IgA NMDA receptor antibodies are markers of synaptic immunity in slow cognitive impairment

H. Prüss, MD*
M. Höltje, PhD*
N. Maier, MD
A. Gomez, MD
R. Buchert, PhD
L. Harms, MD
G. Ahnert-Hilger, PhD
D. Schmitz, MD
C. Terborg, MD
U. Kopp, PhD
C. Klingbeil
C. Probst, PhD
S. Kohler, MD
J.M. Schwab, MD, PhD
W. Stoecker, MD
J. Dalmau, MD, PhD
K.P. Wandinger, MD*

Correspondence & reprint requests to Dr. Prüss: harald.pruess@charite.de

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Supplemental Data

ABSTRACT

Objective: To report that antibodies to synaptic proteins may occur in association with slow, progressive cognitive decline.

Methods: A total of 24 patients with progressive cognitive dysfunction of unclear etiology were examined for onconeural and synaptic receptor antibodies. The effect of serum was examined in cultures of dissociated mouse hippocampal neurons.

Results: Seven patients had immunoglobulin A (IgA), but no immunoglobulin G (IgG), antibodies against NMDA receptor (NMDAR). Anti-NMDAR IgA positive patients' serum, but not serum from control individuals, caused dramatic decrease of the levels of NMDAR and other synaptic proteins in neurons, along with prominent changes in NMDAR-mediated currents. These effects correlated with the titer of IgA NMDAR antibodies and were reversed after removing patients' serum from the culture media. When available, comprehensive clinical assessment and brain metabolic imaging showed neurologic improvement after immunotherapy.

Conclusions: A subset of patients with slowly progressive cognitive impairment has an underlying synaptic autoimmunity that decreases the density of NMDAR and other synaptic proteins, and alters synaptic currents. This autoimmunity can be demonstrated examining patients' serum and CSF for NMDAR IgA antibodies, identifying possible candidates for immunotherapy. *Neurology*® 2012;78:1743-1753

GLOSSARY

ACSF = artificial CSF; **AD** = Alzheimer disease; **FDG** = fluorodeoxyglucose; **FTLD** = frontotemporal lobe degeneration; **GABA** = γ -aminobutyric acid; **GM** = gray matter; **IgA** = immunoglobulin A; **IgG** = immunoglobulin G; **LBD** = Lewy body disease; **NMDAR** = NMDA receptor; **NMDAR-AI** = NMDAR antibody index; **PE** = plasma exchange; **sEPSC** = spontaneously occurring excitatory postsynaptic current; **VGLUT1** = vesicular glutamate transporter-1; **VOI** = volume of interest; **WM** = white matter.

The forming of new memories, attention, and planning require proper neuronal activity in frontal and temporal regions that largely depends on normal fine-tuned ion channel activation and distribution in the brain. For example, dysregulation of one type of excitatory glutamatergic receptors, the synaptic NMDA receptor (NMDAR), can contribute to neuropsychiatric disorders such as dementia or schizophrenia.¹⁻³ Recent studies show that in patients with anti-NMDAR encephalitis the immunoglobulin G (IgG) antibodies cause internalization of NMDAR resulting in a significant decrease of these receptors at synapses.⁴ These patients develop a characteristic clinical picture including psychiatric symptoms, decreased levels of consciousness, hypoventilation, epileptic seizures, and dyskinesias. After the acute stage of the disease patients are often left with chronic frontal and temporal lobe dysfunction (impulsivity,

*These authors contributed equally to this work.

From the Department of Neurology (H.P., L.H., U.K., S.K., J.M.S.), Institute for Integrative Neuroanatomy (M.H., G.A.-H.), Neuroscience Research Center (N.M., D.S.), Department of Psychiatry (A.G.), and Department of Nuclear Medicine (R.B.), Charité University Medicine Berlin, Berlin; Cluster of Excellence (D.S.), NeuroCure and Bernstein Center for Computational Neuroscience Berlin (BCCNB), Berlin; DZNE—German Center for Neurodegenerative Diseases (D.S.), Berlin; Department of Neurology (C.T.), Asklepios Hospital St. Georg, Hamburg; Institute for Experimental Immunology (C.K., C.P., W.S., K.P.W.), affiliated to Euroimmun, Lübeck, Germany; Department of Neurology (J.D.), University of Pennsylvania, Philadelphia; Institut Catalana de Recerca i Estudis Avançats (ICREA) at Institut d'Investigació Biomèdica August Pi i Sunyer (IDIBAPS) (J.D.), Service of Neurology, Hospital Clínic, University of Barcelona, Spain; and Institute for Neuroimmunology and Clinical MS Research (K.P.W.), Center for Molecular Neurobiology Hamburg (ZMNH), University Medical Center Eppendorf, Hamburg, Germany. H.P. is currently affiliated with the Division of Immunology, Harvard Medical School, Boston, MA.

