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Human Anti-prion Antibodies Block Prion Peptide Fibril Formation and Neurotoxicity

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Background: AD and prion diseases both involve conformational changes and deposition of insoluble proteins; similar to anti-Aβ autoantibodies, anti-PrP autoantibodies may be present in healthy individuals.
Results: PrP autoantibodies (PrP-AA) purified from human IgG could significantly block PrP-(106–126) peptide fibril formation and PrP-induced neuronal death.
Conclusion: Human prion autoantibodies reduce prion peptide aggregation and associated neurotoxicity.
Significance: Purified PrP-AA may be a potential treatment for prion diseases.

Prion diseases are a group of rare, fatal neurodegenerative disorders associated with a conformational transformation of the cellular prion protein (PrP^C) into a self-replicating and proteinase K-resistant conformer, termed scrapie PrP (PrP^{Sc}). Aggregates of PrP^{Sc} deposited around neurons lead to neuropathological alterations. Currently, there is no effective treatment for these fatal illnesses; thus, the development of an effective therapy is a priority. PrP peptide-based ELISA assay methods were developed for detection and immunoaffinity chromatography capture was developed for purification of naturally occurring PrP peptide autoantibodies present in human CSF, individual donor serum, and commercial preparations of pooled intravenous immunoglobulin (IVIg). The ratio of anti-PrP autoantibodies (PrP-AA) to total IgG was ~1:1200. The binding epitope of purified PrP-AA was mapped to an N-terminal region comprising the PrP amino acid sequence KTNMK. Purified PrP-AA potently blocked fibril formation by a toxic 21-amino acid fragment of the PrP peptide containing the amino acid alanine to valine substitution corresponding to position 117 of the full-length peptide (A117V). Furthermore, PrP-AA attenuated the neurotoxicity of PrP(A117V) and wild-type peptides in rat cerebellar granule neuron (CGN) cultures. In contrast, IgG preparations depleted of PrP-AA had little effect on PrP fibril formation or PrP neurotoxicity. The specificity of PrP-AA was demonstrated by immunoprecipitating PrP protein in brain tissues of transgenic mice expressing the human PrP(A117V) epitope and Sc237 hamster. Based on these intriguing findings, it is suggested that human PrP-AA may be useful for interfering with the pathogenic effects of pathogenic prion proteins and, thereby has the potential to be an effective means for preventing or attenuating human prion disease progression.

Prion diseases, or transmissible spongiform encephalopathies (TSEs),[2] are rapidly progressive neurodegenerative disorders with untreatable invariably fatal outcomes. Disease caused by altered forms of prion protein (PrP) include scrapie in sheep, bovine spongiform encephalopathy in cattle, as well as the human forms Kuru, Creutzfeldt-Jakob disease (CJD and vCJD), and the Gerstmann-Straussler-Scheinker (GSS) syndrome (1). These diseases are most likely caused by misfolding and aggregation of the normal host protein (PrP^C) into a highly insoluble form PrP^{Sc}. In this process, a portion of the α-helix and random coil structure of PrP^C, which is ubiquitously expressed in neurons and leukocytes, adopts the PrP^{Sc} β-pleated conformation, rendering the protein poorly soluble in water and resistant to protease digestion (1). Autopsy on the brains of prion disease patients has identified amyloid plaques comprised of insoluble PrP^{Sc} aggregates deposited around neurons in affected brain regions, which is thought to induce neuronal dysfunction and death, thus producing the clinical symptoms of infection (1–7). The primacy of a single protein causing disease across species by diverse mechanisms is unique in biology.

To date, there are no therapeutic treatments available for prion diseases. However, recent studies in cultured cells and mice indicate that immunotherapeutic strategies employing antibodies against the cellular form of PrP^C can antagonize prion infectivity and disease development. Monoclonal antibodies (mAbs) or recombinant F(ab) fragments recognizing PrP effectively prevented prion infection of susceptible mouse neuroblastoma cells and abrogated *de novo* PrP^{Sc} formation in chronically infected cells (8–9). In addition, passive transfer of a PrP mAb into scrapie-infected mice suppressed peripheral prion replication and infectivity, and significantly delayed onset of the disease (10–12). Notably, no obvious adverse effects were observed in these studies. These findings suggest that immuno-

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[2] The abbreviations used are: TSE, transmissible spongiform encephalopathies; PrP, prion protein; CJD, Creutzfeldt-Jakob disease; AD, Alzheimer disease; IVIg, intravenous immunoglobulin; PrP-AA, anti-PrP autoantibodies; CGN, cerebellar granule neuron; Aβ, beta amyloid.

therapeutic strategies for human prion diseases are worth pursuing.

Recently, we and others (13–14) have suggested that an impaired or reduced ability to generate antibodies specific for beta amyloid (Aβ) peptides may be one mechanism contributing to Alzheimer disease (AD) pathogenesis. Intravenous immunoglobulin (IVIg) preparations containing natural levels of anti-Aβ antibodies or purified autoantibodies against Aβ have shown beneficial effects in trials with AD patients (13, 15–17). We have demonstrated that these autoantibodies prevent or disaggregate Aβ fibril formation and block their toxic effects in primary neurons (18).

Since the pathogenic mechanisms of AD and prion diseases both involve toxic conformational changes and deposition of insoluble protein aggregates (1, 19–23) and given the early successes with natural Aβ autoantibodies for treatment of AD, we hypothesized that anti-PrP autoantibodies (PrP-AA) may also be present in blood products derived from healthy individuals. The potential for efficacy of PrP-AA is also based on results demonstrating the ability of mouse mAbs to prevent fibril formation, disaggregate already formed fibrils, and inhibit the neurotoxic effect of PrP^{Sc} (24). A benefit of purified human PrP-AA over humanized mouse mAbs is a reduced potential for neutralizing host responses to residual mouse sequences in the chimeric antibody.

