Section 1 Overview

Chapter 1

Importance, Definitions, History, Classification, and Frequency of the Autoimmune Encephalitides

1.1 Importance of the Autoimmune Encephalitides

About 20 years ago, the group of diseases currently known as 'autoimmune encephalitis or encephalitides' or 'antibody-mediated encephalitides' was unknown and the field of 'Autoimmune Neurology' non-existent. Since then, 18 autoimmune encephalitides and the corresponding syndromes have been described, including 16 in which the antigens are expressed on the cell surface of neurons and two on the surface of glial cells.[1–3] In many of these diseases several immunological triggers have been identified – usually tumours or viral infections – and in some a genetic susceptibility linked to distinct human leukocyte antigens (HLA) has been shown.[1,4] These findings, along with the availability of specific diagnostic tests and clinical guidelines,[5] have remarkably changed the diagnostic and treatment approach to patients with encephalitis of unclear aetiology which until recently comprised 45–50% of all cases of encephalitis and now represent only 20–25% of all cases.[6] Neurologic disorders once considered idiopathic, or ascribed to possible infections, or defined with descriptive terms, are now recognized as autoimmune diseases that may lead to psychosis, catatonia, abnormal movements, seizures, memory impairment, cognitive decline, dysautonomia, or spinal cord dysfunction. Due to the severity and duration of these symptoms, up to 50% of patients require and receive prolonged intensive care support that not long ago may have been considered futile.[7,8]

In parallel to these clinical advances, we are learning how antibody-mediated dysfunction of neural cell surface proteins and receptors can alter memory and behaviour, cause psychosis, and probably result in many other symptoms. Cellular and experimental animal models have started to reveal the underlying pathogenic mechanisms and related immunological and neurobiological alterations, providing targets for novel therapies.[1]

Finally, the importance of autoimmune encephalitis is affirmed by two features: first, the rapid cumulative knowledge derived from the exponential growth of scientific publications over the last 10 years; and second, the fact that the translation of results from bench to bedside and vice versa is particularly rapid and effective in these diseases.

Some of the autoimmune encephalitides associated with antibodies against neural surface antigens were briefly discussed in a previous book, *Paraneoplastic Syndromes* by Darnell and Posner in 2011,[9] but much has occurred since. The important clinical implications of understanding these diseases convinced us to write this book.

1.2 Definitions and Nomenclature

General definitions and terms used in the following chapters are shown in Table 1.1. Some of these are obvious and widely used, but we feel others need clarification. For example, although limbic encephalitis is one of the best-defined subtypes of the autoimmune encephalitides, it is often confused with other disorders. First described in 1960 as 'subacute encephalitis mainly affecting the limbic areas',[10] and further defined in 1968 as 'limbic encephalitis',[11] the authors of these seminal studies readily noted the similarity of the distribution of the inflammatory 'reaction concentrated around the limbic areas' with that of herpes simplex encephalitis. Nowadays, despite having clinical criteria and powerful and precise tools

Table 1.1 Definitions

Term	Definition
Encephalopathy	Brain disorder characterized by altered mental functions or decreased level of consciousness with or without focal neurological deficits.
Encephalitis	Brain disorder associated with inflammatory or immune-mediated mechanisms that usually result in encephalopathy. Encephalitis may cause isolated memory deficits or psychiatric symptoms without overt signs of encephalopathy.
Limbic encephalitis	A form of encephalitis with clinical, MRI, EEG, and/or FDG-PET findings showing restricted involvement of the limbic system, mainly the hippocampus. Although many encephalitides affect the limbic system along with other areas of the brain, only a subset corresponds to limbic encephalitis.
Paraneoplastic disorder	A disorder caused by cancer that can affect the nervous system or any other organ or tissue, but is not related to direct cancer invasion of the affected organ or tissue, and is not caused by vascular, metabolic, toxic, or iatrogenic causes.
Classical paraneoplastic neuronal antibody	An antibody that serves as a biomarker of a paraneoplastic syndrome (e.g., its detection implies that in >80% of the cases the neurologic syndrome is associated with an underlying cancer).
Neural antibody	An antibody that reacts with brain cells, either neurons (neuronal antibody) or glia (glial antibody).
Onconeural antibody	An antibody that recognizes a neural antigen expressed by the associated tumour (almost always a systemic tumour). When the onconeural antigen is intracellular, the terms *classical paraneoplastic antibody* and *onconeural antibody* are interchangeable.
Antibody against a cell surface antigen	An antibody that reacts with a protein or receptor expressed on the cell surface. In the central nervous system (CNS), most of the known targets are on the surface of neurons, but some are expressed by glial cells or both. Many neuronal cell surface antibodies associate with encephalitides that occur with or without cancer association (the cancer frequency ranges from 0 to 70%, depending on the antibody). These antibodies do not fulfil the criteria of 'classical paraneoplastic antibodies' or antibodies against intracellular (onconeuronal) antigens in which the cancer association is >80%.
Paraneoplastic encephalitis	A form of encephalitis that in >80% of cases occurs with cancer and usually associates with classical paraneoplastic antibodies.
Autoimmune encephalitis	A form of encephalitis that occurs as a result of a brain-specific immune response with or without a cancer association. It usually associates with an antibody against a neuronal, or glial, cell surface antigen. These features are different from those of paraneoplastic encephalitis, which almost always (>80%) associate with cancer, and the target antigens are usually intracellular.
Antibody-mediated encephalitis	Autoimmune encephalitis for which there is evidence that the antibodies are directly pathogenic (e.g., they change the structure or function of the target antigen or directly cause symptoms). Most antibodies against neuronal cell surface antigens have been shown to be pathogenic. In contrast, most classical paraneoplastic antibodies or onconeural antibodies are not considered pathogenic.

