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Title: Inflammation, Ictogenesis and Epileptogenesis: An Exploration through Human Disease

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Summary

Epilepsy is historically seen as a disease of aberrant neuronal signalling manifesting as seizures. With the discovery of numerous autoantibodies and the subsequent growth in understanding of autoimmune encephalitis, there has been an increasing emphasis on the contribution of the innate and adaptive immune system to ictogenesis and epileptogenesis. Pathogenic antibodies, complement activation, CD8+ cytotoxic T cells and microglial activation are seen, to various degrees, in different seizure associated neuroinflammatory and autoimmune conditions. These aberrant immune responses are thought to cause disruptions in neuronal signalling, generation of acute symptomatic seizures and, in some cases, the development of long-term autoimmune epilepsy. While early treatment with immunomodulatory therapies improves outcomes in autoimmune encephalitides and autoimmune epilepsies, patient identification and treatment selection are not always clear cut. This review examines the role of the different components of the immune system in various forms of seizure disorders including autoimmune encephalitis, autoimmune epilepsy, Rasmussen encephalitis, febrile infection related epilepsy syndrome (FIRES) and new onset refractory status epilepticus (NORSE). In particular, the pathophysiology and unique cytokine profiles seen in these disorders and their links with diagnosis, prognosis and treatment decision making is discussed.

Keywords

Autoimmune Diseases of the Nervous System, Epilepsy, Seizures, Cytokines

Key points

- Both the innate and adaptive immune response are implicated in ictogenesis and epileptogenesis with mechanisms ranging from pathogenic antibodies, cytotoxic T cells, complement activation and reactive microglia
- The diagnosis of autoimmune epilepsy currently requires auto-antibody positivity or syndromic manifestations which are not always present. Novel biomarkers are required to assist accurate diagnosis and allow prompt treatment

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- Optimal and timely selection of immunotherapy and decisions regarding cessation of treatment for autoimmune and neuroinflammation related seizures is unclear; an increased understanding of the underlying pathogenesis will aid rational treatment selection
- Cytokines, a family of small molecules involved in intercellular communication, are potential biomarkers for the characterisation of the aberrant immune response and the identification of ongoing inflammation in various seizure and epilepsy syndromes
- Antibody mediated autoimmune encephalitides have raised levels of B cell chemoattractant CXCL13 but also Th17 related cytokines IL-17 and IL-23; current second line therapies include rituximab and cyclophosphamide, but do not specifically utilise anti-Th17 therapies
- Elevated proinflammatory cytokines such as IL-6, TNF-α and IL-1β are found in NORSE and FIRES and therapies targeting these cytokines have been efficacious in these conditions

Introduction

In recent years, with the expansion of the understanding of autoimmune encephalitis, there has been growing interest in the role of autoimmunity and neuroinflammation in ictogenesis and epileptogenesis. There is a growing understanding that the role of neuroinflammation in these processes exists along a spectrum (FIG. 1). At one end, there are well described autoimmune encephalitides (AEs) that lead to acute symptomatic seizures and SE, with variable long-term epileptogenic potential, where the immune system plays a pivotal pathogenic role. These include antibody positive and antibody negative AEs which can be part of a paraneoplastic syndrome or occur in isolation[1, 2]. There are also syndromes where previously well patients present with refractory status epilepticus (SE) and encephalopathy where no cause is found but an autoimmune or neuroinflammatory mechanism is postulated, such as NORSE (new onset refractory status epilepticus), FIRES (febrile infection related epilepsy syndrome), DESC (devastating epileptic encephalopathy in school-aged children) and Rasmussen encephalitis; these conditions often end in the development of chronic drug resistant epilepsy (DRE)[3-5]. At the other end of the inflammatory spectrum lie the more subtle changes of inflammation mainly involving microglia, astrocytes, neurons and the endothelial cells of the blood brain barrier (BBB) noted on serological, cerebrospinal fluid (CSF) or histopathological testing of patients presenting with SE and DRE where the cause versus effect relationship is more complex[6, 7]. In animal studies, pro-inflammatory cytokines such as interleukin-1β and HMGB1, which are also elevated in brain tissue samples from DRE patients, are not only elevated in the context of seizures, but also appear to contribute to epileptogenesis and worsening of seizure control[8]. This potential positive feedback loop may explain why the risk of epilepsy in those with *de novo* SE rises with SE severity. In particular, there is a general risk of 30-40% of developing epilepsy post *de novo* SE and this rises to

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87.5% in those with refractory status epilepticus, defined as ongoing seizures despite the use of two anti-seizure medications[9-11].

Here, we provide a critical review on the role of inflammation in ictogenesis and epileptogenesis, highlighting the pathogenic mechanisms of the different arms of the immune system through the exploration of representative diseases. In particular, we will discuss various diseases that come under the following two categories: the autoimmune encephalitides and neuroinflammation related syndromes that present with denovo refractory status epilepticus. For each disorder, we review the clinical presentation and disease course, followed by the putative immunological mechanisms.

While it is important to keep in mind that the specificity of antibody detection depends on the laboratory techniques used, with cell-based assays superior to other techniques (e.g. western blot)[12], further discussion is beyond the scope of this review. Furthermore, should the reader wish for more comprehensive clinical information regarding autoimmune encephalitis associated seizures, a number of excellent review articles already exist[2, 13].

