Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies

Josep Dalmau, *Amy J Gleichman, *Ethan G Hughes, Jeffrey E Rossi, Xiaoyu Peng, Meizan Lai, Scott K Dessain, Myrna R Rosenfeld, Rita Balice-Gordon, David R Lynch

Summary

Background A severe form of encephalitis associated with antibodies against NR1–NR2 heteromers of the NMDA receptor was recently identified. We aimed to analyse the clinical and immunological features of patients with the disorder and examine the effects of antibodies against NMDA receptors in neuronal cultures.

Methods We describe the clinical characteristics of 100 patients with encephalitis and NR1–NR2 antibodies. HEK293 cells ectopically expressing single or assembled NR1–NR2 subunits were used to determine the epitope targeted by the antibodies. Antibody titres were measured with ELISA. The effect of antibodies on neuronal cultures was determined by quantitative analysis of NMDA-receptor clusters.

Findings Median age of patients was 23 years (range 5–76 years); 91 were women. All patients presented with psychiatric symptoms or memory problems; 76 had seizures, 88 unresponsiveness (decreased consciousness), 86 dyskinesias, 69 autonomic instability, and 66 hypoventilation. 58 (59%) of 98 patients for whom results of oncological assessments were available had tumours, most commonly ovarian teratoma. Patients who received early tumour treatment (usually with immunotherapy) had better outcome (p=0.004) and fewer neurological relapses (p=0.009) than the rest of the patients. 75 patients recovered or had mild deficits and 25 had severe deficits or died. Improvement was associated with a decrease of serum antibody titres. The main epitope targeted by the antibodies is in the extracellular N-terminal domain of the NR1 subunit. Patients’ antibodies decreased the numbers of cell-surface NMDA receptors and NMDA-receptor clusters in postsynaptic dendrites, an effect that could be reversed by antibody removal.

Interpretation A well-defined set of clinical characteristics are associated with anti-NMDA-receptor encephalitis. The pathogenesis of the disorder seems to be mediated by antibodies.

Funding National Institutes for Health, University of Pennsylvania Institute for Translational Medicine, Lankenau Institute for Medical Research, Foederer Foundation of the Children’s Hospital of Philadelphia.

Introduction

NMDA receptors are ligand-gated cation channels with crucial roles in synaptic transmission and plasticity. The receptors are heteromers of NR1 subunits that bind glycine and NR2 (A, B, C, or D) subunits that bind glutamate.1 NR1 and NR2 combine to form receptor subtypes with distinct pharmacological properties, localisation, and ability to interact with intracellular messengers. Overactivity of NMDA receptors causing excitotoxicity is a proposed underlying mechanism for epilepsy, dementia, and stroke, whereas low activity produces symptoms of schizophrenia.2,4

We recently identified a disorder, designated anti-NMDA-receptor encephalitis, that associates with antibodies against NR1–NR2 heteromers and results in a characteristic neuropsychiatric syndrome.1 The first patients identified were young women with ovarian teratoma who presented with psychosis or memory problems, rapidly progressing to multiple neurological deficits requiring prolonged intensive care support. Despite the severity of the disorder, patients often recovered after tumour removal and immunotherapy, suggesting an immune-mediated pathogenesis. Preliminary studies suggested the target epitopes were located in extracellular regions of NR1–NR2B NMDA receptors.3 However, selective disruption of receptors containing NR2B, which are predominantly expressed in the forebrain and hippocampus, would not explain the extensive deficits of patients. We postulated that the crucial epitopes were present in the more widely expressed NR1 subunit. If the antibodies were pathogenic we reasoned that their effects on NMDA receptors would be reversible because most patients recovered.

We report the clinical features of 100 patients, analysing the frequency and type of tumour association, antibody titres, and response to treatment. We also investigate the epileptic region of the NMDA receptor and how antibodies affect NMDA receptors in primary cultures of hippocampal neurons.