A peptide fragment spanning human PrP sequences 106–126 ($PrP_{106-126}$) possesses several chemicophysical characteristics of PrP^{Sc}, including the propensity to form β-sheet-rich, insoluble, and protease-resistant fibrils similar to those found in prion-diseased brains (25–26). This peptide has been widely used in an *in vitro* model to study PrP^{Sc}-induced neurotoxicity (27–32). A mutation in the prion protein gene (PRNP) leading to a substitution of valine for alanine at peptide position 117 (A117V) is associated with GSS syndrome, an inherited prion disease (33–35) that is characterized by multi-centric amyloid plaques in the cerebellum and cortex (36). The A117V mutation lies within the $PrP_{106-126}$ region. The finding that a modification of $PrP_{106-126}$(A117V) alters the toxic mechanism *in vitro* suggests that there may be heterogeneity in the mechanism of neurotoxicity of PrP^{Sc}. The mechanism underlying the neurotoxic effects of $PrP_{106-126}$(A117V) includes at least two components: The first is similar to that of PrP^{Sc}, which requires the presence of microglia and neuronal PrP^{C} expression; while the second is independent of neuronal PrP^{C} expression or presence of microglia (36).

In this study, we have found evidence that PrP-AA are present in human CSF and serum. These autoantibodies could be successfully purified from IVIg by using affinity chromatography columns conjugated with $PrP_{106-126}$(A117V) peptide. Additionally, we identified a five amino acid binding epitope for PrP-AA. Furthermore, we demonstrated that purified PrP-AA effectively protects cultured cerebral granule neurons (CGN) against wild type and mutant $PrP_{106-126}$ induced neurotoxicity.

EXPERIMENTAL PROCEDURES

Purification of PrP-AA and Autoantibodies against Aβ—The protocol was adapted from a previously described method (13). Disposable chromatography columns were packed with CNBr-activated Sepharose 4B (Amersham Biosciences, Piscataway, NJ). $PrP_{106-126}$(A117V) (Bachem) and $A\beta_{1-40}$ (Invitrogen) were conjugated to Sepharose beads (0.6 mg/ml drained Sepharose) according to the manufacturer's instructions. The labeled Sepharose columns were equilibrated and washed with PBS (pH 7.4). After passing individual donor or commercial pooled human IgG (Baxter or Octapharm) through the columns and collecting the unbound (*i.e.* pass-through) fractions, bound IgG fractions were released by passing elution buffer (50 mM glycine at pH 2.5) through the column. The pH-neutralized fractions were collected and tested by ELISA.

Epitope Mapping of Purified PrP-AA—An array of 11 amino acid peptides, which were sequentially frame shifted by one residue or had single amino acid replacements, were synthesized on a cellulose membrane (Department of Biochemistry, Schulich School of Medicine and Dentistry, University of Western Ontario) using the spot method of multiple peptide synthesis (37–38). During the mapping study, membranes bound with peptides were prepared by washing with 100% ethanol and PBS, three times each, followed by blocking with 5% no-fat milk in PBS overnight at 4 °C. The membrane was then washed with PBS once more before adding 0.2 μg/ml purified PrP-AA and incubating overnight at 4 °C. After incubating with anti-human IgG HRP antibody (1:2000), the blots were visualized with the Super Signal chemiluminescence substrate (Pierce).

ELISA—The ELISA assay for PrP-AA was modified from a previously described method (13). 96-well ELISA plates were coated with $PrP_{106-126}$ (A117V) that was dissolved in a coating buffer (1.7 mM NaH_2PO_4, 98 mM Na_2HPO_4, 0.05% sodium azide, pH 7.4).

Determination of PrP-AA Isotype—The IgG subclasses of purified antibody samples were determined using a Quantibody human Ig isotype array (Raybiotech, INC, cat QAH-ISO-1-1).

Immunoprecipitation of PrP and PrP^{Sc} by Purified PrP-AA—Reaction mixtures of homogenates in buffer containing 100 mM NaCl and 25 mM Tris/HCl (pH 7.4) were prepared from the cerebellum of a PrP(A117V) transgenic mouse and the brain of a hamster inoculated with Hamster Scrapie Strain Sc237 (10% v/v, InPro Biotechnology, South San Francisco, CA) (39). After centrifuging at 11,000 $\times$ *g* for 30 min at 4 °C, the mouse or hamster brain homogenates (2.5 or 100 mg/ml, respectively) were incubated with or without 100 μg/ml proteinase K (PK) at 37 °C for 2 h. PK digestion was terminated with 10 mM phenylmethylsulfonyl fluoride and heated at 100 °C 5 min. Cooled reaction mixtures were incubated overnight at 4 °C with 1 μg of purified human PrP-AA or purified human autoantibodies against Aβ. Protein A-agarose was added, and a second overnight incubation was performed, followed by centrifuging and washing three times with PBS. Immunoprecipitates were loaded into 4–12% NuPage Bis-Tris gel (Invitrogen NP0321) for Western blotting with diluted (1/2000) commercial anti-PrP monoclonal antibodies (3F4, Chemicon, AB1562; and 6D11, Santa Cruz Biotechnology, sc-58581) followed by horseradish-peroxidase-conjugated goat anti mouse IgG. Binding was visualized by enhanced chemiluminescence (Thermo Scientific, 34095). The 3F4 monoclonal antibody was raised against amino acids 109–112 of human PrP. According to the

manufacturer, 3F4 recognizes both protease sensitive and resistant forms of human and hamster PrP, but not mouse PrP after denaturing. Monoclonal antibody clone 6D11 was raised against amino acids 93–109 of human PrP. According to the manufacturer this antibody recognizes PrP^{c} as well as PrP^{Sc} of human, mouse, and hamster origin.

Fluorometric Experiments—Fluorometry has been previously described (18, 40). Synthetic $PrP_{106-126}$ was incubated with or without purified PrP-AA in PBS buffer at 37 °C overnight. Samples were added to 50 mmol/liter glycine pH 9.2, 2 μmol/liter thioflavin T (Sigma) in a final volume of 2 ml. Fluorescence was measured spectrophotometrically at excitation with emission wavelengths of 435 nm and 485 nm, respectively. Samples were run in triplicate and were plotted with the mean ± S.D.