Autoimmune Encephalitides

AEs encompass an expanding group of syndromes in which the immune system plays a key role in the pathogenesis. Seizures, of which SE is the most extreme presentation, is a common feature of these conditions consistent with its inclusion in the diagnostic criteria outlined by Graus et al[14]. There are an increasing number of antibodies being identified in association with AE subtypes. The pathogenicity of these antibodies vary: those targeting neuronal cell surface antigens or synaptic proteins are thought to be directly pathogenic, while those directed against intracellular antigens are postulated to be an epiphenomenon of an underlying autoimmune process in which T cells potentially play a dominant role[13, 15-18]. Treatment responsiveness as well as the occurrence of seizures during AEs and the development of post-AE epilepsy varies between syndromes, with those featuring antibodies directed against cell surface antigens being more responsive to immunotherapy and less likely to develop subsequent epilepsy than those with antibodies directed against intracellular antigens[2, 19]. The development of subsequent epilepsy may be due to ongoing inflammation, or from irreversible changes to neuronal networks persisting after the inflammatory process has resolved and leading to spontaneous seizure occurrence[20].

The risk of seizures is highest with anti-leucine rich glioma inactivated 1 (LGI1), gamma amino butyric acid-A receptor ($GABA_AR$) and gamma amino butyric acid-B receptor ($GABA_BR$) antibody-mediated AE, with around 74-90% of patients with anti-LGI1 AE[21, 22] and over 90% of those with anti-$GABA_AR$ AE[23-25] and anti-

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$GABA_BR$ AE[26-28] developing seizures; SE has been estimated to occur in 6-19% of anti-LGI-1 antibody mediated AE[29, 30] and 20-64% of anti-$GABA_BR$ AE[26, 29, 30]. In one series, SE or epilepsia partialis continua (EPC) occurred in 11 out of 26 patients with anti-$GABA_AR$ AE, although, in a smaller cohort of six patients with high titre antibodies, SE or EPC was reported in 100% [23, 24]. EPC is a subset of focal motor SE presenting as spontaneous focal clonic or myoclonic motor activity continuing for a period of hours to weeks[31, 32]. Up to 80% of individuals with anti-N-methyl-D-aspartate receptor (NMDAR) antibody mediated AE present with seizures[33-35] and 10% with SE[29, 33]. Seizures in the context of paraneoplastic limbic encephalitis is also common, occurring in around 40-60% of patients[36, 37]. Conversely, the risk of central nervous system (CNS) autoimmune disease in patients presenting with SE is around 2.5% and tends to occur in a younger population with more refractory seizures[38]. AEs are important to recognise as management includes early immunotherapy for the acute treatment of seizures and SE and, in some cases, neoplastic workup, rather than reliance on anti-seizure medications alone[30, 39].

Anti-NMDAR antibody and anti-LGI1 mediated AEs are the two most common forms of AEs associated with neuronal cell surface antigens[40]. Despite the high risk of acute symptomatic seizures with these two conditions, the risk of chronic epilepsy is low. Less than 5 to 33% of patients with anti-NMDAR antibody mediated AE have ongoing seizures after over two years follow up[19, 29, 41] and, while up to 30-40% of anti-LGI1 AE patients had ongoing anti-seizure medication use, only 9-22% had ongoing seizures after two or more years follow up[2, 19, 21, 29, 42, 43]. This suggests, at least in the most part, a reversible mechanism of seizure generation. Anti-$GABA_BR$ AE also has a high incidence of acute symptomatic seizures, but unlike anti NMDAR and anti LGI1 antibody mediated AEs, it may have a higher risk for epilepsy with one study including 11 patients with anti-$GABA_BR$ AE reporting a 45% incidence of ongoing seizures at two years[29]. In contrast, in another cohort of 15 patients with anti-$GABA_BR$ AE, five died within six months, two were lost to follow up and seven were seizure free with a follow up time of between three and 72 months, although the use of anti-seizure medications in this cohort was not described. Therefore, the decision to withdraw anti-seizure medications in this group should be approached with this in mind[29]. There is little evidence to guide the choice of anti-seizure medication in these situations, however in a study of 153 patients with anti-LGI1, anti-NMDAR, or anti-$GABA_BR$ AEs, there was a suggestion that carbamazepine was more effective than levetiracetam and valproate for the treatment of seizures related to anti-LGI1 encephalitis, although there was no difference seen for the other two subtypes[30]. Similarly, in a retrospective review, sodium channel blockers (carbamazepine, oxcarbazepine, lacosamide, phenytoin) appeared to be more effective for seizure control with nine out of 50 patients with suspected AE becoming seizure free either following these anti-seizure medications alone or only after they were added to immunotherapy[44]. In contrast, none of the patients taking levetiracetam became seizure free despite it being the most

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commonly used anti-seizure therapy[44]. Of note, the nine responders consisted of one anti-LGI1, one anti-CASPR2 and one high titre GAD65 antibody positive patients, while the remainder were a mix of anti-VGKCc, anti-TPO, anti-ganglionic acetylcholine receptor antibody and antibody negative patients, raising the possibility of a non-immune mechanism in some of these patients. When selecting anti-seizure medications, side effect profiles also need to be considered. In the group with anti-LGI1 AE, there is also a 29% to 41% cutaneous reaction risk, including Steven Johnson Syndrome, to anti-seizure medications such as carbamazepine, phenytoin, valproate and levetiracetam[45-47]. It is unclear why anti-LGI1 AE patients have such a high risk of adverse skin reactions. HLADRB1*07:01 and DQB1*02:02 occur more frequently in anti-LGI1 AE than in healthy controls, suggesting a genetic predisposition to this AE[48-50]. While this may provide insights into mechanisms for autoimmunity, they differ from the HLA subtypes associated with an increased risk for cutaneous drug reactions to anti-seizure medications[51-53]. In addition to cutaneous reactions, hyponatremia occurs in 56-65% of anti-LGI1 AE patients[21, 42], mainly in those with cognitive impairment[46, 47], and the potential for this to be exacerbated by sodium channel blockers needs to be kept in mind. Vigilance for these side effects must be maintained if these therapies are used.

