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Brain-Reactive Autoantibodies Prevalent in Human Sera Increase Intraneuronal Amyloid-$\beta_{1\text{-}42}$ Deposition

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Abstract. Previous studies have reported immunoglobulin-positive neurons in Alzheimer's disease (AD) brains, an observation indicative of blood-brain barrier (BBB) breakdown. Recently, we demonstrated the nearly ubiquitous presence of brain-reactive autoantibodies in human sera. The significance of these observations to AD pathology is unknown. Here, we show that IgG-immunopositive neurons are abundant in brain regions exhibiting AD pathology, including intraneuronal amyloid-β_{42} ($A\beta_{42}$) and amyloid plaques, and confirm by western analysis that brain-reactive autoantibodies are nearly ubiquitous in human serum. To investigate a possible interrelationship between neuronal antibody binding and Aβ pathology, we tested the effects of human serum autoantibodies on the intraneuronal deposition of soluble $A\beta_{42}$ peptide in adult mouse neurons *in vitro* (organotypic brain slice cultures). Binding of human autoantibodies to mouse neurons dramatically increased the rate and extent of intraneuronal $A\beta_{42}$ accumulation in the mouse cerebral cortex and hippocampus. Additionally, individual sera exhibited variable potency related to their capacity to enhance intraneuronal $A\beta_{42}$ peptide accumulation and immunolabel neurons in AD brain sections. Replacement of human sera with antibodies targeting abundant neuronal surface proteins resulted in a comparable enhancement of $A\beta_{42}$ accumulation in mouse neurons. Overall, results suggest that brain-reactive autoantibodies are ubiquitous in the blood and that a defective BBB allows these antibodies to access the brain interstitium, bind to neuronal surfaces and enhance intraneuronal deposition of $A\beta_{42}$ in AD brains. Thus, in the context of BBB compromise, brain-reactive autoantibodies may be an important risk factor for the initiation and/or progression of AD as well as other neurodegenerative diseases.

Keywords: Alzheimer's disease, amyloid, autoantibodies, autoimmunity, blood brain barrier, neurodegenerative disease

INTRODUCTION

Alzheimer's disease (AD) is a progressive and devastating neurodegenerative disorder of the elderly that is highlighted by a dramatic reduction of memory and

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cognition and linked to loss of neurons and synapses [1–5]. Additional key pathological features include the deposition of amyloid-β (Aβ), especially the 42-amino acid peptide ($A\beta_{42}$), within neurons, amyloid plaques and in the walls of brain blood vessels as well as the appearance of neurofibrillary tangles, glial activation and widespread inflammation [6–15]. The deposition of $A\beta_{42}$ within neurons is initiated early in the course of the disease, precedes amyloid plaque and tangle formation and temporally and spatially coincides with loss of synapses in human AD and transgenic mouse brains [6, 8, 14, 16–21]. Mechanisms and factors contributing to Aβ peptide deposition within neurons and amyloid plaques remain elusive.

In healthy individuals, the blood brain barrier (BBB) strictly controls the brain microenvironment by restricting the entry of blood-borne components, including most soluble proteins and blood cells, into the brain parenchyma [22, 23]. Numerous studies have suggested that cerebrovascular compromise plays a role in the initiation and progression of AD pathogenesis [24–26]. In support of this, the presence of immunoglobulin (Ig)-immunopositive neurons in histological sections of postmortem AD brains has been reported in several studies, a phenomena only rarely observed in comparable brain regions of non-demented, age-matched controls [27–29]. In addition, we also recently showed that brain-reactive autoantibodies are both numerous and ubiquitous in human sera, regardless of age or presence of disease [30]. This implies that BBB compromise in AD brains allows brain-reactive autoantibodies [28, 31] from the blood to gain access to neurons within the brain tissue [27, 28, 31–34]. Indeed, our own studies of AD brains have confirmed the presence of plasma components, including soluble Aβ peptides, complement proteins and immunoglobulins leaking from blood vessels and entering into the surrounding brain tissue [35]. Further, we have shown that BBB disruption induced by injection of Pertussis toxin resulted in an influx of blood-borne, fluorescent (FITC)-labeled $A\beta_{42}$ into the mouse brain interstitium, suggesting that, under conditions of BBB breakdown, the blood could be a major and chronic source of the Aβ that deposits in AD brains. In accord with this notion, entry of blood-borne FITC-labeled $A\beta_{42}$ was followed by the binding and accumulation of this labeled peptide selectively within cortical pyramidal neurons, whereas little or no blood-borne $A\beta_{42}$ entered into the brains of healthy mice with an intact BBB [35].

The above findings suggest that there may be a relationship between BBB breakdown, entry of soluble Aβ peptides and immunoglobulins into the brain tissue, the association of Aβ and immunoglobulins with neurons, and the pathogenesis of AD. Therefore, in the present study, we have examined the possibility that blood-borne, brain-reactive autoantibodies contribute to intracellular Aβ deposition in the brains of AD patients. First, we used immunohistochemistry to examine the presence and distribution of IgG in postmortem AD brains and found that IgG-immunopositive neurons are most abundant in brain regions exhibiting typical AD pathological features, including intraneuronal $A\beta_{42}$ and amyloid plaques. Next, Western analysis was used to confirm the ubiquitous presence of brain-reactive autoantibodies in sera from AD patients, age-matched neurologically normal controls and younger healthy subjects. To directly examine the possible relationship between neuronal IgG binding and Aβ-related pathology, we tested the effects of human sera on neuronal IgG binding and intraneuronal deposition of soluble $A\beta_{42}$ peptide in adult mouse neurons *in vitro* (in organotypic brain slice cultures). Binding of human serum IgG to mouse neurons was accompanied by a dramatic increase the rate and extent of neuronal $A\beta_{42}$ accumulation in pyramidal neurons of the cerebral cortex and hippocampal region. Additionally, replacement of human sera with antibodies targeting specific, abundant neuronal surface receptors, e.g., the alpha7 nicotinic acetylcholine and glutamate R2 receptors, resulted in a comparable enhancement of $A\beta_{42}$ internalization and accumulation in mouse neurons, thus confirming the direct involvement of antibodies in this process. Overall, results suggest that brain-reactive autoantibodies are ubiquitous in the blood and that a defective BBB allows these antibodies to access the brain interstitium, bind selectively to the surfaces of certain neuronal subtypes, and enhance intraneuronal deposition of Aβ peptides, particularly $A\beta_{42}$, in these cells in AD brains.

MATERIALS AND METHODS

Animals

Swiss-Webster mice were obtained from Taconic Farms (Hudson, NY) and used for experiments at 3–6 months of age. Sprague-Dawley rats were also obtained from Taconic Farms and used at 7–9 weeks of age. Both were maintained on *ad libitum* food and water with 12-hour light/dark cycle in an AALAC-accredited vivarium. Animals use was reviewed and approved by the UMDNJ IACUC.

Human brain tissue

Brain tissue from patients with sporadic AD ($n = 23$, age range = 71–88) and age-matched, neurologically normal individuals ($n = 14$, age range = 69–83) were obtained from the Harvard Brain Tissue Resource Center (Belmont, MA), the Cooperative Human Tissue Network (Philadelphia, PA), the UCLA Tissue Resource Center (Los Angeles, CA) and Slidomics (Cherry Hill, NJ). Postmortem intervals were <24 h and pathological confirmation of AD was evaluated according to criteria defined by the National Institute on Aging and the Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of AD [36]. Formalin-fixed tissues were processed for routine paraffin embedding and sectioning according to established protocols. Control tissues exhibited minimal localized microscopic AD-like neuropathology.

Antibodies

$A\beta_{42}$ antibodies were obtained from Millipore International (Temecula, CA,) (polyclonal, Cat. No. AB5078 P, dilution = 1:50) and Pharmingen (San Diego, CA) (polyclonal Cat. No. 4767, dilution = 1:50). Biotinylated anti-human IgG antibodies for immunohistochemistry were obtained from Vector Laboratories (Burlingame, CA) (host: goat, Cat. No. PK-6103, dilution = 1:100). Peroxidase-conjugated anti-human IgG antibodies for western blotting were obtained from Thermo Scientific (Rockford, IL) (host: goat, Cat. No. 31410, dilution = 1:200,000). The following antibodies were used for treatments of mouse organotypic brain slice cultures: anti-alpha7 nicotinic acetylcholine receptor (α7 nAChR) (C-20, Santa Cruz Biotechnology, Santa Cruz, CA); anti-GluR2 (polyclonal N19, Santa Cruz Biotechnology, Santa Cruz, CA); anti-beta tubulin (D-10, Santa Cruz Biotechnology, Santa Cruz, CA). The specificity of these antibodies was confirmed by western blotting (data not shown).

