Autoantibodies associated with diseases of the CNS: new developments and future challenges

Angela Vincent, Christian G Bien, Sarosh R Irani, Patrick Waters

Several CNS disorders associated with specific antibodies to ion channels, receptors, and other synaptic proteins have been recognised over the past 10 years, and can be often successfully treated with immunotherapies. Antibodies to components of voltage-gated potassium channel complexes (VGKCs), NMDA receptors (NMDARs), AMPA receptors (AMPArs), GABA type B receptors (GABABRs), and glycine receptors (GlyRs) can be identified in patients and are associated with various clinical presentations, such as limbic encephalitis and complex and diffuse encephalopathies. These diseases can be associated with tumours, but they are more often non-paraneoplastic, and antibody assays can help with diagnosis. The new specialty of immunotherapy-responsive CNS disorders is likely to expand further as more antibody targets are discovered. Recent findings raise many questions about the classification of these diseases, the relation between antibodies and specific clinical phenotypes, the relative pathological roles of serum and intrathecal antibodies, the mechanisms of autoantibody generation, and the development of optimum treatment strategies.

Introduction

The role of autoantibodies in several neuromuscular disorders has been recognised since the 1970s: antibodies to the acetylcholine receptor have a role in myasthenia gravis and antibodies to voltage-gated calcium channels in the Lambert-Eaton myasthenic syndrome. Both antibodies bind to the extracellular domain of essential membrane proteins. Patients with these diseases usually improve rapidly after plasma exchange, which removes circulating antibodies. The pathogenic roles of these antibodies were established by various in-vitro and in-vivo approaches.1 A major subsequent development in the field was the discovery of specific onconeural antibodies in patients with classic paraneoplastic neurological disorders associated with lung or gynaecological tumours, thymomas, or other tumours,2 but unfortunately patients with these disorders did not often respond to immunotherapies.

The excitement over the past 10 years has come from the discovery of diseases, in both adults and children, that are associated with antibodies to cell-surface proteins expressed in neurons, and the fact that these patients can improve with immunotherapies, although usually more slowly than those with peripheral antibody-mediated disorders. Many patients present with amnesia, confusion, seizures, and psychiatric features, and some then develop a generalised encephalopathy with movement disorders, loss of consciousness, and hypothalamic disturbance. Some patients have ovarian teratomas, thymomas, or small-cell lung cancer, but most do not have detectable tumours. The disorders associated with these antibodies have previously been termed autoimmune channelopathies,3,4 even though some of the antigens are not the channels themselves but proteins that are complexed with them on the plasma membrane of neurons, their axons, dendritic spines, or nerve terminals. These proteins are generally expressed throughout the nervous system; nevertheless, the diseases can be quite specific regarding the regions they affect (eg, limbic encephalitis), perhaps owing to the particular vulnerability of some neurons or the increased accessibility of the region to the antibodies.

Although rare, these disorders are beginning to be recognised and treated worldwide, and further specific antibodies will most likely be identified in the future. We describe the main clinical syndromes associated with neuronal antibodies that have been defined in the past few years. We start with limbic encephalitis, which is currently a well recognised syndrome associated with several different antibodies, and then describe other disorders that are less well known and affect wider brain systems. Finally, we briefly discuss the pathogenic roles of the antibodies and propose research priorities.

Autoimmune disorders affecting mainly the temporal lobe or the limbic cortex

Limbic encephalitis was originally described as a rare cliniconeuropathological entity, involving amnesia, seizures, and psychological disturbance, and associated with an underlying neoplasm.4 Since the 1980s, various onconeural antibodies have been discovered, which are biomarkers for these classic paraneoplastic syndromes.2 However, paraneoplastic limbic encephalitis is a rare complication of cancer and patients with a non-paraneoplastic form of limbic encephalitis associated with antibodies to neuronal proteins are now being recognised (table 1 and table 2); they generally have a better prognosis than that of patients with the paraneoplastic form. Guidelines for diagnosis of these patients have been proposed by Bien and Elger,5 and recognition of this syndrome, after exclusion of infective, toxic, or neoplastic causes (table 1), even without demonstrable antibodies,6,7 should prompt consideration of immunotherapy.

Several antibodies (to NMDA receptors [NMDARs], AMPA receptors [AMPArs], GABA type B receptors [GABABRs], and leucine-rich glioma inactivated 1 [LGI1]) were identified by use of indirect immunohistochemistry on rodent brain sections (in which binding to the neuropil of the hippocampus was shown) and by binding to the surface of hippocampal neurons in culture.8


