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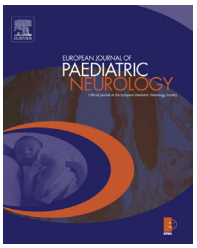

Original article

Antibodies to AMPA receptors in Rasmussen's encephalitis

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ABSTRACT

Rasmussen's encephalitis is a rare progressive childhood disorder characterized by frequent severe seizures, hemiparesis, encephalitis and mental deterioration, and associated with severe unilateral neuroinflammation. Autoantibodies, particularly to the GluA3 subtype of the alpha-amino-3-hydroxy-5-methyl-isoxazole-4-propinonic acid receptor (AMPAR) were reported in the 1990s but not always confirmed. To explore further, sera from 52 patients with Rasmussen's encephalitis were tested by cell-based assays for antibodies to AMPAR GluA1/2/3, N-methyl-D-aspartate (NMDA NR1/2b), γ-aminobutyric acid type A and B ($GABA_AR$ α1/γ2/β2 and $GABA_BR$ b1/b2) receptors, for potassium channel complex proteins, and for binding to live cortical and hippocampal neuronal cultures. Two patients' sera (3.8%) bound to HEK cells co-transfected with the GluA2 and GluA3 subunits. One additional patient had a low level of VGKC-complex antibodies. These three, and seven additional, sera bound to hippocampal cultures. No other antibodies were detected. Thus, despite the rarity of GluA2/3 antibodies, 10 patients (19.2%) had evidence of antibodies to neuronal antigens. Whether these antibodies play a primary role in RE, or appear secondary to the neuro-inflammatory damage in this highly destructive disease, requires further study.

Abbreviations: Abs, antibodies; AED, anti-epileptic drugs; AMPAR, amino-3-hydroxy-5-methyl-4-isoxazolepproprionic acid receptor; BBB, blood brain barrier; CASPR2, contactin-associated protein receptor like 2; CBA, cell based assay; CNS, central nervous system; CSF, cerebrospinal fluid; DC, disease control; EPC, epilepsia partialis continua; $GABA_AR$, gamma-aminobutyric acid receptor A; $GABA_BR$, gamma-aminobutyric acid receptor B; HC, healthy control; HEK, human embryonic kidney cells; HR, hemispheric ratio; LGI1, leucine glioma inactivated protein; MRI, magnetic resonance image; NMDAR, N-methyl-d-asparate receptor; RE, Rasmussen's encephalitis; VGKC, voltage gated potassium channel.

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1. Introduction

Rasmussen's encephalitis (RE) is a severe epileptic syndrome, characterized by unihemispheric brain dysfunction and tissue loss.[1] An inflammatory histopathology, neurological impairment and intractable focal seizures, including epilepsia partialis continua (EPC), are common clinical features. Magnetic Resonance Imaging (MRI) of patients with RE demonstrates the spread of cortical inflammation throughout the hemisphere. The disease almost always leads to hemiparesis and atrophy (reviewed in[2]). Neuronal cell loss, microglial nodules and T cell infiltrates are common pathological features of the affected cerebral cortex.[2,3] Patients with RE experience frequent seizures, and these are poorly responsive to antiepileptic drugs (AEDs). Some patients show a positive response to immunotherapies such as plasma exchange, and tacrolimus.[2,4]

In 1994, rabbits immunized with a recombinant fusion protein of GluA3 (then termed GluR3, an alpha-amino-3-hydroxy-5-methyl-isoxazole-4-propinonic acid receptor (AMPAR) subunit) developed GluA3 antibodies and seizures, and their cerebral histopathology showed features that resembled RE. Five children with RE were found to have similar antibodies, and one child showed a very good response to plasma exchange, suggesting that GluA3 antibodies (Abs) might play a pathogenic role.[4] However, GluA3 Abs, detected by enzyme linked immunosorbent assays (ELISAs) using short peptide sequences of GluA3, were subsequently reported not only in RE patients but also in other epilepsies and controls, and further studies using a range of methods did not identify GluA3 Abs at that time.[5]

In the last 15 years a variety of Abs to neuronal proteins have been identified in central nervous system (CNS) disorders using cell-based and other assays designed to detect Abs that bind to conformational cell-surface exposed antigens,[6] including the AMPA receptor[7] AMPA receptors are of particularly interest as they mediate excitatory transmission in the central nervous system and have been shown to be critical to the generation and spreading of epileptic activity.[8] Here we used these approaches to examine/re-examine 52 RE patients for the newly described Abs using these techniques and investigated further the role of antibodies to the AMPA receptor.