Methods

Patients and procedures

Clinical information was obtained by the authors or provided by referring physicians, and has been partly reported for 21 patients.2,4 The webappendix contains additional information and details of control individuals. Control samples were obtained from 20 healthy individuals and 230 patients with suspected autoimmune or
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Figure 1: Immunohistochemical criteria for the presence of NR1–NR2B antibodies

Sera and CSF from all patients’ with anti-NMDA-receptor encephalitis showed identical antibody reactivity in three different assays. Coronal section of rat brain incubated with a representative CSF (A) shows intense reactivity predominantly involving the hippocampus. Cultures of non-permeabilised live rat hippocampal neurons (B) incubated with the same CSF show extensive cell-surface immunolabelling. HEK293 cells transfected with NR1 and predominantly involving the hippocampus. Cultures of non-permeabilised live rat hippocampal neurons (B) incubated with the same CSF show extensive cell-surface immunolabelling. HEK293 cells transfected with NR1 and

paraneoplastic encephalitis, or patients with tumours without encephalitis examined during the period of this study. Samples were from patients seen at University of Pennsylvania or patients referred to the university for a study of autoimmune disorders. All patients had brain MRI, radiological screening for a systemic neoplasm, and serological or CSF studies that ruled out other disorders (webappendix). Serum and CSF were tested for antibodies against the NMDA receptor, and considered positive if antibodies, rat hippocampal neurons after 14 days in vitro were incubated with patients’ CSF (1:15 dilution in 0–25% Triton X-100) and a rabbit monoclonal antibody against NR1 (1:1000, Chemicon) for 2 h at room temperature followed by the appropriate fluorescent-conjugated secondary antibodies (Jackson Immunologicals, West Grove, PA, USA). Imaging and quantification was done as previously reported (webappendix).

Embryonic rat hippocampal neurons were cultured as previously described. To determine the degree of immunolabelling of NMDA receptors by patients’ antibodies, rat hippocampal neurons after 14 days in vitro were incubated with patients’ sera (diluted 1:200 [0·5%]) or CSF (1:10 [10%]) along with a rabbit monoclonal antibody against NR1 (1:10000, AB9864 Chemicon, Temecula, CA, USA) or rabbit polyclonal antibodies against NR2A (1:200, Upstate, Lake Placid, NY), NR2B (1:200, Zymed, San Francisco, CA) or NR2C (1:200, Chemicon), followed by the appropriate fluorescent secondary antibodies.

To determine the location of the main epitope region, we took advantage of the property of NR1 to stably assemble homomers, and of a modified NR1 subunit (NR1d4), in which amino-acid residues 25–380 are deleted but which still assembles with NR2B (webappendix). The reactivity of patients’ sera with these heteromers (NR1d4–NR2B) was examined by immunocytochemistry as described above.

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Cultures of embryonic rat hippocampal neurons were incubated for 24 h with IgG isolated from serum of patients or control individuals. Cell-surface proteins were biotinylated and then isolated from the whole cell lysate. The cell-surface fraction of NR1 from neurons treated with either patients’ or control IgG were then quantified by immunoblot analysis (webappendix).

Statistical analysis

Statistical analyses were done with SAS 9.1 (version 9.1, SAS Institute, Cary, NC, USA). Contingency tables were analysed with Fisher’s two-sided exact test. Differences in antibody titres among groups were analysed with the Kruskal-Wallis and Wilcoxon sum rank tests, with the Bonferroni correction for pairwise tests. The effects of IgG and CSF on neuronal cultures were analysed with the Kruskal-Wallis non-parametric ANOVA followed by Dunn’s pairwise comparison.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or the writing of the report. The corresponding author had full
access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Table 1 summarises the clinical information. 86 patients who could be assessed had headache, low-grade fever, or a non-specific viral-like illness within 2 weeks before hospital admission. 77 patients presented with prominent psychiatric symptoms, including anxiety, agitation, bizarre behaviour, delusional or paranoid thoughts, and visual or auditory hallucinations. 23 presented with short-term memory loss or seizures alone or associated with psychiatric manifestations.

During the first 3 weeks of symptom presentation, 76 patients had seizures. 88 patients developed decreased consciousness, progressing to a catatonic-like state, with periods of akinesia alternating with agitation, and diminished or paradoxical responses to stimuli (eg, no response to pain but resisting eye opening). Some patients mumbled unintelligible words or had echolalia. Eye contact or visual tracking was absent or inconsistent. During this clinical stage, large proportions of patients developed dyskinesias, autonomic instability, and central hypoventilation (median time of ventilatory support, 8 weeks; range 2–40 weeks). Orofacial dyskinesias were the most common; these included grimacing, masticatory-like movements, and forceful jaw opening and closing, resulting in lip and tongue injuries or broken teeth. 37 patients had cardiac dysrhythmias, including tachycardia or bradycardia, with prolonged pauses in seven patients; four needed pacemakers. 52 patients had dyskinesias,
In six patients, the tumour was diagnosed after recovery from the encephalitis (56–380 months). Ovarian teratoma identified with CT, MRI, or ultrasound was a common tumour type (median size 6 cm, range 1–22 cm). Eight patients had bilateral teratomas; four were synchronous, two had history of a contralateral teratoma, and two developed contralateral teratomas before recurrence of the encephalitis. All teratomas contained nervous tissue; 25 were examined for expression of NMDA receptors, and all were positive (data not shown).