Human sera

Human serum samples [AD ($n = 52$, age range = 61–97 years); age-matched controls ($n = 28$, age range = 51–86); and younger healthy controls ($n = 28$, age range = 19–30 years)] were obtained from Analytical Biological Services Inc. (Wilmington, DE). Samples were numerically coded and included the following information: age and gender of the patient, the presence or absence of a detectable neurological disease, an indication of disease severity via a Mini-Mental Status Exam score (MMSE), and estimated post-mortem interval. Use of these samples was approved by the UMDNJ IRB.

Immunohistochemistry

Immunohistochemistry was carried out using paraffin-embedded human and mouse brain tissues as previously described [6, 8]. Briefly, tissues were deparaffinized using xylene and rehydrated through a graded series of decreasing concentrations of ethanol. Antigenicity was enhanced by microwaving sections in citrate buffer. Endogenous peroxidase was quenched by treating sections with 0.3% H_2O_2 for 30 min. Sections were incubated in blocking serum and then treated with primary antibodies at appropriate dilutions for 1 h at room temperature. After a thorough rinse in PBS, biotin-labeled secondary antibody was applied for 30 min. Sections were treated with the avidin-peroxidase complex (Vectastain ABC Elite, Vector Laboratories, Inc., Foster City, CA) and visualized with 3-3-diaminobenzidine-4-HCL (DAB)/H_2O_2 (Imm-Pact-DAB) (Vector). Sections were then lightly counterstained with hematoxylin, dehydrated through increasing concentrations of ethanol, cleared in xylene and mounted in Permount. Controls consisted of brain sections treated with non-immune serum or omission of the primary antibody. Specimens were examined and photographed with a Nikon FXA microscope, and digital images were recorded using a Nikon DXM1200F digital camera and processed and analyzed using Image Pro Plus (Phase 3 Imaging, Glen Mills, PA) and Cell Profiler image analysis software.

Preparation of adult rat brain proteins

Our previous study [30] demonstrated that rat brain protein fractions can be used for detection of human brain-reactive autoantibodies. To prepare this fraction, fresh rat brain tissue was removed from storage at -80°C and placed in a 1 mM phenylmethylsulfonyl fluoride, 50.0 mM Tris-HCL buffer solution, pH 7.4, at a 10.0 ml/g ratio along with protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO) at a 0.5 ml/g ratio. Using a pre-cooled Dounce homogenizer (Arrow Engineering Co., Inc., Hillside, NJ) at a setting of four, brain samples were subjected to homogenization. Brain samples were then centrifuged at 3,000 rpm

using a Beckman CS-6 R centrifuge (Beckman Coulter Inc, Brea, CA) equipped with a swing-rotor at 4°C for a period of 10 min to remove intact cells and large debris. The supernatant was retained as whole brain protein fraction. Protein concentrations were determined using the Bradford Assay.

Detection of autoantibody targets via western blotting

Western blot analysis was performed to determine the brain targets of serum autoantibodies. First, 12.5% SDS-polyacrylamide separating gels were cast using the Mini PROTEAN 3 System (165–3302, BioRad, Hercules, CA) and overlain with stacking gels (4.0%). 100.0 μg of protein sample was added to sample buffer and applied to the gel alongside PageRuler™ Prestained Protein Ladder Plus (SM1811, Fermentas, Glen Burnie, MD). Proteins were fractionated at 130 V for 7 min, followed by 100 V for the remainder of the resolving time. Proteins were then transferred to Hybond-ECL Nitrocellulose Membrane (RPN3032D, Amersham, Piscataway, NJ) for 75 min at 180 mA. Blots were blocked in 5.0% non-fat dried milk dissolved in PBS-Tween (PBS-T), then transferred to human serum samples (primary antibody), diluted 1:500 in blocking solution, for overnight incubation at 4°C. The following morning, blots were thoroughly rinsed in PBS-T then placed in the appropriately diluted peroxidase-conjugated secondary antibody and incubated for 1 h at 4°C. Blots were thoroughly rinsed in PBS-T and quickly rinsed in dH_2O to remove phosphate buffer. Blots were then developed using the Pierce enhanced chemiluminescence (ECL) substrate (32106, Pierce, Rockford, IL) and autoradiography film (XAR ALF 1824, Lab Scientific, Livingston, NJ). Each western blot for a given serum sample was performed in triplicate.

Mouse organotypic brain slice cultures and treatments

Organotypic adult mouse brain slice cultures (MBOCs) were prepared using the technique of Stoppini et al. [37]. Neurons in these cultures have been shown to accumulate exogenous $A\beta_{42}$ (detectable within 4 h of exposure to 100 nM $A\beta_{42}$) [1, 36–38]. Brains from Swiss-Webster mice (3–6 months old) were isolated under sterile conditions and transverse coronal slices (0.5–0.75 mm thick) through desired brain regions were prepared using a McIlwain tissue chopper, placed on 30 mm Millicell-CM culture inserts (Millicell-CM, Millipore, Bedford, MA, USA), and allowed to stabilize in serum-free medium (Dulbecco's Mimimal Essential Medium, DMEM) briefly (one hour) or in 25% inactivated horse serum, 25% Hanks' BSS, 50% DMEM, 25 mg/l penicillin-streptomycin) overnight prior to treatment. Following stabilization, cultures were exposed to serum-free medium (DMEM alone) or complete medium (25% inactivated horse serum, 25% Hanks' BSS, 50% DMEM, 25 mg/l penicillin-streptomycin) containing $A\beta_{42}$ peptide (100 nM) and anti-GluR2 antibody (diluted 1:250), human serum samples (diluted 1:50), anti-α7 nAChR antibody (diluted 1:1000), or anti-β-tubulin antibody (diluted 1:200). Control slices received medium only. For human serum treatment, we elected to treat with diluted whole human serum, rather than purified IgG fractions in an effort to more closely approximate what is expected to occur in the brain under conditions of BBB compromise. MBOCs were treated for 24 h at 37°C in a 5% CO_2-enriched atmosphere. $A\beta_{42}$ was solubilized to the monomeric form prior to use [39].

Image analysis and statistics

The amount of $A\beta_{42}$ accumulation within neurons in MBOCs treated with 100 nM $A\beta_{42}$ with or without human serum or antibodies directed against α7 nAChR, GluR2 or tubulin was determined using quantitative immunohistochemistry. Briefly, MBOCs treated as described above were embedded in paraffin, sectioned and immunostained with anti-$A\beta_{42}$ antibodies under identical conditions. Five images from each section were recorded under identical illumination, magnification and camera settings using a Nikon FXA microscope equipped with a Nikon CCD camera and image analysis softwares (Image Pro Plus and Cell Profiler). The total amount of $A\beta_{42}$-positive material in each viewing field was measured by pixel counting using Cell Profiler to determine the percentage of the total viewing field occupied by $A\beta_{42}$-immunopositive material. In addition, we also used Cell Profiler to count the number of $A\beta_{42}$-positive cells in the same field. All data were downloaded into Excel Spreadsheets. The relative amount of $A\beta_{42}$ per $A\beta_{42}$-immunopositive neuron among the different treatment groups was calculated. Data is presented in graphs as mean amount of $A\beta_{42}$ per $A\beta_{42}$-positive neuron along with the standard error. The Student's t test was used to test the significance of differences among the different treatment groups.

RESULTS

Immunoglobulin G (IgG)-positive neurons, mostly pyramidal cells, are abundant in brain regions showing typical AD pathology

Previous studies have reported detection of Ig-positive neurons in postmortem AD brains, suggesting that the blood-brain barrier (BBB) is defective and that blood-borne components, including antibodies, can chronically gain access to the brain interstitial space during the course of the disease [22, 24, 27, 29, 31–33, 35, 40, 41]. Here, immunohistochemistry using anti-human IgG antibodies was employed to test for the presence of IgG-immunopositive brain components in 23 AD and 14 age-matched control brains. IgG-positive neurons with labeled cell bodies and main dendrite trunks were found in all brains (control and AD) that were examined (Fig. 1a–f). IgG-positive neurons were far more abundant, widespread and intensely immunostained in AD brains (Fig. 1a–e) than in corresponding age-matched control brains (Fig. 1f). In the latter, IgG-positive neurons were most often encountered as scattered individual cells and small cell clusters separated by relatively large expanses of brain tissue that were completely devoid of IgG-positive cells (Fig. 1f). In AD brains, IgG-positive neurons were particularly abundant in brain regions known to be vulnerable to AD-associated pathological changes [e.g., temporal cortex (Fig. 1a, b), entorhinal cortex (Fig. 1c, d) and hippocampus (Fig. 1e)]. In both AD and control brains, IgG immunoreactivity was associated primarily with pyramidal neurons, and these cells often showed marked individual variations in the intensity of IgG immunolabeling (Fig. 1a–d, f). Most of the smaller neurons, astrocytes, microglia and cells associated with the brain microvasculature were IgG-negative (Fig. 1a–d). In three of the 23 AD brains