<table>
<thead>
<tr>
<th>Common symptoms</th>
<th>VGKC-complex-Ab; mainly LG1 Abs</th>
<th>VGKC-complex-Ab; mainly CASPR2 Abs</th>
<th>Anti-NMDAR encephalitis</th>
<th>AMPAR-Ab limbic encephalitis</th>
<th>GABA-R Ab limbic encephalitis</th>
<th>GAD-Ab limbic encephalitis</th>
<th>GlyR Ab-associated disorders</th>
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<tr>
<td>Predominantly limbic encephalitis with amnesia, seizures, psychiatric disturbance, facial or truncal myokymia, and cognitive symptoms</td>
<td>Morvan’s phenotype with confusion, amnesia, insomnia, autonomic dysfunction, neurovegetative symptoms, and pain</td>
<td>Multistage cortico-subcortical encephalopathy including psychiatric manifestations, dyskinesias, seizures, mutism, reduction in consciousness; occasionally limbic encephalitis</td>
<td>Typical limbic encephalitis (amnesia, seizures), psychosis can dominate</td>
<td>Limbic encephalitis with prominent seizures</td>
<td>Temporal lobe epilepsy with mild cognitive involvement</td>
<td>Combinations of startle (hyperekplexia), stiffness, rigidity, brainstem disturbance, cognitive involvement rare but sometimes seizures</td>
<td></td>
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<tr>
<td>Differential diagnoses</td>
<td>Wernicke-Korsakoff syndrome, infective encephalitis (especially HSV), drug or toxin overdose, Creutzfeldt-Jakob disease, Hashimoto’s encephalopathy, non-convulsive status epilepticus</td>
<td>Motor neuron disease, prion diseases (familial or sporadic), hereditary, or acquired, neurovegetative symptoms, phaeochromocytoma</td>
<td>Encephalitis lethargica, PANDAS, Sydenham’s chorea, infective encephalitis (eg, rabies), neuroleptic malignant syndrome, Kleine-Levin syndrome, non-convulsive status epilepticus, Hashimoto’s encephalopathy, neuropsychiatric lupus, porphyria</td>
<td>As for LG1</td>
<td>As for LG1</td>
<td>As for LG1</td>
<td>Stiff person syndrome, tetanus, hereditary stantle disease</td>
</tr>
<tr>
<td>Main known target(s)</td>
<td>VGKC-complex-associated LG1 Abs more frequent than CASPR2 Abs</td>
<td>VGKC-complex-associated CASPR2</td>
<td>NMDAR (mainly NR1 subunit)</td>
<td>GABA,R1</td>
<td>GAD</td>
<td>GlyR1</td>
<td></td>
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<tr>
<td>Localisation</td>
<td>Strong in hippocampal neuropil</td>
<td>Ubiquitous but strong in hippocampal and cerebellar neuropil</td>
<td>High in hippocampal neuropil</td>
<td>Widespread in CNS but high in hippocampus</td>
<td>Widespread in CNS but high in hippocampus</td>
<td>Widespread in CNS on inhibitory interneurons</td>
<td>Inhibitory interneurons in spinal cord and brainstem</td>
</tr>
<tr>
<td>Antibodies</td>
<td>IgG4&gt;IgG1</td>
<td>IgG4&gt;IgG1</td>
<td>IgG1 predominantly</td>
<td>Not well described</td>
<td>Intrathecal synthesis</td>
<td>Intrathecal synthesis reported</td>
<td>Intrathecal synthesis reported</td>
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<tr>
<td>CSF intrathecal synthesis</td>
<td>Variable intrathecal synthesis</td>
<td>Insufficient data</td>
<td>Almost always intrathecal synthesis, which can be considerable (eg, &gt;20 fold)</td>
<td>Intrathecal synthesis</td>
<td>Intrathecal synthesis</td>
<td>Intrathecal synthesis reported</td>
<td>Intrathecal synthesis reported</td>
</tr>
<tr>
<td>Tumour association or other pathology</td>
<td>Tumours very rare in patients with LG1 Abs</td>
<td>Not invariable; small-cell lung cancer or other rare tumours</td>
<td>Ovarian (or other) teratomas in &lt;50%</td>
<td>In about 50% of cases (thymoma, lung, breast)</td>
<td>Thymoma and lung</td>
<td>Very uncommon but can occur</td>
<td>Typically non-paraneoplastic, one thymoma</td>
</tr>
<tr>
<td>Disease course</td>
<td>Often monophasic without need for continuing immunosuppression</td>
<td>Can be treatment-responsive or have spontaneous improvement, but progression confounded by tumour when present</td>
<td>Responds well to early immunotherapies and early tumour removal but non-paraneoplastic cases can be chronic and tend to relapse</td>
<td>Responds to treatments but relapses common</td>
<td>Responds to treatments</td>
<td>Usually chronic disorders and role of long-term immunosuppression not yet clear</td>
<td>In case reports, immunotherapy led to substantial improvement</td>
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**VGKC= voltage-gated potassium channel. Ab=antibody. LG1=leucine-rich glioma inactivated 1. NMDAR=NMDA receptor. AMPAR=AMPA receptor. GABA,R=GABA type R receptor. GAD=glutamic acid decarboxylase. GlyR=glycine receptor. HSV=herpes simplex virus. PANDAS=pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. CASPR2=contactin associated protein 2.**

Table 1: CNS antibody-associated disorders in adults

Immunoprecipitation of neuronal protein extracts and mass spectrometry permitted subsequent identification of the antigen (eg, AMPAR, GABA,R, and LG1). Binding assays with cells engineered to express the target antigen are now the main methods for detection of these antibodies (figure 1A). Four of these proteins (NMDAR, AMPAR, GABA,R, and glycine receptor [GlyR]) are well known neuronal receptors in the CNS; three less well known (LG1, contactin associated protein-like 2 [CASPR2], and Contactin 2) are described in this Review. Representative structures of CNS antigens and an antibody are described in figure 1B.

The antigens of the voltage-gated potassium channel complex: LG1, CASPR2, and Contactin-2

Recent investigations have shown that most of the antibodies thought previously to be directed towards the voltage-gated potassium channels (VGKCs), are, in fact, binding to other proteins that could be identified in complexes with VGKCs in extracts of mammalian cortical neuronal membranes. The antibodies bind mainly to proteins such as LG1 and CASPR2 or, less frequently, Contactin-2, all of which are components of VGKC complexes. LG1 autoantibodies were initially identified through systematic investigation of proteins known to be complexed with VGKCs and by use of immunoprecipitation and mass spectrometry. Commercial antibodies for LG1 immunoprepcipitate about 70% of the VGKC complexes that were extracted from rabbit brain and identified by use of bound b231-a-dendrotoxin, a snake toxin specific for the Kv1.1, 1.2, and 1.6 subtypes of VGKC, indicating that LG1 is a major component of these complexes. LG1 is a glycoprotein secreted from...
presynaptic terminals that associates with synaptic Kv1 VGKCs and other neuronal proteins. Two specific receptors for LGI1 are the disintegrin and metalloproteinase domain-containing proteins 22 and 23 (ADAM22/23), which are expressed postsynaptically and presynaptically, respectively (for further discussion see Lai and colleagues). LGI1 is highly expressed in the hippocampus and the neocortex, and mutations in LGI1 are associated with autosomal dominant lateral temporal lobe epilepsy. In transgenic mice expressing LGI1 mutations, neurons had abnormal dendritic morphology and seizure phenotypes occurred. Antibodies to LGI1 have been detected predominantly in patients with limbic encephalitis and epilepsy, and in a few patients with Morvan’s disease.