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behavioral disinhibition, poor memory, attention, and planning) that may progressively improve until full or partial recovery.^{4–6} This and other recently identified autoimmune disorders of memory and cognition associated with antibodies against synaptic proteins (AMPA, GABA[B] receptors, mGluR5, LGI1, and Caspr2 proteins) strengthen the concept that autoimmunity can result in progressive cognitive dysfunction.^{7–10} Demonstration of novel antibodies to synaptic proteins can be used as a diagnostic tool to uncover autoimmune, potentially treatable, disorders that otherwise would be considered primary degenerative diseases with limited therapeutic options.¹¹ Here, we report a subset of patients with slowly progressive cognitive decline associated with a humoral immune response that alters the density of several synaptic proteins and synaptic currents. We also show that determination of immunoglobulin A (IgA)-NMDAR antibodies could be used as a serologic test to uncover this autoimmune mechanism.

METHODS Patients. In 2009, the identification of a patient with cognitive dysfunction and IgA-NMDAR antibodies who substantially improved with immunotherapy (see index patient) led to investigate these antibodies in serum and CSF of 23 additional patients with progressive cognitive decline of unclear etiology (15 women). Of these 23 patients, 6 were found to have IgA-NMDAR antibodies. This finding further extended our studies to examine the serum or CSF of 238 individuals, including 75 healthy individuals and 163 patients with several disorders: 70 had neuropsychiatric diseases (15 schizophrenia, 20 bipolar disorder, 20 multiple sclerosis, 15 amyotrophic lateral sclerosis), 29 well-defined dementia (10 Alzheimer disease [AD], 10 Lewy body disease [LBD], 9 frontotemporal lobe degeneration [FTLD]), and 64 anti-NMDAR encephalitis (IgG antibodies to NMDAR-NR1 subunit). Clinical information was obtained by the authors or referring physicians.

Standard protocol approvals, registrations, and patient consents. Studies were approved by the Charité University Hospital Institutional Review Board and written informed consent was obtained from patients or representatives.

Detection of NMDAR antibodies. Testing for NMDAR antibodies was performed with frozen sections of rat hippocampus and cerebellum and recombinant immunofluorescence with HEK293 cells transfected with NR1 or NR1/NR2b subunits of the NMDAR^{4,12} (figure 1). Other antibodies were examined with HEK cells transfected with GAD65, LGI1, CASPR2, AMPAR, and GABA(B1) receptor. Classic paraneoplastic antibodies (i.e., anti-Hu, -Yo, -Ri, -Ma, -CV2, -amphiphysin) were determined by indirect immunofluorescence and line immunoblot (Euroimmun, Lübeck, Germany). NMDAR antibody index (NMDAR-AI) was calculated as the ratio between the CSF/serum quotient for NMDAR-IgA antibodies, and the CSF/serum quotient for

total IgA. Values >4 were considered as evidence of intrathecal NMDAR-specific IgA antibody synthesis.^{13,14}

Primary hippocampal neurons and Western blot. Cultures of dissected mice hippocampal neurons were obtained as previously reported^{15,16}; 8×10^4 cells/well were plated on coverslips precoated with poly-L-lysine/collagen. Time-matched cells were used for electrophysiology, immunocytochemistry ($n = 3$ patients), and protein isolation for Western blot ($n = 4$ patients) between 10 and 20 days in vitro to allow for full synapse maturation. For ECL-Western Blot, membrane preparations were used^{15,16} and monoclonal antibodies against the NR1 subunit of the NMDAR (1:5,000) and synaptophysin (1:5,000) applied (Synaptic Systems, Göttingen, Germany). Actin polyclonal antiserum (1:2,000) served as control (Sigma, Deisenhofen, Germany).

IgA purification. Patient serum IgA was purified using Jacalin-agarose (Sigma); 500 μ L serum (1:5 in PBS) was incubated with 500 μ L equilibrated lectin-agarose for 5 minutes. After centrifugation, the matrix was washed 10 times with PBS and protein eluted with 800 mM D-galactose. IgA antibody reactivity was confirmed with NR1/NR2 transfected HEK-cells.

Immunocytochemistry. Neurons were fixed with 4% formaldehyde, permeabilized for 30 minutes using 0.3% Triton-X100/PBS, and stained with primary antibodies overnight at 4°C: anti-synaptophysin (monoclonal 1:500), synapsin (rabbit polyclonal 1:500), vesicular glutamate transporter-1 (VGLUT1, rabbit polyclonal 1:2,000), and vesicular γ -aminobutyric acid (GABA)-transporter VGAT (rabbit polyclonal 1:1,000; all Synaptic Systems). After washing in PBS, Alexa 488-/594-conjugated secondary antibodies were applied for 1 hour at room temperature (Molecular Probes, Eugene, OR).