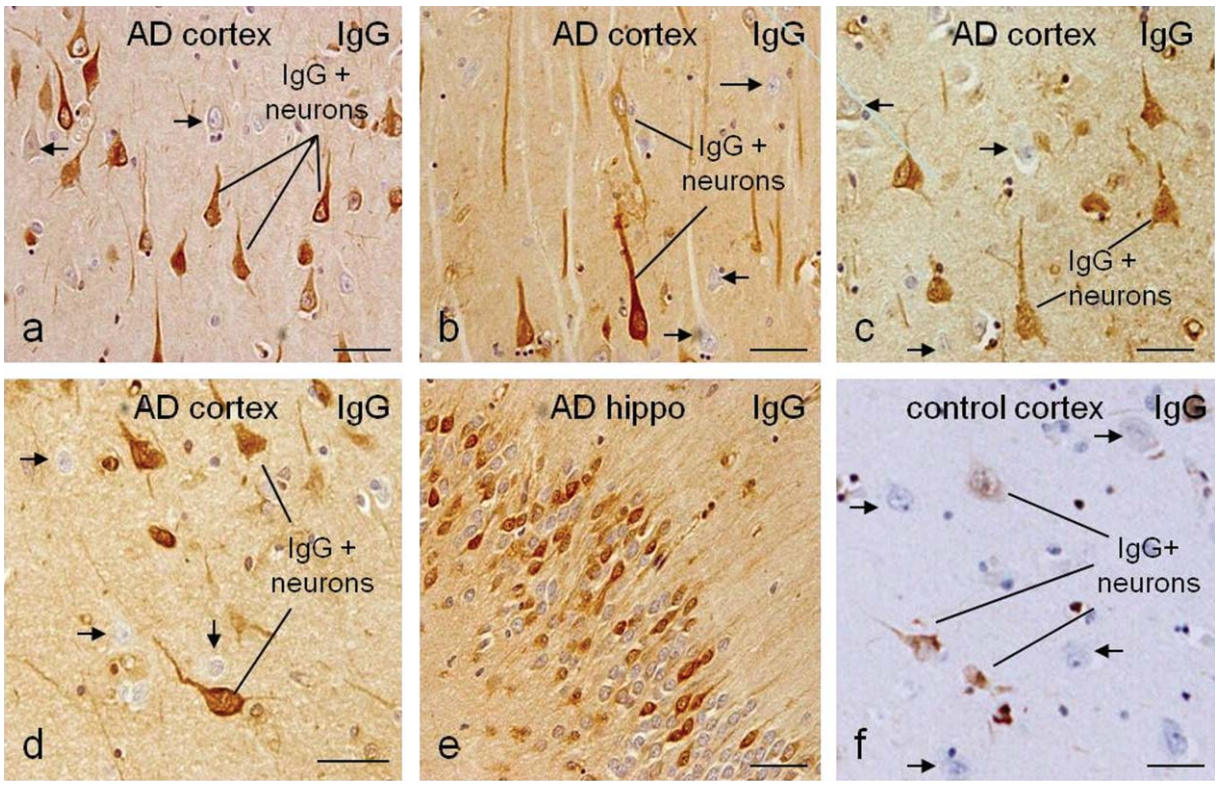

Fig. 1. IgG-immunopositive neurons are common and abundant in postmortem AD brains. a–d) Sections through AD brains showing that IgG-positive and -negative (arrows) neurons can coexist in the same brain region. The intensity of neuronal immunolabeling in AD brains is variable, in part dependent on the extent of postmortem changes. In neurons, IgG is localized to the cell body and main dendrite trunk. In the cerebral cortex, IgG labeling is selective for pyramidal neurons; other smaller local neurons and glial cells are generally IgG-negative. e) Section through the hippocampal region of an AD brain showing numerous IgG-positive neurons. f) Section through the cerebral cortex of an age-matched control brain showing only a few, weakly IgG-positive neurons and the vast majority of neurons IgG-negative (arrows). Bar = 50 μm.

examined, both astrocytes and pyramidal neurons were IgG-positive, but this was not observed in any of the age-matched control brains.

Neurons containing intracellular $A\beta_{42}$ deposits are also IgG-immunopositive in AD brains

To examine a possible interrelationship between the IgG immunoreactivity and intraneuronal $A\beta_{42}$ deposition in neurons, we probed sections of postmortem AD brain tissue with antibodies specific for human IgG and $A\beta_{42}$. In regions of the cerebral cortex (Fig. 2a, b) and hippocampus (Fig. 2c, d) showing mild AD pathology (i.e., with sequestered intraneuronal $A\beta_{42}$ deposits but few amyloid plaques), most $A\beta_{42}$-immunopositive neurons were also IgG immunopositive. Figure 2e, f show consecutive sections (with a number of neurons appearing in both sections) immunostained to reveal the relative distribution of $A\beta_{42}$ and IgG in the cerebral cortex of an AD brain with mild AD pathology. IgG and $A\beta_{42}$ were colocalized in many (but not all) neurons appearing in both sections (Fig. 2e, f). The reason for this discrepancy is not clear, but it may be that IgG is sensitive to post-mortem changes, since both IgG and $A\beta_{42}$ were consistently co-localized in cultured mouse neurons as described below. Nevertheless, the temporal and spatial coincidence of intraneuronal $A\beta_{42}$ deposition and IgG immunolabeling within pyramidal neurons in AD brains suggests that IgG binding to neurons and intracellular $A\beta_{42}$ deposition may be mechanistically linked in these cells.

Brain-reactive autoantibodies are ubiquitous in human sera and immunoreactive with rodent brain proteins

Consistent with the presence of IgG-immunopositive neurons in postmortem AD brains, we have previously shown using western analysis that brain-reactive autoantibodies are nearly ubiquitous in human sera, and that their presence in sera is apparently independent of age or disease [30]. Here, we have confirmed and extended these studies by similarly testing a larger number of sera from AD patients ($n = 52$, age range 61–97 years), age-matched, non-demented control subjects ($n = 28$, age range 51–86 years) and

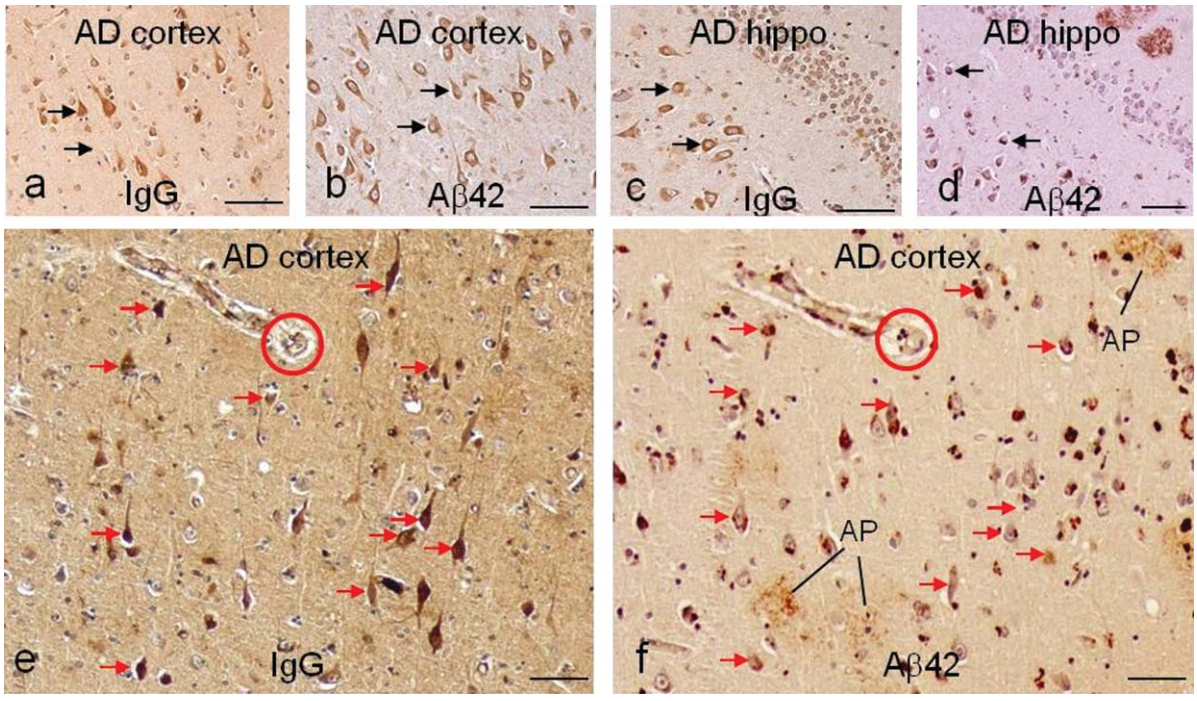

Fig. 2. Neurons containing intracellular $A\beta_{42}$ deposits are also IgG-immunopositive in AD brains. a, b) Sections through the cerebral cortex of an AD brain with mild pathology showing that pyramidal neurons are selectively $A\beta_{42}$- and IgG-immunopositive (arrows). c, d) Sections through the hippocampus of an AD brain with mild pathology showing that hilar neurons (arrows) are both $A\beta_{42}$- and IgG-immunopositive. e, f) Consecutive sections through the cerebral cortex of an AD brain with mild pathology. IgG and $A\beta_{42}$ are colocalized to the same neurons (red arrows) appearing in both sections. Red circles are fiduciary markers. Bar = 100 μm.

younger healthy individuals ($n = 28$, age range 19–30 years). For western analyses, individual sera were tested for the presence of brain-reactive autoantibodies by probing proteins obtained from whole cell homogenate derived from adult rat brain. Results confirmed the presence of brain-reactive autoantibodies in all sera from the three groups tested (Fig. 3). Remarkably, the number of immunoreactive protein bands generated by each serum sample was similar for all three subject groups: mean = 5.1 ± 3.1 for AD sera ($n = 52$); 7.4 ± 4.0 for age-matched control sera ($n = 28$); and 6.0 ± 3.8 for younger healthy control sera ($n = 28$). Comparable results were obtained when human sera were used to probe mouse and human brain proteins as shown previously by [30]. The discovery of brain-reactive autoantibodies in the sera of younger individuals was an unexpected, but consistent, finding, and further work is needed to explain the underlying reasons for their presence in these healthy subjects.

