

Autoantibody testing in encephalopathies

Rachel Lee, Camilla Buckley, Sarosh R Irani, Angela Vincent

Nuffield Department of
Clinical Neurosciences,
Neuroimmunology Laboratory,
University of Oxford, Oxford, UK

Correspondence to

Angela Vincent,
Nuffield Department of
Clinical Neurosciences,
Neuroimmunology Laboratory,
University of Oxford, Level 5/6
John Radcliffe Hospital, Oxford
OX3 9DU, UK;
angela.vincent@clneuro.ox.ac.uk

Received 21 July 2011
Accepted 15 September 2011

The last decade has seen significant advances in our understanding of the spectrum of probable antibody-mediated central nervous system disease and the autoantibodies that can help in their identification. In their recent review for this journal, Paul Gozzard and Paul Maddison detailed the wide range of rare neurological conditions associated with paraneoplastic (onconeural) antibodies, which are usually directed at intracellular antigens. Here, the authors focus on antibodies directed against mainly cell-surface neuronal antigens which are associated with central nervous system conditions that often respond to immunotherapies.

Introduction

Antibodies that target the extracellular domain of neuronal cell-surface proteins and thus are likely to contribute directly to the pathology of the condition. Patients with these antibodies often do not have associated tumours and tend to respond well to immunotherapies, with tumour treatment, if appropriate. Successful treatments are often associated with paralleled reductions in antibody concentrations. Some appear to have a monophasic course but others may relapse and require ongoing immunosuppression.¹

By contrast, onconeural antibodies usually target intracellular proteins and represent markers of an associated cancer but have not, in general, been shown to be pathogenic.² Patients with these onconeural antibodies usually do not respond to immunosuppression even in addition to tumour removal. Nevertheless, because of the

importance of identifying an underlying neoplasm, onconeural antibodies are often also requested when an autoimmune condition is suspected, even when a cell-surface neuronal autoantibody is found.

Although all currently recognised antibody-mediated non-paraneoplastic central nervous system (CNS) disorders are rare, numbering perhaps 5–10 per million per year (based on current results of routine antibody screening from the Oxford Clinical Service), it is important to identify these disorders so that appropriate treatments can be offered. Here, we describe the syndromes and provide guidelines to the antibody tests that are most likely to be helpful in each clinical scenario. It is important to appreciate that there are patients whose syndromes appear to be identical to the established phenotypes, but who are negative on the tests currently available. Moreover, as mentioned below, autoantibodies to specific neuronal proteins are beginning to be recognised in a proportion of patients with otherwise unexplained subacute onset of neurological symptoms, with or without MRI and cerebrospinal fluid (CSF) evidence of inflammation. In all of these patients, immunosuppressive treatments should be seriously considered, although it needs to be made clear that there have been no formal treatment trials in most of the conditions discussed here.

Limbic encephalitis (LE)

LE typically presents with acute to subacute onset of memory loss, confusion and seizures (table 1). Classically, a combination of MRI evidence of hippocampal swelling/inflammation, mediotemporal EEG

abnormalities, abnormal CSF and/or immunohistological evidence of inflammation would be required for the diagnosis. However, with detection of a specific antibody, the diagnosis can be made on clinical, radiological and serological grounds alone.³ Since the previous *Practical Neurology* guide to LE in 2006,⁴ several new antibodies have been identified that are associated with LE. Ideally such patients should now be tested for serum (paired with CSF if possible) antibodies to voltage-gated potassium

channel (VGKC) complex antigens (leucine-rich glioma inactivated protein 1 (LGI1), contactin-associated protein-2 (CASPR2) and contactin-2), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA_Rs), γ -aminobutyric acid-B receptors (GABA_BRs), glutamic acid decarboxylase (GAD), *N*-methyl-D-aspartate receptor (NMDAR) and for onconeural antibodies (particularly anti-Hu, anti-Ma 1/2, CV-2, amphiphysin, but most laboratories will first perform screening tests to look for all

Table 1 Clinical and paraclinical features of limbic encephalitis

Clinical presentation	Specific features that might help in selecting antibody tests	Which antibodies?	Demographics	Imaging and CSF	Most likely tumour (if paraneoplastic)	Outcomes
Limbic encephalitis						
Subacute memory loss, psychiatric/behavioural disturbance, seizures. MRI typically shows high signal in the MTLs. CSF variable but can be pleocytosis, mildly raised protein or oligoclonal bands	Serum hyponatremia is a distinctive feature	VGKC-complex LGI1 in majority but can be CASPR2	Median age: 65 years. Male: female 2:1	MRI: increased MTL signal but can be normal in 40%. Hippocampal atrophy may develop. CSF: Normal or mild lymphocytosis, unmatched oligoclonal bands uncommon.	Uncommon but SCLC, thymoma and other tumours have been reported with VGKC-complex antibodies, usually directed at CASPR2	Good, unless a tumour complicates prognosis. AEDs and immunotherapies can be withdrawn after months to years in many cases
	Rapidly progressing LE with frequent features of acute psychosis	AMPA _R (GluR 1/2)	Median age: 60 years. Female predominance	MRI: typical increased MTL signal. CSF: Lymphocytic pleocytosis, elevated protein.	SCLC, breast, thymoma (70%)	Good but tendency to relapse, without tumour recurrence. Residual memory deficits.
	Seizures as the predominant symptom, usually of temporal lobe onset with secondary generalisation Seizures may present without memory loss. Can be associated with SPS or cerebellar ataxia	GABA _B R GAD	Median age: 62 years. Male: female 1:1 Median age: 23 years. Female predominance	MRI: increased MTL signal. CSF: Lymphocytic pleocytosis; mildly raised protein or oligoclonal bands. MRI: increased MTL signal, hippocampal atrophy often evolves over time. CSF: Normal protein and cell count, unmatched oligoclonal bands can be present.	SCLC (47%) Rare	Good Not satisfactory to date. Chronic, non-remitting LE, often little reduction in antibody titres following immunotherapies. Residual memory deficit and AEDs have to be maintained.
Facio-brachial dystonic seizures (FBDS)	Brief (few seconds), frequent (50/day) dystonic seizures. Described as prodrome to VGKC-complex-Ab LE but may occur independently	VGKC-complex LGI1 in almost all	Median age: 64.5 years. Male > female	MRI: usually normal but not often performed. CSF: Not usually tested.	None described to date	Poor response to normal AED, but clinical improvement with immunotherapies in a few patients identified prospectively