While the development of epilepsy in patients with antibody-mediated AEs is generally low to moderate, the risk of epilepsy following limbic encephalitis associated with antibodies targeting intracellular and glutamic acid decarboxylase (GAD) antigens are more significant. For instance, the majority of anti-Hu and anti-GAD associated autoimmune limbic encephalitis develop epilepsy[54, 55].

Furthermore, there is the concept of autoimmune epilepsy, which according to the 2017 ILAE epilepsy classification system refers to epilepsy 'where there is evidence of autoimmune mediated CNS inflammation'[56]. This classification encompasses patients with epilepsy related to AE and also those who present with epilepsy but do not meet the full diagnostic criteria of AE. In select populations, the incidence of autoimmune epilepsy may be relatively high. For example, in one study of 112 patients presenting with adult onset epilepsy of unknown cause, a serum autoimmune antibody was present in 34.8% of cases – including anti-LGI1, NMDAR, GAD, Hu and voltage gated potassium channel complex (VGKCc) antibodies. However, these antibodies were not tested on CSF and also included low titre serum GAD and VGKCc, both of which have questionable significance in relation to autoimmune neurological syndromes[57].

The ILAE classification system, which emphasises aetiology, reminds clinicians to treat the underlying inflammation and not just the seizures. However, as noted above, many AE cases, especially those featuring neuronal cell surface antibodies, will not develop epilepsy. To account for this, the ILAE has recently proposed separating patients into two groups: a) 'acute symptomatic seizures secondary to autoimmune encephalitis' for those with seizures during the acute inflammatory phase of disease (or with disease relapse) which have the potential to respond to immunotherapy and b) 'autoimmune-associated

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epilepsy' should seizures persist beyond these periods and be refractory to immunotherapy[20]. This will help engender more focussed discussions based on underlying pathophysiology and epileptogenesis which will guide treatment development and better define treatment endpoints.

Immunological Mechanisms of Disease

Anti-NMDAR antibody mediated autoimmune encephalitis

Anti-NMDAR antibody mediated AE is a syndrome that consists of seizures, cognitive dysfunction and psychiatric symptoms that can then progress to stupor, autonomic dysfunction and movement disorders[33, 34]. 30%-58% of patients exhibit extreme delta brush (symmetric, broadly distributed synchronous, 1-3Hz delta activity with overriding 20-30Hz beta frequencies) on EEG early in the course of disease[58, 59] which may correlate with a poorer prognosis[60]. Around 70-90% of adults with anti NMDAR antibody mediated AE patients are female and 20-59% will have an associated tumour of which the vast majority are ovarian teratomas[33, 34, 61, 62]. In these patients, ovarian teratomas were found to contain nervous tissue that express NMDARs and immunofluorescence techniques have demonstrated patient's autoantibodies binding to these receptors[33, 62, 63]. Furthermore, evidence of tumour associated germinal centres synthesising NMDAR (NR1) antibodies[64] suggests that the tumour triggers a breakdown in immune tolerance by allowing the immune system access to antigens previously hidden in an immunopriviledged site such as the CNS[33, 62]. Herpes virus encephalitis and exposure to immune checkpoint inhibitors can also lead to a secondary AE, most commonly anti-NMDAR mediated AE, providing other examples of failure of immune tolerance[65, 66].

Whatever the triggering event may be, subsequent encephalitis also involves intrathecal production of antibodies, with most studies showing high CSF-to-serum concentrations of anti-NMDAR antibodies[33, 67] as well as infiltrating antibody secreting cells in the perivascular and interstitial spaces of the CNS[68]. Once within the CNS, anti-NMDAR antibodies are thought to act via binding to and crosslinking NMDARs on the neuronal cell surface ultimately leading to their internalisation and a loss of function[33, 69] (FIG. 2). This process appears to be concentration-dependent and reversible[69], and the CSF antibody titres generally parallel the disease course[19, 33, 70]. In post mortem pathological studies, IgG deposits are seen throughout the CNS with a predominance in the hippocampus, basal forebrain, basal ganglia and cervical spine, and are mostly of the IgG1 subclass[63] – a subclass that is induced in response to soluble and membrane protein antigens[71]. While IgG1 can activate complement in the periphery and complement deposition has been shown in the neural component of the teratoma[68, 72], this does not appear to occur in the central nervous system in anti-NMDAR mediated AE[18, 63].

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In addition, microglial activation, as defined by alterations in microglial morphology and immunoreactivity to CD68 antibody, is also seen with the same preference for the hippocampus, basal forebrain, basal ganglia and spinal cord in autopsy specimens[63]. Microglia are the resident immune cells of the CNS and have phagocytic, cytokine secreting and T cell activating as well as antigen presenting properties which vary depending on the antigenic stimulus. They can be neuroprotective or induce changes that could ultimate lead to neurodegeneration in various human CNS diseases[73, 74]. In terms of adaptive cellular immunity, pathological examination of biopsy and autopsy specimens obtained from anti-NMDAR mediated AE reveals minimal T cell infiltration into the brain parenchyma and a paucity of cells expressing cytotoxic markers (e.g. T cell intracytoplasmic antigen-1, granzyme B, perforin)[18, 33, 63] indicating that cellular immunity plays a lesser role than antibody mediated processes (table 1).