Human brain-reactive autoantibodies also selectively immunolabel the same subset of living neurons in adult mouse brain

To test for the reactivity (i.e., binding) of human serum antibodies with the surfaces of living neurons, we maintained adult mouse brain organotypic (brain slice) cultures (MBOCs) in medium with or without diluted human serum for up to 72 h, although 24 h was chosen for the experiments outlined below. MBOCs can retain the adult brain histological architecture for up to several weeks under proper conditions and contain neurons that can internalize and accumulate exogenous, soluble $A\beta_{42}$ peptide [37, 38, 42, 43]. Immunohistochemistry using anti-human IgG antibodies revealed that addition of human serum to the culture medium for 24 h resulted in intense and selective IgG immunolabeling of living adult mouse neurons, whereas controls not exposed to serum showed no detectable IgG immunoreactivity (Fig. 4a–c). Interestingly, as was shown in postmortem human AD brains described above, pyramidal neurons of the cerebral cortex of MBOCs were consistently the most intensely IgG-immunopositive cells (Fig. 4b, c).

Human autoantibodies increase the internalization of exogenous $A\beta_{42}$ in adult mouse neurons in vitro

As shown above, the abundance of IgG-immunopositive pyramidal neurons in human post-mortem AD brain raises the interesting possibility that binding of autoantibodies to neuron cell surfaces may be mechanistically linked to the internalization of exogenous $A\beta_{42}$ in these cells. To directly test this possibility, we took advantage of the cross-reactivity of the antibodies in human serum with rodent brain proteins as demonstrated above in western blots and in brain tissue in immunohistochemical preparations. MBOCs were

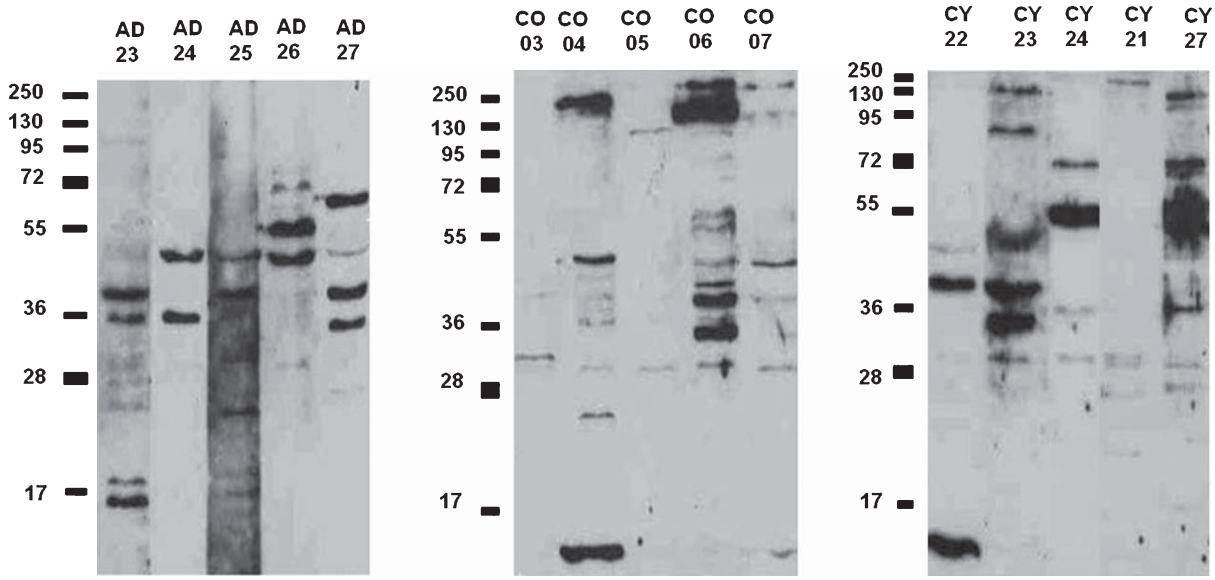

Fig. 3. Brain-reactive autoantibodies are nearly ubiquitous in human sera. Western analysis of sera from AD patients, age-matched, non-demented control subjects (CO) and younger healthy individuals (CY). Individual sera (diluted 1:500) were tested for the presence of brain-reactive autoantibodies by probing proteins obtained from whole cell homogenate derived from adult rat brain. Proteins were separated by 12% SDS-PAGE. Results confirmed the presence of brain-reactive autoantibodies in all sera from the three groups.