AED, antiepilepsy drug; AMPAR, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors; CASPR2, contactin-associated protein-2; CSF, cerebrospinal fluid; GABA_BR, γ -aminobutyric acid-B receptors; GAD, glutamic acid decarboxylase; GluR, glutamate receptor; LE, limbic encephalitis; LGI1, leucine-rich glioma inactivated protein; MTL, medial temporal lobe; SCLC, small cell lung cancer; SPS, stiff person syndrome; VGKC, voltage-gated potassium channel.

onconeural antibodies and then confirm with specific immunoblotting).² However, to avoid cost and delay, until wide panels of the new antibodies are available routinely, it is reasonable to limit the antibody request to those that are most likely to be relevant. In table 1 we emphasise those clinical and epidemiological features that may help to distinguish between the LE patients with different antibodies.

The VGKC-complex antigens: LGI1, CASPR2 (and contactin-2)

VGKCs are found throughout the brain and are important in restoring the membrane potential during hyperpolarisation. Antibodies to the VGKC (VGKC-Abs) have been identified since the 1990s using a ¹²⁵I- α -dendrotoxin-labelled radio-immunoprecipitation assay (RIPA). Dendrotoxin labelled VGKCs were extracted from rabbit brain tissue and positive results were first described in patients with acquired neuromyotonia.⁵ This condition includes spontaneous and continuous muscle fibre activity, painful cramps and impaired muscle relaxation; electromyography (EMG) shows doublet, triplet or multiplet single unit discharges with high intraburst frequency.⁶ Some patients with neuromyotonia also have autonomic dysfunction.

VGKC-Abs were then identified in the rare Morvan's syndrome, where symptoms of

neuromyotonia are accompanied by florid autonomic features and CNS involvement.⁷ Subsequently, VGKC-Abs were identified in a form of LE^{8,9} and more recently in patients with faciobrachial dystonic seizures (FBDS).¹⁰

It is now clear that the VGKC-Abs detected by RIPA are not in fact directed at the VGKCs themselves (except in rare cases and perhaps in patients with low titres). They mainly bind to other components of the VGKC-complexes that are present in mammalian brain extracts, and are now better termed VGKC-complex antibodies. The main VGKC-complex proteins identified as antibody targets so far are LGI1^{11,12} and CASPR2.^{11,13} It is likely that antibodies to these proteins will also be found in some patients who are not positive for VGKC-Abs by RIPA (E Becker, P Pettingill, A Vincent, unpublished), but since there may well be more VGKC-complex proteins to identify, and until more sensitive and reliable commercial antibody testing becomes available, we continue to use the RIPA for VGKC-complex antibodies, available as a commercial kit (RSR Ltd, Cardiff, UK), with assays for the specific antigens (see below) if appropriate. Figure 1 illustrates the possible localisations of the VGKC-complex proteins, and figure 2 demonstrates binding of serum antibodies to LGI1 detected using a cell-based assay.