It is not completely understood how seizures occur in anti-NMDAR AE. A study of six patients with drug-resistant epilepsy which utilised intrahippocampal microdialysis to measure extracellular CNS glutamate and depth electrodes to record seizure activity, showed that glutamate, the major CNS excitatory neurotransmitter and NMDAR agonist, increases in concentration in the extracellular fluid during and just prior to seizures[75]. While glutamate has excitatory activity, it may also be involved in negative feedback mechanisms that limit seizure activity. In rodent models, glutamate has been shown to promote microglial process extension towards neuronal elements in an NMDAR dependent process that results in the release of ATP and activation of P2Y12 receptors on microglia[76]. Reduction of microglial process extension in P2Y12 knockout mice is associated with prolongation of induced seizures suggesting that this NMDAR dependent microglia-neuronal interaction has anti-seizure effects[76]. Another proposed mechanism is the reduction of NMDARs on inhibitory GABAergic interneurons resulting in a disinhibition of excitatory neurons and reduction in seizure threshold[77, 78]. Further supporting the role of NMDARs in seizure generation, the *GRIN1*-related neurodevelopmental disorder which is caused by loss or gain-of-function mutations in *GRIN1* (encoding the GluN1 subunit of the NMDAR) is associated with seizures in approximately 65% of patients[79]. This disorder also mimics other aspects of anti-NMDAR mediated AE such as movement disorders and cognitive dysfunction[80].The fact that both loss and gain-of-function mutations can lead to seizures highlights the complexity surrounding seizure generation and the role of the NMDAR[80].

Overall, human pathological studies on biopsy or autopsy specimens do not reveal prominent neuronal death [18]. While routine MRI scans usually do not display significant long term structural brain changes[81], more detailed volumetric, functional and diffusion tensor imaging (DTI) MRI studies have revealed hippocampal atrophy, changes in hippocampal functional connectivity and widespread white matter damage which correlated with disease severity and long term cognitive outcomes[81, 82]. The relatively subtle changes on MRI and the minimal neuronal death on pathology along with the high risk of acute seizures

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but low risk of long term epilepsy supports a mainly reversible antibody mediated process with acute functional changes in neuronal networks and low epileptogenic potential. Nevertheless, the chronic changes on detailed imaging studies explain the longer term functional and cognitive deficits that the majority of patients suffer[33, 83].

Anti-LGI1 antibody mediated autoimmune encephalitis

Limbic encephalitis is the most common manifestation of anti-LGI1 AE which is more common in males, with an onset usually between 60 to 70 years of age and is associated with underlying malignancy in around 10% of cases[21, 22, 42, 43, 84]. This syndrome classically presents over weeks to months with early faciobrachial dystonic seizures (FBDSs) and later development of other seizure types, memory disturbance, behavioural changes, insomnia, autonomic dysfunction and hyponatremia[21, 22, 42, 45].

FBDSs are pathognomonic for this condition and occur in 25% to 47% of patients[21, 29, 30, 42] and usually precede the development of other symptoms by several weeks to a month[21, 45]. They are described as brief dystonic contractions usually involving the face and arm, lasting one to five seconds and occurring anywhere between two to 960 times per day, with an average frequency of between 10 to 66 times per day[21, 43, 46, 84]. FBDSs can be accompanied by other features such as dysphasia, oral automatisms, vocalisations and loss of awareness in a quarter of patients[45, 47, 84]. In the majority of FBDSs, there is no surface EEG correlate [21, 43, 84], which has contributed in the past to debates surrounding their epileptic aetiology. However, in one series, 7 out of 7 patients with FBDSs had a slow wave detected from the contralateral frontal region and preceding motor activity implying a cortical origin[85]. MRI shows T1 or T2 changes in the basal ganglia in 42% of cases which is not usually seen in patients without FBDSs[43]. PET scan findings in FBDS include basal ganglia hypermetabolism, and less frequently hypometabolism, with associated motor and sensory cortex hypermetabolism[42, 85, 86]. The semiological features, together with the EEG findings, basal ganglia and cortical changes on PET and the basal ganglia abnormalities on MRI suggests a deep seizure generator with a network that involves the basal ganglia and the primary motor cortex. Without immunotherapy, only 10-42% of faciobrachial dystonic seizures resolve, compared with 89-100% with immunotherapy, emphasising the importance of immune dysfunction in the pathogenesis of this condition[43, 46]. Early recognition of this symptom, especially prior to onset of cognitive impairment, allows for earlier immunotherapy initiation which is associated with halting of symptom progression and prevention of long term cognitive deficits[46, 87].

