 knockdown by using siRNA decreased Aβ efflux and IVIG-stimulated Aβ efflux through the formed confluent layer of Z310 cells compared with scrambled siRNA controls (C). Aβ in outer chamber media was quantified by the ELISA method. Control: scrambled siRNA without IVIG treatment, LRP1 (−): LRP1 knockdown without IVIG treatment, IVIG: scrambled siRNA with IVIG treatment, IVIG + LRP1 (−): LRP1 knockdown cells with IVIG exposure. Data represent mean ± SD of three independent experiments, $n = 3$. $^{*}p < 0.05$, $^{***}p < 0.001$.

overexpress Aβ in the brain. Similar to our human studies, mouse data clearly show that after 1-week treatment with IVIG containing Aβ autoantibodies, the plasma level of $A\beta_{total}$ was dramatically higher in IVIG-treated mice than the saline-treated and IVIG w/o anti-Aβ-treated mice. In contrast, 2 weeks of IVIG treatments significantly decreased brain and CSF levels of $A\beta_{total}$. The activities of anti-Aβ in the IVIG and IVIG without anti-Aβ antibodies were detected by ELISA (Dodel et al., 2002; Du et al., 2003). Naturally occurring antibodies directed against amyloid were detected in IVIG. There was a strong signal in IVIG, however, no signal detected in the IVIG without anti-Aβ antibodies. There was little difference in the levels of Aβ between the IVIG w/o anti-Aβ group and saline-treated group, suggesting that Aβ autoantibodies in IVIG may play a key role in inducing efflux of Aβ from the brain into the blood.

Since the BCB is a major mechanism mediating efflux of molecules from CSF to the blood, we used an established *in vitro* BCB model (Zheng and Zhao, 2002; Shi and Zheng, 2005; Shi et al., 2008a) to investigate if and how the BCB was involved in IVIG-induced Aβ mobilization from the brain. Additionally, as LRP1 was reported to possibly contribute to the regulation of Aβ mobilization in the BCB, we used RAP, a specific LRP1 receptor antagonist, to further confirm the role of the BCB in Aβ efflux. It was reported that RAP could bind Aβ at above 500 nM (Kanekiyo and Bu, 2009), therefore in this study, we used 100 nM at which RAP was demonstrated to show little binding affinity to Aβ and still could significantly block LRP1 function (Shakibaei and Frevert, 1996; Hayashi et al., 2009). As expected, RAP significantly blocked both basal and IVIG-induced Aβ efflux from inner to outer chambers. For further confirmation, we performed LRP1 knockdown experiments by using LRP1 siRNA. The results revealed that inhibition of LRP1 expression significantly reduced basal and IVIG-induced Aβ efflux. These results suggest that LRP1 should play an important role in regulating Aβ mobilization in this *in vitro* BCB model. Results reveal that IVIG did induce Aβ efflux through the LRP1 receptor in BCB cells. Our data suggest that the CP played an important role in removing Aβ from the brain during IVIG treatments and LRP1 in the BCB was specifically involved at least partially, if not completely, in this process. Interestingly enough, two AD risk factors, apoE and A2M, are agonists for LRP1 receptor and they may affect IVIG- and other anti-Aβ antibody-involved therapy.

In our previous study, we found that Aβ autoantibodies in IVIG were mainly present in the blood after i.v. injection (Bacher et al., 2009). Similar to studies using Aβ-antibody 266 (DeMattos et al., 2002), Aβ autoantibodies in IVIG induced Aβ efflux across the CP from brains into blood also possibly by the sink mechanism. Additionally, as suggested by Yamada et al. (2009) who reported that only soluble-free Aβ, not the 266 antibody complexed with Aβ might move through the LRP1 in the CP, Aβ complexed with IVIG in peripheral also prevented transport of Aβ back into the brain that could further enhance Aβ efflux. Furthermore, we found that CP levels of both LRP1 and RAP were not affected by *in vivo* IVIG treatments indicating that binding between IVIG and Aβ in peripheral might be the major force to induce Aβ efflux out of brains.

It should be noted that blockade of LRP1 only partially inhibited Aβ efflux across the BCB indicating other transport mechanisms may also be involved in IVIG-induced Aβ efflux. P-glycoprotein (P-gp), an ATP-driven efflux transporter in the CP, was reported to be involved in Aβ clearance from the brain (Hartz et al., 2010; Pascale et al., 2011). Additionally, there are other receptors that help transport Aβ from the CSF into the CP epithelium, such as the RAGE and the low density LRP-2 (Pascale et al., 2011). Both receptors were localized in the CP and linked to Aβ transport from CSF into CP cells (Maslinska et al., 2011; Pascale et al., 2011) (Hammad et al., 1997). Therefore, further studies regarding the roles of other transporters/receptors in IVIG-induced Aβ efflux are necessary.

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