One boy (11 years old, without tumour) and 21 women and girls were younger than 19 years (median 15 years, range 5–18 years); 12 had an ovarian teratoma (five with immature features), and nine had no tumour. Metastases were identified only in one man with immature teratoma of the testis.

Seven patients with cancer did not have tumour resection (one small-cell lung cancer, two teratomas found at autopsy, four not removed). Six patients who had tumours removed did not receive immunotherapy (table 2). 40 of 42 patients without tumour had immunotherapy and two had supportive care.

Median follow-up was 17 months (1–194 months): 47 patients had full recovery, 28 mild stable deficits, 18 severe deficits, and seven died as a result of the neurological disorder. Patients whose tumour was identified and removed within the first 4 months of the onset of the neurological disease had better outcome than the rest of the patients (figure 2). The median time from symptom presentation to initial signs of improvement was 8 weeks (range 2–24 weeks) for the group of patients with early tumour treatment, 11 weeks (4–40 weeks) for the group whose tumour was treated late or not treated, and 10 weeks (2–50 weeks) for the group without tumour (Kruskal-Wallis, p=0·10).

The median duration of hospitalisation was 2–5 months (range 1–14 months). While hospitalised, seven patients had high levels of serum creatine kinase, six developed pulmonary embolism, six transient aphasia, four hemiparesis, and four tetraparesis. After discharge, 64 (85%) of the 75 patients who were left with mild deficits or eventually attained full recovery had signs of frontal-lobe dysfunction including poor attention and planning, impulsivity, and behavioral disinhibition; 20 (27%) had prominent sleep dysfunction, including hypersonnia and inversion of sleep patterns.

15 patients had one to three relapses of encephalitis (webappendix). The median time between initial presentation and last relapse was 18 months (1–84 months). Relapses were less common in patients with early tumour treatment (1 of 36) than in other patients (14 of 64; p=0·009), including patients whose tumour was treated late (six of 22; p=0·009) and patients without tumour (eight of 42; p=0·03). None of the patients was receiving immunotherapy at the time of the neurological relapse.

Seven patients died of the neurological disorder (webappendix), although the diagnosis was established.
retrospectively by examining archived CSF for all of them.

Analysis of the reactivity of patients’ sera or CSF against the indicated NMDA-receptor subunits or heteromers showed that antibody reactivity was not modified by changing the NR2 subunit (A, B, C, or D) and was retained by homomers of NR1 (webtable 3). Having established that NR1 was recognised by all patients’ antibodies, we investigated the epitope region by use of a plasmid (NR1d4) that encodes an NR1 subunit lacking amino-acid residues 25–380 that can nevertheless assemble with NR2B. The successful expression of NR1d4–NR2B in HEK293 cells was confirmed by immunocytochemistry with the indicated mouse and rabbit antibodies against NR1 and NR2B (data not shown). The use of these heteromers abrogated the reactivity of sera or CSF from 92 patients, and substantially decreased the reactivity of the samples of the remaining eight cases. Hence the main epitope region recognised by all patients’ antibodies lies within the extracellular region of the NR1 subunit (webtable 3).

To determine whether patients had intrathecal synthesis of antibodies, we first measured the integrity of the blood–brain barrier. Of 58 patients with paired serum and CSF available, 53 had preserved integrity of the blood–brain barrier. Analysis of normalised concentrations of IgG showed that all 53 patients had higher concentrations of antibodies in CSF than in serum (A). In 83 patients with anti-NMDA-receptor encephalitis (54 with tumour, 29 without tumour) and 50 controls (B), those with tumours had higher titres than those without (Wilcoxon rank, p<0.0001) and controls (p<0.0001). Six patients (one with tumour, five without tumour) had very low ELISA readings that overlapped with the signal given by negative controls. These six patients had low antibody titres; in contrast, the 50 controls were negative. Solid lines indicate the mean of the titres in each group. The dotted line indicates three SD above the mean value given by background signal of negative controls. Follow-up of serum antibody titres (C) in 14 representative patients who had neurological improvement (black lines) and four who did not (red lines); the second time-point is the sample obtained at the last follow-up (median 5.6 months, range 2–83 months). The dotted line indicates three SD of the mean value given by background signal of 50 negative control sera. Similar results were obtained by ELISA with NR1–NR2 heteromers (data not shown). Values in A, B, and C are given in relative fluorescence units (rfu) from the ELISA reader, and plotted in a logarithmic scale.