LGI1 antibodies are thought to be pathological and their titres correlate with disease activity and disease relapse[47, 88]. Unlike anti-NMDAR antibody mediated AE, antibodies in anti-LGI1 AE are predominantly of the IgG4 subtype[89], although complement fixing IgG1 can be present and may be related to more severe

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disease manifestations[46]. In surgical and autopsy derived pathological specimens, complement deposition was seen on neurons in association with acute neuronal death[18] suggesting that complement mediated neuronal cell loss likely plays a role in the pathogenesis of disease (table 1). Consistent with this, there is frequent progression to hippocampal atrophy on routine MRI as well as whole brain atrophy on volumetric studies [47, 88]. Given neuronal cell death is an irreversible process, this could partly account for the higher rate of long-term epilepsy when compared with anti NMDAR antibody mediated AE.

In terms of acute seizure generation, there are also non-inflammatory, direct antibody mediated actions that are postulated to play a role. LGI1 is a protein secreted by neurons that forms a trans-synaptic protein complex with binding to presynaptic a disintegrin and metallopeptidase domain 23 (ADAM23) and postsynaptic a disintegrin and metallopeptidase domain 22 (ADAM22). These interactions are important for presynaptic potassium channel (Kv1 subunit) and post synaptic α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid receptor (AMPAR) localisation and function which are important for fast excitatory synaptic transmission and hippocampal long term synaptic plasticity[17, 90, 91]. In rodent antibody transfer studies, anti-LGI1 antibodies prevent LGI1-ADAM22/23 interactions which in turn reversibly reduces the levels of presynaptic Kv1.1 channels leading to increased glutamatergic activity [92] and neuronal hyperexcitability. There is also subsequent reduction in postsynaptic AMPAR[46, 92, 93] which are present on excitatory and inhibitory neurons. Loss of AMPAR on inhibitory interneurons may also contribute to neuronal disinhibition and seizure generation[93] (FIG. 2).

Anti-$GABA_BR$ antibody mediated autoimmune encephalitis

Anti-$GABA_B$R AE presents as a limbic encephalitis with early recurrent seizures and not uncommonly SE[26-28, 94]. Seizures are followed by the development of memory impairment, confusion and behavioural change in over 80-90%[26, 28, 95]. Cancer, most commonly small cell lung cancer (SCLC), is diagnosed in half of the patients and usually follows neurological disease onset[27, 28, 96]. Males make up over 60% of the presentations, and onset is most common between 50 to 70 years of age, although earlier onset is seen in those without concomitant small cell lung cancer[26-28, 96]. Mortality ranges from 30-60% most commonly from cancer progression, but also secondary to SE and infectious complications[28, 95, 97]. In those who survive, functionally limiting cognitive complaints persist in majority[28, 95].

SCLC tissue from patients both with and without anti-$GABA_B$R AE express $GABA_B$R[26, 97]. This shared antigen could explain the loss of immune tolerance in paraneoplastic cases. $GABA_B$R antibodies can be detected in both serum and CSF, although in some cases is found only in CSF supporting intrathecal production[26]. Antibodies bind to extracellular B1 subunit domain of the $GABA_B$R[26, 27] which also contains the GABA binding site[98]. Unlike anti-NMDAR antibodies, anti-$GABA_B$R antibodies do not cause internalisation of the

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receptors[99] and therefore are postulated to act via interference with $GABA_BR$ function. $GABA_BR$ mediate pre- and post-synaptic inhibition and interference with this function would promote neuronal hyperexcitability and seizures[98] (FIG. 2).

Antibody mediated pathology may not be the sole contributor to disease. Autopsy performed on one patient one-month post presentation revealed bitemporal, parenchymal, CD4+ and CD8+ T lymphocytic infiltrates, microgliosis and astrogliosis. Perivascular T and B cells and macrophages were also seen. Neuronal damage and necrosis was found within the hippocampus and colocalised with cytotoxic T cells, suggesting cell mediated damage[95]. In contrast, pathology results from a patient 20 months following diagnosis only showed reactive astrogliosis[26]. In addition, anti-$GABA_BR$ antibodies are mainly of the IgG1 subtype, and can fix complement as demonstrated in mice brain exposed to patient serum[100]. This may also contribute to neuronal death and hence a propensity to seizures. In addition, the resultant permanent alterations in neuronal networks could explain the persistent cognitive deficits that are seen in most patients with anti $GABA_BR$ mediated AE.

Paraneoplastic limbic encephalitis associated with onconeural antibodies

Autoimmune limbic encephalitis presents with subacute working memory deficits, seizures and psychiatric symptoms and can be associated with both cell surface or synaptic antibodies such as anti-LGI1 antibodies as discussed above, or onconeural antibodies directed against intracellular antigens[15]. There are a number of onconeural antibodies that are directed against intracellular neural antigens with varying tumour associations – for example, anti-Ma2 is associated with cancer in 90% of cases, most commonly testicular cancer[101], and anti-Hu is associated with cancer in approximately 85% of patients, most commonly small cell lung cancer[102, 103]. In the majority of cases, oncological diagnosis follows neurological presentation and antitumour therapies where cancer is detected appears more effective than immunotherapy[101, 103]. Therefore, the knowledge of neuronal antibody-tumour associations guides oncological work-up allowing prompt anti-tumour therapy initiation with the potential for improved functional outcomes[101, 103]. Antibody identification also has prognostic significance. For instance, in 16 patients with small cell lung cancer and limbic encephalitis, eight patients with anti-Hu antibodies were less responsive to cancer treatment compared to eight patients without anti-Hu antibodies[104]. However, unlike anti-NMDAR and anti-LGI1 antibodies, these antibodies do not appear to be pathogenic with no significant immunoglobulin deposition or signs of complement activation on pathological studies[18] and a general lack of response to antibody depleting therapies[104, 105]. Instead, cellular immunity appears to be more important with cytotoxic T cell infiltration, granzyme B mediated neuronal damage[18] and significant neuronal loss with gliosis on biopsy and autopsy specimens[36, 104] (table 1). In another study of three patients with either anti-Ma2 or