CASP2 is a membrane protein with a large extracellular domain (unlike VGKCs, which have three small extracellular loops; figure 1B) and coinmunoprecipitates with VGKCs. It acts as a cell-adhesion molecule essential for the localisation of VGKCs at neural juxtaradonades; it interacts with another adhesion protein, contactin-2 (called Tag-1 in mice). Contactin-2 has a large extracellular domain and is expressed in axons and ensheathing glial cells throughout the nervous system. It interacts directly with CASPR2 forming a link between the axon and the glial membranes. Contactin-2 forms a rather small (<10%) component of the VGKC complexes compared with CASPR2 (20%), and few autoantibodies for Contactin-2 have been detected so far in patients. Both of these targets (Contactin-2 and CASPR2) are defined components of the nodes of Ranvier, in which they interact with VGKCs, but other roles in the nervous system cannot be excluded. Mutations that cause truncation and reduced expression of CASPR2 were described in a family with cognitive impairment, seizures, and absent deep tendon reflexes, confirming that CASPR2 has physiological roles throughout the nervous system. Antibodies to CASPR2 have been identified in patients with Morvan’s disease or neuromyotonia, and also in some cases of limbic encephalitis.

Antibodies to LGI1, CASPR2, and Contactin-2 can be detected in patients’ serum samples by use of cell-based assays (figure 1A). The cell-based assays for LGI1, CASPR2, and Contactin-2 and the radioimmunoassay for VGKC-complex antibodies are becoming commercially available. The radioimmunoassay measures antibodies to all components of the VGKC complexes and this Review will generally refer to VGKC-complex antibodies, unless otherwise specified.

**Limbic encephalitis associated with antibodies to the VGKC complex**

Limbic encephalitis is the most common syndrome associated with high concentrations of serum VGKC-complex antibodies. This form of limbic encephalitis is now diagnosed regularly in Europe, the Americas, and Australasia. High titres (≥400 pmol/L; normal values <100 pmol/L) were detected in serum samples of patients in a UK study at the rate of about 1–2 per million per year, and in this retrospective study, 64 (67%) of 96 representative patients with these high titres had limbic encephalitis. The rest had neuromyotonia (11%), Morvan’s syndrome (5%), epilepsy only (4%), or CNS features that could not be categorised (12%). Tan and colleagues suggested a wider range of clinical features in patients with VGKC-complex antibodies in serum samples that were referred for paraneoplastic antibody screening. As a consequence, the cohort included a high proportion of patients with paraneoplastic syndromes (37% of 72). LGI1 antibodies were detected in all 57 patients with limbic encephalitis in one study, but in another antibodies to LGI1 were detected in 49 (77%) of 64 cases, with CASPR2 antibodies in 7 (11%), and the VGKC-complex protein undefined in the remaining 8 (12%). Other antigenic components of the VGKC complex might therefore still await identification.

Typically, patients with limbic encephalitis and VGKC-complex antibodies present with acute to subacute onset

<table>
<thead>
<tr>
<th><strong>Limbic encephalitis</strong></th>
<th><strong>Onconeural antibodies (anti-Hu, Ma2/2, CV2, amphiphysin)</strong></th>
<th><strong>GAD antibodies</strong></th>
<th><strong>VGKC-complex antibodies (LGI1, CASPR2, Contactin-2)</strong></th>
<th><strong>NMDAR antibodies</strong></th>
<th><strong>AMPA antibodies</strong></th>
<th><strong>GABA,R antibodies</strong></th>
<th><strong>Amphiphysin antibodies</strong></th>
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<td>+</td>
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**Progressive cortic-subcortical encephalopathy**

|                         | +                                                           | +                 | +                                                           | +                | +                 | +                 | +                 |

**Rapidly progressive abnormal behaviour resembling acute psychosis**

|                         | +                                                           | +                 | +                                                           | +                | +                 | +                 | +                 |

**Stiff person syndrome complex**

|                         | +                                                           | +                 | +                                                           | +                | +                 | +                 | +                 |

Table 2: Suggested testing for autoantibodies in patients with different presentations

Australasia. High titres (>400 pmol/L; normal values <100 pmol/L) were detected in serum samples of patients in a UK study at the rate of about 1–2 per million per year, and in this retrospective study, 64 (67%) of 96 representative patients with these high titres had limbic encephalitis. The rest had neuromyotonia (11%), Morvan’s syndrome (5%), epilepsy only (4%), or CNS features that could not be categorised (12%). Tan and colleagues suggested a wider range of clinical features in patients with VGKC-complex antibodies in serum samples that were referred for paraneoplastic antibody screening. As a consequence, the cohort included a high proportion of patients with paraneoplastic syndromes (37% of 72). LGI1 antibodies were detected in all 57 patients with limbic encephalitis in one study, but in another antibodies to LGI1 were detected in 49 (77%) of 64 cases, with CASPR2 antibodies in 7 (11%), and the VGKC-complex protein undefined in the remaining 8 (12%). Other antigenic components of the VGKC complex might therefore still await identification.

Typically, patients with limbic encephalitis and VGKC-complex antibodies present with acute to subacute onset
memory loss, confusion, mediotemporal lobe seizures, agitation, and other psychiatric features evolving over several days or weeks. Sometimes, the patients have a preceding history of systemic infection. Most patients are older than 40 years, and men predominate (2:1 ratio). Some patients are diagnosed at initial presentation with psychosis or cryptogenic epilepsy. Rapid eye movement sleep behaviour disorder is common in these patients when investigated with appropriate sleep studies. Other sleep disturbances, startle syndrome, hypothermia, and ataxia have been reported in individual patients. Some patients with limbic encephalitis associated with antibodies to the VGKC complex develop intestinal pseudo-obstruction, probably due to antibodies accessing the myenteric plexus.