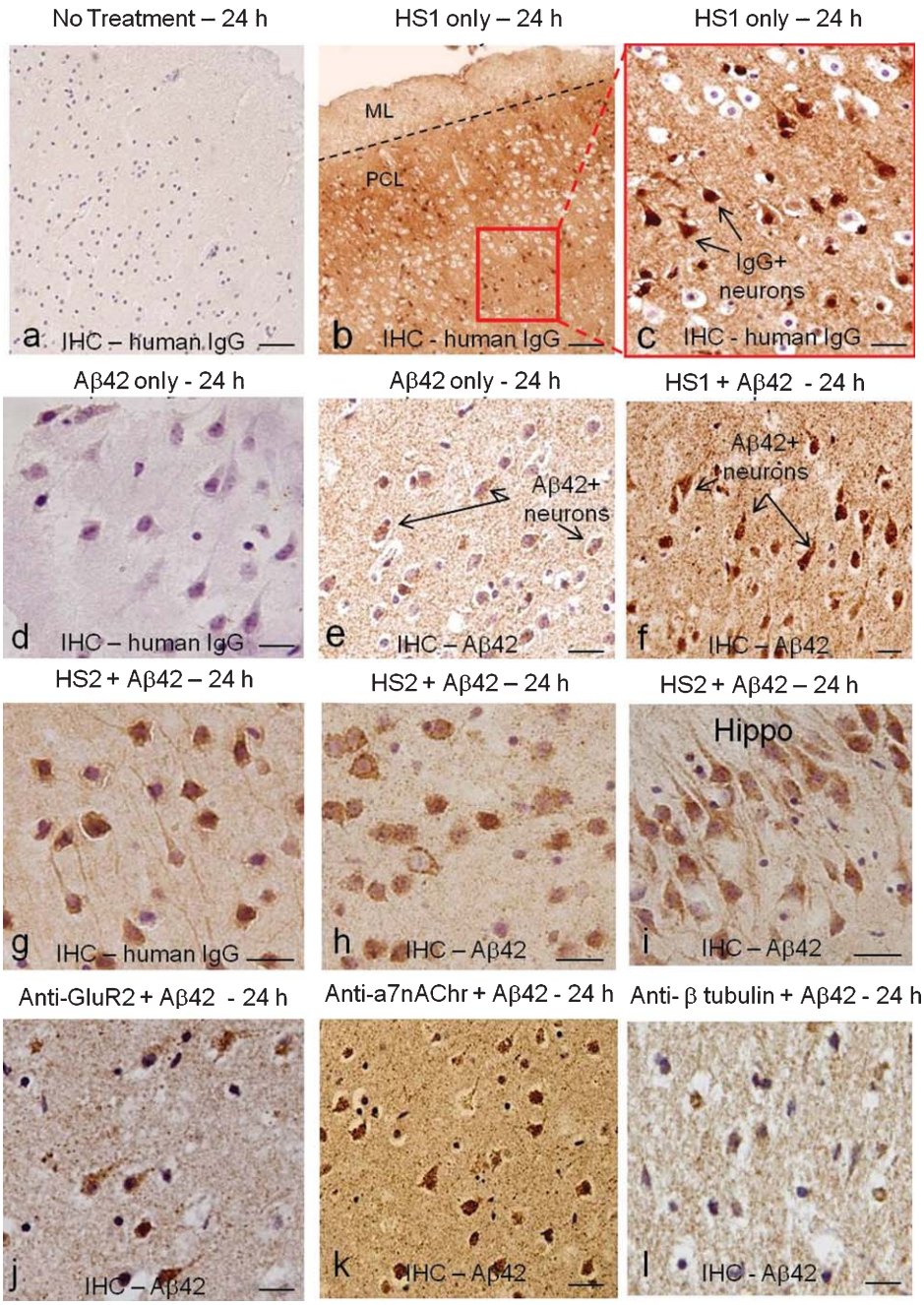

Fig. 4. Human autoantibodies dramatically increase the rate and extent of exogenous $A\beta_{42}$ internalization in neurons in mouse brain organotypic cultures (MBOCs). a) Section of control culture (no treatment) maintained for 24 h showing no detectable human IgG. b) Low magnification view of MBOC (cerebral cortex) treated for 24 h with human serum #1 (HS1) showing intense human IgG-positive immunolabeling of neurons in cortical layers 2–3 and 4–6 (red box). c) Enlarged portion of (b) showing intensely IgG-positive pyramidal neurons of the cerebral cortex. d) Section of MBOC treated with 100 nM $A\beta_{42}$ alone for 24 h showing no detectable IgG immunolabeling of pyramidal neurons. e) Section of MBOC treated with 100 nM $A\beta_{42}$ alone for 24 h showing a low level of $A\beta_{42}$ within pyramidal neurons. f) Section of MBOC treated with 100 nM $A\beta_{42}$ plus human serum (HS1) for 24 h showing a dramatic increase in neuronal $A\beta_{42}$ immunostaining. g, h) MBOCs treated with 100 nM $A\beta_{42}$ plus HS2 for 24 h showing neurons that are both intensely IgG- (g) and $A\beta_{42}$ (h)-positive. i) Hippocampal region of MBOC treated with HS2 plus 100 nM $A\beta_{42}$ for 24 h showing neuronal $A\beta_{42}$ accumulation. j, k) Cortical region of MBOCs treated with polyclonal anti-GluR2 (j) or anti-α7 nAChR (k) antibodies plus 100 nM $A\beta_{42}$ showing increased $A\beta_{42}$ immunostaining compared to sections treated with 100 nM $A\beta_{42}$ alone (e). l) Cortical region of MBOC treated with anti-beta-tubulin antibodies plus 100 nM $A\beta_{42}$ showing low levels of intraneuronal $A\beta_{42}$ immunostaining comparable to that seen in MBOCs treated with 100 nM $A\beta_{42}$ alone. (a+b) Bar = 100 μm; (c–l) Bar = 50 μm.

treated with 100 nM $A\beta_{42}$ in the presence or absence of individual human serum samples diluted 1:50 in otherwise serum-free medium for 24 h, and the relative amounts of intraneuronal $A\beta_{42}$ were quantified using image analysis. MBOCs treated with 100 nM $A\beta_{42}$ alone for 24 h showed no human IgG immunostaining (Fig. 4d) and only minimal $A\beta_{42}$ immunoreactivity (Fig. 4e). On the other hand, when MBOCs were exposed to human serum autoantibodies plus $A\beta_{42}$ peptide for 24 h, pyramidal neurons selectively showed a dramatic increase in intracellular $A\beta_{42}$ accumulation over that of controls treated with $A\beta_{42}$ peptide or serum alone for the same time period (Fig. 4f). Within these neurons, $A\beta_{42}$-positive material was localized to dense cytoplasmic granules that were concentrated in the neuronal perikaryon and proximal dendrite trunk (Fig. 4f). In addition, the increased extracellular $A\beta_{42}$ "background" staining as shown in Fig. 4e, f was highly granular, suggesting that this granularity may reflect increased $A\beta_{42}$ localization to synapses. Measurements of the relative amounts of intraneuronal $A\beta_{42}$ in MBOCs after 24 h of treatment revealed that the addition of human serum to medium containing 100 nM $A\beta_{42}$ caused a sharp increase in neuronal $A\beta_{42}$ immunoreactivity over that in cells treated with $A\beta_{42}$ alone (Fig. 5). Surprisingly, despite this increase, there was no morphological evidence of significant cell death and loss of $A\beta_{42}$-burdened neurons in MBOCs during the 24 h treatment window.

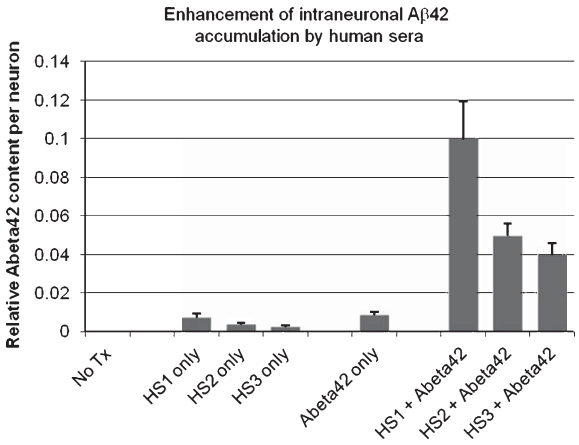

Fig. 5. Graph showing the enhancement of intraneuronal $A\beta_{42}$ deposition mediated by human sera. Relative $A\beta_{42}$ content per immunopositive neuron was calculated using the image analysis program, Cell Profiler. Each bar represents data obtained from analysis of 20 randomly selected images of the cerebral cortex derived from five separate MBOCs. Low levels of intraneuronal $A\beta_{42}$ were detected in neurons exposed to different human sera (HS1, HS2, and HS3) or $A\beta_{42}$ only, whereas treatment of MBOCs with human serum plus 100 nM $A\beta_{42}$ resulted in a dramatic, several-fold increase in the extent of intraneuronal $A\beta_{42}$ deposition ($p < 0.05$). Individual sera showed different potencies with regards to the level of their enhancement of intraneuronal $A\beta_{42}$ deposition.

The extent of serum-mediated enhancement of neuronal $A\beta_{42}$ internalization and accumulation can vary among individual sera

We next asked whether individual human sera exhibit different potencies in terms of their ability to facilitate the accumulation of exogenous $A\beta_{42}$ peptide in neurons. To address this, MBOCs were treated with 100 nM $A\beta_{42}$ with or without one of three serum samples (HS1, HS2, and HS3) at identical dilutions for 24 h as described above. The relative amount of neuronal $A\beta_{42}$ was determined by measuring the amount and intensity of $A\beta_{42}$ immunostaining using image analysis. All three human sera were found to contain brain-reactive autoantibodies with selective affinity for pyramidal neurons (e.g., Fig. 4b, c, g). Comparison of neuronal $A\beta_{42}$ immunostaining revealed that the extent of $A\beta_{42}$ internalization and accumulation in cortical pyramidal neurons varied considerably among the three sera tested, with increases of 11.8-, 5.9-, and 4.7-fold, respectively for HS1, HS2, and HS3 over MBOCs treated for 24 h with 100 nM $A\beta_{42}$ alone (e.g., Figs 4f–h, 5). Neurons in the hippocampal region also showed human serum-mediated enhancement of $A\beta_{42}$ accumulation (Fig. 4i).

Human sera immunolabel neurons in AD brain sections with different potencies that correlate well with their efficacy at enhancing intraneuronal $A\beta_{42}$ deposition in MBOCs

We next examined whether the observed differential potency of individual human sera to enhance intraneuronal $A\beta_{42}$ accumulation in MBOCs is related to the ability of these sera to immunolabel neurons in histological sections of AD brain tissue. To test this, we first identified regions of the cerebral cortex and hippocampus of AD brains that lacked inherent neuronal IgG immunolabeling, and then reacted histological sections taken through these brain regions with each of the three sera mentioned above. As shown in Fig. 6, sections of both the cerebral cortex and hippocampus immunostained with HS1 and HS2 showed a greater IgG immunostaining intensity than similar sections treated with HS3, a result which correlates well with measurements of the relative capacity of each of these sera to enhance intraneuronal $A\beta_{42}$ deposition in MBOCs (c.f., Fig. 5). Also, despite individual differ-

