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Case report

A clinical and neurobiological case of IgM NMDA receptor antibody associated encephalitis mimicking bipolar disorder

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ABSTRACT

Autoimmune encephalitis associated with IgG antibodies to the *N*-methyl-D-aspartic acid receptor subunit NR1 (NMDAR) presents with neurological symptoms, such as seizures, and especially psychiatric symptoms, such as hallucinations, psychosis, agitation and anxiety. To date, however, the pathological relevance of IgM NMDAR antibodies remains elusive. Here, we describe clinical, neuroradiological and neurobiological findings of a 28-year-old male presenting with IgM NMDAR antibodies coincident with autoimmune encephalitis characterized by symptoms of bipolar disorder. After repeated steroid treatment, cognitive and psychiatric abnormalities improved and no NMDAR antibody was detectable. Using primary neuronal cultures, we demonstrate that patient's serum containing IgM NMDAR antibodies reduced the detection of NMDAR on neuronal cells and decreased cell survival. Although NMDAR encephalitis with IgG antibodies is increasingly recognized and diagnosed, atypical presentations with NMDAR antibodies with immunoglobulin subclasses other than IgG pose a diagnostic and therapeutic challenge. Further clinical and neurobiological studies are needed to study the pathophysiological relevance of IgM NMDAR antibodies.

1. Introduction

A recently described subtype of autoimmune encephalitis is defined by the detection of IgG antibodies to the *N*-methyl-D-aspartic acid receptor subunit NR1 (NMDAR) (Dalmau et al., 2011). The clinical presentation has been well defined for acute and chronic courses. The early features include seizures, higher cognitive dysfunction and especially psychiatric symptoms, like hallucinations, psychosis, agitation, depression and anxiety. In up to 60% of the cases, a tumor association has been observed, most commonly teratomas of the ovaries but also with small lung carcinoma, neuroblastoma, breast carcinoma, thymoma, testicular cancers and teratomas and non-gonadal teratomas (Irani et al., 2010). Most patients respond well to immunosuppressive treatments with steroids, human immunoglobulins/plasmapheresis, CD20 depleting antibodies or cyclophosphamide. However, the relevance and pathogenicity of NMDAR antibodies of the IgM subtype are unknown.

In February 2009, a 28-year-old Caucasian presented to our clinic with a first episode of depression. He reported depressed mood, anhedonia, decreased drive, and reduced alertness and concentration. The symptoms responded well to medical therapy with quetiapine 100 mg. Fourteen months later, a first manic episode with logorrhea, aggressive and disinhibited behavior completely remitted after oral treatment with quetiapine 1000 mg. Therefore, a diagnosis of bipolar I disorder was made (DSM-IV). Two months later, the patient presented with another depressive episode associated with slowed and inhibited thinking, dysphoria and reduced energy to our clinic. Despite medical treatment with quetiapine, aripiprazole, lithium, valproate and escitalopram, the patient did not improve. Neurological examination was remarkable for extrapyramidal symptoms with left-sided rigor and bradykinesia. On initial and concurrent magnetic resonance imaging (MRI), numerous T2-hyperintensive subcortical lesions in the frontal lobes were detected (Fig. 1a). Cerebrospinal fluid (CSF) analysis showed isolated intrathecal oligoclonal bands with normal cell count and protein concentration. Screening for autoimmune antibodies detected a positive titer for IgM NMDAR antibodies in serum using transfected HEK293 cells, whereas CSF was negative (titer 1:32). No significant immunolabeling on rat brain was observed due to low titer in serum. IgG NMDAR antibodies were not detected. No signs for viral infections with EBV, HSV, VZV, HIV, hepatitis A, hepatitis B, or hepatitis C were found,