For quantification of synapsin immunosignals, 10–15 view-fields at 40 $\times$ magnification ($350 \times 262 \mu$ m) were evaluated per condition in each individual experiment. Thresholded images were analyzed by Scion Image software (Scion, now Bio-Soft Net) according to intensity and size criteria, and total cells were counted.

PET. PET acquisition was started 40 minutes after IV injection of 250 MBq [F-18]-fluorodeoxyglucose (FDG) and follow-up PET images were coregistered to baseline.¹⁷ Transaxial images were reconstructed and stereotactically normalized (for details see e-Methods on the *Neurology*[®] Web site at www.neurology.org). Each FDG-PET image was compared with corresponding images of a group of 28 normal control subjects on a voxel-by-voxel basis. Only effects in clusters of at least 125 voxels (~ 1 mL) were considered. For direct visualization of changes between baseline and follow-up PETs, voxel-based subtraction was performed (figure 2E).¹⁸

MRI-based volumetry. High-resolution T1-weighted MRIs were segmented and stereotactically normalized (see e-Methods for details).¹⁹ Tissue probability maps for gray matter (GM), white matter (WM), and CSF generated from 662 healthy elderly subjects aged 63–75 years were used.²⁰ Total volume of GM, WM, and CSF was obtained by summing over all voxel intensities. Predefined volumes of interest (VOIs) for frontal, parietal, occipital, and temporal lobe, hippocampus and ventricles were obtained.^{20,21}

Electrophysiologic recordings. Coverslips with age-matched hippocampal cells exposed to human control serum or anti-NMDAR-IgA serum of the index patient (dilution 1:200, 3 days) were transferred to a submerged recording chamber reper-fused with artificial CSF (ACSF) (see e-Methods for details). Recordings were done at 31°C–32°C using a Multiclamp-700A

(A) Immunostaining of HEK cells transfected with the NR1 subunit of NMDAR and probed with patient serum and anti-IgA secondary antibodies. (B) No staining was observed using an anti-immunoglobulin G (IgG) secondary antibody. (C) Nontransfected HEK293 cells served as negative control. Strong immunopositive staining of primate cerebellum (D) and rat hippocampus (E) with IgA-positive serum ($n = 3$), but not with control serum (F). (G) Primary mouse hippocampal neurons were incubated for 3 days with patient serum (1:100 dilution). Following removal of incubation medium cells were cultured for further 4 days with growth serum alone (rescue). A membrane fraction was obtained from harvested cells and processed for Western blotting. Staining against NR1 subunits revealed a strong downregulation of NMDAR following incubation with index patient serum. Removal of patient serum restored initial NMDAR levels. Incubation with control serum (CTL) or media (no additions) had no effect on NMDAR expression. Actin was used for loading control. (H) Example trace illustrating the NMDAR current response following a brief UV flash-triggered glutamate uncaging (arrow) onto the cell soma. Cells had been incubated for 3 days in control serum. (I) Note smaller synaptic NMDAR current as a response to the UV flash stimulus after 3-day exposure to serum from the index patient. (J) Grand averages of NMDAR-mediated synaptic responses triggered to UV flash onset including all cells investigated (black and red for control and index patient serum, respectively). (K, left) Cumulative probability plots showing the peak amplitude distributions of NMDAR-mediated currents following UV pulse-triggered glutamate uncaging. Note the systematic reduction of responses in index patient serum treated cells (red distribution) compared to control ($p = 0.0008$, Kolmogorov-Smirnov test). (K, right) Comparison of group averages demonstrates a ~90% reduction of NMDAR currents upon glutamate uncaging (111.3 ± 19.4 pA vs 11.7 ± 8.1 pA for control and patient sera; $p = 0.0007$, rank sum test; $n = 10$ and $n = 7$ cells, respectively).

Figure 2 Immunoglobulin A (IgA) antibody titers, immunomodulatory treatment, and titer-dependent pathogenic effect of antibodies