that help to localize the main burden of the disease in the limbic system, there are still many reports in which the term limbic encephalitis is used for any inflammatory process above the foramen magnum, as long as the patient has cancer or a neuronal antibody. In fact, among the autoimmune encephalitides, there are only a few disorders that manifest with clinical, MRI, EEG, or fluorodeoxyglucose-positron emission tomography (FDG-PET) features with predominant involvement of the limbic system.[12] We will follow the concept of limbic encephalitis developed by Corsellis et al.,[11] who defined this disorder as a clinico-pathological inflammatory entity predominantly 'focused on the limbic grey matter'. Thus, we will apply this concept using clinical, neuroimaging, or EEG findings to demonstrate the focused limbic involvement. Moreover, within the group of limbic encephalitides there are several autoimmune mechanisms that define different disorders, each characterized by distinct clinical accompaniments and different probabilities of tumour association (or specific tumour histologies), response to treatment, and outcome. For example, patients with antibodies against leucine-rich glioma-inactivated protein 1 (LGI1) often develop prodromal faciobrachial dystonic seizures or hyponatraemia but rarely have cancer,[13,14] whereas those with α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) or gamma-aminobutyric acid b receptor (GABAbR) antibodies do not develop faciobrachial dystonic seizures or hyponatraemia, and 50–70% of patients have cancer.[15,16] These three disorders respond to immunotherapy, but for those that occur with tumours the degree of neurological improvement and outcome are influenced by the degree of tumour control.[17,18]

As a group, the autoimmune limbic encephalitides should be differentiated from other more diffuse, non-focal encephalitides such as those associated with antibodies against the

N-methyl D-aspartate receptor (NMDAR) or metabotropic glutamate receptor 5 (mGluR5), among others.[19,20] For example, anti-NMDAR encephalitis manifests with clinical and paraclinical features indicating diffuse involvement of the brain (including the limbic system), but only exceptionally develops as pure limbic encephalitis. Additionally, many features of patients with anti-NMDAR encephalitis (age, symptoms, frequency of tumour association, need for intensive care or aggressive immunotherapy, duration of hospital admission, and long-term prognosis) are different from those associated with the autoimmune limbic encephalitides.

The terms 'paraneoplastic encephalitis' and 'autoimmune encephalitis' also need clarification. It has become customary to use the term autoimmune encephalitis for all types of encephalitis associated with antibodies against neuronal cell surface proteins, whereas the concept of 'paraneoplastic' is applied only when the syndromes are associated with antibodies against intracellular neuronal proteins. The likely origin of these concepts is the fact that antibodies against intracellular neuronal proteins were the first to be identified among the paraneoplastic syndromes of the central nervous system (CNS) and in most cases the antigens are also expressed by the associated tumour.[21,22] Hence, the concepts of 'classical paraneoplastic antigens', 'intracellular neuronal antigens', or 'onconeural antigens' are used interchangeably. However, not all antibodies against intracellular antigens associate with cancer. For example, patients with neurologic syndromes related to glutamic acid decarboxylase 65 (GAD65) antibodies or adenylate kinase 5 (AK5) antibodies rarely have cancer.[23,24] Furthermore, the autoimmune encephalitides with antibodies against neuronal cell surface proteins can also occur in association with a tumour, and in these cases the tumour usually expresses the target neuronal surface antigen.

Therefore, as far as tumour association is concerned, the main difference between the classical paraneoplastic syndromes with antibodies against intracellular (onconeuronal) antigens and the autoimmune encephalitides with antibodies against cell surface proteins is that the former occur with cancer in more than 80% of the patients, whereas the latter have a variable association with cancer ranging from 0 to 70% depending on the antibody. To avoid confusion, we will apply the term 'paraneoplastic' in two settings: (1) the neurological syndrome is linked to the presence of an antibody against an intracellular (onconeural) antigen, with a cancer probability >80%; and (2) the immune-mediated neurologic syndrome is causally linked to the presence of a tumour, regardless of the type of antibody.

Finally, we will use the term 'antibody-mediated encephalitis' for any antibody-associated encephalitis in which the antibodies have been shown to alter the structure or function of the target antigen in models using cultured brain cells that express the antigen (e.g., neurons, astrocytes, oligodendrocytes), or cause similar alterations and symptoms in animal models. Most antibodies against cell surface proteins have these properties.

1.3 Historical Overview

1.3.1 Paraneoplastic Syndromes of the CNS

The identification of the autoimmune encephalitides was undoubtedly influenced by pioneering work done with the paraneoplastic diseases of the CNS, particularly those occurring with classic paraneoplastic or onconeuronal antibodies, and with the antibody-mediated diseases of the peripheral nervous system, including myasthenia gravis, Lambert–Eaton myasthenic syndrome (LEMS), and neuromyotonia.

The first suggestion that paraneoplastic diseases of the CNS could be immune-mediated came from Dorothy Russell in 1961.[25] In the book *The Encephalitides* she wrote, 'yet the possibility still remains that a carcinoma, even of small size, elaborates some product which in certain subjects provoke the formation of antibodies. If so the neural lesions might represent the interaction of antigen and antibody at this level … these speculations merely underline the necessity for further investigation of the problem.' Four years later, Wilkinson and Zeromski identified, using immunofluorescence techniques, neuronal antibodies in the serum of four patients (two also in cerebrospinal fluid (CSF)) who had sensory neuronopathy and small-cell lung cancer (SCLC).[26] The antibody reactivity was noted in sections of brain and dorsal root ganglia but not peripheral nerves; it was not species-specific (there was reactivity with guinea pig, chicken, and human tissue); and it did not occur with tumour tissue. Attempts to reproduce these findings by other investigators[27] were unsuccessful until 1985, when Graus et al. identified a neuronal antibody reacting with the nuclei of neurons in the serum of two patients with paraneoplastic sensory neuronopathy and SCLC.[28] A follow-up investigation demonstrated that the neuronal antigen recognized by the patients' antibodies was also expressed by the tumour.[29] This finding provided robust support, for first time, to the theory that tumour expression of an ectopic neuronal protein could potentially initiate an immune response that cross-reacted with the same protein expressed by neurons of the dorsal root ganglia and brain. This antibody, named anti-Hu[29] (the first two letters of the index patient) and later called by others anti-nuclear neuronal 1 antibody or ANNA1, is probably the same as that reported by Wilkinson and Zeromski, and was eventually found to occur in association with paraneoplastic encephalomyelitis and sensory neuronopathy, usually in patients