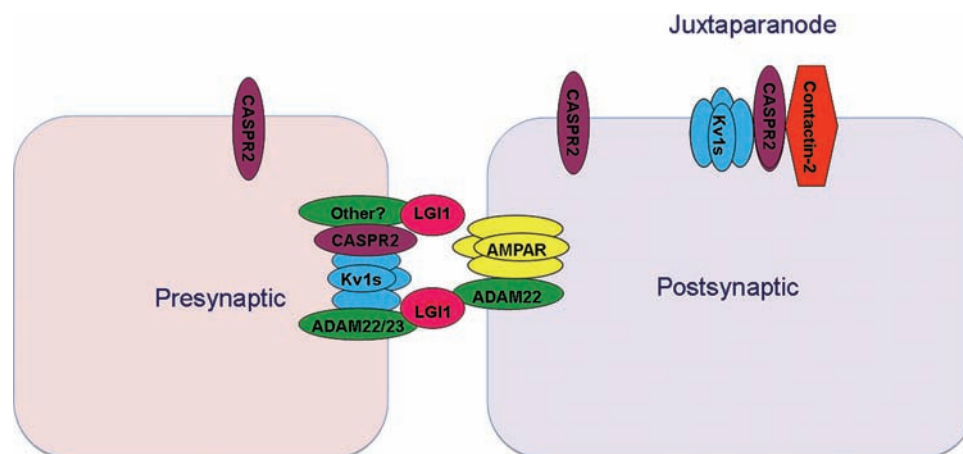


Figure 1 Targets for VGKC-complex antibodies. VGKC antibodies are now recognised to bind mainly to proteins that are complexed with the VGKCs, rather than to the VGKCs themselves. These proteins have extracellular domains and play important roles in localisation of the VGKCs (CASPR2) at the juxtaparanodes adjacent to the nodes of Ranvier, or in modifying neuronal function (LGI1). CASPR2 binds to contactin-2 a less frequent target for VGKC antibodies.²⁶ VGKC Kv1 complexes containing CASPR2, contactin-2 and other proteins are present at the juxtaparanodes in myelinated axons as illustrated top right.²⁶ At the synapse, there are presynaptic Kv1 complexes that may contain both CASPR2 and LGI1²⁶ (left), but it has also been proposed that LGI1 links ADAM22 and ADAM23 transsynaptically as illustrated, ADAM22 interacting with postsynaptic AMPAR.¹² There is also preliminary evidence that antibodies bind to CASPR2 and perhaps LGI1 at sites where they are not complexed with VGKCs. Not all VGKC-complex antibodies bind to these proteins, and doubtless there will be further developments in this field. ADAM, a disintegrin and metalloprotease domain; AMPAR, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors; CASPR, contactin-associated protein; LGI1, leucine-rich glioma inactivated protein; VGKC, voltage-gated potassium channel.

LE with VGKC-complex antibodies

Patients with VGKC-complex-antibodies typically present with the triad of memory loss, confusion and seizures.^{8 9 14} They were first defined on the basis of high titres of VGKC-complex-antibodies (>400 pM). However, lower levels can be found in patients with typical LE¹⁵ as well as in around half the cases with neuromyotonia. An important and diagnostically useful feature of LE patients is serum hyponatremia (in around 60%),^{9 11 12 16} the CSF is usually normal and seldom with oligoclonal bands.^{8 16} EEG may show diffuse slowing with occasional epileptogenic foci. The MRI brain scan typically shows increased bilateral medial temporal lobe (MTL) signal,^{11 12} but with increased recognition it is clear that this occurs in only around 50% of patients and some have unilateral involvement.¹¹

VGKC-complex antibodies are remarkably susceptible to immunotherapies and the antibody titres tend to fall rapidly with effective immunotherapies (eg, high dose oral corticosteroids

or pulsed methylprednisolone, plasma exchange and/or intravenous immunoglobulins).^{8 9 11 14} The seizures usually cease and the hyponatraemia resolves. Cognitive changes often take longer to resolve but moderate to substantial functional improvements can be achieved.¹¹ The MRI changes may resolve but hippocampal atrophy is a common observation at follow-up. Late onset of hippocampal sclerosis and ensuing temporal lobe epilepsy have been identified in adults whose MRIs suggest that they had a preceding form of LE.¹⁷

Most patients with typical LE and VGKC-complex antibodies have antibodies directed against LGI1 and usually do not have a tumour.^{11 12} By contrast, patients with antibodies to the VGKC-complex-protein CASPR2 commonly have neuromyotonia or Morvan's syndrome and a significant incidence of tumours particularly thymomas (often associated with previous or concurrent myasthenia gravis).^{11 13} However, there is some overlap and a few patients with LE do have CASPR2 antibodies.¹¹

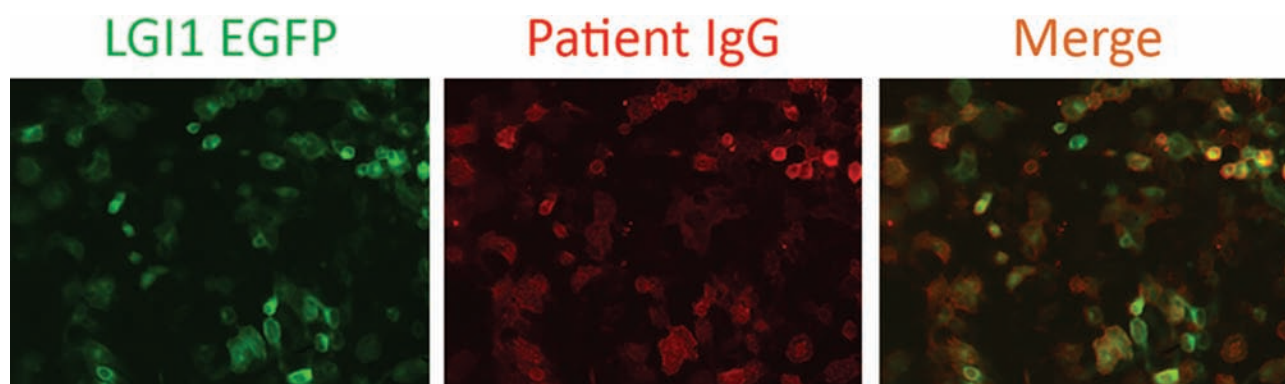


Figure 2 The cell based assay for measuring antibodies binding to cell-surface neuronal antigens. LGI1 antibodies detected by red fluorescent anti-human immunoglobulin G bind to HEK cells transfected with cDNAs for an LGI1 construct and for enhanced green fluorescent protein. Results of a strong positive serum from a patient with typical limbic encephalitis and high titre VGKC-complex antibodies. Control samples do not bind to the transfected HEK cells or give only a faint and non-specific signal. HEK, human embryonic kidney; LGI1, leucine-rich glioma inactivated protein; VGKC, voltage-gated potassium channel.

Table 2 Clinical and paraclinical features of NMDAR-antibody encephalitis

Clinical features	Demographics	Imaging and CSF	Most likely tumour (if paraneoplastic)	Outcomes
Psychosis, seizures, cognitive and behavioural changes evolving to choreoathetoid movement disorders, orofacial dyskinesia, dysautonomia, mutism and catatonia. May have prodromal viral-like symptoms but preceding infections uncommon.	Median age: 23 years but wide age range from 1 to 70 years. Male: Female 1:2.5	EEG: generalised slowing, possible epileptiform activity. MRI: normal, mild abnormalities usually outside the MTL. CSF: lymphocytic pleocytosis, possible raised protein.	Ovarian teratoma (20–59%), rarely testicular teratoma or SCLC.	Better outcomes with early tumour removal and immunotherapy than in patients with no tumour identified or late treatment. Possible frontal lobe dysfunction but may have full recovery

CSF, cerebrospinal fluid; MTL, medial temporal lobe; NMDAR, N-methyl-D-aspartate receptor; SCLC, small cell lung cancer.