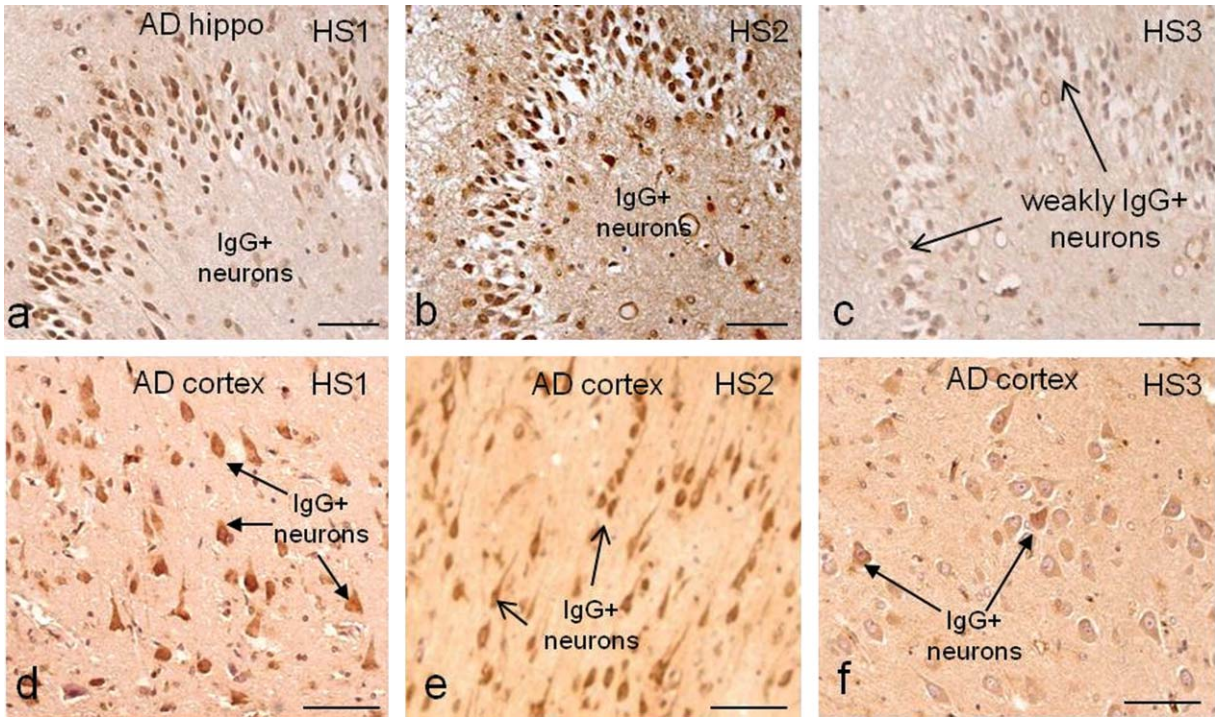

Fig. 6. Human sera exhibit individual differences in the potency of neuronal IgG immunolabeling when these individual sera are used as primary antibodies to probe IgG-deficient regions of AD brains. Sections of the same human AD hippocampus (a–c) and cerebral cortex (d–f) immunostained with sera from three individuals (HS1, HS2, and HS3, respectively), showing marked individual differences in the intensity of neuronal immunolabeling. Although HS1 and HS2 exhibit relatively intense neuronal immunostaining in both brain regions, HS3 shows a much weaker immunostaining of these same brain regions. Bar = 100 μm.

ences in the potency of neuronal immunolabeling, the specific neuronal subtypes that were IgG immunopositive were consistent among three serum samples tested (Fig. 6a–f).

Exposure of adult mouse brain neurons to purified antibodies targeting abundant neuronal surface proteins resulted in a comparable enhancement of intraneuronal $A\beta_{42}$ accumulation in vitro

The above findings have led us to propose a generalized mechanism whereby autoantibodies that target essentially any antigen exposed on the surface membrane of a neuron can potentially facilitate and enhance the internalization and accumulation of exogenous $A\beta_{42}$ within neurons in the brain. To test this possibility, MBOCs were treated for 24 h with commercially available antibodies directed against two neuronal receptors that are known to be abundantly expressed on neuronal cell surfaces, α7 nAChR and the glutamate R2 (GluR2) receptor. Both antibodies were found to be effective in increasing intraneuronal $A\beta_{42}$ accumulation, again selectively in pyramidal neurons and well above levels seen in cultures treated with $A\beta_{42}$ alone (Figs 4j, k, 7). To explore whether neuronal cell surface reactivity of IgG is required for enhancement of exogenous $A\beta_{42}$ internalization, we also treated MBOCs with an antibody directed against the common intracellular protein, beta-tubulin, along with 100 nM $A\beta_{42}$. Treatment with beta-tubulin antibody resulted in levels of neuronal $A\beta_{42}$ accumulation that were comparable to treatment with 100 nM $A\beta_{42}$ alone (Figs 4l, 7).

DISCUSSION

The results of the present study highlight the potentially important role of autoimmunity and blood-borne, brain-reactive autoantibodies in the pathogenesis of AD. The major findings of this study are the demonstration that brain-reactive autoantibodies are essentially ubiquitous in the blood, independent of age or the presence of disease, and that these autoantibodies can enhance $A\beta_{42}$ peptide deposition, a pathological hallmark of AD, within the same neurons in adult

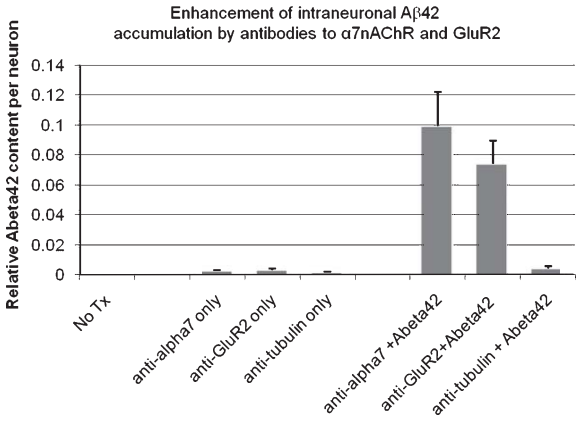

Fig. 7. Graph showing an enhancement of intraneuronal $A\beta_{42}$ deposition mediated by antibodies to α7 nAChR and GluR2. Relative $A\beta_{42}$ content per immunopositive neuron was calculated using Cell Profiler. Each bar represents data obtained from analysis of 20 randomly selected images of the cerebral cortex derived from five separate MBOCs. Low levels of intraneuronal $A\beta_{42}$ were detected in neurons exposed only to antibodies specific for α7 nAChR, GluR2 and tubulin. However, addition of 100 nM $A\beta_{42}$ to each of the antibody treatment groups resulted in a marked increase in intraneuronal $A\beta_{42}$ deposition in all but the anti-tubulin group ($p < 0.05$).

mouse brains that are well-known to harbor extensive intracellular $A\beta_{42}$ deposits in human AD brains. These results suggest that a defective and permeable blood-brain barrier (BBB), which has been linked to a number of neurodegenerative diseases including AD, allows blood-borne, brain-reactive autoantibodies to access the brain interstitium, bind selectively to exposed neuronal surface targets and enhance intraneuronal deposition of Aβ peptides, especially $A\beta_{42}$, in AD brains. Thus, we propose that the spectrum of brain-reactive autoantibodies and the specific location and extent of the BBB breakdown are important risk factors for the initiation and/or progression of neurodegenerative diseases, including AD.

IgG-immunopositive neurons are abundant in regions of AD pathology in postmortem AD brains

Several previous studies have reported the presence of IgG-positive neurons in postmortem AD brain tissue [28, 30]. Here, we show that IgG-immunopositive neurons, especially pyramidal cells, are abundant in the cerebral cortex and hippocampus of AD brains and particularly prevalent in brain regions displaying AD-associated pathological features, including intraneuronal $A\beta_{42}$ deposition and amyloid plaques. These observations imply the common occurrence of BBB breakdown in regions of evolving and ongoing AD pathology. Further, they suggest that neurons are chronically accessible to brain-reactive autoantibodies that leak into the brain tissue through a defective and permeable BBB in the living brain.

Brain-reactive autoantibodies are ubiquitous in human sera and can react with neurons in the brain

Our previous study has demonstrated the widespread presence of BBB compromise in AD brains through detection of the telltale extravasation of plasma components, including Aβ peptides, complement proteins and immunoglobulins (e.g., IgG) and their entry into the surrounding brain tissue [35]. The consistent finding of BBB breakdown in AD brains naturally points to the blood as the source of brain-reactive autoantibodies. Supporting this possibility, several previous studies have reported the presence of brain-reactive autoantibodies in the serum of AD patients [27, 33, 44–46]. More recently, we [30] have provided evidence that brain-reactive autoantibodies may be ubiquitous in human sera, with their presence in sera unexpectedly independent of age or the presence or absence of disease. In the present study, we have extended this work by increasing the number of serum samples that were evaluated using western analysis. Results confirm our earlier findings, including the demonstration that the average number of brain-reactive autoantibodies in human sera, surprisingly, is similar among AD patients, age-matched, neurologically normal controls and younger healthy individuals. In addition, we show here that, when human sera are allowed to react with histological sections taken through AD brain regions that happen to lack inherent neuron-bound IgG, these sera immunolabel the same neuronal subtypes that are IgG-immunopositive in postmortem AD brains. Lastly, we show that individual sera exhibit marked differences in their potency for neuronal immunolabeling in these sections. Such differences invite the prediction that, under conditions of otherwise equal BBB compromise, individuals possessing serum capable of a more robust neuronal immunostaining in histological sections would be at greater risk for earlier disease initiation and/or a later increased rate of disease progression.