(A) Plasma exchange (PE) was most efficient for short-term reduction of serum IgA antibodies (note log scale of antibody titer). (B) Marked atrophy of the temporal lobes in brain MRI at clinical presentation of the index patient (left). Following immunotherapy, no significant increase in brain atrophy was observed during 6 months (right; identical timescale as in A) as judged by MRI-based volumetry of gray matter (GM) and CSF (C, left). (C, right) Compared to healthy age-matched controls, the reduction of GM volume was most pronounced in the hippocampus (detail) and in frontal and parietal cortex areas (orange). (D) The pathogenic effect of the patient serum on synaptic protein expression was largely reversible after PE: cultivated hippocampal neurons were incubated for 3 days with control serum (left), index patient serum before (middle; 1:100 dilution) and after PE (right). Fixed cells were stained for synapsin (arrows indicate synapsin dots). Incubation with serum before PE resulted in a markedly reduced synapsin expression compared with control serum (similar to figure 3B; $p = 0.000008$), while serum after PE exhibited only a very slight reduction in the synapsin immunoreactivity, thus rescuing the effect of the pre-PE serum ($p = 0.000016$). Scale bar: 50 μm . (E) Temporal and frontal brain hypometabolism (FDG-PET) in the index patient and partial improvement following immunotherapy. (a) Transversal slices through the scaled and stereotactically normalized FDG-PET images of the index patient at baseline (before PE) showing frontal and temporal areas of hypometabolism. (b) Comparison of the index patient with stereotactically normalized FDG-PET images of the control group revealed significant hypometabolism (blue) in the frontotemporal brain areas. (c) Transversal images of the index patient immediately after PE (3 weeks after baseline PET in [a]) using the same color table as in (a). (d) Subtraction analysis of changes in scaled FDG uptake from before PE/after PE ([a] – [c]). The cold color table indicates decrease of scaled FDG uptake, whereas the hot color table indicates increase, e.g., arrows pointing to increased metabolism after PE in the temporal region. The subtraction maps are given in units of the SD of change in brain regions with stable FDG uptake and are thresholded at 2.0 SD. The subtraction maps are overlaid to the baseline image. (e) Subtraction analysis of the baseline PET and follow-up imaging 4 months later with increase of metabolism in posterior brain areas.

amplifier (Axon Instruments, Union City, CA). Laser-induced photolysis of caged glutamate was performed in the presence of 20 μM NBQX (Sigma-Aldrich, Germany) and 1 μM gabazine (Biotrend, Germany).

RESULTS Index patient. In December 2009, a 65-year-old woman with a 2-year history of cognitive impairment was admitted for walking difficulties, frequent falls, slight dysarthria, and urine incontinence. At examination, she had poor short-term

memory and marked frontal lobe dysfunction. She had saccadic pursuits with lateral gaze, increased muscle tone, spastic right hemiparesis, and positive Babinski reflexes. Intermittently, she presented with nihilistic thoughts, mistrust and emotional blunting, systematized delusions, acoustic hallucinations, and stereotypy of speech. Brain MRI showed marked temporal and frontal atrophy (figure 2, B and C). CSF showed 3 WBC/ μL , no oligoclonal bands, total

Table Demographic features and symptoms of the 7 patients with serum IgA-NMDAR antibodies^a

Symptoms	Patient 1, F/65 y	Patient 2, F/81 y	Patient 3, M/73 y	Patient 4, F/57 y	Patient 5, F/70 y	Patient 6, F/71 y	Patient 7, M/49 y
Leading symptoms	Cognitive impairment, gait instability, sudden falls, loss of interests	Cognitive impairment, frequent falls, dizziness	Cognitive impairment, disorientation, decreased consciousness	Cognitive impairment, depression	Cognitive impairment, gait instability	Cognitive impairment, loss of interests	Cognitive impairment, speech problems
Duration of cognitive impairment	2 y	3 y	3 mo	5 y	1 y	6 mo	6 mo
Initial working diagnosis by referring physicians	Frontotemporal dementia	Cognitive impairment of unexplained etiology	Cognitive impairment of unexplained etiology	(Depressive) pseudodementia	Hydrocephalus	Dementia of unknown origin	Hashimoto encephalopathy
Psychiatric symptoms	Auditory hallucinations, nihilistic thoughts, systematized delusions		Increased suggestibility, disoriented to time, confabulations, perseverations	Depression was not confirmed with clinical scales	Apathy, depression	Apathy	Apathy, irritability
Neurologic symptoms	Right spastic hemiparesis, primitive reflexes, urine incontinence	Dysarthria, left hemiparesis (from previous stroke), urine incontinence	Absent Achilles tendon reflexes	Normal	Apraxia, increased muscle tone	Urine incontinence, Alzheimer suspected, but no cortical signs	Speech apraxia, increased muscle tone
Seizures	No	Focal	No	No	No	No	No
Diagnostic workup							
CSF	3 WBC, no oligoclonal bands, protein 63.5 mg/dL	0 WBC, no oligoclonal bands, protein 47.3 mg/dL	1 WBC, identical oligoclonal bands in CSF and serum, normal protein	4 WBC, identical oligoclonal bands in CSF and serum, protein 27.8 mg/dL	0 WBC, no oligoclonal bands, protein 26.9 mg/dL	2 WBC, no oligoclonal bands, protein 53.6 mg/dL	1 WBC, no oligoclonal bands, protein 55.9 mg/dL
NMDAR antibodies							
IgA (serum)	1:32,000	1:320	1:32	1:320	1:32	1:100	1:100
IgA (CSF)	1:3.2	ND	ND	ND	ND	1:3.2	1:10
NMDAR-IgA Ab index	<1	—	—	—	—	22.1	43.6
Antineuronal antibodies	Negative for Anti-Hu, anti-Yo, anti-Ri, anti-Ma, anti-CV2, anti-amphiphysin, anti-Lgi1, anti-Caspr2, anti-GAD65, anti-AMPA, anti-GABA _B R						
Neuropsychological testing	Short-term memory loss, deficits of attention, disinhibition	Short-term memory loss, poor attention and planning	Short-term memory loss, MMSE 21/30	Progressive short-term memory loss	Poor attention and planning, MMSE 25/30	Dysexecutive syndrome, short-term memory loss, MMSE 16/30	Progressive short-term memory loss
Dementia markers (CSF)							
Phospho-tau (normal <61 ng/L)	16	42	22.9	114	Not available		
Tau, ng/L (normal 100–300 ng/L)	1,300	2,622	133	726		124	152
Ptau/tau, ng/L (normal approximately 0.15)	0.012	0.016	0.17	0.16			