FBDS as an epileptic prodrome to LGI1/VGKC-complex antibodies LE

Most recently, high VGKC-complex Ab titres (238 to 8800 pM) have been found in adult patients presenting with dystonic seizures involving the arm and face. These FBDS are very brief (a few seconds) but occur at a median of 50 times a day and have been identified both prospectively and retrospectively in around 20% of VGKC-complex-antibody LE patients.¹⁰ Ninety per cent of the patients had LGI1 antibodies. Interestingly, many patients developed FBDS before the onset of amnesia or confusion. In addition, a few cases were treated with immunotherapies and never developed cognitive dysfunction. Ninety-six per cent of patients showed a greater than 20% reduction in FBDS frequency with these treatments. By contrast, their response to standard antiepileptic drugs was poor.¹⁰ Therefore, FBDS may represent an immunotherapy-responsive prodrome to VGKC-complex LGI1-antibody associated LE.

Indeed, we are now aware of UK clinicians who have diagnosed and treated this syndrome prior to the antibody result without the patients developing amnesia (SRI, AV, MR Johnson, unpublished observations).

Morvan's syndrome

Morvan's syndrome is not diagnosed frequently. It is defined as a combination of muscle fasciculations and cramps, autonomic disturbance and CNS involvement with frequent insomnia and hallucinations.⁷ It can be misdiagnosed as schizophrenia until the muscle involvement is recognised.¹⁸ VGKC-complex antibodies are present in most reported cases, with a high proportion directed at CASPR2 and the patients can improve dramatically after immunotherapies.^{7 18} Thymomas are a commonly associated malignancy in the cases reported to date, and the patients may have co-existing, or previous histories of myasthenia gravis.^{11 13}

Table 3 Clinical and paraclinical features in patients with mainly subcortical features

	Specific clinical features associated with antibody type	Which antibodies?	Demographics	Imaging	Most likely tumour if paraneoplastic	Outcomes
Stiff person syndrome	Stiffness, rigidity, CNS involvement not uncommon. Absent during sleep. May have IDDM and/or other autoantibodies.	GAD but onconeural antibodies particularly to amphiphysin should be requested	Median age: 45. Female predominance	MRI: normal, non-specific. EMG: continuous motor unit activity. CSF: Oligoclonal bands common but not invariable.	Not usually paraneoplastic (rarely breast, lung) with GAD antibodies	Marked clinical improvement but immunotherapy probably needs to be maintained for years
PERM	Rigidity, myoclonus, brainstem and cranial nerve involvement. Progressive and severe.	GAD or Glycine	Median age: 46 years. Female predominance	MRI: normal. EMG: as for SPS. CSF: Lymphocytic pleocytosis, oligoclonal bands possible.	Not usually paraneoplastic	May have lasting remission
Neuromyotonia and Morvan's syndrome	Fasciculations, stiffness and muscle cramps. Peripheral nerve hyperexcitability (neuromyotonia). Autonomic and CNS dysfunction, insomnia (Morvan's).	VGKC-complex (CASPR2 in 37% of cases)	Median age: 54 years. Male predominance	EEG: generalised slowing. EMG: doublet, triplet, multiplet single unit discharges. MRI: usually normal. CSF: Not usually tested.	Thymoma, SCLC	Patients without tumours may respond better to immunotherapy
Cerebellar ataxia	Subacute or chronic onset, slow progression. Limb ataxia, dysarthria, nystagmus. May have diabetes/autoimmune thyroid disease.	GAD or VGKC-complex/CASPR2	Generally middle-aged. Female predominance	MRI: cerebellar atrophy. CSF: May have oligoclonal bands	Not usually paraneoplastic	Clinical improvement and increased cerebellar perfusion

CASPR2, contactin-associated protein-2; CNS, central nervous system; CSF, cerebrospinal fluid; EMG, electromyography; GAD, glutamic acid decarboxylase; IDDM, insulin dependent diabetes mellitus; PERM, progressive encephalomyelopathy with rigidity and myoclonus; SCLC, small cell lung cancer; SPS, stiff person syndrome; VGKC; voltage-gated potassium channel.

LE with AMPAR antibodies

The AMPAR is a subtype of the glutamate receptor (GluR), composed of tetramers of the GluR types 1, 2, 3 or 4 and known to mediate fast excitatory neuronal transmission. Antibodies to the AMPA GluR1 and GluR2 subunits have been found in patients with LE, many of whom have thymus, breast or lung tumours, but some patients had no tumours identified.¹⁹ Unlike the monophasic VGKC-Ab LE, these patients have a tendency to relapse, and some require longer term immunosuppression with azathioprine. CSF may show leucocytosis and elevated protein.

A further report of patients with AMPAR-Ab LE noted two patients who presented with rapidly progressing abnormal behaviour similar to an acute psychosis, who had normal CSF and MRI.²⁰ Physicians need to be aware that atypical psychosis may be linked to AMPAR-Ab and other autoantibodies (see below).