Brain-reactive autoantibodies have been linked to neurological and neuropsychiatric diseases

The presence and abundance of autoantibodies on the surfaces of neurons in regions of AD brains show-

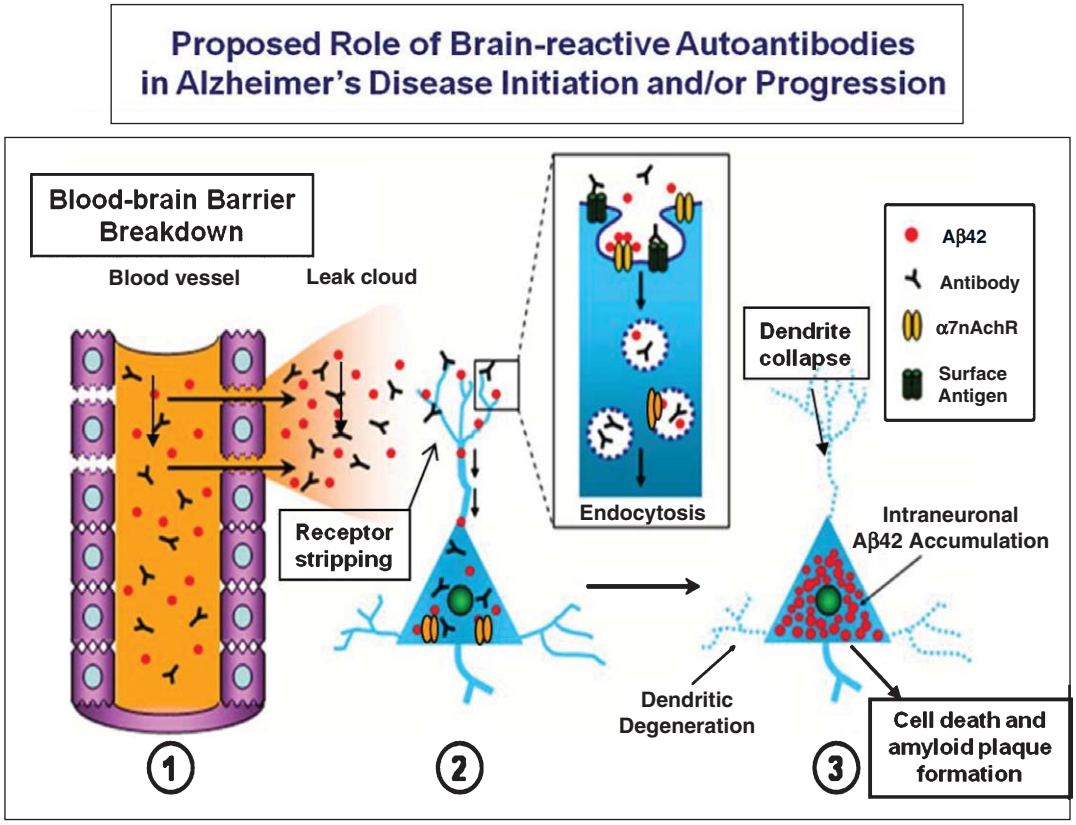

Fig. 8. A generalized mechanism for AD pathogenesis with two major contributing factors: breakdown of the blood-brain barrier (BBB) and the presence of neuron-binding autoantibodies in the serum. In STEP 1, breach of the BBB results in chronic extravasation of plasma components including autoantibodies and soluble $A\beta_{42}$ peptide. Autoantibodies bind to targets on neuronal surfaces; soluble exogenous $A\beta_{42}$ has selective affinity for the surfaces of neurons that express the alpha7 nicotinic acetylcholine receptor (a7 nAChR). In STEP 2 , neurons continually attempt to clear their surfaces of bound autoantibodies by receptor-mediated endocytosis, which drives internalization of autoantibodies and $A\beta_{42}$ and stripping of other key cell surface membrane proteins, both of which jeopardize neuronal function. Neurons attempt to replace lost receptors, which increases metabolic stress. Endocytic vesicles fuse with lysosomal elements. Autoantibodies are readily degraded within lysosomes, but $A\beta_{42}$ is non-degradeable and thus progressively accumulates over time. In STEP 3 , excessive intaneuronal $A\beta_{42}$ accumulations lead to inability of neurons to support their large dendrite trees, causing dendrite collapse, synaptic loss and eventual cell death. Local release of intraneuronal $A\beta_{42}$ contributes to the formation of amyloid plaques.

ing pathological changes raises the possibility that they are involved in at least some of these changes associated with AD. Indeed, an abundant literature strongly supports the potential involvement of serum antibodies in a wide variety of neurological diseases and syndromes (reviewed in [47]). For example, schizophrenia has been linked to the appearance of a number of different antibodies including anti-cardiolipin [48, 49], anti-nuclear protein [50], anti-DNA [51], and anti-histone antibodies [52, 53] as well as antibodies directed against several different types of neuronal surface receptors [54, 55]. Neuron-binding autoantibodies have also been detected in sera after streptococcal infections, particularly in individuals that later develop obsessive compulsive disorder, Sydenham's chorea, Tourette syndrome, pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection (PANDAS), and Hashimoto's encephalopathy [56–59]. They are also found in sera from elderly patients with systemic lupus erythematosus (SLE) that exhibit cognition and memory loss [60, 61]. In fact, in SLE, neurological problems are detected in nearly 75% of elderly patients including seizures, various degrees of cognitive dysfunction and psychopathology [62–64]. Diamond and coworkers have shown that, in some SLE patients, a subset of DNA-specific antibodies crossreacts with the NR2 a and NR2 b subunits of the N-methyl-D-aspartate receptor (NMDAR) [61, 65]. They have also shown that binding of this antibody activates NMDAR in mouse brain slices *in vitro* and elevates calcium levels in neurons that promote excitotoxic cell death.

Brain-reactive autoantibodies are normally confined to the blood, but can gain access to neurons in the brain through local defects in the BBB

Our results demonstrating the binding of serum IgG to neurons in the AD cerebral cortex compliments previous work by others implicating cerebrovascular compromise in AD pathogenesis [24–26] and points to one's brain-reactive autoantibody profile and BBB integrity as factors of major importance to the initiation and progression of neurodegenerative diseases. Our observation of the widespread presence and abundance of IgG-positive neurons in postmortem AD brains implies that autoantibodies are able to chronically gain access to neurons in the brain through a compromised BBB. The normal function of the BBB is to restrict the entry of soluble molecules, including immunoglobulins, and blood-borne cells into the brain parenchyma [22, 23]. We have previously reported that the vast majority (possibly all) of AD brains exhibit morphological evidence of BBB breakdown in the form of perivascular plasma leak clouds containing antibodies, complement and soluble Aβ peptides such as $A\beta_{42}$ [35]. In the present study, our data suggest that a defective BBB plays a key role in the initiation and/or progression of AD. Although, for obvious reasons, it is difficult to demonstrate this directly in the human brain, there is some supporting evidence for a similar mechanistic scenario in mice. For example, when mice expressing NMDAR-specific antibodies are treated with lipopolysaccharide to disrupt the BBB, they show an influx of antibodies and loss of neurons in the hippocampus [60]. Similarly, our previous study has shown that little or no blood-borne, fluorescent (FITC)-labeled $A\beta_{42}$ enters into the brain in healthy mice with an intact BBB. However, BBB disruption induced by injection of *Pertussis* toxin resulted in an influx of $A\beta_{42}$ into the mouse brain interstitium and selective binding and accumulation of this peptide within cortical pyramidal neurons [35]. Thus, it is reasonable to expect that a chronic BBB compromise in elderly humans would have a similar outcome.

Brain-reactive autoantibodies and BBB permeability - a "two hit" scenario for neurodegenerative disease

Unexpectedly, we found that brain-reactive autoantibodies are also common in the blood of younger healthy individuals, and sera from many of these individuals show robust neuronal immunolabeling comparable to sera from elderly, nondemented, and AD patients. It is reasonable to speculate that many of the younger healthy and older non-demented individuals are currently free of detectable pathology because they possess an intact BBB that prevents access of their complement of blood-borne, brain-reactive autoantibodies to neurons in the brain. Accordingly, we are also led to predict that younger individuals with robust brain-reactive autoantibodies may be particularly prone to express neurological sequelae in clinical situations that cause transient or permanent BBB compromise, such as head trauma or certain infections that can secondarily render the BBB permeable. Likewise, it might also be expected that the presence of robust brain-reactive autoantibodies in the sera of elderly individuals with relatively poor vascular health would predispose these individuals to neurological complications that are exacerbated by the chronic binding of autoantibodies to targets (especially neurons) in the brain. Additionally, since many brain neurodegenerative diseases are accompanied by inflammation that could jeopardize the integrity of the BBB [12], a compromised BBB may play a key and hitherto generally unappreciated role in the progression of many brain neurodegenerative diseases. If this proves to be the case, it becomes even more critical to identify factors that can modulate the integrity and permeability of the BBB with respect to transport of brain-reactive autoantibodies and Aβ peptides into the brain tissue. Certainly, vascular pathologies within the brain, such as cerebrovascular amyloidosis, would be expected to have a negative influence on BBB integrity [15, 66, 67]. Lastly, the requirement for BBB permeability would explain why the presence of brain-reactive autoantibodies in the sera alone would not necessarily correlate with CNS disease. It also would provide a reasonable explanation for the preferential expression of these diseases in the elderly, who are more vulnerable to microvascular disease and consequent BBB compromise.