with SCLC.[30] The second neuronal antibody identified with immunohistochemical methods was reported in 1976 by Trotter et al.[31] in a patient with cerebellar ataxia and Hodgkin's lymphoma; this antibody was named anti-Tr[32] (after Dr Trotter) and was recently found to be directed against the Delta and Notch-like epidermal growth factor-related receptor (DNER).[33] In 1983, Greenlee et al. reported another antibody reactive with Purkinje cells and deep cerebellar nuclei of the cerebellum in two patients with ovarian cancer and paraneoplastic cerebellar degeneration, and also in 2 of 14 patients with ovarian cancer without neurological symptoms.[34] The same type of Purkinje cell antibodies were described by Jaeckle et al. in 1985 in 6 of 12 patients with paraneoplastic cerebellar degeneration;[35] among the antibody-positive cases, 3 had breast cancer, 2 ovarian cancer, and 1 abdominal adenopathies of unknown primary cancer. The antibody was later named anti-Yo or Purkinje cell antibody (PCA)1 (Figure 1.1).

The description of these three antibodies (Hu, Tr, and Yo) in paraneoplastic syndromes of the CNS was followed by many other neuronal antibodies in different paraneoplastic syndromes, all having in common that the target antigens were intracellular neuronal proteins and the patients almost always had systemic cancer or lymphoma (described in Chapter 4).[36] In these disorders plasma exchange or intravenous immunoglobulin were usually ineffective,[37,38] and attempts to model the symptoms in animals using passive transfer of patients' serum antibodies were unsuccessful, suggesting that the antibodies were unlikely pathogenic.[39,40]

1.3.2 Antibody-Associated Diseases of the Peripheral Nervous System

Different from the classical paraneoplastic syndromes of the CNS, where the initial findings that suggested an immune aetiology came from neuropathological observations demonstrating extensive inflammatory infiltrates and immunological studies showing neuronal-specific autoantibodies, for the antibody-associated diseases of the peripheral nervous system (myasthenia gravis, LEMS, neuromyotonia) the discovery of their immune aetiology came from different observations.

1.3.2.1 Myasthenia Gravis

The occurrence of myasthenia gravis in young women, often in association with other autoimmune diseases, the presence of thymic hyperplasia or thymoma, and the transfer of symptoms from mothers to their neonates, led Simpson in 1960 to hypothesize that myasthenia gravis was mediated by antibodies against an 'endplate' protein.[41] This theory was confirmed by Patrick and Lindstrom in 1973 when they demonstrated that immunization with acetylcholine receptors (AChR) produced weakness and paralysis in rabbits that, like the human disease, were responsive to edrophonium.[42] Further support for an antibody-mediated pathogenesis was provided by Toyka et al. in 1975 in studies showing that passive transfer of patients' IgG to mice reproduced the electrophysiological features of the disease that were reversed by neostigmine.[43] Lindstrom et al., using passive transfer of IgG from rats immunized with AChR to naive litter mates, showed that recipient rats developed severe weakness and fatigability along with electrophysiological features of myasthenia.[44] These authors also demonstrated the presence of antibodies bound to AChR extracted from rat muscle and a quantitative decrease of extractable AChR that paralleled in time the course of clinical and electrophysiological features. The disproportionate reduction of AChR relative to the amount of antibodies injected suggested that complement-related mechanisms along with inflammatory infiltrates were also pathogenically involved.

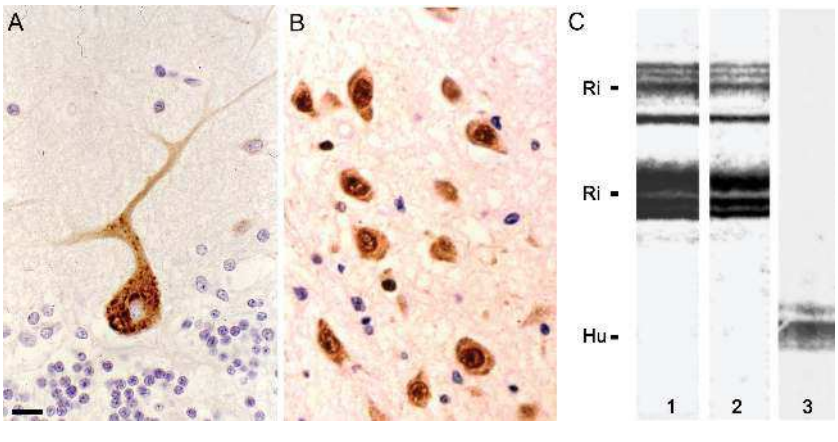

Figure 1.1 Immunohistochemical and immunoblot determination of classic paraneoplastic antibodies. Immunohistochemical reactivity of Yo (A) and Hu (B) antibodies with sections of human cerebral tissue; the reactivity is shown as brown staining in both panels. In early studies the antibody specificity of paraneoplastic antibodies was established using immunoblot of human brain protein extracts (C). Scale bar, 10 μm.