Viral encephalitis, toxin or drug-induced encephalopathies, and Wernicke's encephalopathy must be considered in the differential diagnosis, and little doubt exists that similar cases in the past were presumed to have an unproven viral infection. In a recent multicentre UK study of encephalitis, 7 (3%) of 203 patients had antibodies to VGKC complexes. Although estimates vary, a fifth of non-prion cases of rapidly progressive dementia might be accounted for by autoimmune causes, and some cases that meet WHO criteria for Creutzfeldt-Jakob disease have been shown to have VGKC-complex antibodies and an immunotherapy-responsive syndrome, widening the differential diagnosis of this untreatable condition. Low sodium concentrations in plasma (between 115 mmol/L and 130 mmol/L) before antiepileptic therapy or other treatments have been started should alert the clinician to the diagnosis of limbic encephalitis with VGKC-complex antibodies; low sodium concentrations have been reported in 38 (59%) of 64 patients. Concentrations of VGKC-complex antibodies are typically high (>400 pmol/L) in patients with limbic encephalitis, often higher than 1000 pmol/L, but lower values (100–400 pmol/L) can be detected in patients with similar clinical presentations including children, in those sampled later in the disease when some recovery has occurred, or in patients who improve spontaneously. Additionally, titres lower than 400 pmol/L are frequent in neuromyotonia.
detected in some patients with epilepsy (see later), in 8 (5%) of 164 elderly patients, and have been reported in patients with tumours, particularly in those with thymomas, or lung or other carcinomas. Thus, although in the Oxford study only three of the 64 patients with limbic encephalitis and high-titre VGKC-complex antibodies had past histories of tumours, and none had detectable tumours despite a mean follow-up of 3 years, paraneoplastic forms of limbic encephalitis must be excluded with serology for onconeural antibodies (table 2) and appropriate body imaging.

High signal in the mesial temporal lobes, either unilaterally or bilaterally, is common, particularly on T2 or fluid-attenuated inversion recovery (FLAIR) MRI, but up to 45% of patients with limbic encephalitis with VGKC-complex antibodies can have normal MRI at onset or through the disease course. The amygdalae are sometimes affected, occasionally without further temporal lobe involvement. Fluorodeoxyglucose PET can be more sensitive in detecting hippocampal dysfunction than MRI, and can show altered metabolism (hypermetabolism early in the disease; hypometabolism at late stages) in the limbic areas. Electroencephalography usually shows interictal foci of epileptiform activity or slowing over antero-temporal or mid-temporal (sometimes also frontal) regions and ictal activity in the same areas.

Seizures often show a poor response to antiepileptic drugs, but respond well to immunotherapies such as steroids, plasma exchange, and intravenous immunoglobulins (IVlg). Plasma sodium concentrations often normalise, and VGKC-complex antibodies are usually undetectable within a few months in patients who are treated adequately. In many cases, the antibodies will not reappear following careful weaning of steroids, and these patients often seem to have a monophasic rather than chronic or relapsing-remitting disease, but, in a small proportion of patients, the antibodies persist or reappear and the patients improve slowly or relapse. On the basis of modified Rankin scores, many patients with typical limbic encephalitis associated with VGKC-complex antibodies are left with restricted deficits that usually do not impinge on daily living, or only modestly impair function. Figure 2 shows the treatment responses for a cohort of patients, according to the presence of antibodies for LGI1 or CASPR2. Almost all patients in this cohort responded well, except those with thymomas who were positive for CASPR2 antibodies (see below).

**Faciobrachial dystonic seizures and other epilepsies associated with VGKC-complex antibodies**

Several studies were designed to show the presence of VGKC-complex antibodies in patients with antiepileptic drug (AED)-refractory or otherwise unexplained epilepsy. Raised concentrations were detected in 22 (9%) of 245 patients in two different cohorts with the higher values usually associated with a subacute onset of encephalitis, with or without limbic features. Additionally, high VGKC-complex antibodies (>400 pmol/L) have been identified in patients who present with frequent, brief dystonic limb movements, especially of the face and arms. We termed these faciobrachial dystonic seizures (FBDS). These patients can respond well to immunotherapies, with falls in concentrations of VGKC-complex antibodies in parallel with their clinical improvement, but most patients who are not offered immunotherapies at onset progress to typical limbic encephalitis and almost all have LGI1 antibodies. Although it is not yet clear how many patients with VGKC-complex or LGI1 antibodies and FBDS will not progress to limbic encephalitis, since they often respond poorly to antiepileptic medication, recognition of these distinctive seizures (see videos) should prompt consideration of immunotherapies.

**Limbic encephalitis associated with AMPAR antibodies**

Antibodies to AMPARs have been recently described in patients with an otherwise typical limbic encephalitis...
often associated with relapses; the AMPAR antibodies have so far been detected mainly in female patients with different tumours, and who responded well to tumour treatment and immunotherapies.\textsuperscript{39–42} It is too early to know whether, in the future, other patients without neoplasms might be identified (as for the other syndromes described here), whether the relapses are a distinctive feature of this disorder, and if the phenotype will also widen as more cases are identified (as is happening in the clinical syndrome associated with NMDAR antibodies). Psychosis has been reported as a presenting and dominating syndrome.\textsuperscript{51} One patient had coexisting GAD antibodies, but this patient had stiff-person syndrome as well as limbic encephalitis.

**Limbic encephalitis associated with GABA\textsubscript{R} antibodies**

Patients with GABA\textsubscript{R} antibodies present with early or prominent seizures but otherwise have the typical features of limbic encephalitis. Antibodies to the GABA\textsubscript{R} receptor were detected in 15 individuals with limbic encephalitis.\textsuperscript{53} Seven of these patients had tumours, mainly small-cell lung cancer. They improved with immunotherapies, unlike many of the patients with paraneoplastic forms of limbic encephalitis. As with AMPAR antibodies, it is not yet clear how prevalent this syndrome is or if the spectrum of clinical presentations will expand. Interestingly, in this cohort, three of the patients with GABA\textsubscript{R} antibodies also had GAD antibodies, but their clinical syndromes were not different.

