Abstract

Contrary to established wisdom, there now appear to be antibody-mediated central nervous system (CNS) disorders. Over the last few years, a number of patients have been defined with antibodies to voltage-gated (VGKC) or ligand-gated (NMDAR, GlyR) ion channels or ungated water (AQP4) channels. Some of the disorders improve spontaneously over time, others may be more chronic and relapsing-remitting, but immunotherapies reduce antibody levels and improve clinical outcomes. These are exciting developments that herald a new era of immunotherapy-responsive CNS diseases, and they raise interesting questions regarding the aetiological and pathogenic mechanisms mediating these conditions.

Introduction and context

Autoimmune channelopathies are becoming one of the exciting areas of neurological diseases in clinical practice because, though relatively uncommon (collectively perhaps 20 per million per year), diagnosis of these conditions usually indicates a significant clinical improvement following immunotherapies that reduce autoantibody levels. The field stems from three decades of research into myasthenia gravis and the Lambert-Eaton myasthenic syndrome [1,2]; in these conditions, autoantibodies to muscle nicotinic acetylcholine receptors (AChRs) or voltage-gated calcium channels (P/Q-type), respectively, are the main pathogenic agents and cause destruction and/or downregulation of their targets, leading to neuromuscular junction transmission failure (Table 1) which can be demonstrated in animal models. Newer disorders of peripheral neurotransmission include (a) peripheral nerve hyperexcitability syndromes with antibodies binding to [125I]-dendrotoxin-labelled shaker-type (Kv1) voltage-gated potassium channels (VGKCs) extracted from mammalian cortex [3] and (b) autonomic neuropathies with antibodies to [125I]-epibatidine-labelled ganglionic nicotinic AChRs [4].

Over the last decade or so, a new family of antibody-associated diseases has emerged that is beginning to overturn previous concepts that regarded the brain as immune-privileged and protected by an impermeable blood-brain barrier. First, glutamate receptor (GluR3) antibodies were present in children with the very rare but devastating form of epilepsy called Rasmussen encephalitis [5], but these findings were not always confirmed in other cohorts of patients [6], and the main pathology is now thought to be cellular rather than antibody-driven [7]. The paradigm shift really began with the finding of very high VGKC antibody levels in patients with limbic encephalitis – which includes seizures, psychological disturbance, memory loss and high signal on magnetic resonance imaging (MRI) in the medial temporal lobes – who responded convincingly to immunotherapies such as plasma exchange (which removes circulating plasma components such as antibodies and replaces them with substitute plasma proteins; see Figure 1) [8-10]. Until then, limbic encephalitis was almost always recognised as ‘paraneoplastic’ (that is, associated with a T cell-mediated immune response to a tumour [11]) and with a poor response to treatments. The VGKC
antibody-associated central nervous system (CNS) phenotypes are now recognised widely, are usually nonparaneoplastic and include patients to the proximal dendrites of the hippocampal neuropil [16], distinct from the binding of VGKC antibodies more distally [8,16]; many of these antibodies were subsequently shown to be directed against N-methyl-d-aspartate receptors (NMDARs) (NR1/NR2B) [17], with NR1 as the main target [18]. Most of these patients progressed to a more complex phenotype with movement disorders or catatonia, mutism, sleep disturbance and autonomic dysfunction [17,18]. At first, the syndrome was associated with ovarian teratomas in young women, but in these cases, unlike the traditional paraneoplastic disorders [11], the conditions improved when the tumour was removed and immunotherapies given [17]. Now many nonparaneoplastic cases are being identified and the phenotype is widening to include both male and female adults, teenagers, and even young children [18,19] (S Irani, A Vincent, unpublished data). These NMDAR antibodies may be different from those measured by binding to linear peptide sequences of NR2A/NR2B seen in neuropsychiatric patients [20] and have the potential to be pathogenic since they target extracellular domains on NR1/NR2B transfected human embryonic kidney cells and substantially reduce the expression of these subunits in primary cultures of hippocampal neurons [18]. The NMDAR antibodies were most easily detected in the cerebrospinal fluid (CSF) (at 1:10) compared with serum (at 1:400), and there is substantial intrathecal synthesis of the specific antibody [18] (Table 2); nevertheless, in absolute terms, serum levels are higher than CSF levels. Recently, antibodies to AMPAR (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) GluR1/GluR2 were identified in another form of limbic encephalitis that was mostly cancer-related. These patients also showed treatment responses but tended to relapse [21].