In 1978, Drachman et al. demonstrated that the reduction of AChR in myasthenic patients was in part due to the ability of the antibodies to crosslink the receptors.[45] To show this, the authors compared the effects of IgG or its divalent fragment F(ab′)2, which both caused AChR degradation, with the effects of the monovalent fragment Fab, which had no effects. They observed that adding a second 'piggyback' antibody to crosslink the Fab–receptor complexes accelerated the rate of AChR degradation, indicating the need for two arms of the antibody for crosslinking and internalization of the receptor. Many years later, this study inspired investigators to demonstrate that the antibodies of patients with anti-NMDAR encephalitis crosslink and cause internalization of NMDAR, altering synaptic transmission and plasticity (Figure 1.2).[46] Finally, Engel et al. used peroxidase-conjugated α-bungarotoxin (which binds to AChR) to determine the levels of these receptors in the muscle endplates of patients and experimental rats; they found a correlation between the severity of symptoms and the levels of AChR (sometimes barely detectable) in the terminal expansions of the postsynaptic folds.[47]

Overall, these pioneering investigations demonstrated that myasthenia gravis is predominantly an antibody-mediated disease with several accompanying mechanisms (e.g., complement, inflammatory infiltrates) that result in a reduction of AChR and flattening of the postsynaptic folds.

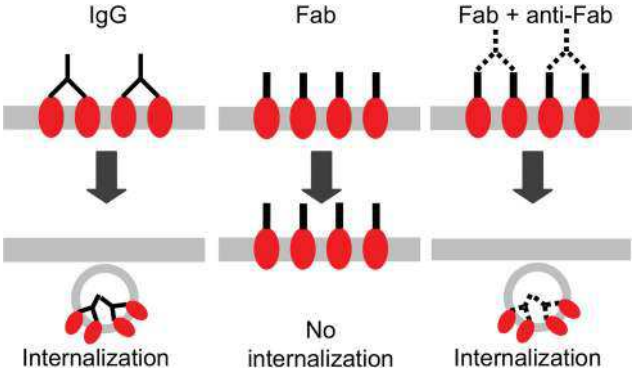

Figure 1.2 Patients' IgG1 antibodies bind, crosslink, and internalize receptors. Diagram showing how the two (Fab) arms of an IgG1 autoantibody are needed to crosslink and internalize the target protein or receptor. Left panel: intact IgG antibodies crosslink and internalize receptors. Middle panel: Fab (single arm) fragments prepared from patients' antibodies bind but do not cause internalization of receptors. Right panel: if two single Fab arms are linked with an antibody, the two-arm construct recapitulates the receptor internalization caused by intact antibodies. This experiment applies to different IgG1 antibodies from distinct diseases such as myasthenia gravis, LEMS, or anti-NMDAR encephalitis, among other disorders (modified from reference 46 with permission).

More recent studies have shown that 20% of patients with myasthenia gravis lack antibodies against AChR antibodies (seronegative myasthenia gravis), and that about 40% of these patients carry autoantibodies against a receptor tyrosine kinase named muscle-specific kinase (MuSK).[48] These antibodies are also pathogenic, as shown by passive transfer of patients' IgG4 antibodies to mice[49] and the clinical response to immunotherapy,[50] but the mechanisms are different from those related to antibodies against AChRs.[51]

1.3.2.2 Lambert–Eaton Myasthenic Syndrome

Another disease of the neuromuscular junction that was initially suspected to be autoimmune, based on clinical observations, is LEMS. About 50% of patients with LEMS have a systemic tumour, usually SCLC, and the coexistence of other autoimmune disorders such as thyroid diseases, pernicious anaemia, or vitiligo, among others, is frequent, particularly in patients without a tumour. In 1982, Lennon et al. found one or more organ-specific autoantibodies in 29 of 64 (45%) patients with LEMS.[52] The evidence of an antibody-mediated pathogenesis was provided by Lang et al. in 1981,[53] through studies showing that plasma exchange led to clinical and electrophysiological improvement and that passive transfer of patients' plasma or IgG fractions into mice resulted in neurophysiological changes similar to those of LEMS.[54,55] Quantitative freeze–fracture electron microscopy in mice injected with LEMS IgG resulted in a decrease in the number of active zones, which was similar to what was observed at the nerve terminals of patients.[56] These findings suggested that LEMS IgG decreased acetylcholine release by binding to presynaptic voltage-gated calcium channels (VGCC) or to structures closely associated with them. Subsequent studies using divalent F(ab′)2 antibody fragments showed that they caused a reduction in the distance between particles in the presynaptic active zones that preceded the clustering of the active zone particles. These effects were not observed when a monovalent Fab fragment of the antibodies was used, supporting a mechanism of antibody-mediated crosslinking of adjacent particles, independent of complement.[57,58] Additional data supporting that VGCC were the target of LEMS IgG were obtained in experiments in which the function of SCLC cells (that normally generate $Ca2^{+}$ spikes that can be blocked by calcium channel blockers) were also affected when the cells were grown in the presence of patients' IgG.[59]

These observations eventually led to experiments that showed that patients' antibodies precipitated solubilized N-type and P/Q-type VGCC pre-labelled with ^{125}I neurotoxins, thus confirming that the antibodies are directed against these channels,[60,61] and more specifically against the P/Q-type VGCC.[61,62]

1.3.2.3 Neuromyotonia, Peripheral Nerve Hyperexcitability, or Isaacs' Syndrome

Neuromyotonia, peripheral nervous system hyperexcitability, or Isaacs' syndrome is another disease that for a long time was known to occur in patients with thymoma, myasthenia gravis, or AChR antibodies without myasthenia gravis.[63] In addition, neuromyotonia had been described in patients with other tumours, mainly lung cancer,[64,65] and as a complication of penicillamine, which can trigger autoimmune disorders.[66] These findings led to the hypothesis that neuromyotonia was an autoimmune disease, and in 1991 Sinha et al. used plasma exchange to treat a young man who had had severe symptoms for seven years that were unresponsive to pharmacologic treatments.[67] After two sessions of plasma exchange, his symptoms almost completely resolved for 2–3 weeks in parallel with a notable improvement of the electrographic neuromyotonic discharges. Moreover, injection of the patient's plasma or purified IgG into mice enhanced *in-vitro* resistance to d-tubocurarine (a neuromuscular blocker) in experiments examining the innervation of diaphragm preparations. These findings suggested an antibody-mediated reduction of voltage-gated potassium channels (VGKC) that normally regulate nerve excitability. Subsequent experiments showed an increase of the quantal release of acetylcholine at endplates in diaphragms of mice infused with patients' plasma or IgG, and a marked increase in repetitive firing of action potentials in cultures of dorsal root ganglia neurons exposed to patients' IgG.[68]