—Continued

Table Continued

	Patient 1, F/65 y	Patient 2, F/81 y	Patient 3, M/73 y	Patient 4, F/57 y	Patient 5, F/70 y	Patient 6, F/71 y	Patient 7, M/49 y
β -Amyloid 1-42 (normal 680-1,200 ng/L)	652	337	929	2,186		809	
14-3-3, ng/L	Negative	Negative		Slightly positive		Negative	Negative
Cerebral MRI	Frontotemporal atrophy, some cerebellar atrophy	Previous MCA infarction	Normal	Slight global atrophy, unspecific small white matter lesions	Slight global atrophy	Leukoencephalopathy, gliotic white matter changes	Slight global atrophy
EEG	Normal	Slow activity, right focal epileptic discharges	Normal	Normal	Normal	Not performed	Normal
Tumor	No	Suspicious of renal cell carcinoma (CT)	Basal cell carcinoma (tongue)	No	No	No	No
Smoker	No	No	Yes	No	No	No	Yes
Family history of cancer	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Immune treatment (in patients 2-7 independent of the knowledge of IgA NMDAR antibodies)	Methylprednisolone, PE, cyclophosphamide, rituximab	Prednisolone	Tumor resection	Prednisolone (for rheumatoid arthritis)	No	No	Methylprednisolone (for suspected Hashimoto encephalopathy)

Abbreviations: IgA = immunoglobulin A; MMSE = Mini-Mental State Examination; ND = not detected; NMDAR = NMDA receptor; PE = plasma exchange; protein = total CSF protein (normal <45 mg/dL); WBC = white blood cells (normal <5 per μ L).

^a Median age was 70 years (range 49-81); 5 patients were women. IgA antibody index >4 indicates intrathecal synthesis of NMDAR-IgA antibodies.

protein was 63.5 mg/dL, and glucose 127 mg/dL. Tau, phospho-tau, and β -amyloid were normal (table). Tests for paraneoplastic antibodies, HIV, vasculitis, thyroid autoantibodies, chest CT, and whole body 18F-FDG PET were normal or negative. Brain PET showed frontal and temporal hypometabolism exceeding the grade of atrophy, suggestive of a neurodegenerative disease.

Considering that patients with anti-NMDAR encephalitis usually develop frontal and temporal lobe dysfunction, these antibodies were investigated in patient's serum and CSF. IgG-NMDAR antibodies were not detected, but IgA-NMDAR antibodies were detected at 1:32,000 in serum and 1:3.2 in CSF (figure 1).

The patient was treated with immunotherapy (figure 2A) including plasma exchange (PE) and corticosteroids. Clinical findings before and immediately after the 3-week PE period demonstrated dramatic improvement of speech, alertness, muscle spasticity, and reduction of psychiatric symptoms (mistrust, delusions, hallucinations). Moreover, PE resulted in a substantial decrease of the IgA-NMDAR antibody titer (figure 2A, note log scale). This decrease was associated with improvement of cerebral metabolism in temporal brain areas in a PET after PE (figure 2E, arrows). Cyclophosphamide was started and—as antibody titers did not further decline—changed for rituximab. Although the patient's overall condition eventually deteriorated, anterograde memory deficits and cognition did not worsen for about 6 months. In most brain areas, the metabolic activity assessed by PET did not further decline and increased activity was noted in some areas (figure 2E). Additionally, a follow-up brain MRI 6 months after initiation of immunotherapy showed no progression of atrophy (figure 2B).