Electron Microscopy—Synthetic $PrP_{106-126}$ was incubated with or without purified PrP-AA in PBS buffer at 37 °C overnight. 2 μl of each sample were dropped onto 300 mesh carbon/formvar-coated grids and allowed to absorb for 3 min. A drop of the negative stain (NanoVan, Nanoprobes, Inc. Yaphank, NY) was placed on the grid for 8–10 s and then wicked off for drying. Images were taken using a Tecnai G12 BioTwin transmission electron microscope (FEI, Hillsboro, OR) with an AMT CCD camera (Advanced Microscopy Techniques, Danvers, MA).

Mass Spectrometry—Electrospray ionization mass spectrometry (ESI-MS, API 4000, Applied Biosystems) was used to detect the monomer of PrP. The instrument was equipped with a Z-spray ionization source. Both nebulizer and desolvation gases were nitrogen and the collision gas was argon. Mass spectrometric parameters were set as follows: collision gas (CAD) 8, curtain gas (CUR) 10, ion source gas 1 (GS1) 15, ion source gas 2 (GS2) 35, electrospray voltage 5000 in positive ion scan mode, and dry temperature at 500 °C. The mixture of methanol, water, and formic acid (90:10:0.1, v/v/v) were used as the mobile phase with a flow rate 0.2 ml/min. Synthetic $PrP_{106-126}$ was incubated with or without purified PrP-AA in PBS buffer at 37 °C overnight. The samples were filtered and directly infused into the mass spectrometer (10 μl) through a LC system (Agilent 1100) with an auto sampler. All data were acquired at least in triplicate to confirm the reproducibility of the results.

Primary Rat Neuronal Culture and Neurotoxicity Assays—CGN were prepared from 7-day-old Sprague-Dawley rats as described previously (41). Briefly, rat CGN cells were prepared and seeded into 48-well poly-l-lysine-coated culture plates at a cell density of 2×10^5 cells/well in the BME medium with 10% fetal bovine serum and 25 mM KCl (Sigma). After incubating for 24 h, 10 μM cytosine arabino-furanoside (Sigma) was added to prevent glial proliferation. These cultures contain about 95% neurons (95% granule cells) with the remaining 5% of non-neuronal cells, mainly of astrocytic type (42–43). Treatments were performed after 14 days *in vitro*. $PrP_{106-126}$ (A117V or 117A) or scrambled $PrP_{106-126}$ (NGAKALMGGHGATKVMVGAAA) was pre-incubated in PBS, pH 7.2 at 37 °C for 48 h in the absence or presence of purified PrP-AA *in vitro* and was then added to cells. After the exposure of the cells to these incubates for 3 days, cell viability was determined by staining neurons with fluorescein diacetate/propidium iodide.

Glial Cell Culture—Primary cultures of rat cerebellar astroglial cells were prepared from the cerebellum of 7-day-old Sprague-Dawley rats as previously described (44–45). Cells dissociated from cerebella were plated at a density of 5×10^5/well on 24-well plates coated with poly-L-lysine and cultured in a complete medium containing 10% FBS. After 3 days, the medium was replaced with a fresh one containing 10% FBS, and the cells were cultured for additional 3–4 days before treatment until they were more than 90% confluent. As previous reports state, these cultures are composed of up to 90% of astrocytes positive for glial fibrillary acidic protein (44, 46).

Generation of Mice Heterozygous for the $PRNP^{A117V}$ Allele—The plasmid expression vector (pProPrpHGSal) (47), containing the proximal half of genomic mouse PNRP, including the promoter and coding sequences of exon 1, intron 1, and exon 2 fused to exon 3, was used to create the chimera. We inserted the hamster open reading frame (ORF) in place of the murine ORF. The hamster ORF sequence was amplified using PCR with hamster cDNA as the template and GCTATGTGGACTGATGTCGGC; CAGGGCCCACTAGTGCCAAG as the forward and reverse primers. The PCR fragment was cloned initially into pIRESneo. An A117V mutation (A117→V) was introduced by using the Quick Change Mutagenesis Kit (Stratagene). The mutation and absence of polymerase errors were verified by sequencing. The ApaI/PshA I insert was released and inserted in place of the murine ApaI/PshA I within the pProPrpHGSal vector, leading to a construct termed SHa-Mo PrP. An 11-kb DNA fragment containing the A117V mutant allele of the PNRP gene was excised from vector pProPrpHGSal by Not/SalI digestion and injected into the pronuclei of fertilized oocytes from PNRP knock-out mice (47). Genomic DNA, isolated from tail tissue of weanling animals, was screened for the presence of incorporated mouse/hamster chimeric PRNP transgene using PCR primers. The forward primer sequence (5′-CAA CCG AGC TGA AGC ATT CTG CCTT-3′) is in the mouse PrP region and the reverse primer sequence (5′-CAC GCG CTC CAT TAT CTT GAT G-3′) is in the hamster PrP region.

RESULTS

After identifying PrP-AA in all human CSF and serum samples from five normal individuals by using ELISA, we developed and used an affinity column coated with the mutant human PrP sequence encompassing residues 106–126 (KTNMKHMAGAA**V**AGAVVGGLG), which is termed $PrP_{106-126}$ (A117V), to isolate human PrP-AA from IVIg or serum from individual blood specimens. An intense signal was observed with antibody capture of $PrP_{106-126}$(A117V) in an ELISA assay using bound PrP-AA (Fig. 1). The non-binding fraction ("pass-through" (PT)) was depleted of antibodies which bound $PrP_{106-126}$(A117V) (Fig. 1). In contrast, purified PrP-AA could not be detected by ELISA coated with the unrelated $A\beta_{1-40}$ peptide (data not shown).