LE with GABA_BR antibodies

A further target for antibody-mediated LE is the GABA_{B1}R, which mediates synaptic inhibition. Of the fifteen reported patients with these antibodies, the predominant presenting symptom was seizures of temporal lobe onset.²¹ Patients had increased MTL signal on MRI and CSF often showed lymphocytic pleocytosis. There was some overlap with other antibodies: a few had antibodies to GAD. Half had tumours, mostly small cell lung cancer, but most (9/15) responded to immunotherapy.²¹

GAD-antibodies encephalitis

GAD is an enzyme required to convert the excitatory neurotransmitter glutamate to the inhibitory neurotransmitter GABA. Lack of this conversion would be expected to lead to motor and CNS hyperexcitability. Autoantibodies to GAD were first described in stiff person syndrome (SPS).²² Unlike the previously discussed antibody targets, GAD is located intracellularly, but high titres of GAD antibodies appear to be markers for immune-mediated diseases including SPS, cerebellar ataxia, epilepsy and a form of LE.²³ Few of these patients have tumours but many have other autoimmune diseases.^{15 23}

There are several reports of patients with GAD-Abs and LE or epilepsy. In one study of nine patients with intractable seizures seen at an epilepsy centre, the patients with GAD-Ab LE were mostly young adult females who presented with seizures without memory loss.¹⁵ CSF was often normal although some had oligoclonal bands and

MRI showed increased amygdalohippocampal signal, which was included in the selection criteria, usually bilaterally. Unlike VGKC-complex antibodies LE patients, those with GAD-Ab LE followed a chronic course, and serum antibody titres did not fall substantially following the immunotherapies used.¹⁵

Encephalitis with NMDAR antibodies

This syndrome really needs to be considered as a distinct entity from LE because of the clinical involvement of much wider brain functions and frequent lack of MRI evidence for temporal lobe swelling or inflammation (table 2).

NMDARs are ligand-gated ion channels, known to be major mediators of excitatory neurotransmission. Antibodies to the NMDAR (the main target being the NR1 subunit) were initially described in a paraneoplastic encephalitis associated with ovarian teratomata, with neurological symptoms presenting prior to tumour diagnosis (range, 1–380 weeks).^{24 25} The first symptoms were usually psychiatric, behavioural disturbances and seizures, with clinical worsening over 10–20 days to generate dyskinesias (orofacial grimacing, dystonic posturing and choreoathetoid movements), autonomic instability and reduced consciousness, often requiring ventilatory support.^{26 27} The two-stage progression of these symptoms may involve cortical and subcortical functions^{26 27} but this pattern may not be as common in children.²⁸ Pleocytosis and NMDAR antibodies in the CSF, with high intrathecal antibody synthesis are very frequent^{25–27} but serum NMDAR-antibody levels are higher in absolute terms.²⁶ MRI findings vary from normal to mild abnormalities, usually outside the MTLs, in the cerebral cortex, overlying meninges or basal ganglia.^{25–27} A recent review covers the clinical and paraclinical findings in more detail.²⁷

With increasing clinical awareness of the syndrome, the proportion of patients with an ovarian tumour has decreased and a higher proportion of children and men are being identified with the syndrome.^{26 27} Paraneoplastic cases are infrequent in children^{26–28} and are very unusual in men.²⁶ In those patients with ovarian or other tumours, the syndrome responds to immunotherapy once the tumour is removed; later removal can be associated with poor outcomes.^{25–27} Although there is no formal evidence for the effects of immunotherapies or for which treatment is most appropriate, patients do seem to benefit from aggressive immunotherapy.^{26 27} Patients may recover completely

or be left with residual cognitive impairment and usually all have amnesia for the events of the acute illness. Relapses occur in up to 25% of non-paraneoplastic patients suggesting that long-term immunosuppression may be helpful.^{25 26}

Antibody-mediated encephalomyelopathies and cerebellar ataxia

SPS and progressive encephalomyelopathy with rigidity and myoclonus (PERM)

Antibodies have been identified in a range of conditions associated with rigidity, spasms and abnormal movements. In these patients it may be relevant to request testing of antibodies to GAD, glycine receptor and the onconeural antibodies (table 3).

Antibodies to GAD

SPS is a rare disorder characterised by muscle rigidity and episodic spasms, which typically affect the lumbar, thoracic, paraspinal and proximal leg muscles. Respiratory function can be compromised leading to increased mortality in this patient group. Patients can have high levels of anxiety and panic attacks and are often initially thought to have a non-organic disorder. Antibodies to GAD are found in the non-paraneoplastic form of the disease,²⁹ whereas amphiphysin antibodies are found in forms associated with neoplasm, most commonly of the breast or lung.² EMG demonstrates continuous low frequency firing of motor units. Treatment includes medication directed against the muscle spasm and rigidity such as benzodiazepines and baclofen, antiepileptics and immunotherapies.³⁰

Antibodies to GAD are also found in PERM as well as other forms of SPS such as stiff limb syndrome. In patients with PERM, rigidity and spasms are preceded or accompanied by brainstem signs (often ataxia and vertigo) and sensory disturbance, followed by generalised myoclonus and autonomic features.³¹ Progression of symptoms can be variable, from a protracted course of typical SPS with some features of encephalomyelitis, to a more rapid deterioration involving the brainstem and cranial nerves until death occurs. For this reason, it is important to monitor patients closely to observe for such deterioration. In PERM, CSF may show lymphocytic pleocytosis and oligoclonal bands, while imaging, EMG findings and treatment approaches are similar to those in SPS.³¹

Antibodies to glycine receptor

Antibodies to the glycine receptor were first identified in one case of PERM, associated with an

excessive startle response. The patient responded to aggressive immunotherapy with intravenous immunoglobulin, prednisolone, plasma exchange and intravenous cyclophosphamide and returned to work part-time with only some residual spinal rigidity and bilateral ptosis.³² Several other SPS or PERM patients with antibodies to glycine receptors have now been identified.^{33–35} The clinical spectrum and treatment responses are variable; only one patient had a tumour and he made a complete recovery after thymectomy.³⁴ One young man, who also had NMDAR-Abs, died unexpectedly.³⁵