Detection of anti-NMDAR antibodies of the IgA subclass. Serum samples of the index patient and 6 additional patients showed IgA NMDAR antibodies, but not IgG or IgM antibodies (figure 1). The IgA antibodies of all 3 tested patients reacted strongly with primate cerebellum (figure 1D) and rat hippocampus (E), whereas control samples did not (figure 1F). IgA-NMDAR antibodies were detected at low titer in the CSF of 3 of the 7 positive patients. The IgA antibody index was >4 in 2 patients, demonstrating intrathecal NMDAR-IgA antibody synthesis. Among the 238 control samples IgA NMDAR antibodies were identified in 20 of 64 (31%) patients with anti-NMDAR encephalitis and 1 patient diagnosed with FTLT.

Pathogenic relevance of patients' IgA antibodies. Whereas the presence of anti-NMDAR antibodies of the IgG subtype is well established in anti-NMDAR

encephalitis, and these antibodies are known to be pathogenic,^{4,16,22,23} the relevance of IgA antibodies was hitherto unclear. We therefore extensively examined patients' sera and purified IgA for any possible effects using hippocampal primary cultures. Neurons incubated with patient serum showed a substantial decrease of membrane NMDAR that was titer-dependent, not detectable with control serum, and reversible (figure 1G). These findings suggest that antibodies contained in IgA-NMDAR-positive serum are potentially pathogenic.

Subsequently, we assessed the effects of patients' IgA-NMDAR antibodies using whole-cell recording on neuronal cultures. Neurons from the same preparation (identical synapse maturation) were treated for 3 days with patient or control serum, and NMDAR-mediated currents were evoked by laser-induced somatic uncaging of glutamate (figure 1, H–K). Indeed, patients' antibodies profoundly decreased NMDAR-mediated currents (figure 1K).

The effect of patients' sera and purified IgA was not confined to loss of NMDAR but also affected the expression of other synaptic proteins (figure 3). We detected robust reduction of synaptophysin expression, a standard synaptic marker. Similar to NMDAR, the effect was reversible (figure 3A). Also, incubation with patient serum (but not control sera) resulted in clear reduction of reactivity of the synaptic marker protein synapsin (figure 3B) and the synaptic transporters VGLUT1 and VGAT (figure 3B).

These findings were confirmed using electrophysiologic recordings. In cells treated with control and patient sera we analyzed spontaneously occurring excitatory postsynaptic currents (sEPSCs; figure 3C). Cells treated with patients' serum showed impaired network-driven, spontaneous activity with highly significantly reduced incidence (figure 3D) and mean amplitude of sEPSCs (figure 3E). The disturbed network function after NMDAR downregulation indicates significant impairment of structure and synaptic function in cells exposed to IgA-NMDAR antibody-positive sera.

We also detected presynaptic binding of IgA antibodies as a possible mechanism for changes in synapsin expression (figure 4B). In cultures incubated with control serum, no immunosignal for human IgA antibodies was detectable (figure 4C). Synapsin downregulation was also observed after incubation of hippocampal neurons with purified IgA (figure 4D), thus ruling out involvement of IgG or IgM antibodies.

To determine whether the effect correlated with titer changes during disease course, hippocampal neurons were cultured with serum samples obtained before and after PE of the index patient, during

which the IgA antibody serum titer declined from 1:32,000 to 1:1,000 (figure 2). Indeed, the initial serum reduced the levels of synapsin (arrows) to a markedly greater extent than the serum with lower titer (figure 2, D and E).

Clinical course, imaging findings. The table summarizes demographic features and symptoms of the 7 patients with serum IgA-NMDAR antibodies. The common symptom was an otherwise unexplained cognitive impairment interfering with normal life. Four patients showed signs of generalized cerebral atrophy, most prominent in the index patient with predominant frontotemporal involvement (figure 2). No increase of fluid-attenuated inversion recovery or T2 signal was noted in any of the patients. CSF studies were normal except for increased protein in 4 patients. EEG recordings showed signs of slowing and epileptiform discharges in 1 patient. Two patients had a tumor, detected at the time of neurologic symptom presentation, and the others had negative cancer screening including body CT or PET.

Working diagnoses included frontotemporal dementia (1 patient), cognitive impairment of unexplained etiology (3 patients), hydrocephalus (1 patient), Hashimoto encephalopathy (1 patient), and pseudodementia (1 patient). In the latter patient, analysis of archived serum and CSF obtained 3 years earlier during initial assessment of dementia revealed a serum titer of IgA-NMDAR antibodies of 1:320, similar to the last follow-up. During these 3 years the only neurologic finding was progressive memory loss that was confirmed with repeated neuropsychological testing. Two patients had previous history of autoimmune disorders (rheumatoid arthritis, Hashimoto thyroiditis). None had acute symptoms that would resemble classic anti-NMDAR IgG encephalitis. However, the deficits in the neuropsychological assessment of all 7 patients revealed a frontotemporal (executive) dysfunction that typically occurs during the process of recovery from anti-NMDAR encephalitis. If patients underwent immunotherapy, it was before identification of NMDAR-IgA antibodies (table). Because of this and lack of follow-up of treatment-induced changes of antibody titers, the effects of treatment could not be assessed except for the index patient.