The specificity of PrP-AA was evaluated by immunoprecipitating PrP(A117V) from homogenates of brains from transgenic mice that express the human sequences encompassing residues 106–126. This was accomplished by knocking-in a hybrid mouse/hamster PNRP gene containing the A117V sub-

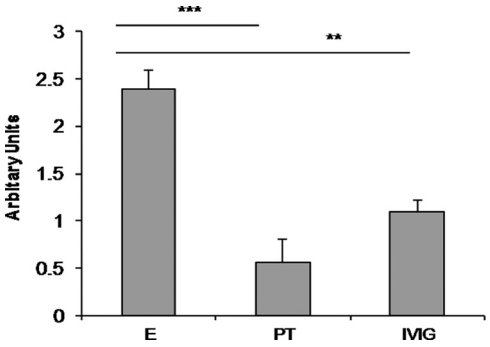

FIGURE 1. **Analysis of $PrP_{106-126}$(A117V) binding by purified PrP-AA in an ELISA assay.** Purified PrP-AA, non-binding, pass-through IgG (*PT*) or original IVIg (all at 1 μg) were added to $PrP_{106-126}$(A117V) peptide-coated wells. After washing, bound antibodies were detected with horseradish peroxidase-conjugated secondary anti-human IgG antibodies. Purified PrP-AA showed an enhanced signal compared with the original IVIg; whereas, the PT IgG was greatly diminished in binding capacity. *E*, PrP-AA; *PT*, pass-through IgG depleted of PrP-AA; *IVIg*, original IVIg used to purified PrP-AA; **, $p < 0.01$; ***, $p < 0.001$.

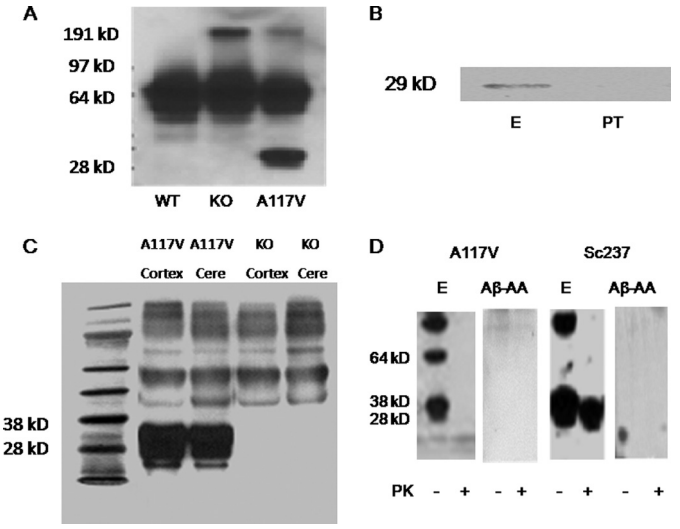

FIGURE 2. **Characterization of PrP-AA specificity for PrP.** *A*, A117V transgenic mice, but not wild-type (*WT*) nor PNRP knock-out (*KO*) mice, were shown to express the PrP protein, which was detectable in brain homogenates using the murine monoclonal antibody 3F4. *B*, visualization of PrP(A117V) in brain homogenates (500 μg protein) of transgenic mice by immunoprecipitation with purified PrP-AA (*E*) or PT. Immunoprecipitated complexes were subjected to Western blot analysis with 3F4 antibody. *C*, purified PrP-AA recognized the PrP protein in Western blots of brain cortex and cerebellar (*Cere*) homogenates of A117V transgenic mice but not KO mice. Although, multiple bands were observed with overexposure, the strongest signal corresponded to the approximately band of 29 kDa PrP (A117V) observed in PrP(A117V) transgenic mice. *D*, Western blot analysis of immunoprecipitates from brain homogenates (1 mg transgenic mouse cerebellum and 10 mg Sc237 hamster brain) pretreated with or without proteinase K using PrP-AA or autoantibodies against Aβ. An anti-PrP antibody 6D11 which detects both mouse and hamster PrP, was used for detecting antibody. Numbers adjacent to horizontal lines indicate positions of molecular mass markers (kDa). 10 μl samples were loaded in each lane. Purified PrP-AA recognized both PrP and PK-resistant PrP^{Sc} (27–30kDa). Autoantibodies against Aβ did not recognize PrP nor PK-resistant PrP^{Sc} (27–30kDa). The photo was selected from a single representative experiment that was repeated three times with similar results. *PT*, pass-through IgG depleted of PrP-AA. *Aβ-AA*, autoantibodies against Aβ

stitution, which has been used previously to investigate GSS (48). It has already been established that hamster PrP binds a human single chain PrP antibody (49), suggesting that brains expressing the coding region of the hamster protein could bind to human PrP antibodies. We confirmed the expression of PrP(A117V) in transgenic mouse brain homogenates using the commercially available mouse monoclonal antibody 3F4. This antibody recognized a protein of the correct mobility (~29 kDa) in brain homogenates from transgenic PrP (A117V) mice, but not wild type or PNRP knock-out mice (Fig. 2*A*). Immunoprecipitation of ~29 kDa proteins from brain homogenates of PrP(A117V) transgenic mice was accomplished with purified PrP-AA; whereas, no protein bands where observed after immunoprecipitation with PrP-AA-depleted IVIg (PT) (Fig. 2*B*). Western blotting of homogenates from brains of PrP(A117V) or PNRP knock-out mice demonstrated a major band corresponding to PrP only in the cortex and cerebella of the transgenic mice (Fig. 2*C*). Of note, although other minor protein species were evident upon detection of PrP-AA immunoprecipitates with the unrelated 3F4 antibody, PrP(A117V) was by far the predominant protein band observed (Fig. 2*C*). Taken together, these data indicate that PrP-AA binds PrP(A117V) with high specificity and affinity. Additionally, to examine whether PrP-AA could bind to protease-resistant PrP^{Sc} conformers, brain homogenates isolated from a Sc237 hamster pretreated with or without PK were immunoprecipitated by PrP-AA or autoantibodies against Aβ as a negative control. We clearly demonstrated that purified PrP-AA recognized both PrP and PK-resistant PrP^{Sc} (27–30 kDa) (Fig. 2*D*).