Meanwhile, a completely different condition was found to be associated with antibodies to a water channel. Neuromyelitis optica (NMO, or Devic disease) has usually been considered to be part of the spectrum of inflammatory demyelinating disorders, of which multiple sclerosis is the best known. However, NMO is a distinct inflammatory condition of the optic nerves which involves severe visual failure and inflammation of the spinal cord causing longitudinally extensive transverse myelitis (at least three spinal cord segments with high signal on MRI), that leads to para- or tetraparesis, sensory deficits and bladder disturbances. Patients show variable recovery with immunomodulatory treatments but accumulate disability over time, and mortality is high if the disease is not appropriately treated [22]. In 2004, antibodies binding around small vessels, under

| Table 1. Peripheral nervous system autoimmune channelopathies |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Typical symptoms                | Muscle weakness and fatigue | Muscle weakness | Muscle twitching, cramps, sweating | Hypotension, constipation, papillary abnormalities, sicca syndrome (dry eyes and mouth) |
| Target                          | Muscle nicotinic AChR | VGCC P/Q-type | Dendrotoxin-binding VGKC Kv1.1, I.2, I.6 complexes extracted from mammalian cortex | Ganglionic nicotinic AChR (alpha 3) |
| Tumour association or other pathology | Thymoma, thymic hyperplasia or idiopathic complement-mediated damage, increased AChR degradation, some direct block of AChR function | Small-cell lung cancer common in adults (about 50%) Increased VGCC degradation, no evidence of complement-mediated damage | Small-cell lung cancer or other tumours uncommon Direct block of function and increased degradation |
| Main pathogenic mechanism       | Usually chronic, rare spontaneous remissions | Usually chronic, may improve with tumour removal | Can be chronic or monophasic, postinfectious or postallergic | Monophasic or chronic, postinfectious |
| Disease course                  |                  |                  |                  |                  |

AChR, acetylcholine receptor; VGCC, voltage-gated calcium channel; VGKC, voltage-gated potassium channel.
the pia and in Virchow-Robin spaces were defined by immunofluorescence [23], and the target was subsequently identified as aquaporin-4 (AQP4), the only water channel expressed strongly in the brain (and also in kidney and stomach) [24]. Antibodies to AQP4 bind to the astrocyte endfeet that abut CNS blood vessels and are thought to be important contributors to the integrity of the blood-brain barrier. The antibodies lead to substantial loss of surface AQP4 by internalisation and activate complement with formation of the membrane attack complex, leading to cellular damage [25]. They also reduce astrocyte expression of excitatory amino acid transporter 2 (EAAT2) with reduced reuptake of glutamate [26] and hence potential excitotoxic damage. Interestingly, it seems that AQP4 and EAAT2 are part of a macromolecular complex [25]. Whether these changes alone lead to the substantial inflammatory infiltrates, areas of demyelination, loss of AQP4 and sometimes necrosis that are found in lesions [27,28] is not yet clear, but increases in antibody levels are associated with clinical relapses, and AQP4 antibodies decrease in parallel with clinical improvement after

Figure 1. Aspects of the new autoimmune channelopathies

(a) VGKC-Ab limbic encephalitis

High signal in hippocampal regions

Antibodies to VGKC identified by immunoprecipitation of 125I-alpha-dendrotoxin-VGKCs in patient with limbic encephalitis