These findings suggested that VGKC antibodies played a pathogenic role in the symptoms of some patients with neuromyotonia. A 2002 study of a series of 60 patients with acquired neuromyotonia showed that 35% had antibodies against VGKC that were detected with an (125)I-alpha-dendrotoxin immunoprecipitation assay.[69] In addition to neuromyotonia, 20% of the patients had neuropathy, 18% myasthenia gravis, 17% thymoma, and 8% lung cancer.[69] Recent investigations have demonstrated that the antibodies of patients with neuromyotonia do not in fact react with the VGKC but are directed against a protein that interacts with these channels, named contactin-associated protein-like 2 (Caspr2).[13,70] The frequency of these antibodies also appears to be lower than that suggested in initial reports. A study focused on patients with isolated neuromyotonia (e.g., without electrophysiologic features of axonopathy) showed that only 10% of the patients had antibodies against VGKC-related proteins.[71]

1.3.3 The Autoimmune Encephalitides

Clinical, pathological, and immunological studies in paraneoplastic syndromes of the CNS and antibody-mediated diseases of the peripheral nervous system provided a framework that facilitated the clinical and immunological recognition of the autoimmune encephalitides. Yet, important differences should be noted between these groups of disorders.

First, myasthenia gravis, LEMS, and neuromyotonia had well-defined clinical syndromes and electrophysiological features long before their autoimmune cause was discovered (e.g., 'the disease was known, the cause not'). In contrast, most autoimmune encephalitides were not recognized as distinct entities ('they were unknown, thus the cause was not sought'). The only exception was a subgroup of patients with limbic encephalitides that was seronegative for classical paraneoplastic antibodies against intraneuronal or onconeuronal antigens.[72] Other types of encephalitides without the characteristic clinical or MRI features of limbic encephalitis were not recognized as distinct syndromes or clinical entities due to the substantial clinical overlap between these entities and also with encephalitis of other aetiologies (viral, secondary to systemic diseases), including some that until now had unclear pathogenic mechanisms such as Hashimoto encephalitis or encephalitis lethargica.[73,74]

Second, many autoimmune encephalitides do not associate with other autoimmune diseases; thus, if other autoantibodies are detected (anti-nuclear, DNase or streptolysine O (ASLO), thyroid peroxidase) they usually misdirect the diagnosis towards disorders such as neurolupus, paediatric autoimmune neuropsychiatric disorder post-streptococcal infection (PANDAS), or Hashimoto encephalitis, among others. Therefore, contrary to the myasthenic syndromes or neuromyotonia, whose frequent association with other autoimmune diseases contributed to the discovery of their immune aetiology, in the autoimmune encephalitides the occasional association with other autoimmune disorders (or biomarkers of autoimmunity) had the opposite effect, diverting attention from the main disease. This problem still occurs despite the availability of diagnostic tests and clinical guidelines for the diagnosis of autoimmune encephalitides (see Clinical Vignette 1.1).

Third, most autoimmune encephalitides can develop with or without a tumour association. This is similar to what occurs with the myasthenic syndromes or neuromyotonia, but different from the classical paraneoplastic syndromes that almost always associate with cancer.

Fourth, autoimmune encephalitides can affect patients of any age; thus, they show a much wider age range than that of the antibody-mediated diseases of the peripheral nervous system (20–70 years) or classical paraneoplastic syndromes of the CNS (usually older than 50). Except for paraneoplastic opsoclonus-myoclonus that can affect both children and adults, other classical paraneoplastic syndromes are exceptional in children. On the other hand,

some autoimmune encephalitides with antibodies against neuron or glial surface antigens (NMDAR, mGluR5, GABAaR, myelin oligodendrocyte glycoprotein (MOG)) predominantly affect children and young adults.

Overall, the combination of the four factors discussed above likely contributed to the difficulty in recognizing the multiple diseases currently grouped under the term autoimmune encephalitides, and may explain the substantial lag of time between the recent characterization of these diseases and the much earlier characterization of classical paraneoplastic syndromes and antibody-mediated diseases of the peripheral nervous system.

Considering the key role that the coexistence of other autoimmune diseases played in the discovery of the immune aetiology of the myasthenic syndromes and neuromyotonia, it is not surprising that the first clues suggesting that some autoimmune encephalitis were antibody-mediated came from studies in patients with Morvan's syndrome. This disorder associates with neuromyotonia, hyperhidrosis, and other dysautonomic features along with CNS dysfunction that may include confusion, hallucinations, and severe insomnia. Previous observations that patients with isolated neuromyotonia improved with plasma exchange led Madrid et al., in 1996, to use plasma exchange in two patients with Morvan's syndrome (one with sigmoid cancer) that in both cases resulted in marked clinical improvement.[75] In 1998, Lee et al. reported a patient with Morvan's syndrome associated with myasthenia gravis, thymoma, psoriasis, atopic dermatitis, and antibodies against AChR, titin, N-type VGCC, and VGKC.[76] Plasma exchange, thymectomy, and immunosuppression induced a dramatic resolution of symptoms.