**Limbic encephalitis and epilepsy associated with GAD antibodies**

High titres of GAD antibodies (>1000 U/mL) are associated with various neurological syndromes, including stiff-person syndrome, cerebellar ataxia, limbic encephalitis, and epilepsy.\textsuperscript{44} Here, we will focus on epilepsy and limbic encephalitis, because the other syndromes have been reviewed elsewhere.\textsuperscript{45–47} Reports so far are scarce, numbering 31 patients overall (webappendix pp 1–2) but, by contrast with the forms of limbic encephalitis discussed earlier in this Review, the patients are often young adult women, they do not have tumours, and temporal lobe epilepsy usually dominates the clinical presentation.\textsuperscript{47} All patients with limbic encephalitis associated with GAD antibodies, whose epilepsy has been studied in detail, had temporal lobe seizures and seem to have been classified as limbic encephalitis on the basis of the subacute onset (<6 months) and the relative degree of encephalopathy. In our opinion, hyperexcitability of the temporal lobe is likely to underlie both limbic encephalitis associated with GAD antibodies and epilepsy, which, respectively, might represent the acute and chronic stages of the same disorder. GAD antibodies are directed to an intracellular enzyme and are therefore unlikely to be the key pathogenic moiety themselves because antibodies for intracellular antigens are rarely thought to be pathogenic. However, since these diseases are associated with high signal on T2 MRI in the medial temporal lobes (figure 3A–D), indicating inflammation (as confirmed by immunohistopathology in patients undergoing biopsies),\textsuperscript{47,48} and patients sometimes respond to immunotherapies, GAD antibodies seem to be markers for an immune-mediated process. An explanation could be that other antibodies might coexist in these patients: cell-surface antibody binding was identified in some patients with limbic encephalitis positive for GAD antibodies,\textsuperscript{49} and GAD antibodies were detected in three patients with GABA\textsubscript{R} antibodies and limbic encephalitis.\textsuperscript{51} It is possible that these antibodies might have caused the disease and this needs to be explored further.

Limbic encephalitis or temporal lobe epilepsy associated with GAD antibodies has a chronic, non-remitting course that finally stabilises.\textsuperscript{47} Individual case reports\textsuperscript{50–52} suggest that some immunotherapies or plasma exchange might have a beneficial effect. In a recent study\textsuperscript{50} of nine patients with seizure-related limbic encephalitis and high titres of GAD antibodies, the patients did not respond well to treatment with monthly intravenous steroid pulses, and seizure frequencies were only modestly reduced (figure 3G). Even when antibody titres substantially fall, the improvement in seizure frequency might not be that great (figure 3H). It must be appreciated, however, that prompter recognition might enable better outcomes.

Titres of GAD antibodies are usually higher than 1000 U/mL\textsuperscript{43,45} compared, for instance, with the lower titres in typical type 1 diabetes. CSF oligoclonal bands and intrathecal synthesis of GAD antibodies are common.\textsuperscript{44,47} Development into hippocampal atrophy, and persistent signal increase are the MRI features characteristic of hippocampal sclerosis, which are also present in some patients with chronic temporal lobe epilepsy (figure 3A–D).\textsuperscript{44}

GAD antibodies are not a marker for paraneoplastic disorders, but have been detected during routine screening for onconeural antibodies by use of indirect immunohistochemistry and immunoblotting (figure 3E), and have been reported in patients in association with lung, renal, pancreatic, or thymic tumours.\textsuperscript{44,53–56} Therefore, comprehensive tumour screening is recommended after diagnosis.

**Syndromes with diffuse involvement of the CNS and characteristic subcortical dysfunction**

**Anti-NMDAR-encephalitis**

NMDAR antibodies are detected in patients with a newly-described syndrome, termed anti-NMDAR encephalitis by the original authors.\textsuperscript{18–20} This syndrome is a severe encephalopathy that generally follows a characteristic temporal sequence of features.\textsuperscript{18–20} Some old case reports probably described the same disease.\textsuperscript{21} The outcome of these patients is often good, but recovery can be slow and 15–25% of patients relapse.\textsuperscript{18–20} A recent review\textsuperscript{22} discusses the clinical experience of 400 patients and the pathological...
mechanisms of the disease. Initially, reported patients were women between 15 and 45 years of age, all of whom had ovarian teratomas.66 In the first large US series of 100 patients, the median age of disease onset was 23 years (range 5–76 years) with 91% being women.65 In a more recent UK study,68 however, the first 44 patients were similar in age range, but only 32 (73%) were women. Importantly, children are beginning to form a substantial subgroup of these patients,67,68 and tumours are present in less than 10% of patients in this age group, compared with 40–50% in women older than 18 years.68,70 Men are much less likely to have tumours. Six cases, about 20% of encephalitides in a tertiary intensive care unit,71 have been shown to patients with NMDAR antibodies, and patients with these antibodies represented nine (4%) of 203 of all acute encephalitides cases in a UK study.72 Although the initial description involved anti-NMDAR-encephalitis with ovarian teratoma,66 other types of malignancies (small-cell lung, pancreatic, and breast cancer,73 and Hodgkin’s lymphoma74) have occasionally been identified. Moreover, there is increasing evidence that some patients do not have an underlying neoplastic disease.66,68,73,74 Nevertheless, all patients should undergo extensive tumour screening, repeated at yearly intervals.70 Altogether, three large patient series have been reported (clinical data on 225 patients65,67,68 and many others reviewed by Dalmau and colleagues70), as well as many smaller case series,72,73,74 and several single-case reports.73,77,78 The clinical presentation of anti-NMDAR-encephalitis can be very characteristic, with the following stages recognised: (1) in a few patients, diverse infections occur in a prodromal phase; (2) an early stage with psychosis, confusion, amnesia, and dysphasia—some patients might be seen first by psychiatrists; and (3) within 1–2 weeks, patients progress to a later stage characterised by movement
disorders, autonomic instability, hypoventilation, and often reduced consciousness, requiring admission to intensive care (figure 4A). Choreoathetoid involuntary movements are characteristic, but some patients become mute and catatonic.73 Recovery is often slow, even with immuno therapies, and during recovery the clinical features tend to remit in the reverse sequence of their appearance.70 Although the sequential presentation is common, the symptoms in some patients with less well defined syndromes can overlap with those in patients with psychogenic movement disorders, opsoclonus-myo clonus,87 or psychiatric syndromes.70 There are a few patients who have epilepsy as their main or presenting feature. 68,77 Spontaneous recovery can occur but is seldom complete.74 Recent series suggest advantages of treatment, especially if started promptly. Patients with paraneoplastic aetiology seem to have better outcomes than those with non-paraneoplastic disease,66,68 particularly if they undergo tumour removal (and usually immunotherapy) within 4 months of presentation. Early and multiple treatments including corticosteroids, IVlg, and plasma exchange as first-line therapies, with rituximab and cyclophosphamide subsequently, if required, seem to give good outcomes.68,70 In some patients, recovery starts within a few days of teratoma removal68 or plasma exchange,75 but the response often takes weeks to months. In some cases, the antibody titres might still be rising during the early stage of the disease, and treatments might only succeed in prevention of clinical deterioration, rather than lead to rapid improvement. Unfortunately, despite treatment efforts, the median time for ventilatory support was 8 weeks with a range of 2–40 weeks in the first major study.66 Good correlations have been shown between concentrations of serum NMDAR antibodies and clinical improvement,65 or change in disability as measured by the modified Rankin scale.68,73,81 but persistence of antibodies after full recovery has been also noted.68,77 Importantly, and by contrast with most patients with VGKC-complex antibodies, 15 (15%) of 100 and 11 (25%) of 44 patients had a relapsing disease course.65,68 Relapses often occur in patients without a tumour, and seem to include similar clinical features to the primary episode.68 Inadequate treatment of the first episodes (often before the condition was first reported), or occult tumours, might have been partly responsible for those relapses.