2. Material and methods

2.1. Patients details

Informed consent was obtained from all patients, or their parents, seen in Oxford (see[5]), Bonn or other neurological centers over many years with approval from the local ethics committee as appropriate at the time. A diagnosis of Rasmussen's encephalitis was made using standard criteria.[9] Samples from children (<18 years) with other forms of epilepsy (n = 106) and healthy controls (n = 112) were also tested, as approved by the Oxfordshire Regional Ethical Committee A (07/Q1604/28).

2.2. Diagnostic assays

In this cross-sectional study fifty-two patients with RE, 26 previously published[5] and 26 new patients were tested for Abs to voltage-gated potassium channel (VGKC)-complex and glutamic acid decarboxylase (GAD) by radioimmunoassays; N-methyl-D-aspartate receptor (NMDAR), leucine-rich, glioma inactivated protein1 (LGI1), contactin-associated protein-like 2 (CASPR2), γ-aminobutyric acid type A (α1/γ2/β2) and B receptor ($GABA_AR$ and $GABA_BR$) and AMPAR were tested on live cell based assays (CBA) as previously described.[10] Surface expression of all proteins in HEK cells was established using specific commercial antibodies (data not shown). For the AMPAR CBA transfection, human embryonic kidney (HEK) cells were transfected with plasmids encoding AMPAR GluA1, GluA2 or GluA3, separately.[7] Reactivity to GluA1 and GluA3 HEK cells was also tested when cells were co-transfected with GluA2 at a 1:1 DNA content (forming the most common dimer–dimer formation of the AMPAR). Patient sera were diluted to 1:20, while CSFs were tested undiluted. Any positive samples were serially diluted to obtain end-point titers (dilution at which reactivity was weak but still clearly detectable). Reactivity of sera (1:100) and CSFs (1:2) to live primary neuronal cultures (hippocampal and cortical) were investigated at 14 days in vitro (div) using methods described previously.[10]

3. Results

3.1. Patient cohort

Fifty two patients with RE (n = 38 childhood-onset and n = 14 adult-onset) were studied (see Table 1). The median age of diagnosis of the childhood-onset patients was 7 years (range: 1–18), and of adult patients was 32 years (range: 19–41). The median duration of disease at time of sampling was 2.2 years (range: 0.1–27). Two patients had paired serum and CSF samples and one additional patient had an unpaired CSF. Results were compared to samples from disease and healthy controls.

3.2. Antibody results

Although some (n = 20) of the samples had been previously tested by other approaches for GluR3 (GluA3), GAD and VGKC-complex autoantibodies,[5] in this study all samples were retested with the full panel of autoantibodies now available. All samples were negative for NMDAR, LGI1, CASPR2, $GABA_AR$ and $GABA_BR$ Abs. Only one patient had low serum VGKC complex antibodies (153 pM, normal<100 pM), however this patient was negative for LGI1 and CASPR2 antibodies.

None of the RE patient sera bound HEK cells transfected with DNAs for single AMPAR subunits (GluA1, A2 or A3), or co-transfected for GluA1 and GluA2. However, when HEK cells were co-transfected with GluA2 and GluA3 (GluA2/3), two of 52 patient samples showed reactivity (Fig. 1A). These sera had end-point titrations of 1:540 and 1:180. For one of these patients (#1) a serum collected one year previously was negative for GluA2/3 Abs. None of the three CSFs (from other patients),

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