Recombinant immunofluorescence assay for the detection of anti-glutamate receptor (type NMDA) antibodies in the differential diagnosis of autoimmune encephalopathies

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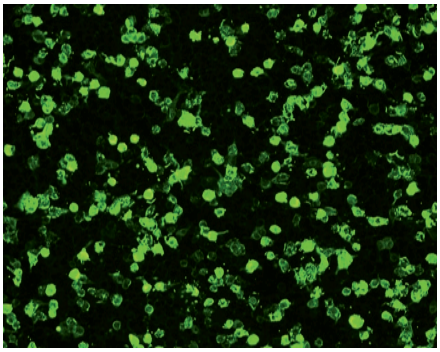
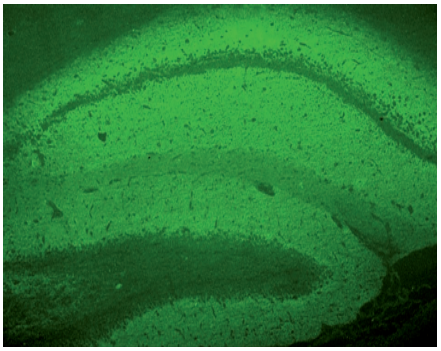
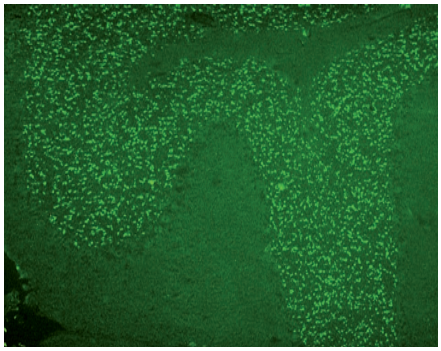


HEK293 cells transfected with recombinant NMDA receptor



Neuropilic staining of the molecular layer on rat hippocampus



Staining of the granular layer on rat cerebellum

Introduction

Anti-**N-methyl-D-aspartate receptor (NMDAR)** encephalitis is a severe and considerably underdiagnosed disorder that frequently affects young women with teratomas of the ovary, but is also observed in females without tumors as well as in men and children. Patients usually present with symptoms like memory loss, disorientation, confusion, paranoid thoughts, visual or auditory hallucinations, dyskinesias, decrease of consciousness, lethargy, seizures and autonomic instability. Early diagnosis is crucial since patients often improve with immunotherapy and removal of the tumor. Final diagnosis is based on the determination of anti-glutamate receptor (type NMDA) antibodies in serum or **cerebrospinal fluid (CSF)**. Here we report a recombinant assay for standardized detection of anti-glutamate receptor (type NMDA) antibodies applicable in each laboratory familiar with indirect immunofluorescence.

Methods

cDNAs for the glutamate receptor (type NMDA; subunbits NR1/NR1 and NR1/NR2, respectively) were inserted into eukaryotic expression vectors and transfected into HEK293 cells. Recombinant cells were grown on slides of cover glass, followed by fixation with acetone. Substrates were fragmented to BIOCHIPs and used in a mosaic which contained additionally frozen sections of rat hippocampus and cerebellum for the determination of autoantibodies in the indirect immunofluorescence test.

47 serum and 23 CSF samples from patients with anti-NMDAR encephalitis and controls with other disorders, including, anti-VGKC and AMPA receptor encephalitis, were examined. In addition, sera of 100 healthy blood donors were analyzed.

Results

All samples from patients with anti-NMDAR encephalitis (29 serum, 10 CSF) were tested positive with the transfected cells, while all disease control samples (18 sera, 13 CSF) and healthy blood donors (100 sera) were negative. In addition, all anti-glutamate receptor (type NMDA) positive samples showed a neuropilic staining of the molecular layer on rat hippocampus and of the granular layer on rat cerebellum in a highly characteristic, although less specific manner. Moreover, the rat brain immunohistochemistry served to identify other antibodies (e.g. VGKC and AMPA receptor) in 23% of the patients that were negative for anti-glutamate receptor (type NMDA) antibodies.

Conclusion

Indirect immunofluorescence using glutamate receptors (type NMDA) recombinantly expressed in human cells as antigenic substrate is highly competent in diagnosing NMDAR encephalits. The combined use of transfected cells with hippocampal and cerebellar tissue substrates allows the detection of other autoantibodies implicated in the differential diagnosis of autoimmune encephalopathies, such as antibodies to VGKC and AMPA receptors or uncharacterized antigens of the neuropil of hippocampus.

	n	Recombinant NMDAR	Hippocampus	Cerebellum
Anti-NMDAR encephalitis	39	100%	100%	100%
Other disorders	31	0%	23%	23%
Healthy blood donors	100	0%	0%	0%

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