In many patients, the severe encephalopathy has no correlate on MRI. If abnormalities are present, they are either seen cortically (usually in the limbic mediotemporal cortex)65,79,88 or subcortically in the brainstem, the basal ganglia, or the cerebellum.65,68 Rarely, T2 and FLAIR hyperintensities or contrast enhancement (in cortical meninges or basal ganglia) are detected.66 In some patients, mediotemporal or frontotemporal atrophy can develop,65,68,77 but few reports of longitudinal MRI studies exist.

Moderate lymphocytic pleocytosis (median 32 cells per μL, range 5–480 cells per μl) is frequent at onset.66,68 In many patients, oligoclonal bands are detected in the CSF, but these are not necessarily present at onset.66

Figure 4: Anti-NMDA receptor encephalitis
(A) Time course of first presentation of neuropsychiatric and other clinical features. Data are presented as Kaplan Meier cumulative plots. Day 0 corresponds to the day of first presentation of any neurological feature. Note that, in adults, the neuropsychiatric features almost always precede presentation of movement disorders, autonomic dysfunction, or reduced consciousness by about 10 days or more. Data modified from Irani and colleagues.68 In children, the progression is less characteristic.70 (B) Values from endpoint titrations of 14 serum samples and paired CSF samples. Note the different vertical scales and that serum levels of NMDAR antibodies are higher than CSF levels; these values have not been first normalised to the total IgG concentration as was done, for instance, in the study by Dalmau and colleagues.65 *One data pair could not be plotted as the CSF was negative. Reproduced from Irani and colleagues68 by permission of Oxford University Press.
Concentrations of NMDAR antibodies in serum are in our experience higher than those in the CSF (figure 4B), but when normalised to total IgG concentrations (which are about 300–400 times higher in the serum), almost all patients show high intrathecal synthesis of NMDAR antibodies.\(^{65,68}\) Whereas no electroencephalographic abnormalities might be present at onset, widespread interictal and ictal epileptiform activity arising from the cortex can be seen in some patients during the early stage of the disease. More commonly, and at later stages, this activity is replaced by generalised diffuse dysrhythmic, high-amplitude slowing.\(^{38}\)

**Morvan's syndrome**

This syndrome is a rare disorder, first described in 1890,\(^{48}\) and was thought to be caused by heavy metals or other neurotoxins. The association with neuromyotonia, myasthenia,\(^{46–49}\) thymomas, and reported improvement after plasma exchange\(^{49,53}\) has led to its recognition as a potentially treatable autoimmune disease. No established definition of Morvan’s syndrome exists, but characteristic cases present with dysfunction of the cortex, thalamus, hypothalamus, or brainstem, and autonomic and peripheral nerve involvement. Reported cases are rare but the disease seems to occur worldwide.

Patients with Morvan’s syndrome can present with a subacute or insidious onset, including insomnia, psychiatric disturbance, memory loss, confusion and, in two well studied cases,\(^{91,94}\) evidence of dysregulation of neuroendocrine and circadian rhythms. In the face of these overt symptoms, the presence of fasciculations, cramps, and sweating might be overlooked. Pain, which could be of a neuropathic nature or manifest as arthritis or myalgia, is common, and dysautonomia leads to variable hyperhidrosis, cardiac arrhythmias, haemodynamic disturbances, impotence, constipation, urinary problems, excess salivation, and lacrimation.\(^{93–98}\)

MRI is often normal. Oligoclonal bands can be detected in the CSF in some patients. Two patients\(^{94,98}\) presented with psychiatric features and had increased metabolic activity in the basal ganglia, which normalised with immunotherapies in one case.\(^{94}\) Tumour screening with CT or MRI frequently shows the presence of a thymoma, or occasionally another tumour. VGKC-complex antibodies are raised in most patients with Morvan’s syndrome, although their true incidence is unclear, since some patients have similar clinical presentation but are negative for VGKC-complex antibodies.\(^{91,94}\) The co-existing presence of high signal in the medial temporal lobes on MRI suggests an occasional overlap with features of limbic encephalitis.\(^{97}\) A high proportion of the VGKC-complex antibodies in Morvan’s syndrome are directed against CASPR2, but some patients have LGI1 antibodies.\(^{13}\) Some cases resolve spontaneously,\(^{99}\) but most will be treated with symptomatic treatments and immunotherapy, and the results are often impressive clinically\(^{91,96}\) and serologically.\(^{94}\) However, if a thymoma is present, the prognosis can be poor.\(^{51,100}\)