Cerebellar dysfunction

GAD-Ab cerebellar ataxia

Cerebellar ataxia has many causes, of which paraneoplastic cerebellar degeneration is the best known, typically associated with antibodies to the onconeural antigens Yo or Hu (and less frequently amphiphysin or Tr), and with a poor outcome.² However, paraneoplastic cerebellar ataxia can also be associated with antibodies to the voltage-gated calcium channel. These are usually in the context of small cell lung cancer, and treatment responses are disappointing.^{2 36} Nevertheless, cerebellar ataxia is the second most common neurological condition associated with antibodies to GAD.²³ The patients predominantly present with gait ataxia, some also have limb ataxia, dysarthria and nystagmus. Although antibodies to GAD are also noted in insulin dependent diabetes mellitus, the levels are typically much higher in patients with neurological diseases (>1000 pM but not always measured quantitatively in routine laboratories). Clinical improvement and increased cerebellar perfusion have been found in some patients with GAD-Ab and cerebellar ataxia treated with intravenous immunoglobulins.³⁷

A recent study of 52 patients with subacute onset of ataxia identified antibodies to CASPR2 in seven, antibodies to voltage-gated calcium channels in three, and to GAD in only one (E Becker, L Zuliani, A Vincent, unpublished data). Interestingly, only one patient (who also had LE) had high VGKC-complex antibodies by the RIPA. None of the seven patients had tumours identified, including four who had been followed up for many years. This work needs to be extended but it seems that there may be several different antibodies associated with autoimmune non-paraneoplastic forms of ataxia, and this is an area that is likely to expand in the future.

Wider implications, general considerations and immunotherapies

Increasingly, each of the antibodies discussed above is beginning to be found in patients with a wider spectrum of clinical features, or in those in whom an autoimmune aetiology might not have been expected. First, there has been a steady trickle of cases of patients with cryptogenic epilepsy who have VGKC or GAD antibodies,^{38 39} although in many of these studies the long-duration of the condition at serum testing raises questions regarding a primary or secondary role of the antibodies. Nevertheless, we have encountered patients with an acute onset of temporal lobe epilepsy (TLE) and highly positive VGKC-complex antibodies directed against both LGI1 and CASPR2 (C Buckley, S R Irani, A Vincent, unpublished data). In a recent study of new onset epilepsy, 15% had VGKC-complex Abs, strongly suggesting that these may play a primary role in some forms of epilepsy, probably both in children^{40 41} and adults (T Brenner, B Lang, unpublished observations). This field has been thoroughly reviewed recently.⁴²

Clinicians should consider antibody testing in patients presenting with acute encephalitis. In a UK study of encephalitis presenting to district hospitals,⁴³ 16 of the 44 patients (36%) without an identifiable infectious aetiology, had autoantibodies to either NMDAR (9 patients) or VGKC-complexes (7 patients), making autoimmunity the third highest association with acute encephalitis. Furthermore, in a series of patients from the Queen Square intensive care unit, around a fifth of the patients with encephalopathies had NMDAR-Abs.⁴⁴

Children with encephalitis lethargica associated with NMDAR-Ab have been described in a non-paraneoplastic setting. These patients presented with dyskinesia, seizures, agitation and somnolence and many of their features were also immunotherapy-responsive.⁴⁵ Although clinically similar to typical NMDAR-Ab encephalitis, their prominent dyskinesias, lack of marked cognitive changes and lowered consciousness meant that the diagnosis could have been missed.

There may also be cases with a relatively pure psychiatric disturbance. Three of 46 patients with first episode psychosis were found to have NMDAR-Ab (n=2) or VGKC-complex Ab (n=1).⁴⁶ Their response to immunotherapies was variable and more detailed studies are required.

It seems likely that these observations are just the tip of the iceberg and that further studies and

time will demonstrate that autoimmunity with antibodies to cell-surface receptors will become an important part of the differential diagnosis of many more neurological and neuropsychiatric syndromes.

Serum or CSF antibodies for diagnosis and management

In our experience, absolute antibody levels are almost always higher in the serum than in the CSF. Only in very rare cases are the CSF levels positive while serum levels are undetectable, and this may be because the serum is usually diluted, at least 1:20 and frequently more, whereas CSF can be tested undiluted (because of the much lower total immunoglobulin G concentration). Much more commonly, the serum values are similar or higher despite a relative serum dilution. Nevertheless, there is often (VGKC-complex antibodies) or almost always (NMDAR, GAD, glycine receptor antibodies) intrathecal synthesis of the specific antibody which means that some of the specific plasma cells have gained access to the intrathecal compartment and are synthesising the antibody there. How early in the disease intrathecal synthesis occurs and whether CSF levels are more informative than serum levels, as some believe, is difficult to say at present, especially without a direct multicentre comparison of the assays. There is at least some evidence that plasma exchange, and probably many other treatments, directly or indirectly affect both serum and CSF levels in parallel, suggesting that serum evaluation should be sufficient. These observations, however, are confounded by different timing of paired samples, and it would be helpful to have regular paired serum and CSF samples at onset, at nadir, and after 'successful' treatment for careful antibody measurements in order to establish the most useful measurements for clinical management in the future. For routine diagnosis in Oxford, serum is sufficient but CSF will always be tested in parallel if provided. For other centres, it would be wise to enquire before sending the samples.