The titer of PrP-AA in IVIg was determined to be 1:1200 within a total IgG concentration of 10 g/100 ml. The distribution of different IgG subclasses in the purified PrP-AA were as follow: IgG1 74.2%, IgG2 12%, IgG3 11.4%, and IgG4 2.4%. Thus, the IgG subclasses of purified PrP-AA are similar to the distribution of IgG subclasses in IVIg products and human serum. Furthermore, the PrP-AA binding *epitope* was determined using an array displaying a series of modified $PrP_{106-126}$ peptides (Fig. 3, *A* and *B*). Binding of PrP-AA occurs at the extreme N terminus of $PrP_{106-126}$, and requires, at a minimum residue KTNMK (106–110) as demonstrated in Fig. 3, *C* and *D*. Both lysines in this motif are critical for high affinity antibody binding since substitution or deletion of either completely abolished PrP-AA binding (Fig. 3).

Next we investigated by electron microscopy, mass spectrometry, and fluorometric measurement using a Thioflavin T (ThT) reagent that binds specifically to fibrillar structures whether purified human PrP-AA could block PrP fibril formation as well as disaggregate preformed fibrils (Fig. 4). Dose-response and kinetic studies showed that pre-incubating $PrP_{106-126}$ monomers or preformed peptide fibrils with purified human PrP-AA dose-dependently prevented fibril formation and disrupted preformed fibril structures in a time-dependent manner, as evidenced by a substantial decrease in ThT fluorescence (Fig. 4, *A* and *B*) compared with the control using PT. These findings were confirmed in independent experiments using various concentrations of PrP-AA and reaction time (Fig. 4, *C* and *D*).

To confirm findings obtained from the ThT fluorescence assay and to exclude interference of ThT bound with PrP fibrils by antibodies, fibrils, and monomers were visualized by electron microscopy and measured by mass spectrometry. The mass spectra of PrP monomers incubated with (Fig. 4*D*) or

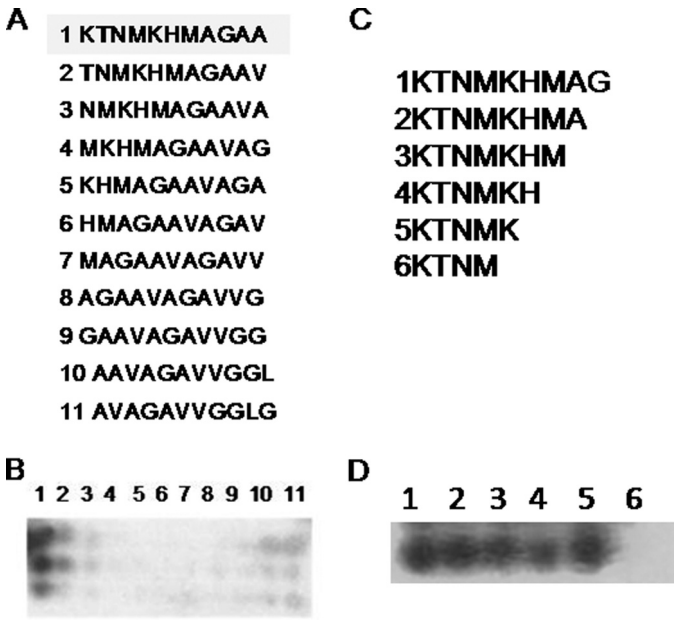

FIGURE 3. **Mapping PrP-AA binding epitopes.** Domain specificities of PrP-AA were determined using a peptide microarray. Sequences of either sequentially one amino acids shifted (*A*) or single amino acids deletions (*C*) peptides within region $PrP_{106-126}$ which were synthesized and spotted on membranes are displayed in *A* and *C*. Membranes were then probed with PrP-AA (2 μg/ml) and then HRP conjugated anti-human-IgG antibody (triplicate membranes were probed). The sequence motif KTNMK appeared to be highly important since only peptide 1 is bound by PrP-AA, as shown in panel *B*. Further validation came from experiments shown in *panel D*, which show strong binding only when residues 1–5 are present, implying the two lysines (K*XXX*K) are key elements for binding.

without (Fig. 4*E*) PrP-AA revealed that PrP-AA treatments significantly increased the well-resolved PrP monomer peak, indicating that PrP-AA blocked PrP fibril formation. Electron microscopic examination of these reactions confirmed the data from mass spectrometry (Fig. 4, *E* and *F*).

We next assessed whether PrP-AA could protect cultured primary rat neurons from toxicity of predominantly fibrillar $PrP_{106-126}$, as determined by measuring viability using FDA/PI stains (Figs. 5 to 7). The addition of $PrP_{106-126}$ was allowed to form fibrils before addition induced neuronal death in a dose-dependent fashion (Fig. 5*A*). In contrast, a control peptide with a scrambled $PrP_{106-126}$ sequence showed no neurotoxicity compared with $PrP_{106-126}$ (Fig. 5*B*). These data demonstrate the specific toxicity to CGN of $PrP_{106-126}$, which had been allowed to form fibers before addition.

Next we tested whether neurotoxicity of $PrP_{106-126}$ monomers or fibers could be blocked by pre-incubating with purified PrP-AA before adding to cultures of CGN. Human PrP-AA almost completely prevented neurotoxicity of the mutated $PrP_{106-126}$(A117V) added as monomers (Fig. 6*A*) or preformed fibrils (Fig. 6*B*). In addition, PrP-AA also potently blocked wild-type $PrP_{106-126}$-induced neurotoxicity (Fig. 6*C*). Conversely, PrP-AA-depleted fraction of IVIg failed to protect against neurotoxicity produced by either peptide.