**Disorders associated with GlyR antibodies**

A rare disorder, part of the stiff-person syndrome spectrum, is progressive encephalomyelitis with rigidity and myoclonus (PERM). The clinical features of this disorder include stiffness and rigidity, excessive startle in response to various stimuli, and brainstem involvement with oculomotor dysfunction. Respiratory arrest can occur and the disease is potentially life-threatening. Several post-mortem studies have been reported in the literature.\(^{56,102,103}\) Some patients have antibodies to GAD, as do those with stiff-person syndrome.\(^{104}\) One patient with PERM and a typical history of auditory and tactile-stimulated startle, rigidity, myoclonic jerks, and brainstem involvement, and no evidence of GAD or paraneoplastic antibodies, was treated aggressively with immunotherapies over 2 years and made a good recovery despite initial worsening.\(^{104}\) He had antibodies to glycine receptor alpha 1 at concentrations that correlated with his clinical features, and the autoantibody titre was lowered after successful treatment. Other patients with these antibodies have been identified, with features of hyperekplexia, stiff-person syndrome, or PERM,\(^{105}\) usually without co-existing GAD antibodies. The clinical spectrum is expanding\(^{106}\) and, in one patient, immunotherapies and removal of a thymoma resulted in complete recovery.\(^{107}\)

**Challenges and questions for the future**

All these findings in patients with disorders associated with autoantibodies raise crucial questions. How should the diseases be classified—on the basis of clinical presentation or the associated specific antibody? What are the causal mechanisms in patients without tumours? How do the antibodies access the brain parenchyma? Which cells are affected and by which mechanisms? What are the best treatment strategies?

**Are the antibodies pathogenic?**

All the diseases considered above, except limbic encephalitis with GAD antibodies, are associated with antibodies to proteins that are expressed on the plasma membrane of neurons. As in myasthenia gravis, which still provides the paradigm for the study of these disorders, irrespective of the precise mechanisms, loss of function of the specific target is likely to be the major pathophysiological mechanism in vivo. VGKC-complex antibodies from patients with neuromyotonia have been shown to act mainly by downregulation of VGKC currents and do not seem to activate complement,\(^{108}\) consistent with the predominance of IgG4 antibodies. However, these conclusions from in-vitro experiments on VGKC-complex antibodies in neuromyotonia might not apply to all the antibodies from patients with CNS syndromes. Nevertheless, the pathogenicity of the VGKC-complex
induced currents in hippocampal cultures, of the LGI1 antibodies on VGKCs. Other mechanisms effect of α-dendrotoxin and supports a modulatory effect excitability in hippocampal slices. This is similar to the with limbic encephalitis and LGI1 antibodies on neuronal epileptogenic effects of purified IgG from one patient antibodies in limbic encephalitis is supported by the epileptogenic effects of purified IgG from one patient with limbic encephalitis and LGI1 antibodies on neuronal excitability in hippocampal slices. This is similar to the effect of α-dendrotoxin and supports a modulatory effect of the LGI1 antibodies on VGKCs. Other mechanisms have not yet been investigated and further possibilities are discussed elsewhere.

Experiments with anti-NMDAR purified IgGs or CSF samples led to a substantial reduction in surface expression of NR1 (and NR2B) subunits and in NMDAR induced currents in hippocampal cultures, and reduced NMDAR immunostaining in the hippocampus after in-vivo infusion of the antibody in rats. The loss of NMDARs was shown to be dependent on divalent IgG, as previously shown for AChR antibodies in myasthenia gravis. Post-mortem pathology also showed reduced NMDAR staining, and did not suggest a major role for complement, which is surprising since NMDAR antibodies are IgG1 and capable of activating complement on the surface of NRI-transfected HEK293 cells and hippocampal neurons. CSF and purified IgGs also increased corticmotor hyperexcitability in rats.

GAD antibodies are unlikely to be pathogenic, although in-vitro studies suggest that IgGs from patients can mediate effects on cerebellar neurons, and intracerebellar injection of IgG can alter corticmotor responses and induce continuous motor activity. One possibility to explain these findings could be that the antibodies are taken up into the neurons, where they inhibit GABA synthesis, similar to what has been shown for amphiphysin antibodies associated with paraneoplastic stiff-person syndrome. Another and perhaps more likely explanation is that other unknown antibodies in sera positive for GAD antibodies are the pathogenic moiety; the possible co-existence of antibodies for GABA, R, ClyR, or other membrane antigens warrants further study.

In the future, it will be important to perform systematic experiments with purified IgG from patients with the different antibodies or, better still, with affinity-purified antibodies, on cultured neurons, brain slices, and with both intrathecal and systemic injection in vivo in animals. For these experiments, ways to increase blood–brain barrier permeability in animals should be explored. Active immunisations to study both T-cell and B-cell responses and induce continuous motor activity. One intracerebellar injection of IgG can alter corticmotor reactivity towards the antigen should be established, and cellular immunity to the antigens investigated. The relative roles of neuronal atrophy and plasticity in disease and during recovery will also need to be dissected in patients and in animals.

Figure 5: Compartment model of the blood-CSF-CNS parenchyma relation
(A) Schematic anatomy of the spaces containing antibodies and antibody-producing plasma cells within the three-compartment model. (1–4) Potential locations of antibody-producing plasma cells (1) in the bloodstream, (2) in Virchow-Robin spaces, (3) in the subarachnoid (CSF) space, and (4) within the brain parenchyma. (a–d) Potential ways of antibody penetration into the CNS parenchyma: (a) directly from the bloodstream, (b) from the Virchow-Robin spaces, (c) from the subarachnoid space, and (d) from intraparenchymal plasma cells. (B) Concentration gradients of antibodies in a simplified four-compartment model (the subarachnoid and Virchow-Robin spaces are grouped together). Whereas the normal ratios between blood and CSF IgG concentrations is known (400:1), the equivalent ratio between Virchow-Robin spaces are grouped together). Whereas the normal ratios between blood and CSF IgG concentrations is known (400:1), the equivalent ratio between Virchow-Robin spaces are grouped together). Whereas the normal ratios between blood and CSF IgG concentrations is known (400:1), the equivalent ratio between Virchow-Robin spaces are grouped together). Whereas the normal ratios between blood and CSF IgG concentrations is known (400:1), the equivalent ratio between Virchow-Robin spaces are grouped together). Whereas the normal ratios between blood and CSF IgG concentrations is known (400:1), the equivalent ratio between Virchow-Robin spaces are grouped together). Whereas the normal ratios between blood and CSF IgG concentrations is known (400:1), the equivalent ratio between Virchow-Robin spaces are grouped together). Whereas the normal ratios between blood and CSF IgG concentrations is known (400:1), the equivalent ratio between Virchow-Robin spaces are grouped together). Whereas the normal ratios between blood and CSF IgG concentrations is known (400:1), the equivalent ratio between Virchow-Robin spaces are grouped together).