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anti-Hu antibody positive AE who underwent epilepsy surgery for DRE, biopsy specimens showed neuronal loss and gliosis and two out of three specimens also contained lymphocytic infiltrates[106]. The patient who did not have lymphocytic infiltrates was operated on 11 years after the acute encephalitis episode compared with within one year or less in those with inflammatory infiltrates[106]. This limited data may suggest that neuronal damage and gliosis with consequent alterations in neuronal networks, as opposed to active inflammation, are important epileptogenic mechanisms in patients with a longer duration of seizures. This could have implications for immunotherapy responsiveness in the chronic versus acute cases, although more studies are needed.

Predicting the development of autoimmune epilepsy: an autoimmune fingerprint

Only a subset of patients with AEs will develop epilepsy, and the subsequent 'autoimmune-associated epilepsy' may be driven by either ongoing inflammation or permanent neuronal and glial network alterations, or both[20]. The differences in the underlying mechanisms driving the long-term epilepsy is important to define given that one scenario would theoretically respond to ongoing immunotherapy whereas the other may only require anti-seizure medications, sparing patients from side effects of long-term immunosuppression. Improved biomarkers are needed to assist clinicians in differentiating between the two.

Furthermore, it is not uncommon for clinicians to encounter a patient with an explosive onset, in adulthood, of DRE in whom autoimmunity is suspected. In one study, 23 out of 112 patients with epilepsy of unknown aetiology had positive serum anti-VGKCc, anti-LGI1, high titre anti-GAD, anti-NMDAR or anti-Hu antibody suggesting an autoimmune aetiology[57]. While immunotherapy in this cohort resulted in a better seizure outcome at short term follow up (four to six weeks), antibody positivity was not necessarily predictive of response and there were seronegative patients who showed similar benefit[57]. That is, identifying those who will respond to immunotherapy proves to be challenging and additional diagnostic instruments need to be developed.

Using antibodies as biomarkers for autoimmune epilepsies is problematic for several reasons. Firstly, not all autoimmune epilepsies will be antibody mediated and not all antibodies are known. Secondly, multiple tests are required to generate an inclusive antibody panel. Thirdly, in chronic epilepsy, antibody titres do not always correlate with inflammatory activity or response to immunotherapy [70, 107, 108]. Finally, the detection of antibodies, especially on serum, does not necessarily imply causation – for instance, low levels of anti-glutamic acid decarboxylase-65 antibodies can be present in up to 8% of people without associated neurological conditions[109]. All of these limit the sensitivity, specificity and utility of using antibody testing for detecting an autoimmune process. Given that antibodies are the specific targeted downstream

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products of a cascade of immune activation, it could be postulated that patterns of 'less specific', but more generalizable upstream immune alterations could be more sensitive for the overall detection of autoimmune epilepsies with ongoing active inflammation. Several studies have looked at various serum and CSF cytokines and their potential as biomarkers into disease activity, prognosis and treatment responsiveness. B cell related chemokines such as C-X-C motif chemokine ligand 13 (CXCL13) have been found to be elevated in the CSF of patients with anti-NMDAR and anti-LGI1 AE – disease processes with pathogenic antibodies [110-112]. In anti-NMDAR antibody mediated AE, CXCL13 levels were positively correlated with poorer functional status at 8 months post-disease onset and increased again with subsequent relapses[111]. C-X-C motif chemokine ligand 10 (CXCL10), which has T cell, macrophage as well as B cell chemoattraction properties, has also been found to be elevated in the CSF of anti-NMDAR AE patients during the early and most symptomatic period between 4 to 60 days post disease onset[112] highlighting the complex interplay between the different immune cell types. In particular, Th17 cells may play an important pathogenic role in AE as Th17 related serum cytokines, Interleukin 17 (IL-17) and interleukin 23 (IL-23), were found to be higher in anti-NMDAR and anti-LGI1 AE compared to encephalitis with intracellular antigens and healthy controls[113]. This is particularly interesting given that therapies against IL-17 and IL-23 are already in clinical use in conditions such as psoriasis and Crohn's disease[114, 115]. In anti-NMDAR and anti-LGI-1 AE, rituximab (anti-B cell therapy) and cyclophosphamide (suppresses both B and T cell activity) have been used as effective second line therapy where first line treatments have been failed[61, 116]. Other molecules have been inconsistently found to be elevated with active disease in mixed populations of AE including proinflammatory interleukin 6 (IL-6), tumour necrosis factor α (TNF α) and high mobility group box 1 (HMGB1) and the Th1 related cytokines interferon γ (IFNγ) and interleukin 12 (IL-12)[112, 113, 117, 118]. In contrast, Th2 related cytokines interleukin-4 (IL-4) and Treg related cytokines interleukin-10 (IL-10) and transforming growth factor β (TFG-β) are not elevated in AE[113, 118] (table 2). Of note, enzyme linked immunosorbent assays (ELISA) or multiplex bead immunoassays were used to evaluate the concentrations of these cytokines and have a reported sensitivity in the picogram per millilitre range[119]. It will be interesting to see whether future trials, utilising more sensitive detection methods such as single molecule array assays which have higher sensitivities in the femtogram per millilitre range[120], will reveal more subtle changes that have been beyond the resolution of the ELISA and multiplex bead immunoassays.