Clinical Vignette 1.1

A six-year-old boy with a history of isolated mild language delay developed fever and right facial clonic movements that lasted 10 minutes and were followed by difficulty speaking that took approximately 30 minutes to recover. The patient was being evaluated at an outpatient clinic when he developed acute onset of headache and clonic movements of the right arm without change of the level of consciousness, followed by right arm rigidity. He was admitted to the hospital, and during the next 48 hours he had several similar episodes involving the right side of the face that were accompanied by increased irritability. An EEG showed continuous left frontal delta activity, without epileptic activity, that did not correlate with the facial and arm movements. The patient was started on valproic acid and levetiracetam, without effect on the movements. He was transferred to the intensive care unit of another hospital where he was started on lacosamide. On admission to the unit, he had lingual dyskinesias, right facial weakness, and slow rhythmic bilateral eye opening and closing. A brain MRI was normal, and the CSF showed 8 WBC/mm^3, with normal glucose and protein concentrations. PCR for herpes simplex virus was negative.

Over the next few days he developed insomnia, episodes of agitation and irritability alternating with drowsiness, right upper and lower extremity weakness, and choreic–dystonic movements of the right arm. Because there was no evidence of seizures, levetiracetam was discontinued and the dose of lacosamide was decreased. Five days after admission, he developed sore throat and mild fever. A rapid strep test was positive, ASLO antibodies were negative, and he was started on amoxicillin and diagnosed with PANDAS. The patient was then started on carbamazepine and intravenous immunoglobulins (IVIg), resulting in improvement of the choreic–dystonic movements, but without change in the irritability, aggressive behaviour, and insomnia. Fourteen days after admission a CSF study for NMDAR antibodies came back positive, leading to initiation of rituximab (375 mg/m^2 per week for four weeks). Ten days after the initial dose, the abnormal movements became worse, along with increased aggressive behaviour, and severe insomnia that required treatment with benzodiazepines. These worsening symptoms prompted the use of plasma exchange (seven exchanges), which resulted in partial symptom improvement (mainly behavioural).

He was discharged to a rehabilitation centre due to persistent right hemiparesis. Three months after discharge, he continued to have right hemiparesis and dystonic posture of the right arm that was accompanied by mild choreic movements when he tried to extend the arm. In addition, he had severe dysphonia that substantially affected his speech. At this time, the EEG showed bilateral frontal slow activity, with occasional frontal–occipital epileptiform discharges involving the left or right side without evidence of clinical seizures. A follow-up 10 months after symptom onset showed remarkable general improvement with full recovery of all motor deficits (paresis, dystonic postures, and dysphonia) but residual cognitive and memory impairment.

Comment

This case illustrates a frequent problem in clinical practice: when a test is positive, it often overrides the clinical assessment, even if the test result does not fit with the patient's syndrome. In this six-year-old patient the presentation of neurological symptoms preceded any evidence of streptococcal infection, and the clinical picture was highly suggestive of anti-NMDAR encephalitis.

The authors indicated that Morvan's syndrome falls in the range of paraneoplastic syndromes associated with thymoma, and briefly commented that some previously reported limbic encephalitis in patients with thymoma had antibodies reacting with the brain (in reference to two cases that probably had a paraneoplastic antibody currently known as collapsing-response mediated protein 5 (CRMP5)).[77] In 2001, Liguori et al. described another patient with paraneoplastic Morvan's syndrome and VGKC antibodies, whose tumour (adenocarcinoma of the lung) was found at autopsy.[78] Neuropathological studies showed the presence of IgG in the thalamus, leading the authors to postulate that VGKC or perhaps other undefined antibodies had effects on the brain. In the same year, Buckley et al. reported two patients with limbic encephalitis, one with thymoma and myasthenia gravis, who also had antibodies against VGKC; one improved with plasma exchange and the other spontaneously.[79] These findings led to the publication of a series of 10 patients in 2004, with clinical or radiological features of limbic encephalitis, frequent hyponatraemia, and serum VGKC antibodies.[80] The clinical features responded to steroids, plasma exchange, or IVIg, but varying degrees of cerebral atrophy and residual cognitive impairment were noted.

Given that the serum of patients with neuromyotonia, Morvan's syndrome, and limbic encephalitis all seemed to react with the Shaker-type-K+ channels (Kv1.1, Kv1.2, or Kv1.6 VGKC), a follow-up study investigated the possibility of VGKC subunit-disease specificity using HeLa cells transfected with each VGKC subunit.[81] Serum from patients with limbic encephalitis labelled more prominently the Kv1.1 subunit than serum from patients with Morvan's syndrome or neuromyotonia; in contrast, serum from patients with neuromyotonia bound more strongly to Kv1.2 subunits. The results of this study could not be reproduced, and several years later the target antigens of the antibodies related to these diseases were found to be two proteins (LGI1 and Caspr2) that interact with the VGKC but not the channels themselves.[13,14]

By 2004 most of the classical paraneoplastic encephalitis and other neurological disorders associated with antibodies against intracellular neuronal proteins known today had been described, and the only encephalitis associated with antibodies against neuronal cell surface proteins was considered to be antigenically related with the VGKC. As a result, the limbic encephalitides were grouped into two broad categories: paraneoplastic (or associated with intracellular onconeural antibodies) and non-paraneoplastic (or associated with VGKC antibodies). This overly simplified classification of the limbic encephalitides was soon challenged by two studies.