Figure 3: Analysis of NR1 antibody titres
In 53 patients with anti-NMDA-receptor encephalitis, antibody titres were higher in CSF than in serum (A). In 83 patients with anti-NMDA-receptor encephalitis (54 with tumour, 29 without tumour) and 50 controls (B), those with tumours had higher titres than those without (Wilcoxon rank, p=0.0001) and controls (p=0.0001). Six patients (one with tumour, five without tumour) had very low ELISA readings that overlapped with the signal given by negative controls. These six patients had low antibody titres; in contrast, the 50 controls were negative. Solid lines indicate the mean of the titres in each group. The dotted line indicates three SD above the mean value given by background signal of negative controls. Follow-up of serum antibody titres (C) in 14 representative patients who had neurological improvement (black lines) and four who did not (red lines); the second time-point is the sample obtained at the last follow-up (median 5.6 months, range 2–83 months). The dotted line indicates three SD of the mean value given by background signal of 50 negative control sera. Similar results were obtained by ELISA with NR1–NR2 heteromers (data not shown). Values in A, B, and C are given in relative fluorescence units (rfu) from the ELISA reader, and plotted in a logarithmic scale.

Figure 4: Immunolabelling of neuronal NR1 clusters
Left: Hippocampal neurons (14 days in vitro) immunostained with antibody against NR1 (black and white and green) or a patient’s CSF (black and white and red). Right: 91% of NR1 clusters are colabelled with patient’s CSF (yellow puncta in overlay), less than 9% of NR1-positive puncta remain unlabelled (green in overlay). Kruskal-Wallis non-parametric ANOVA followed by Dunn’s pairwise comparison, p<0.01.
whereas the seven patients with milder syndromes had the lowest titres (data not shown). Patients who improved had a parallel decrease of serum titres, whereas those who did not improve maintained high titres in CSF and serum (figure 3). Follow-up CSF titres were not obtained in most patients after improvement.

To assess the effect of patients’ antibodies on neuronal cultures, we first determined the extent of immunolabelling of NR1 (or NMDA receptor) clusters in postsynaptic dendrites. Patients’ antibodies labelled nearly all clusters of NMDA receptors (figure 4). This antibody binding did not cause apoptosis (data not shown). However, adding patients’ IgG to rat hippocampal neuronal cultures produced a concentration-dependent decrease of the cell-surface fraction of NMDA receptors (figure 5). IgG from patients with high antibody titres produced a greater decrease of NMDA receptors than IgG from patients with low antibody titres (data not shown).

The effect of patients’ antibodies on clusters of NMDA receptors in postsynaptic dendrites was quantified by confocal microscopy. Neurons treated with patients’ CSF for 3 days or 7 days had fewer clusters of NMDA receptors per length of postsynaptic dendrite than neurons treated with control CSF. By contrast, neurons treated for 3 days with patients’ CSF followed by 4 days with control CSF had similar numbers of clusters of NMDA receptors to those in neurons treated only with control CSF (figure 5).

Patients’ antibodies did not change the concentrations of the postsynaptic protein PSD-95 (figure 5). Together, these findings show that patients’ antibodies produce a selective and reversible decrease of NMDA-receptor clusters in postsynaptic dendrites.

**Discussion**

Of 100 patients with anti-NMDA-receptor encephalitis, a disorder that associates with antibodies against the NR1 subunit of the receptor, many were initially seen by psychiatrists or admitted to psychiatric centres but subsequently developed seizures, decline of consciousness, and complex symptoms requiring multidisciplinary care. While poorly responsive or in a catatonic-like state, 93 patients developed hypoventilation, autonomic imbalance, or abnormal movements, all overlapping in 52 patients. 59% of patients had a tumour, most commonly ovarian teratoma. Despite the severity of the disorder, 75 patients recovered and 25 had severe deficits or died.

This disorder largely affects young people, and its diagnosis is facilitated by the characteristic clinical picture that develops in association with CSF pleocytosis. By contrast to the consistency of the clinical picture, MRI findings are less predictable; only 55% of patients had increased FLAIR or T2 signal in one or several brain regions, without significant correlation with patients’ symptoms (data not shown). Our study indicates that 41% of patients with anti-NMDA-receptor encephalitis do not have a clinically detectable tumour, and that men and children can also be affected. Therefore, although the presence of a tumour that expresses NMDA receptors likely contributes to breaking immune tolerance, other unknown immunological triggers seem to be involved. This paradigm is similar to the Lambert-Eaton myasthenic syndrome, an antibody-mediated disorder of the neuromuscular junction that can occur with or without tumour association.16 In Lambert-Eaton myasthenic syndrome the presence of a small-cell lung cancer confers a poor neurological prognosis; however, in anti-NMDA-
receptor encephalitis, detection of teratoma is a good prognostic factor, probably because this tumour is curable.