Previous studies reported that, unlike the wild-type peptide, $PrP_{106-126}$(A117V) fibrils induce inflammation-mediated neurotoxicity (36). To confirm that purified human PrP-AA protected against inflammation related neurotoxicity of $PrP_{106-126}$(A117V), we applied co-culture system of CGN combined with glia cells. Consistent with the previous report, treatment of these mixed cultures with preformed $PrP_{106-126}$(A117V) fibrils led to markedly greater CGN death (Fig. 7) compared with treatment of CGN monocultures (Fig. 6). This toxicity was greatly reduced with PrP-AA pretreatment of fibrils (Fig. 7). In contrast, PT demonstrated no neuroprotective effects.

DISCUSSION

We have identified specific prion protein-binding antibodies in both sera and CSF from normal individuals and have demonstrated neutralization of PrP toxicity in primary cerebellar neurons. This is the first identification and isolation of PrP antibodies from subjects with no documented exposure to prion antigens. Both immunoprecipitation and Western blot data suggest that PrP-AA strongly binds to the PrP monomer and PrP^{Sc}. We speculate that these autoantibodies may have normal physiological functions of immune-mediated PrP replication control or clearance, similar to what we have previously postulated for circulating Aβ antibodies (13, 15). Our results demonstrate that human PrP-AA can be isolated from currently marketed IVIg; thus, the potential for producing a consistent product to test in the clinic is enhanced.

It has been previously suggested that PrP antibodies may be an effective immunotherapy for prion diseases (50). Interestingly, even though TSE is a CNS disease, PrP^{Sc} accumulates in lymphoid tissues before CNS involvement. Accordingly, lymphoid PrP^{Sc} represents an early primary target for therapeutic strategies, given the greater accessibility of peripheral tissues compared with privileged CNS system which significantly impedes penetration of the antibodies through the blood brain barrier. Possible immunotherapies are active immunization with a PrP antigen or passive immunization with selective antibodies. Development of an active immunization therapy may be problematic since prion infections do not elicit a classical immune response and there likely would be great reticence to immunize asymptomatic or uninfected individuals given the known infectivity of this peptide (50). In addition, a phase II clinical trial in AD patients testing active immunization with the Aβ epitode, AN1792, failed due to severe side effects. Passive immunization, on the other hand, may represent a better approach given the lack of issues cited above.

Our present finding of fairly abundant levels of PrP-AA in normal human sera and concentrated pooled IgG, which can be purified and concentrated, represents a new opportunity for rapidly developing an effective and relatively safer immunotherapy for prion diseases. Alternatively, a humanized monoclonal antibody targeting the PrP epitope could be developed based on the binding sequence of PrP-AA. Although monoclonal antibodies may be viewed as more optimal than purified polyclonal antibodies from the standpoint of consistency of preparation, there is still concern that chronic dosing with humanized antibodies may generate anti-idiotypic responses directed to the residual mouse CDR sequences.

We demonstrate that purified PrP-AA dramatically inhibit PrP fibril formation and disrupt preformed PrP fibrils, as reported in previous studies using mouse PrP antibodies (8–9). The epitope for human PrP-AA is a unique, within the human genome, five-amino acid sequence located at the N terminus of