Overall, refinement of cytokine and inflammatory molecular panels are still needed prior to clinical usage. Nevertheless, identifying various profiles and their correlation with inflammatory disease activity, prognosis and relapse will be useful in terms of helping to refine clinical treatment pathways – for instance, the cessation of immunotherapy in patients with autoimmune-associated epilepsy and the

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commencement of immunotherapy in those with drug-resistant epilepsy where underlying autoimmune aetiology is postulated; decisions which remain difficult and could benefit from supplementary biomarkers. In addition to determining when to treat with immunotherapy, a better understanding of the immune milieu will also guide the selection more targeted therapeutics.

Inflammatory Status Epilepticus

There are several syndromes that present with de novo refractory SE in previously well patients. These include Rasmussen encephalitis, new onset refractory status epilepticus (NORSE), febrile infection-related epilepsy syndrome (FIRES), devastating epilepsy in school-aged children (DESC) and acute encephalitis with refractory, repetitive partial seizures (AERRPS)[121-125], with DESC and AERRPS representing the same syndrome (described by the French and Japanese groups, respectively) and currently grouped under the umbrella term FIRES.

NORSE is a syndrome composed of a group of heterogenous conditions with different aetiologies[4]. FIRES can be viewed as a subcategory of NORSE that is associated with a preceding fever and, while it is most commonly featured in the paediatric literature, can occur at all ages[121]. In a multi-centre study of 130 patients with NORSE, the majority did not have an identifiable aetiology. Of those who did, 23 (18%) had antibodies known to associate with autoimmune/paraneoplastic encephalitis (anti-NMDAR, anti-GAD65, anti-Hu and anti-CRMP5 antibodies) and 25 (19%) had antibodies or diagnoses of unclear relevance such as anti-VGKC complex, anti-striational and anti-Ro antibodies or diagnoses such as steroid responsive encephalopathy associated with autoimmune thyroiditis (SREAT), cerebral lupus or seronegative paraneoplastic cases[4]. These latter cases may represent forms of seronegative autoimmune encephalitis but it is difficult to be certain without knowing the details of the cases. Other than autoimmune/paraneoplastic aetiologies, the remainder of the identified aetiologies consisted of atypical infections and a mix of other conditions in 15 patients (12%)[4]. Taking this diagnostic uncertainty into account, between 29% (38/130) to 48% (63/130) of patients with NORSE will have an identifiable aetiology and between 18% (23/130) and 37% (48/130) will have an autoimmune/paraneoplastic cause[4]. Given that the majority of cases where a cause is identified consist of autoimmune/paraneoplastic aetiologies, cryptogenic NORSE patients are often treated empirically with immunotherapy, albeit with variable results[126-129]. While the range of aetiological mechanisms at play in cryptogenic disease may explain some of the variability there is currently no biomarker to accurately predict response to therapy. Rasmussen encephalitis (RE) is associated with an aberrant immune reaction to an unknown trigger which, on histopathology, involves cytotoxic T cells, reactive microglia and significant neuronal loss without evidence of viral infection[130-132].

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Compared to non-paraneoplastic AE, NORSE, FIRES and RE have higher mortality and DRE rates. NORSE has a reported mortality of 22%, and 92% of survivors remain on anti-seizure medications with 37% experiencing ongoing seizures despite medication at an average of 6 months follow-up[4]. FIRES has a mortality rate of 9-14%[5, 129, 133] and DRE develops in over 90% of survivors; cognitive impairment is found in over 80% of cases[5, 123, 133, 134]. RE also results in significant morbidity with neurological sequelae and chronic epilepsy in the majority of patients[135]. A better pathophysiological understanding of these conditions at the most severe end of the epilepsy spectrum, will not only aid in the production of better diagnostic and prognostic biomarkers and more efficacious treatments, but can also add to our general understanding of epileptogenesis.

Immunological Mechanisms of Disease

NORSE and FIRES

There are no universally accepted diagnostic or treatment protocols for these conditions which result in significant variability between different clinicians in their approach to treatment[136]. In one survey involving 107 neurocritical care practitioners, the majority of whom worked in tertiary hospitals in the United States, 25% would not routinely perform an autoimmune workup in patients presenting with NORSE despite the prevalence of autoimmune or paraneoplastic aetiologies[4]. While most practitioners would consider using corticosteroids and plasma exchange if an autoimmune or paraneoplastic cause was suspected, or when the patient failed to improve after a week, 29% of respondents would never use intravenous immunoglobulin (IVIG) and 24% would not use plasma exchange[136]. Furthermore, while 29% would add steroid sparing immunosuppressants, 42% would never use these medications[136] highlighting the uncertainties facing clinicians managing these patients. The lack of uniformity regarding therapeutic approaches likely stems partly from the lack of diagnostic certainty in cryptogenic cases as well as the lack of robust therapeutic trials, with the majority of recommendations based on expert opinion, case series or cohort studies[137]. Adding to this uncertainty, success with immunotherapy has been variable across case series with far greater success in NORSE compared to FIRES. The lower success rates seen in certain case series are likely secondary to a mixture of non-immune aetiologies, subtherapeutic or delayed commencement of immunosuppression and heterogenous immunotherapy regimens[4, 5, 134, 138, 139]. For instance, in the majority of cases where immunotherapy had been deemed unsuccessful, only first line immunotherapy such as IVIG, steroids and plasma exchange were tried[5, 134]. This may represent undertreatment as second line immunosuppressants such as rituximab[139], cyclophosphamide[140] or tocilizumab[128] have been used with improved seizure control or general clinical improvement when first line agents alone have failed. The reluctance to escalate immunotherapy may, in part, be due to clinician

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concern regarding side effects of these agents weighed against uncertainty about the underlying aetiology. Therefore, improved biomarkers are needed to enable clinicians to identify the subset with an immune aetiology with more confidence. This will not only enable earlier treatment which may have prognostic benefits akin to AE[30, 46, 87, 141], but also to enable more aggressive immunotherapy and prevent undertreatment.