In anti-NMDA-receptor encephalitis the high prevalence of prodromal viral-like symptoms is intriguing. Direct viral pathogenesis is unlikely because extensive studies of CSF samples, brain biopsies, and autopsies were negative for viruses (data not shown). Whether the prodromal symptoms form part of an early immune activation,\(^7,8\) or result from a non-specific infection that facilitates crossing of the blood–brain barrier by the immune response is unknown.\(^9,10\) Nevertheless, the immune response eventually predominates in the nervous system as suggested by the high frequency of pleocytosis, oligoclonal bands, and intrathecal synthesis of NR1 antibodies. In general, patients with an underlying tumour develop more robust immune responses than those without a tumour.

A pathogenic role of patients’ antibodies is suggested by the correlation between antibody titres and neurological outcome and by the decrease in number of postsynaptic clusters of NMDA receptors caused by patients’ antibodies. The latter effect was reversed by removing the antibodies from the cultures, explaining the potential reversibility of patients’ symptoms. Consistent with this antibody-induced decrease in the numbers of NMDA receptors, several NMDA-receptor antagonists such as MK801, ketamine, and phencyclidine cause symptoms similar to anti-NMDA-receptor encephalitis, including psychotic behaviour,\(^11,12\) signs of involvement of dopaminergic pathways (rigidity, dystonia, orofacial movements, tremor),\(^12–21\) and autonomic dysfunaction (cardiac dysrhythmia, hypertension, hypersalivation).\(^22,23,26,27\) Furthermore, disruption of NR1 antibodies in animals results in hypoventilation.\(^28\)

A characteristic feature of patients who recover from anti-NMDA-receptor encephalitis is a persisting amnesia of the entire process (data not shown). This feature is compatible with disruption of the mechanisms of synaptic plasticity, thought to underlie learning and memory, in which the NMDA receptors play a key part.\(^4\)

Recovery from this disorder is typically slow, and symptoms may relapse, especially in patients with undetected or recurrent tumours and patients with no associated tumours. Some of these patients may have an occult tumour, however, in only one of seven patients who underwent exploratory laparotomy was a tumour found (sex-cord stromal tumour; data not shown). A possible explanation for the slow recovery could be the inability of most commonly used treatments (corticosteroids, plasma exchange, intravenous immunoglobulin) to result in a rapid and sustained control of the immune response within the CNS. For example, in a few patients whose CSF was obtained during neurological improvement, the decrease of CSF antibody titres was substantially slower than that of serum titres.\(^7\) Furthermore, 13 of 17 patients unresponsive to the above therapies, responded to cyclophosphamide (five), rituximab (six), or both (two; data not shown), drugs that are effective in other immune-mediated disorders of the CNS.\(^9,10\)

Anti-NMDA-receptor encephalitis represents a new category of immune-mediated disorder that is often paraneoplastic, treatable, and can be diagnosed serologically. Future studies should clarify the best type and duration of immunotherapy, the role of prodromal events in triggering the immune response, and the molecular mechanisms involved in decreasing the number of NMDA receptors.

**Contributors**
JD was involved in study design, clinical assessments of patients, data analysis, and writing of the report. AJG, EGH, JER, XP, ML, and SKD did the laboratory studies and prepared the figures. MRR, RB-G, and DL were involved in study design, data analysis, and writing of the report.

**Conflict of Interest**
A patent has been filed by JP and DL for the detection of antibodies against NR1–NR2 subunits of the NMDA receptor. We have no other conflict of interest.

**Acknowledgments**
We thank the physicians, patients, and family members that provided clinical information; Christina Chu (Department of Gynecology and Obstetrics, University of Pennsylvania, PA, USA) for providing sera of patients with teratoma of the ovary; Francesc Graus (Hospital Clinic, University of Barcelona, Spain) for helpful discussions and advice. This work was supported in part by ZR56CA098054, ROICA107192, and the Institute for Translational Medicine and Therapeutics (JD); R21 MH057683 (RB-G); Institutional funds from the Lankenau Institute for Medical Research (SKD), NSR36-49586 and Feederer Foundation of the Children’s Hospital of Philadelphia (DL).

**References**