One of these studies, reported in 2005, was performed in a single institution and described seven patients with different types of encephalitides suspected to be immune-mediated; six of the patients had antibodies against neuronal cell surface proteins that produced several distinct patterns of immunostaining of the neuropil of rat brain (only one resembling that attributed to VGKC).[82] In four of these six patients the clinical picture was typical of limbic encephalitis, suggesting that multiple antibodies, and not only those attributed to VGKC, could result in this syndrome; the other two patients had clinical features of multifocal or diffuse encephalitis beyond the limbic system. Despite the severity of the symptoms and presence of a tumour in four patients (two had thymic tumours, one teratoma of the mediastinum, and one teratoma of the ovary), all showed remarkable neurologic improvement after immunotherapy and treatment of the tumour. This was in contrast with the findings and outcome of the seventh patient, who developed multifocal paraneoplastic encephalitis associated with papillary carcinoma of the thyroid gland and antibodies against intracellular neuronal proteins; the patient did not respond to treatment and died of the neurologic disease. When considering the six patients with antibodies against neuronal cell surface proteins, two additional features stood out: four were younger than 50 years, and in some their tumours (e.g., teratomas) were unusual among the, until then, neoplasms described in association with paraneoplastic syndromes of the CNS. In fact, the youngest of the six patients was a 26-year-old woman with an ovarian teratoma. At that time, the identity of the autoantigens was unknown for all six patients, although it was revealed in subsequent investigations. This study, however, represented a paradigm change for two reasons; it demonstrated that the repertoire of neuronal cell surface antigens was more extensive than previously thought, and indicated that prompt recognition of these disorders was important because patients usually improved with treatment.[82]

The second study, also reported in 2005, focused on four young women (one included in the previous report[82]) who presented with encephalitis of unclear aetiology characterized by prominent psychiatric symptoms and rapid development of severe neurologic deficits, decreased level of consciousness, and central hypoventilation. Extensive investigations to identify the cause of the encephalitis were negative except for the detection of an ovarian teratoma in all four patients.[83] The four patients were treated with immunotherapy and three also had tumour removal (in one the tumour was not removed) leading to pronounced clinical recovery in three; the fourth patient, who had tumour removal and immunotherapy, died after being ventilator-dependent for several months. The extraordinary clinical similarity of the four patients suggested they had the same disease, leading to studies that revealed, in all four, the presence of autoantibodies that produced an

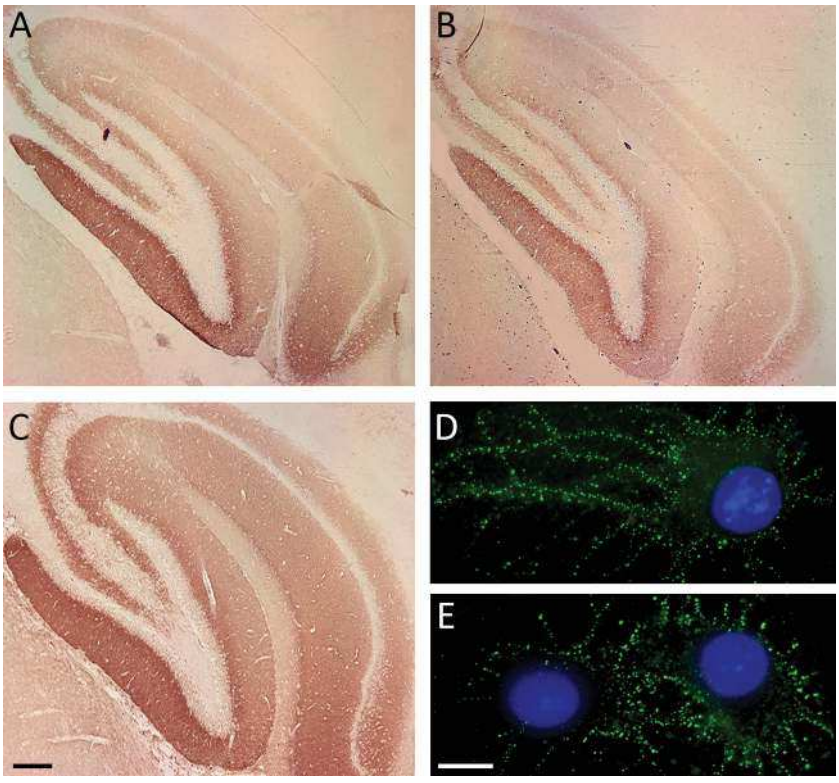

Figure 1.3 Demonstration of NMDAR antibodies using rat brain and cultured neurons. The figure comes from the first study to demonstrate that CSF of patients with a disorder characterized by 'encephalitis, psychiatric symptoms, and hypoventilation' in association with ovarian teratoma had antibodies that produced an identical pattern of reactivity with the neuropil of hippocampus of rat brain and surface of live neurons. Panels A–C show the reactivity of CSF of three patients and panels D and E demonstrate the neuronal surface immunolabelling of two of them (from reference 83 with permission). In subsequent studies the target antigen was characterized as the NMDAR.[84] Scale bar in C, 200 μm; scale bar in E, 10 μm.

identical pattern of reactivity with the neuropil of rat brain and cell surface of live neurons (Figure 1.3). In 2007, the target antigen was characterized as the NMDAR and the disorder was named anti-NMDAR encephalitis.[84] The predictability of the clinical syndrome and availability of a specific diagnostic test (based on the demonstration of NMDAR antibodies) helped to further characterize the disease. The identification of additional patients occurred at a speed not previously experienced in the fields of paraneoplastic or autoimmune encephalitis, suggesting that this disorder was more frequent than initially thought. One year later, a series of 100 patients with anti-NMDAR encephalitis was reported, describing the syndrome as it is currently known, demonstrating that the autoantibodies were more frequently detected in CSF than serum, and indicating that the target epitopes were located in the GluN1 subunit of the NMDAR.[19]

The next autoimmune encephalitis discovered was a subtype of limbic encephalitis that associates with antibodies against the GluA1 or GluA2 subunits of the AMPAR.[15] The characterization of this disorder was made through a set of clinical–immunological steps that have since been used in other autoimmune encephalitides (Figure 1.4). They include (1) identification of a distinct group of patients with similar symptoms, sometimes associated with a similar histologic type of tumour; (2) demonstration of antibodies in patients' serum or CSF producing a similar pattern of reactivity with rodent brain tissue; (3) confirmation that the target antigen is on the cell surface using immunostaining of live cultured neurons or glial cells; (4) immunoprecipitation of the antigen bound to patients' antibodies and subsequent sequencing to reveal the antigen's identity; and (5) development of specific diagnostic tests (e.g., cell-based assays) that in turn help to identify more patients and further refine the description of the syndrome.