After exclusion of immune-mediated causes, most of the 17 patients initially considered with cognitive decline of unclear etiology were eventually diagnosed with AD or LBD.

DISCUSSION We report in 7 patients the novel association of synaptic autoimmunity with progressive cognitive impairment resembling a primary degenerative disorder. Our findings identify IgA-NMDAR

Figure 3 Effect of immunoglobulin A (IgA)-positive serum is not confined to NMDA receptors (NMDAR)

(A) The same hippocampal cultures used in figure 3A were stained for synaptophysin, a general synaptic vesicle protein. The effects observed were qualitatively the same as observed for NMDAR, namely downregulation after 3 days of incubation with index patient serum and restoration of immunoreactivity following a 4-day rescue with growth medium. (B) Downregulation of synaptic proteins by anti-NMDAR antibodies of the IgA class. Cultured hippocampal neurons were incubated for 3 days with no additions (no add.), control (CTL), or index patient serum (1:100). Fixed cells were stained for the general

antibodies as a serologic marker of this autoimmunity that alters the density of synaptic proteins and synaptic currents. The profound morphologic and electrophysiologic effects of patients' sera and purified IgA in cultured rodent hippocampal neurons strongly argue for an immune-mediated cause of cognitive decline. This hypothesis is supported by data obtained during a close follow-up of symptoms, brain metabolic activity, and serum antibody titers of one of the patients. In this case, a reduction of serum antibodies after plasma exchange resulted in partial clinical improvement, increased metabolic activity in the temporal lobes, and lesser reduction of synaptic proteins of neurons exposed to patient's serum.

While anti-NMDAR encephalitis results from an acute IgG antibody-mediated decrease of NMDAR clusters and synaptic currents, the effects of serum of patients with IgA-NMDAR antibodies are broader, altering not only the density of NMDAR but also other synaptic proteins such as synaptophysin and vesicular transmitter transporters. Alternatively, these effects could be due to the presence of a more extensive repertoire of antibodies against other yet unknown synaptic proteins. Furthermore, while anti-NMDAR encephalitis associates with intense intrathecal synthesis of antibodies, the slow cognitive decline related to IgA-NMDAR antibodies associates with undetectable or low levels of intrathecal antibodies. This suggests a model whereby small amounts of serum IgA antibodies against NMDAR and perhaps other synaptic targets may "leak" through the blood-brain barrier, as it occurs for all types of serum antibodies. The current study shows that 31% of patients with anti-NMDAR encephalitis have both IgG and IgA antibodies in their serum; whether the IgA antibodies are also present in CSF, and the relative contribution to the syndrome, are questions that deserve further study.

Keeping in mind the titer-dependent IgA effect in cultured neurons, small levels of antibodies in the brain likely interfere with neuronal function without causing acute neuronal degeneration or inflammatory changes. This continuous interference with neuronal function and synaptic transmission may

underlie the slowly progressive cognitive impairment of the patients. The presence of NMDARs was recently shown to be required for the development of functional dendritic spines,²⁴ and the downregulation of NMDARs might explain a lack of spine formation resulting in progressive cognitive decline. Therefore the absent or limited brain atrophy in the 6 patients with lower levels of IgA-NMDAR antibodies, compared with the more marked brain atrophy of the index patient who had the highest level of antibodies, might result from the low (even if continuous) IgA titers in the CSF compartment.

Given that IgA-NMDAR was found in the serum of 1 patient with FTLD, we cannot exclude that IgA-NMDAR antibodies in patients with slowly progressive cognitive decline represent a secondary immune response against synaptic proteins modified or released as a result of a primary neurodegenerative process. Yet the pathogenic effect of patients' IgA on synaptic proteins and the absence of IgA-NMDAR antibodies in a substantial number of individuals used as controls argue for a primary immune-mediated disorder, or at least a contributory pathogenic mechanism to a primary neurodegenerative disorder.