(b) NMDAR-Ab encephalitis

Patient’s serum IgG (1:40) binding to NR1/NR2b/EGFP transfected cells

Patient’s serum IgG (1:250) binding to 17 day live RAT hippocampal neurons

(c) Neuromyelitis optica

Patient’s serum IgG (1:20) binding to AQP4-EGFP expressing cells

Antibodies to AQP4 identified by immunoprecipitation of EGFP-tagged AQP4

In the images above, antibodies (red anti-human IgG) bind to human embryonic kidney cells expressing N-methyl-D-aspartate receptors (NMDARs) (coexpressed with enhanced green fluorescent protein [EGFP]; some cells express EGFP without detectable NMDARs) in a young girl who developed an encephalopathy with mutism and catatonia and made a complete recovery after immunotherapies (see [32] and the video available online). In the image below, NMDAR antibodies (green) bind to unpermeabilised hippocampal neurons in culture, which were then fixed, permeabilised and stained for synaptophysin (blue) and NR1 (red) (the binding of antibodies to the live cell surface does not colocalise well with the intracellular NR1 which appears throughout the neuron). (c) In the images above, antibodies to aquaporin-4 (AQP4) in a patient with neuromyelitis optica (NMO) bind to cells transfected with AQP4-EGFP. In this case, the directly tagged AQP4-EGFP can also be solubilised from the cells and, as seen in the graph, the antibodies can be measured quantitatively by counting the fluorescence in the immunoprecipitates [34]. In these assays, sera from healthy individuals and from patients with unrelated diseases are negative. Ab, antibody; NDs, neurological diseases. Images courtesy of S Irani, L Zuliani, M I Leite, P Waters and B Lang.
Future directions

There are some important lessons that arise out of these exciting advances. Once defined, the antibodies are best identified by binding to native proteins extracted from mammalian tissue in mild detergents (VGKCs), or better still to the native protein expressed in an appropriate human cell line (NMDARs, AMPARs, AQP4, GlyR), rather than to short peptides that do not represent the native conformation of the target antigen. The protein must be expressed on the cell surface and the cells should be unpermeabilised so that only cell surface-binding antibodies are detected (this ensures that they are potentially pathogenic, in contrast to those antibodies to intracellular components found in paraneoplastic disorders). Clustering of the antigen by use of intracellular scaffolding proteins can increase sensitivity and specificity as recently demonstrated for AChR antibodies [31]. In addition, the antibodies should be shown to bind to the extracellular surface of neurons or astrocytes cultured from mammalian tissues and to induce relevant biological changes in such cultures. In the future, one hopes that these studies will extend to examining the effects of these recently discovered antibodies on neuronal activity in brain slices in vitro and in animal models in vivo.

Considering the diversity of ion channels and receptors in the nervous system, it would be strange if there were no other autoimmune channelopathies to be discovered.
diagnosed and treated. Until now, most of the target channels have been identified by a candidate approach, but if the target for binding to the cultured cells is sufficiently abundant, as appears to be the case for AMPARs [21], it is possible to immunoprecipitate the target using the relatively pure CSF IgG from the patients [21]: this technique has potential for identifying new targets in the future. Even the total patient plasma IgG can be used to identify antigens by this approach when a suitable cell preparation or cell line is identified [32].

In each of these diseases, CSF antibodies are found, and there is often evidence of high concentrations of CSF-specific antibody relative to CSF IgG concentration when compared with similar measurements in serum (‘intrathecal synthesis’, Table 2), but the absolute concentration of antibody is still higher in serum than in CSF. A major question, therefore, is whether the antibodies that are pathogenic come directly from the blood into the CNS parenchyma via a ‘leaky’ or damaged blood-brain barrier or whether the disorders require the presence of specific antibodies in the CSF. The latter could be the result of passive diffusion across the choroid plexus and/or intrathecal synthesis by B cells that have gained entry to the CNS and synthesise the antibodies in the intrathecal compartment. These considerations are not purely academic. Does intrathecal synthesis decrease with current systemic treatments and increase if the patient relapses? Do immune responses ever begin in the CNS and remain undetectable in the serum? And importantly, should drugs and therapies be specifically targeted to the CSF compartment rather than to the systemic immune system? These are just some of the questions that arise from the identification of these new autoimmune disorders, and the answers will likely come from both focused human studies and new autoimmune disorders, and the answers will likely come from both focused human studies and for providing their unpublished images and data for this review.

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References


Abbreviations

AChR, acetylcholine receptor; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid receptor; AQP4, aquaporin-4; Caspr2, contactin-associated protein 2; CNS, central nervous system; CSF, cerebrospinal fluid; EAAT2, excitatory amino acid transporter 2; GluR, glutamate receptor; GlyR1, glycine receptor alpha 1 pentamer; MRI, magnetic resonance imaging; PERM, progressive encephalomyelitis with rigidity and myoclonus; NMDAR, N-methyl-D-aspartate receptor; NMO, Neuromyelitis optica; VGKC, voltage-gated potassium channel.

Competing interests

The author and her department receive royalties and income for antibody immunoassays.


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