These initial studies on anti-NMDAR and AMPAR encephalitides showed that the techniques needed for antigen characterization in the encephalitides associated with antibodies against cell surface proteins were different from those previously used for classical paraneoplastic encephalitides. In the latter, the antibodies are directed against linear epitopes of intracellular proteins that can be identified using cDNA library screening.[85–89] This technique, however, does not work for the conformal epitopes of most encephalitides associated with antibodies against cell surface proteins or receptors. In these disorders, direct precipitation of the antigen from cultures of neurons or from brain tissue using patients' antibodies is the technique most frequently used.[15,16] Less frequently, the identity of the antigen was suggested by the clinical features; for example, in patients

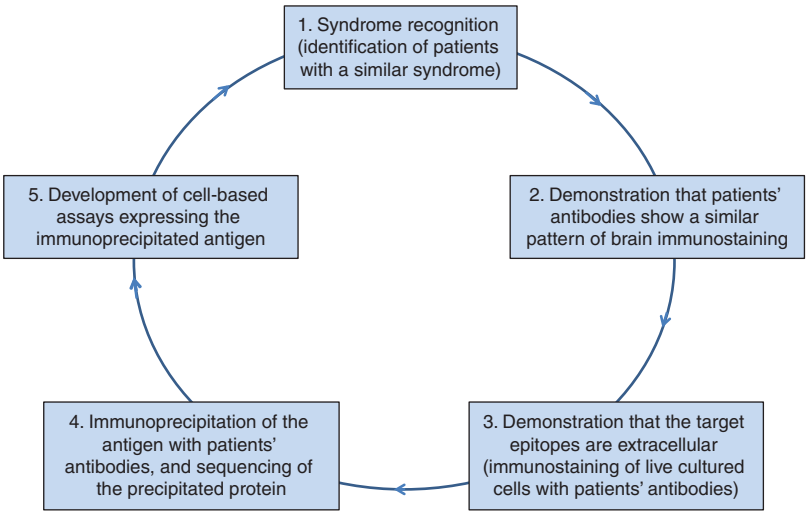

Figure 1.4 Steps in the clinical–immunological characterization of most autoimmune encephalitides. Diagram showing the sequence of steps most frequently followed in the discovery and characterization of most autoimmune encephalitides.

with anti-glycine receptor (GlyR) antibodies, the presence of hyperekplexia, muscle rigidity, and severe spasms, which resemble the symptoms caused by GlyR antagonists (strychnine poisoning) or GlyR mutations pointed to the identity of the antigen.[90] In other instances, the candidate antigen was suggested by the pattern of reactivity of patients' antibodies; for example, in patients with anti-NMDAR encephalitis, the reactivity of the antibodies with the hippocampus and granular cells of the cerebellum was similar to that of monoclonal antibodies against NMDAR.[84]

In 2010, investigations using both approaches, direct antigen precipitation with patients' antibodies[14,70] and screening of VGKC-interacting proteins as potential candidate antigens,[13] demonstrated that the antibodies initially considered against VGKC were in fact directed against LGI1 or Caspr2.

1.4 Frequency of the Autoimmune Encephalitides

Up to 12.6 per 100,000 persons are affected by encephalitis annually.[6] Of these, it has been estimated that 40–50% are caused by infectious agents, 20–30% by autoimmune mechanisms, and the aetiology of the rest is unknown. These aetiological estimates are largely based on a prospective study carried out in the UK over two years during which investigators from 24 hospitals recruited patients of all ages with suspected encephalitis.[91] These cases were investigated using systematic laboratory testing, a specific case definition, and classification of cases by a multidisciplinary expert panel. The cause of encephalitis was infectious in 42%, unknown in 37%, and immunomediated in 21% (including acute disseminated encephalomyelitis (ADEM) in 11%, and neuronal antibody-associated in approximately 8%). In a subsequent study by the same authors,[92] these data were linked to a dataset of Hospital Episode Statistics, restricting both datasets to patients admitted between 1 November 2006 and 31 October 2007. The authors determined the number of cases that matched between the two datasets and applied capture–recapture models, which provided a best estimate of encephalitis incidence of 5.23 cases/100,000 persons/year, although the models indicated that the incidence could be as high as 8.66 cases/100,000 persons/year. However, the results of these two studies, reported in 2010[91] and 2013,[92] probably under-represented the overall frequency of encephalitides, particularly those related to autoimmune mechanisms. A required inclusion criterion was the presence of encephalopathy, which we currently know does not always occurs in patients with autoimmune encephalitis. Indeed, some patients can present with pure psychiatric symptoms,[93,94] seizures,[95,96] memory deficit, or cognitive decline without clinical, CSF, or MRI features suggesting an acute encephalitis or encephalopathy.[97] The authors acknowledged a lower than expected recruitment of children; this was attributed to the fact that CSF analysis was a main method of case ascertainment in the study, and children are less likely than adults to have a lumbar puncture in the UK.[91] Therefore, this inclusion criteria may have excluded a substantial number of patients with anti-NMDAR encephalitis, given that 37% of patients with this disease are children,[98] as well as patients with anti-GABAaR encephalitis (42% are children),[99] and some atypical cases of ADEM or encephalitis with MOG antibodies.[100,101] Only two neuronal autoantibodies were examined, NMDAR and VGKC, the latter now known to be of unclear