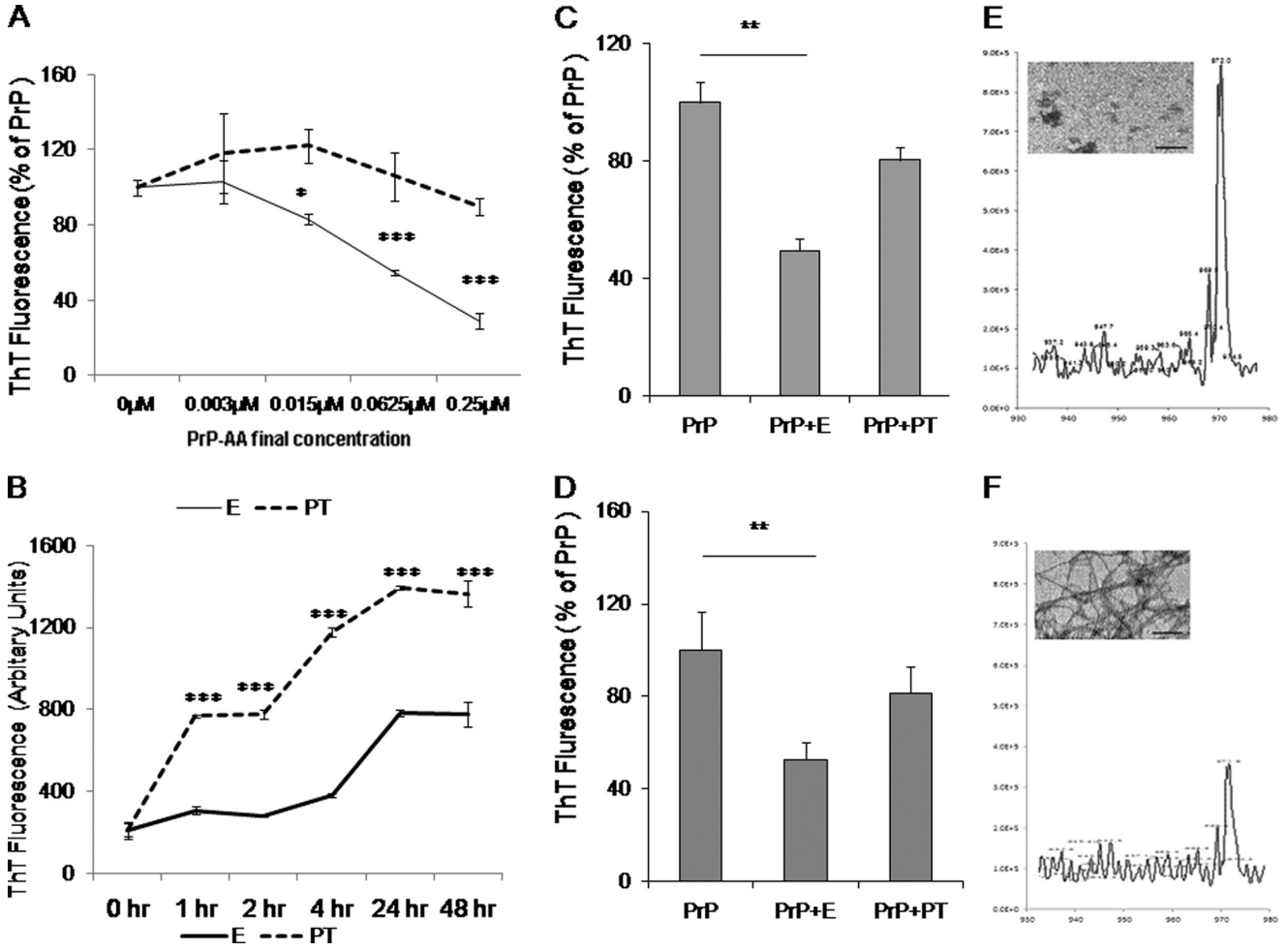

FIGURE 4. **Effects of PrP-AA on PrP peptide's fibril formation.** *A*, dose-response study of $PrP_{106-126}$(A117V) fibril formation and PrP-AA effects. *B*, kinetic study of 50 μM $PrP_{106-126}$(A117V) fibril formation and 0.07 μM PrP-AA effects. *C*, incubation of 50 μM $PrP_{106-126}$(A117V) peptides with or without purified PrP-AA in PBS. Purified PrP-AA significantly inhibited $PrP_{106-126}$(A117V) fibril formation. *D*, incubation of preformed fibrils from 50 μM $PrP_{106-126}$(A117V) peptides with purified PrP-AA (*E*, 0.07 μM) or pass-through IgG (PT, 0.07 μM) in PBS for 48 h. Purified PrP-AA significantly disaggregated preformed $PrP_{106-126}$(A117V) fibrils as measured by ThT staining. Samples were run in triplicate and plotted as the mean ± S.D. (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$ compared with PrP only, one-way ANOVA). Representative data from triplicate mass spectra of the $PrP_{106-126}$(A117V) monomer with (*E*) or without (*F*) PrP-AA were inserted to *E* and *F*. Electron micrographs of the products from experiments are shown in *E* and *F* (scale bar = 500 nm). *E*, PrP-AA; *PT*, pass-through IgG depleted of PrP-AA.

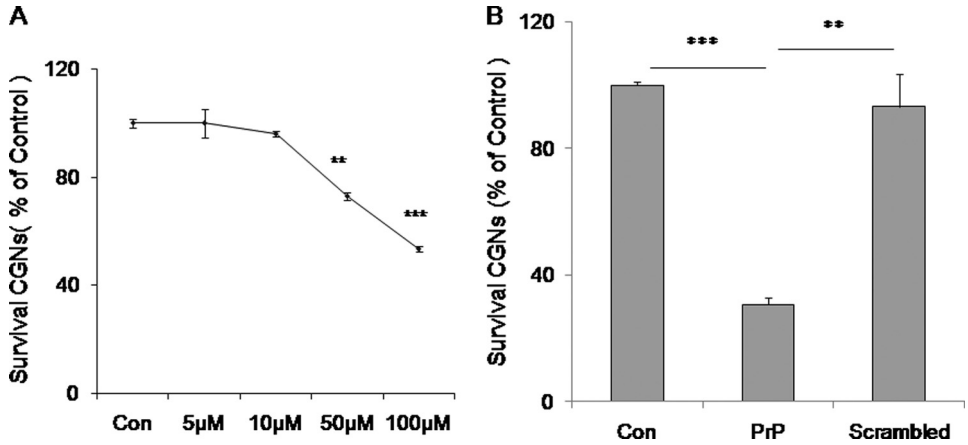

FIGURE 5. **Neurotoxicity of PrP peptides on CGN.** Dose-dependence of $PrP_{106-126}$(A117V) fibril neurotoxicity was examined in CGN. The neurons were exposed to different dosages of $PrP_{106-126}$(A117V) (5 μM to 100 μM) (*A*) or $PrP_{106-126}$(A117V) (100 μM) and scrambled control peptide (100 μM) (*B*) for 3 days. Cell viability was determined by staining neurons with fluorescein diacetate/propidium iodide. Values are expressed as percentages (%) of control (untreated). The data represent the mean ± S.D. (*bars*) values of triplicate determinations from a single but representative experiment, which has been repeated three times with similar results (**, $p < 0.01$; ***, $p < 0.001$ by one-way ANOVA).