The impact of immunotherapy could be assessed in the index patient. A rapid and important reduction of serum antibody levels (from 1:32,000 to 1:1,000) after 10 cycles of PE was associated with improvement of metabolic activity in predominantly temporo-posterior brain regions along with substantial clinical improvement that albeit transient, kept the patient stable, with improved anterograde memory and cognitive functions for 6 months. Although cyclophosphamide and rituximab were used, the antibody levels remained elevated; whether a more intense and persistent reduction of antibodies with more aggressive immunotherapy would have resulted in a sustained improvement remains unknown. This question should be clarified in future studies with larger number of patients. Additionally, these studies should determine the frequency of IgA-NMDAR antibodies in subsets of patients with mild cognitive impair-

synaptic proteins synapsin and synaptophysin (shown in green) as well as for the vesicular GABA transporter (VGAT) and the vesicular glutamate transporter 1 (VGLUT1), markers of inhibitory or excitatory synaptic inputs (shown in red). Incubation with patient serum resulted in a reduced expression of all 4 proteins. Incubation with control serum did not affect expression of the proteins. Insets show the merged details of the boxed areas depicted in the immunofluorescence and phase contrast images. Scale bar: 50 μ m. (C) Example current traces to illustrate differences in spontaneously occurring excitatory postsynaptic currents (sEPSCs) from cells exposed to control serum (left panel) and index patient serum (right panel). Both recordings were performed at -67 mV to isolate excitatory, presumably AMPAR-mediated, synaptic inputs. (D) Group data analysis: cumulative probability plots of the cell-wise averages for sEPSCs. Note the systematic reduction of values from cells incubated with serum from the index patient (red distribution) compared to control (4.0 ± 1.5 Hz vs 1.0 ± 0.4 Hz, $p = 0.02$, Kolmogorov-Smirnov test). (E) Cumulative distributions of sEPSC amplitudes. The systematic negative shift of values corresponds to highly significantly different distributions (57.4 ± 6.2 pA vs 40.1 ± 7.9 pA, $p = 2.3 \times 10^{-5}$, Kolmogorov-Smirnov test; $n = 18$ and $n = 11$ cells for control and patient serum-treated cells).

(A) Double staining of hippocampal neurons with antisynapsin antibodies (red) and human serum followed by antihuman IgA (green). (B) Enlarged insets from A demonstrate colabeling of human IgA antibodies with the presynaptic marker synapsin (arrowheads). (C) In contrast, incubation with control serum (CTL) or media (no add.) did not result in positive staining. (D) Downregulation of synaptic proteins by purified IgA antibodies (1:20) after 3 days of incubation. No effect was observed after incubation with elution buffer (buffer) or control serum (CTL). (D, right) Quantification of synapsin downregulation ($p < 0.001$) and cell counts. No differences in the number of cells were observed.

ment or dementia, and whether antibodies against synaptic targets other than NMDAR are involved.

The current findings have several important implications: 1) synaptic autoimmunity may be responsible not only for acute and severe changes of memory, behavior, and cognition as in encephalitis associated with IgG antibodies to NMDAR, AMPAR,

or GABA(B) receptors, but also for a more chronic, slow, but progressive cognitive decline as the patients reported here; 2) IgA-NMDAR antibody determination can be used as a surrogate marker of the later group of disorders; 3) IgA-NMDAR antibodies can be found in patients with normal CSF; and 4) detection of these antibodies should prompt consideration

of immunotherapy in patients who until now had no specific therapeutic options.

AUTHOR CONTRIBUTIONS

Design or conceptualization of the study: Dr. Prüss, Dr. Dalmau, Dr. Wandinger. Analysis or interpretation of the data: Dr. Prüss, Dr. Hoeltje, Dr. Maier, Dr. Gomez, Dr. Buchert, Dr. Harms, Dr. Ahnert-Hilger, Dr. Schmitz, Dr. Terborg, Dr. Kopp, C. Klingbeil, Dr. Probst, Dr. Kohler, Dr. Schwab, Dr. Stoecker, Dr. Dalmau, Dr. Wandinger. Drafting or revising the manuscript: Dr. Prüss, Dr. Hoeltje, Dr. Maier, Dr. Gomez, Dr. Buchert, Dr. Ahnert-Hilger, Dr. Schmitz, Dr. Klingbeil, Dr. Schwab, Dr. Dalmau, Dr. Wandinger.

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DISCLOSURE

Dr. Prüss, Dr. Hoeltje, Dr. Maier, Dr. Gomez, Dr. Buchert, Dr. Harms, Dr. Ahnert-Hilger, Dr. Schmitz, Dr. Terborg, and Dr. Kopp report no disclosures. C. Klingbeil is a full-time employee of Euroimmun AG. Dr. Probst is a full-time employee of Euroimmun AG. Dr. Kohler and Dr. Schwab report no disclosures. Dr. Stoecker is a full-time employee of and holds stock in Euroimmun AG. Dr. Dalmau has received royalties from a patent re: Ma2 autoantibody test and has patents pending re: NMDA and GABAB receptor autoantibody tests (license fee payments received from Euroimmun AG); and receives research support from funding from Euroimmun AG and the NIH/NCI [RO1CA107192 (PI) and RO1CA89054–06A2 (PI)]. Dr. Wandinger is a full-time employee of and holds stock in Euroimmun AG. **Go to [Neurology.org](#) for full disclosures.**

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