Clinical phenotyping and antibody testing

Although the specificity of the antibodies for their cell-surface target appears to be very high (ie, sera positive for binding to VGKC-complex proteins, do not in the great majority of cases bind to NMDAR or GlyRs), there are rare case reports of patients who have more than one antibody³⁵ and even with careful clinical phenotyping, it is clear that there is considerable overlap between the syndromes associated with the different

Further reading

Hadavi S. Stiff person syndrome. *Pract Neurol* 2011; 11:272–82

antibodies. For this reason in particular, and in order to reduce costs and delay in diagnosis, multiple antigen arrays for testing should be available in the future.

Management of patients with antibody-mediated CNS disease

With such a wide spectrum of disease and range of antibodies, the management of these patients is complex. As previously explained, it is crucial to rule out an underlying malignancy, with CT or positron emission tomography scanning, if appropriate. It may take time for antibody test results to become available and this has been highlighted in questions regarding patient care, particularly in an intensive care setting. Once an infective cause of encephalitis has been ruled out (ie, PCR for herpes simplex virus) immunotherapy should be considered, irrespective of cancer diagnosis or antibody results, as patients tend to respond better and may have fewer long-term sequelae with early aggressive immunotherapy.^{10 25–27} Corticosteroids, intravenous immunoglobulin and plasma exchange are suggested but other drugs including rituximab are increasingly used.

Conclusion

The field of antibody-mediated CNS disease is rapidly expanding. Thorough clinical assessment and appropriate antibody testing are crucial for patient care and future research. Ongoing studies should focus on identifying antibodies to these cell-surface neuronal antigens in patients with a wide range of neurological presentations, finding new antigenic targets, demonstrating antibody pathogenicity by in vitro and in vivo studies, determining the origin of antibody synthesis, and improving clinical care through appropriate immunotherapy management and formal clinical trials.

Acknowledgements SRI was supported by the National Institute of Health Research (NIHR), Department of Health, UK, and CB by the Medical Research Council, Great Britain. The Neuroimmunology Laboratory is supported by the NIHR Oxford Biomedical Research Centre.

Competing interests AV and the Department of Clinical Neurology in Oxford receive royalties and payments for antibody assays. AV is the inventor on patent application WO/2010/046716 entitled 'Neurological Autoimmune Disorders'. The patent has been licensed to Euroimmun

AG for the development of assays for LGI1 and other VGKC-complex antibodies. AV acts as a paid consultant for Athena Diagnostics and is employed by Oxford University and University College London. AV and SRI may receive royalties for testing of VGKC-complex antibodies.

Provenance and peer review Commissioned by Charles Warlow; this paper was reviewed by Neil Scolding.

Contributors Drafted by RL, critically reviewed and amended by CB, SRI and AV. Reviewed and submitted by AV.

References

1. Vincent A, Bien CG, Irani SR, *et al.* Autoantibodies associated with diseases of the CNS: new developments and future challenges. *Lancet Neurol* 2011;10:759–72.
2. Gozzard P, Maddison P. Which antibody and which cancer in which paraneoplastic syndromes? *Pract Neurol* 2010;10:260–70.
3. Bien CG, Elger CE. Limbic encephalitis: a cause of temporal lobe epilepsy with onset in adult life. *Epilepsy Behav* 2007;10:529–38.
4. Schott JM, 2006 Limbic encephalitis: a clinician's guide. *Pract Neurol* 2006;6:143–53.
5. Shillito P, Molenaar PC, Vincent A, *et al.* Acquired neuromyotonia: evidence for autoantibodies directed against K⁺ channels of peripheral nerves. *Ann Neurol* 1995;38:714–22.
6. Hart IK, Maddison P, Newsom-Davis J, *et al.* Phenotypic variants of autoimmune peripheral nerve hyperexcitability. *Brain* 2002;125(Pt 8):1887–95.
7. Liguori R, Vincent A, Clover L, *et al.* Morvan's syndrome: peripheral and central nervous system and cardiac involvement with antibodies to voltage-gated potassium channels. *Brain* 2001;124(Pt 12):2417–26.
8. Buckley C, Oger J, Clover L, *et al.* Potassium channel antibodies in two patients with reversible limbic encephalitis. *Ann Neurol* 2001;50:73–8.
9. Vincent A, Buckley C, Schott JM, *et al.* Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. *Brain* 2004;127(Pt 3):701–12.
10. Irani SR, Michell AW, Lang B, *et al.* Faciobrachial dystonic seizures precede Lgi1 antibody limbic encephalitis. *Ann Neurol* 2011;69:892–900.
11. Irani SR, Alexander S, Waters P, *et al.* Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactivated 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan's syndrome and acquired neuromyotonia. *Brain* 2010;133:2734–48.
12. Lai M, Huijbers MG, Lancaster E, *et al.* Investigation of LGI1 as the antigen in limbic encephalitis previously attributed to potassium channels: a case series. *Lancet Neurol* 2010;9:776–85.
13. Vincent A, Irani SR. Caspr2 antibodies in patients with thymomas. *J Thorac Oncol* 2010;5(10 Suppl 4):S277–80.
14. Thieben MJ, Lennon VA, Boeve BF, *et al.* Potentially reversible autoimmune limbic encephalitis with neuronal potassium channel antibody. *Neurology* 2004;62:1177–82.
15. Malter MP, Helmstaedter C, Urbach H, *et al.* Antibodies to glutamic acid decarboxylase define a form of limbic encephalitis. *Ann Neurol* 2010;67:470–8.
16. Graus F, Saiz A, Lai M, *et al.* Neuronal surface antigen antibodies in limbic encephalitis: clinical-immunologic associations. *Neurology* 2008;71:930–6.
17. Bien CG, Urbach H, Schramm J, *et al.* Limbic encephalitis as a precipitating event in adult-onset temporal lobe epilepsy. *Neurology* 2007;69:1236–44.