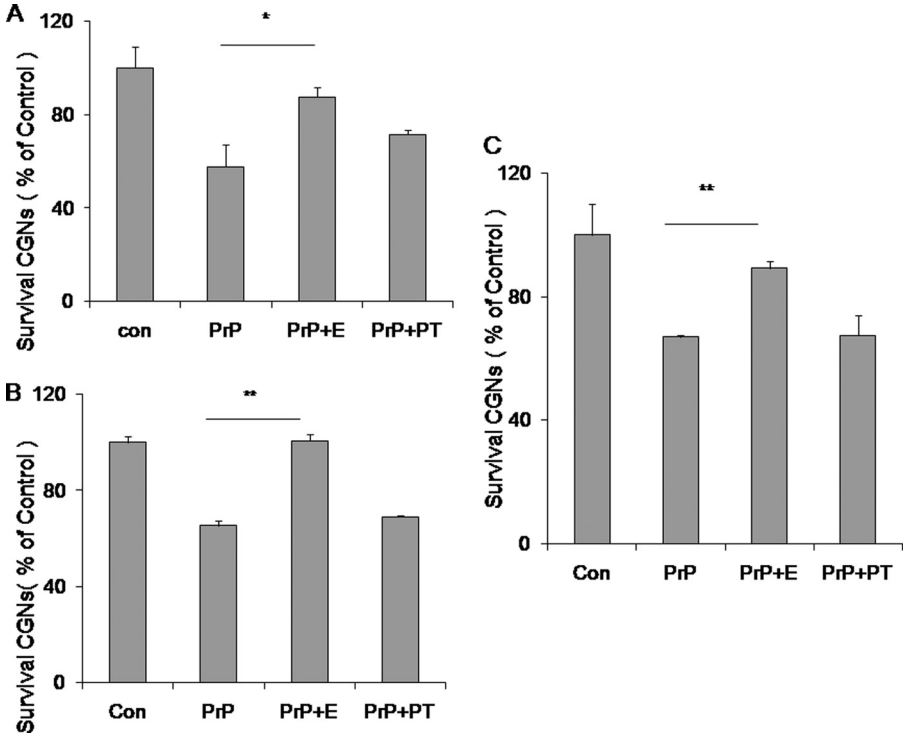

FIGURE 6. **Effects of PrP-AA on wild type or mutant $PrP_{106-126}$ induced neurotoxicity.** Exposure of rat CGN to 50 μM $PrP_{106-126}$(A117V or wild type) fibril resulted in a reduction of neuronal survival during a 3 day incubation period. Purified PrP-AA (0.07 μM) significantly attenuated $PrP_{106-126}$(A117V) fibril-induced neuronal death. *A*, $PrP_{106-126}$(A117V) peptides (50 μM) were incubated with PrP-AA (0.07 μM) before being exposed to neurons. *B*, preformed $PrP_{106-126}$(A117V) fibrils were incubated with PrP-AA (0.07 μM) before being exposed to neurons. *C*, preformed wild-type $PrP_{106-126}$(117A) fibrils were incubated with PrP-AA (0.07 μM) before exposed to neurons. Cell viability was determined by staining neurons with fluorescein diacetate/propidium iodide. The data represent the mean ± S.D. of triplicate determinations from a representative experiment repeated at least three times with similar results (*, $p < 0.05$; ***, $p < 0.001$, compared with $PrP_{106-126}$ only, one-way ANOVA). *Con*, untreated cultures; *PrP*, $PrP_{106-126}$ (A117V or wild type) peptides; *E*, PrP-AA; *PT*, pass-through IgG depleted of PrP-AA.

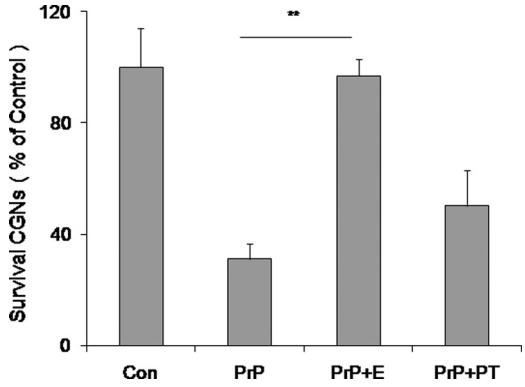

FIGURE 7. **Analysis of PrP-AA in the culture system.** The PrP-AA prevented $PrP_{106-126}$(A117V) induced neurotoxicity in a neuron-glia co-culture system. Purified PrP-AA significantly blocked $PrP_{106-126}$(A117V) fibril-induced neuronal death in the co-cultured system. CGN-glia were treated with 50 μM $PrP_{106-126}$(A117V) fibril only and $PrP_{106-126}$(A117V) fibril that had been preincubated with 0.07 μM PrP-AA for 24 h. Cell viability was determined by staining neurons with fluorescein diacetate/propidium iodide. Values are expressed as percentages (%) of control (untreated). The data represent the mean ± S.D. (*bars*) values of triplicate determinations from a single but representative experiment, which has been repeated three times with similar results (**, $p < 0.01$, by one-way ANOVA). *PrP*, $PrP_{106-126}$(A117V) peptides; *E*, PrP-AA; *PT*, pass-through IgG depleted of PrP-AA.

the PrP106–126 peptide, which is conserved between humans and hamster PrP. Human PrP-AA recognizes the full-length hybrid hamster/mouse prion protein containing the A117V mutation when expressed in a transgenic mouse line. Interestingly enough, human PrP-AA also directly and strongly binds to a well known hamster protease-resistant PrP^{Sc} protein, SC237, indicating human IgG, somehow, may be involved in protecting humans to resist prion infections at a certain degree. The finding that PrP-AA binding is disrupted by mutating a small stretch of amino acids exclusively, suggests that the pool of purified IgG is comprised of only a small number of antibody clones. Furthermore, it identifies a discrete region within the full-length peptide that is crucial for fibril formation and neurotoxicity. Since binding occurs at a region of the PrP protein (*e.g.* 106–110) without known mutations, this purified PrP-AA should be effective for treatment of all prion diseases. Indeed, we have demonstrated prevention of both wild type and $PrP_{106-126}$(A117V) fibril formation and peptide-induced neurotoxicity. In addition, the different pathways of neuronal death induced by these two peptides suggest that PrP-AA may have a broad function to treat prion diseases besides GSS. Additionally, since PrP-AA could interact with PrP^{Sc}, it is necessary to perform a future study to show whether the human PrP-AA can interfere with human PrP^{Sc} formation, replication, and PrP^{Sc}-induced neurotoxicity in the brain. Additionally, it is also important in future studies to test the effect of the PrP-AA on aggregation of full-length PrP or the N-terminal domain of wild-type PrP. Experiments are currently underway in transgenic models expressing various forms of the full-length protein to test this prediction.

This study provides strong evidence that PrP-AA is found in normal human blood and CSF and can be easily purified from pooled IgG. The similar features of PrP-AA to autoanti-Aβ antibodies suggests treatment of prion diseases with PrP-AA is

highly feasible, especially since whole IVIg clinical trials for AD are currently ongoing and have demonstrated some efficacy (51). Thus, administration of purified human PrP-AA or IVIg may be used some day to prevent or slow down prion disease progression.

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