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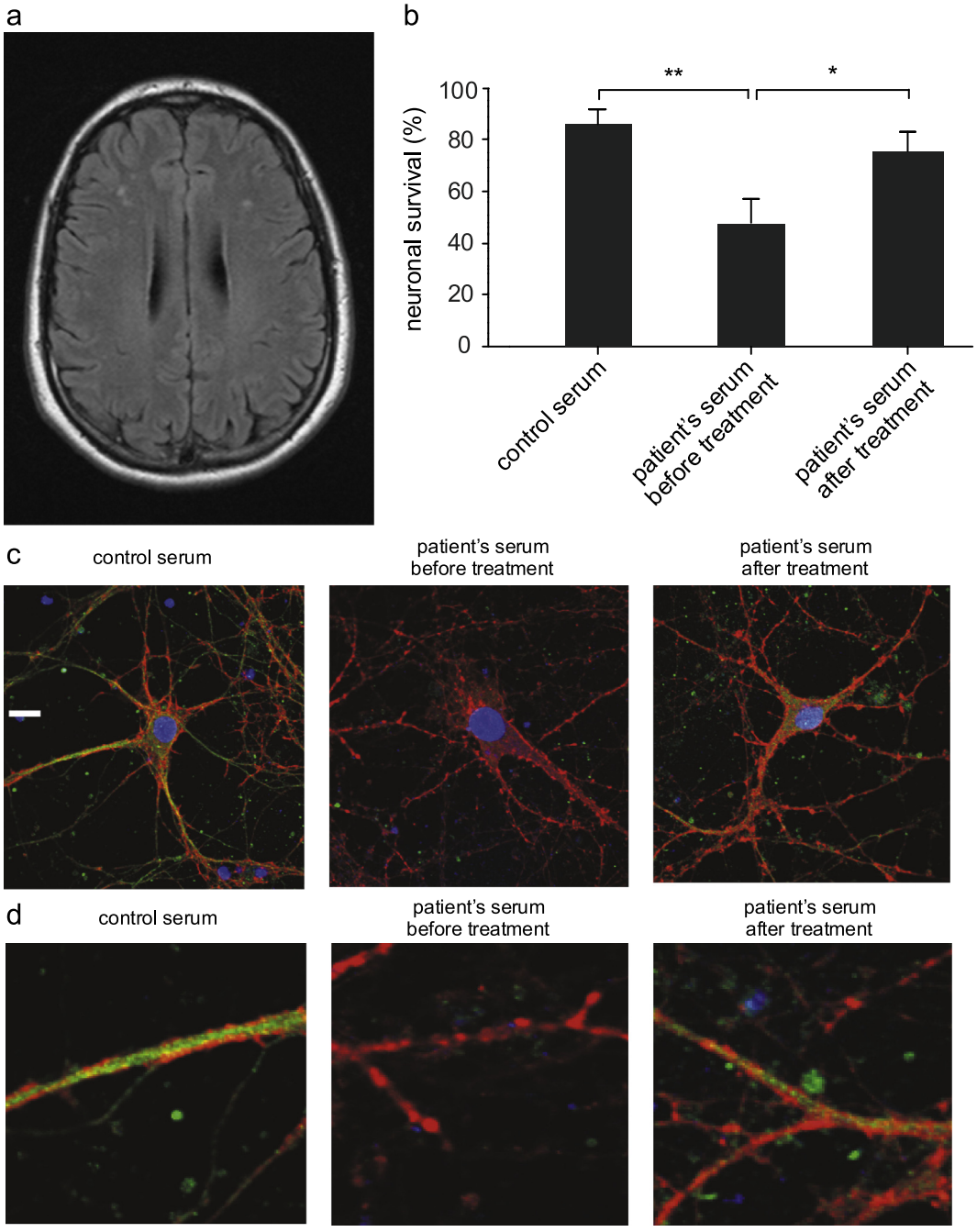

Fig. 1. (a) Fluid attenuated inversion recovery MR image showing bifrontal subcortical lesions suspicious for encephalitis. (b) Neuronal cell survival was evaluated after 3 days of serum incubation. Mean percentages of surviving neuronal cells compared to untreated controls are shown. Results from one representative experiment out of three are presented as mean values ± S.E.M.; asterisks indicate statistical significance of Student's *t*-test; $^{*}P < 0.05$, $^{**}P < 0.001$. (c) Representative and (d) high-resolution stainings of NMDAR NR1 subunit (green) with an antibody, actin filaments by phalloidin (red) and DNA (blue) in cultured murine hippocampal neurons after incubation with respective serums (right). High-resolution insets of neuronal branches (left). (Scale bar: 30 μm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

which could cause unspecific polyclonal stimulation. A diagnosis of autoimmune encephalitis associated with IgM class NMDAR-antibodies was made. Whole body fluoro-deoxy-glucose positron emission tomography with coregistered computed tomography, testis ultrasound and tumor markers (PSA, AFP, β–HCG) did not detect underlying tumors. The patient was treated by two intravenous steroid pulses (3 × 1 g followed by 3 × 2 g methylprednisolone 14 days later). Clinical symptoms slowly improved within 6 weeks. Neither isolated oligoclonal bands nor titers of NMDAR antibody in CSF and serum could be detected 3 weeks after intravenous steroid therapy.

2. Methods

2.1. IgM NMDAR antibody frequency and NMDAR antibody detection

A retrospective analysis at the psychiatric ward PS2 of the University Medical Center Hamburg from September 2009 until July 2012 identified 70 patients who were evaluated for IgM NMDAR antibodies. The median age of our subjects was 32 years (range: 18–88 years). Testing for NMDAR antibodies was performed by recombinant immunofluorescence as previously described (Prüss et al., 2012a). Briefly, plasmids encoding the NMDA type glutamate receptor (using NR1a subunit homodimers and equimolar NR1a/NR2b heterodimers in parallel experiments) were transfected into HEK293 cells. Transfected cells were grown on cover slides, followed by acetone fixation. Coated cover glasses were cut into millimeter-sized fragments

(biochips) and used side by side with cells transfected with an 'empty' plasmid in a mosaic which contained additionally HEK 293 cells transfected with glutamate receptor (type AMPA; GluR1/GluR2), GABA receptor (B1), LGI1, CASPR2, and GAD65 and frozen sections of rat hippocampus and cerebellum. Slides were incubated with 'blinded' patient samples at starting dilution of 1:10 (serum) or undiluted (CSF). After 30 min at room temperature, slides were washed with PBS-Tween for >5 min. Bound antibodies were labeled using individual stainings with Fluorescein-conjugated goat-anti-human IgG, IgA or IgM antibodies for 30 min. Coded samples were classified positive or negative by two independent assessors based on intensity of immunofluorescence of transfected cells in direct comparison with control-transfected cells and control samples.