Antibodies in serum are usually higher in serum than in the CSF. Moreover, VGKC-complex antibodies are not always detectable in the CSF. Ideally, both serum and CSF samples should be sent for antibody testing, but their relative utility in follow-up of patients is under debate. Suggestions for antibody testing in patients with different clinical presentations are given in table 2, and since some syndromes can be associated with different antibodies (eg, AMPAR, VGKC-complex, or anti-NMDAR antibodies in limbic encephalitis, isolated psychosis, or epilepsy), the advent of multiple-antigen testing is welcome. Since this is an evolving specialty, it is always best to check with the local referral laboratory before requesting the tests.
Intrathecal synthesis of IgG and oligoclonal bands can help point to an immune-mediated disorder, before the results of specific antibodies can be obtained, but in the diseases discussed here, oligoclonal bands are not always present at onset or even thereafter, and whether their presence is evidence of ongoing pathology or merely a secondary epiphenomenon is not yet clear. Now, however, the intrathecal synthesis of antigen-specific antibodies can be assessed. This is done by calculation of the amount of specific antibodies in the CSF relative to the total CSF IgG, and by comparison with similar calculations in the serum. The ratio represents intrathecal synthesis and is often, but not always, high in the diseases discussed here. But does this mean that these conditions are dependent on intrathecal synthesis of antibodies? First, one might expect intrathecal synthesis of any antibody for which the target antigen is expressed in the CNS—irrespective of whether these antibodies are pathogenic—since antibody-secreting cells might migrate to the CNS and secrete antibodies there. Second, although the antibodies undoubtedly need to access the brain parenchyma to induce pathophysiological effects, how this occurs is not entirely clear. Some potential pathways are summarised in figure 5. In favour of a role for systemic rather than intrathecal antibodies, animal experiments have shown that certain regions of the brain can become leaky—ie, when systemic inflammation exists or when senescence is accelerated in mice. Moreover, the hippocampus and the hypothalamus seem to be particularly vulnerable and it is notable that limbic encephalitis with VGKC-complex antibodies, and anti-NMDAR encephalitis, usually do not start with symptoms that could originate in the temporal lobe cortex, even though the target antigens (LGI1, CASPR2, NR1) are present more widely in the nervous system. Further CSF and animal studies exploring these issues should be informative.

Optimisation of treatments
Patients with CNS diseases associated with autoantibodies, by contrast with those with myasthenia, usually do not respond quickly to treatment, although in some cases the improvement is noted within a week. Is response slow because the CNS takes time to recover, or because reduction of antibody concentrations in serum is not sufficient to speed up recovery? If intrathecal synthesis and concentrations of antibodies in the CSF are the driving force in these diseases, why does plasma exchange seem to work in some patients? Surely, one should be using drugs that gain access to the CNS. Rituximab, which is becoming popular for rapidly depleting B cells in patients with various autoimmune disorders, persisted in the CSF of patients with multiple sclerosis in one study. Cyclophosphamide might also be suitable for CNS disorders, although few reports exist. Now that we know that specific antibodies are associated with these disorders, it will be important to understand whether successful treatments need to act directly on the intrathecal production of antibodies, rather than on serum concentrations, and which correlates best with changes in clinical severity. For these studies, available diagnostic assays are seldom sufficiently accurate. In our experience, many treatments seem to affect both serum and CSF antibodies in parallel, but others have found a better correlation with CSF concentrations. Clinical trials for these disorders that include systematic titrations of paired serum and CSF samples over the disease course should be established.

Conclusions
Patients with disorders of the CNS associated with autoantibodies can now be diagnosed and treated. Importantly, anti-NMDAR antibodies have been identified in paediatric patients, and VGKC-complex antibodies are beginning to be detected in children with limbic encephalitis or status epilepticus. The known antibodies might only be the tip of the iceberg—with others waiting to be identified in well recognised disorders or even in some not yet considered to have immune aetiology.

How can these diseases be recognised in the clinic? From the evidence presented here, one can suggest as initial diagnostic steps a combination of subacute (occasional acute) onset of a neurological disorder over days or weeks, changes in MRI or CSF, and the exclusion of other diagnoses (eg, viral encephalitis, tumours, and metabolic, drug, or toxic effects). In the absence of detection of an antibody specific for a neuronal protein, the proof that the disease is antibody-mediated will come from the response to immunotherapies, but it is important to realise that this response might not be rapid and, in some cases, immunotherapies might need to be aggressive and sustained.

Contributors
AV, CGB, and SRI contributed sections to the paper and PW created figure 1. AV, CGB, SRI, and PW were all fully involved in the editing of the report and in the submission process. AV had full access to all data and final responsibility for the decision to submit for publication.

Conflicts of interest
AV and the University of Oxford hold patents and receive royalties for antibodies assays. AV, SRI, and PW may receive royalties for use of LGI1, CASPR2, and Contactin-2 in antibody assays; CGB acts as a consultant for UCB, Monheim, Germany, and receives occasional lecture fees from companies producing antiepileptic drugs.

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Search strategy and selection criteria
We searched the authors’ own databases and Pubmed for reports in English published in the past 15 years, focusing on limbic encephalitis, stiff-person syndrome, PERM, Morvan’s syndrome, potassium channels, autoimmunne, VGKC, LGI1, CASPR2, GAD, GlyR, NMDAR, AMPA receptor, and GABA<sub>R</sub> receptor. We included the papers that we felt added to the descriptions of the diseases.
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