Distinguishing "cause" and "effect" autoantibodies

The disease initiating scenario described above suggests that autoantibodies that react with certain abundant targets exposed on the surfaces of neurons are more likely to play a key role in the initial phases of disease pathology than those binding to more rare or cytoplasmic targets. On the other hand, other brain-reactive autoantibodies associated with neurodegenerative diseases may arise secondarily as

a consequence of ongoing pathology. For example, the production of specific autoantibodies has been reported in many neurodegenerative diseases including Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis, Guillain-Barre syndrome, chronic peripheral neuropathy, optic neuritis, vascular dementia, and AD [44, 47, 68]. In AD, studies have demonstrated a link between this disease and the presence of high titers of autoantibodies directed against cytoplasmic proteins that include aldolase, the heavy neurofilament subunit, histones, tubulin, glial fibrillary acid protein, and S-100 [44, 68–70]. The identification of disease-specific, "secondary" antibodies would certainly have clear benefits relating to their potential use as diagnostic biomarkers for confirmation of the disease. However, the detection of these autoantibodies would likely be limited to individuals with the disease already well underway. This is especially true for AD, where symptoms that bring the patient to the doctor's office do not generally emerge until the pathology has already been well underway for years or perhaps even decades [71]. On the other hand, the identification of autoantibodies that play a role in the initiation phases of disease could be of possible therapeutic benefit. Not only could these autoantibodies be used to identify individuals at risk for developing AD prior to disease initiation, but they may also provide future therapeutic targets when strategies become available for selectively removing or suppressing the production of specific immunoglobulins.

Human antibodies accelerate the rate of exogenous $A\beta_{42}$ internalization in adult mouse neurons

Aβ accumulation within neurons has been shown to occur before the appearance of amyloid plaques in both human and transgenic mouse brains, and there is evidence that the eventual death and lysis of $A\beta_{42}$-overburdened neurons can lead to the appearance of amyloid plaques [6, 8, 17, 72, 73]. The factors that cause certain types of brain neurons to begin to accumulate $A\beta_{42}$ in the elderly and throughout the course of AD are unknown. In the present study, we show that many pyramidal neurons in the cerebral cortex of AD brains are both IgG- and $A\beta_{42}$-immunopositive, a coincidence suggesting that these two phenomena may be mechanistically linked. To investigate a possible relationship between neuronal IgG binding and Aβ-related pathology, we took advantage of the cross-reactivity of human serum autoantibodies with comparable neurons in mouse brain and tested the effects of these human autoantibodies on intraneuronal deposition of soluble, exogenous $A\beta_{42}$ peptide in adult mouse neurons *in vitro* (in organotypic brain slice cultures). Our results demonstrate here that binding of human IgG autoantibodies to mouse neurons dramatically increased the rate and extent of neuronal $A\beta_{42}$ accumulation in neurons in the mouse cerebral cortex and hippocampus. Not only does this finding suggest that the binding of brain-reactive autoantibodies can enhance intraneuronal $A\beta_{42}$ deposition many-fold, it also suggests that the influx of soluble exogenous Aβ peptide from the blood may be a major and chronic source of the intraneuronal $A\beta_{42}$ that accumulates within pyramidal neurons throughout the course of the disease. In support of this latter possibility, our previous study has shown that intravascular $A\beta_{42}$ can deposit within pyramidal neurons in the adult mouse brain when the integrity of the BBB is transiently compromised by treatment with *Pertussis* toxin [35]. More recently, we have found that, under conditions of chronic or long-term (i.e., 3 months) BBB compromise via *Pertussis* toxin, pyramidal neurons in the brains of 9–12 month old mice accumulate intravascularly administered $A\beta_{42}$, and behavioral studies have shown that these mice exhibit long-term memory deficits (Manuscript in Preparation). The means by which the binding of autoantibodies to neurons enhances or facilitates the internalization of $A\beta_{42}$ is unknown. However, it is reasonable to propose that this occurs through chronically increased endocytosis, stimulated by the incessant autoantibody-mediated crosslinking of membrane-associated cell surface targets, and representing an attempt on the part of the cell to continually clear surface-bound Ig entering into the brain interstitium through a defective and permeable BBB. Additional evidence for the role of endocytosis in neuronal $A\beta_{42}$ accumulation comes from the recent demonstration that Low-Density Lipoprotein Receptor-Related Protein 1 (LRP1) may be involved in uptake and accumulation of $A\beta_{42}$ in neuronal lysosomes [74]. This suggests that there may be more than one mechanism originating at the cell surface that can induce neuronal endocytosis and contribute to neuronal $A\beta_{42}$ accumulation.

Conclusions and perspectives

A working model for the proposed role of brain-reactive autoantibodies in AD initiation and progression is presented in Fig. 7. In this model, local BBB breakdown allows chronic access of blood-borne, brain-reactive autoantibodies and $A\beta_{42}$ to the brain

tissue in proportion to the extent of the leak. Soluble $A\beta_{42}$ preferentially accumulates on the surfaces of certain subtypes of neurons, including those expressing the $\alpha 7$ nAChR for which $A\beta_{42}$ has been shown to have strong binding affinity [15, 75–79]. The latter provides a possible explanation for the fact that AD shows some selectivity for affecting cholinergic and cholinoceptive pathways. We suggest that binding of autoantibodies to neuronal cell surfaces sets into motion a mechanism aimed at clearing surface-bound antibody via antibody-induced endocytosis that also facilitates the chronic internalization and accumulation of cell surface-associated $A\beta_{42}$. Indeed, it has long been known that antibody-mediated crosslinking of antigen targets on cell surfaces can induce endocytosis [80–82]. Thus, under conditions of chronic BBB compromise and antibody binding, it might be expected that autoantibodies and their antigen targets would be continually stripped from the cell surface and internalized, with the resulting endocytic vesicles eventually fusing with elements of the lysosomal compartment for degradation [43, 83–85]. Under these conditions, intraneuronal $A\beta_{42}$ accumulations would increase over time by virtue of the fact that $A\beta_{42}$ is largely non-degradeable within the lysosomal compartment due to its tendency to self-assemble into insoluble fibrils [86–88]. Chronic binding of autoantibodies to cell surfaces could also have dire consequences to the functional integrity of neurons, since their function depends on maintaining the proper number of specific cell surface membrane proteins and receptors. In the long-term, increased endocytosis would result in a chronic loss of surface receptors (i.e., receptor stripping) that would not only impair neuronal function, but would also induce affected cells to attempt to replace lost receptors, thus imparting additional metabolic stress on neurons that could further escalate the pathology and exacerbate the disease.

The mechanistic scenario presented here can account for many previously unexplained features of AD; e.g., its clear association with aging, its delayed onset in active and healthy individuals (individuals presumably in good microvascular health with an intact BBB) and its strong association with vascular trauma (as in cases of stroke or head impact injuries). It also provides a plausible explanation for the fact that elderly individuals with autoimmune diseases such as SLE often exhibit a pronounced, AD-like decline in memory and cognition. The clinical importance of our results lies in the verification of autoimmunity as an important causative factor in the initiation and/or progression of AD as well as other neurodegenerative diseases, and the recognition that some autoantibodies and individual autoantibody profiles may pose a greater disease risk than others in terms of their potential to contribute to disease initiation and/or progression. Conceivably, an autoantibody targeting any exposed neuronal cell surface protein could be effective in mediating the internalization and accumulation of exogenous, cell surface-associated $A\beta_{42}$ in neurons. In support of this, we show here that antibodies directed against the specific, abundant neuronal surface membrane proteins, $\alpha 7$ nAChR and GluR2, are also capable of dramatically increasing the rate and extent of neuronal $A\beta_{42}$ accumulation in the same neurons in adult mouse brain that accumulate $A\beta_{42}$ in AD brains. Evidence that the mechanism described above could contribute directly to dementias, including AD, is also provided by the phenomenon of paraneoplasia, where the presence of a cancer leads to the production of an autoantibody that happens to target the nervous system, including specific neurons in the brain. It is probably no coincidence that the best examples of this involve autoantibodies directed against the nAChRs [89–91]. Our data suggests that, under conditions of BBB breakdown, the influx of paraneoplastic nAChR autoantibodies into the brain would enhance intraneuronal $A\beta_{42}$ deposition selectively in neurons that express this receptor. Given this mechanism, the rate at which AD progression occurs in patients would depend, at least in part, on the identity and titer of the autoantibody(s) in their blood, the extent and specific brain location of the BBB breach, and the relative abundance of the target protein on the surfaces of vulnerable neurons in the vicinity of the vascular leak.

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