2.2. Primary mouse hippocampal cultures

Primary mouse hippocampal cultures were prepared from pregnant C57BL/6 mice at gestational day E16. The hippocampi were isolated, dissociated, and then plated on poly-D-lysine coated coverslips (5 μM; Sigma-Aldrich) at a density of 1×10^5 cells/1.9 cm^2 well. The hippocampal cells were maintained in neurobasal media (supplemented with B27, penicillin/streptomycin and L-glutamine) at 37 °C, 5% CO_2 and a relative humidity of 98%. After 3 days in culture, 3 μM cytosine β-d-arabinofuranoside (Sigma) was added to the medium to inhibit glial cell proliferation. Prior to immunocytochemistry or cell death assay, 21-day-old hippocampal cultures were incubated for 72 h with the respective sera at final dilutions of 1:100.

2.3. Immunocytochemistry

For immunocytochemistry, treated hippocampal cells were fixed with 4% PFA and permeabilized with ice cold 80% methanol. An antibody against NMDAR NR1 subunit (Millipore) and Rhodamine Phalloidin (Molecular Probes) were incubated overnight at 4 °C. As secondary antibody, DyLight 488 donkey anti-rabbit IgG (Jackson ImmunoResearch) was incubated together with 10% normal donkey serum and 4′,6-diamidino-2-phenylindole dihydrochloride (Sigma) at 20 °C for 1 h. After mounting, coverslips were examined with an Olympus FV1000 confocal microscope.

2.4. Neuronal cell death

To assess neuronal cell death, intact neuronal cell bodies were counted in three random fields (150,000 μm^2) per cover slip. Nine coverslips per condition from two independent experiments were analyzed. Untreated cultures were set to 100% neuronal survival.

3. Results and discussion

To evaluate a putative pathogenic relevance of IgM NMDAR antibodies, we analyzed cell survival in murine neuronal cultures after exposure to control as well as patient's serum before and after treatment. Cell survival was significantly reduced to $47\pm9\%$ (mean $\pm$ standard deviation) after exposure to patient's serum before treatment (Fig. 1b). Furthermore, patient's serum before treatment resulted in reduction of NMDAR clusters positive for NR1-subunits (Fig. 1c). This result is in line with recent findings that IgM NMDAR antibodies were shown to reduce NMDAR expression causing neuronal dysfunction (Prüss et al., 2012b). In contrast, NMDAR clusters and cell viability were not decreased by patient's serum after treatment, and they were indistin guishable from control serum (Fig. 1b and c). Furthermore, we analyzed retrospectively the relative frequency of IgM NMDA receptor antibodies in the serum of 70 patients with a clinical diagnosis of psychosis (DSM IV), who were previously tested for these antibodies in our department. Including the index patient, we found a positive IgM NMDAR antibody titer in serum of 3/70 patients (4.3%). In the other two patients, cerebral MRIs were unremarkable, and CSF analysis, including NMDAR antibodies and oligoclonal bands, was negative. Subcortical frontal lesions did not change after corticosteroid treatment; therefore a non-specific MRI finding cannot be ruled out.

To our knowledge, this is the first case describing IgM NMDAR antibodies coincident with autoimmune encephalitis mimicking bipolar disorder. IgM NMDA antibodies lead to a reduction of surface NMDAR, which has also been shown in IgG NMDAR encephalitis. Whether IgG and IgM NMDAR antibodies detect different epitopes of the NR1 subunit of the NMDAR is unknown. Interestingly in this case, no class switch from IgM to IgG was observed. Hypothetically, a non-NMDAR antigen triggers autoimmunity by antigenic mimicry. Eventually, specificity against NMDAR could be lost during maturation of initially unselective IgM towards antigen-specific IgG.

Although autoimmune encephalitis with IgG antibodies against glutamate receptors is increasingly recognized and diagnosed, neuropsychiatric disorders associated with other immunoglobulin subclasses pose a diagnostic challenge and warrant a high level of vigilance. Generally, autoimmune-mediated neurological disorders should be considered in neuropsychiatric patients. Currently, non-IgG isotypes are not examined in clinical routine. Therefore, further analyses of disease association and diagnostic relevance of IgM NMDAR antibodies are needed.

Contributors

CUC, EK, FL, GL, MAF, and CM cared for the patient. BS and MAF conceived, designed and performed the experiments. CUC, EK, BS, FL, GL, CG, MAF and CM wrote the manuscript.

Disclosure of conflict of interest

The authors declare no conflicts of interest.

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