18. Spinazzi M, Argentiero V, Zuliani L, *et al.* Immunotherapy-reversed compulsive, monoaminergic, circadian rhythm disorder in Morvan syndrome. *Neurology* 2008;71:2008–10.
19. Lai M, Hughes EG, Peng X, *et al.* AMPA receptor antibodies in limbic encephalitis alter synaptic receptor location. *Ann Neurol* 2009;65:424–34.
20. Graus F, Boronat A, Xifró X, *et al.* The expanding clinical profile of anti-AMPA receptor encephalitis. *Neurology* 2010;74:857–9.
21. Lancaster E, Lai M, Peng X, *et al.* Antibodies to the GABA(B) receptor in limbic encephalitis with seizures: case series and characterisation of the antigen. *Lancet Neurol* 2010;9:67–76.
22. Solimena M, De Camilli P. Autoimmunity to glutamic acid decarboxylase (GAD) in Stiff-Man syndrome and insulin-dependent diabetes mellitus. *Trends Neurosci* 1991;14:452–7.
23. Saiz A, Blanco Y, Sabater L, *et al.* Spectrum of neurological syndromes associated with glutamic acid decarboxylase antibodies: diagnostic clues for this association. *Brain* 2008;131(Pt 10):2553–63.
24. Dalmau J, Gleichman AJ, Hughes EG, *et al.* Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *Lancet Neurol* 2008;7:1091–8.
25. Dalmau J, Tüzün E, Wu HY, *et al.* Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Ann Neurol* 2007;61:25–36.
26. Irani SR, Bera K, Waters P, *et al.* N-methyl-D-aspartate antibody encephalitis: temporal progression of clinical and paraclinical observations in a predominantly non-paraneoplastic disorder of both sexes. *Brain* 2010;133(Pt 6):1655–67.
27. Dalmau J, Lancaster E, Martinez-Hernandez E, *et al.* Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. *Lancet Neurol* 2011;10:63–74.
28. Florance NR, Davis RL, Lam C, *et al.* Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis in children and adolescents. *Ann Neurol* 2009;66:11–8.
29. Brown P, Marsden CD. The stiff man and stiff man plus syndromes. *J Neurol* 1999;246:648–52.
30. Dalakas MC. Stiff person syndrome: advances in pathogenesis and therapeutic interventions. *Curr Treat Options Neurol* 2009;11:102–10.
31. Meinck HM, Thompson PD. Stiff man syndrome and related conditions. *Mov Disord* 2002;17:853–66.
32. Hutchinson M, Waters P, McHugh J, *et al.* Progressive encephalomyelitis, rigidity, and myoclonus: a novel glycine receptor antibody. *Neurology* 2008;71:1291–2.
33. Mas N, Saiz A, Leite MI, *et al.* Anti-glycine-receptor encephalomyelitis with rigidity. *J Neurol Neurosurg Psychiatry* 2010; Published Online First: 10 December 2010 doi:10.1136/jnnp.2010.229104.
34. Clerinx K, Breban T, Schrooten M, *et al.* Progressive encephalomyelitis with rigidity and myoclonus: resolution after thymectomy. *Neurology* 2011;76:303–4.
35. Turner MR, Irani SR, Leite MI, *et al.* Progressive encephalomyelitis with rigidity and myoclonus: glycine and NMDA receptor antibodies. *Neurology* 2011;77:439–43.
36. Honnorat J, Saiz A, Giometto B, *et al.* Cerebellar ataxia with anti-glutamic acid decarboxylase antibodies: study of 14 patients. *Arch Neurol* 2001;58:225–30.
37. Nanri K, Okita M, Takeguchi M, *et al.* Intravenous immunoglobulin therapy for autoantibody-positive cerebellar ataxia. *Intern Med* 2009;48:783–90.
38. McKnight K, Jiang Y, Hart Y, *et al.* Serum antibodies in epilepsy and seizure-associated disorders. *Neurology* 2005;65:1730–6.
39. Majoie HJ, de Baets M, Renier W, *et al.* Antibodies to voltage-gated potassium and calcium channels in epilepsy. *Epilepsy Res* 2006;71:135–41.
40. Haberlandt E, Bast T, Ebner A, *et al.* Limbic encephalitis in children and adolescents. *Arch Dis Child* 2011;96:186–91.
41. Suleiman J, Brenner T, Gill D, *et al.* VGKC antibodies in pediatric encephalitis presenting with status epilepticus. *Neurology* 2011;76:1252–5.
42. Irani SR, Bien CG, Lang B. Autoimmune epilepsies. *Curr Opin Neurol* 2011;24:146–53.
43. Granerod J, Ambrose HE, Davies NW, *et al.* Causes of encephalitis and differences in their clinical presentations in England: a multicentre, population-based prospective study. *Lancet Infect Dis* 2010;10:835–44.
44. Davies G, Irani SR, Coltart C, *et al.* Anti-N-methyl-D-aspartate receptor antibodies: a potentially treatable cause of encephalitis in the intensive care unit. *Crit Care Med* 2010;38:679–82.
45. Dale RC, Irani SR, Brilot F, *et al.* N-methyl-D-aspartate receptor antibodies in pediatric dyskinetic encephalitis lethargica. *Ann Neurol* 2009;66:704–9.
46. Zandi MS, Irani SR, Lang B, *et al.* Disease-relevant autoantibodies in first episode schizophrenia. *J Neurol* 2011;258:686–8.