While the majority of known non-cryptogenic NORSE cases are due to immune mediated disease, this has not been the case with FIRES, although testing of antibodies in this cohort is variable and often does not include many of the newly discovered autoantibodies. In one of the largest cohorts of 77 FIRES patients, 35% had immunological testing which was limited to oligoclonal bands, anti-GAD antibodies, anti-VGKC antibodies and anti-glutamate receptor type 3 (GluR3) antibodies of which the majority were negative[5]. Similarly, another case series of 12 patients in which three had CSF and all had serum analysed, found that no antibodies to NMDAR, AMPAR, $GABA_BR$, LGI1 or CASPR2 were present in any of the samples[129]. Furthermore, clinical improvement with use of first line immunotherapy, such as glucocorticoids, IVIG and plasma exchange (PLEX) has been disappointing in many cases, although the same reservations limiting immunotherapy in NORSE is likely to play an even larger role here given the lack of robust evidence for autoimmunity[5, 134]. In terms of second line therapies, the evidence rests mainly on case reports. One report describes a 50% seizure reduction in response to rituximab, but treatment had to be stopped after two doses due to lymphopenia[142]. Another case treated with rituximab, after the failure of intravenous methylprednisolone, IVIG and PLEX to control seizures, has been described in which the patient died five days post treatment due to ongoing seizures, cerebral oedema and multiorgan failure, limiting comment on the potential effectiveness of this therapy[133]. Also described is a case of AERRPS that failed to adequately respond to IVIG and intravenous methylprednisolone in which tacrolimus resulted in control of seizures, although pathology revealed infiltrative T cell, neutrophil and microglia which is atypical of the majority of FIRES cases[143].

Most pathological studies in FIRES do not demonstrate inflammatory cellular infiltrate. If any abnormality is detected, it is usually neuronal cell loss and reactive gliosis[5, 133, 144] with only mild inflammation such as scattered T cells[145] or leptomeningeal inflammation[5]. Limited pathological studies in NORSE show rare inflammatory changes with non-specific reactive microglia[146] or bilateral hippocampal inflammation[138] although there is a lack of significant immune cellular infiltrate in most reports[146, 147] (table 1). This is somewhat at odds with the fact that the majority of non-cryptogenic NORSE are immune mediated and the frequent, possibly overestimated, assumption that a significant proportion of cryptogenic cases are also immune mediated[4]. However, not all AE cases have prominent cellular infiltrate[33, 62] and diagnostic biopsies are not required where patients already fulfil the diagnostic criteria for AE or show a good

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response to empirical immunotherapy which leads to selection bias. These factors may partly explain the lower levels of inflammation on biopsy specimens than expected for NORSE patients.

Overall, it appears that FIRES has less inflammatory infiltrate and is less responsive to first line immunotherapy than NORSE. Interestingly, a few studies, mainly in FIRES, demonstrate a pro-inflammatory alteration in CSF cytokine profiles although, without comparator non-inflammatory SE groups within these studies[143, 148]. Whether this alteration is a cause or effect of SE is still unclear and the clinical utility of these cytokines to predict response to immunotherapy is unknown[128, 143, 148-152].

In FIRES, proinflammatory cytokines such as IL-6[143, 148, 149, 151, 152] and cytokines involved in the innate immune response such as C-X-C motif chemokine ligand 8/interleukin-8 (CXCL8/IL-8)[151, 152] a neutrophil chemoattractant, C-C motif ligand 4 (CCL4) and C-C motif ligand 3 (CCL3)[149] involved in macrophage recruitment, are elevated in CSF. Interestingly, IL-6 tended to be elevated in CSF more than plasma suggesting CNS specific inflammation[148, 152]. IL-1β is a proinflammatory cytokine which is upregulated in brain tissue from drug resistant epilepsy patients[8]. In animal models, it is involved in the generation of fever, neuronal hyperexcitability and seizures[8, 153]. In FIRES, IL-1β was not consistently elevated in CSF or serum between studies, although this finding was limited by detection thresholds and wide concentration variability[148, 151]. Other proinflammatory molecules such as TNF-α[143, 148, 149] and IFN-γ[143, 148] are variably elevated in CSF when compared with healthy controls or patients with non-inflammatory neurological conditions. Anti-inflammatory cytokines such as IL-1 receptor antagonist (IL-1RA)[149, 150] and IL-10[143, 148, 151] are also inconsistently elevated. Even when elevated in CSF and serum, IL-1RA appears to be functionally deficient as shown via a cell-based assay in one patient and this was associated with multiple polymorphisms of uncertain significance in the non-coding regions of the *IL1RN* gene[150]. Given that the IL-1RA in febrile SE is reduced[154] instead of being elevated as in FIRES, it may be that genetic factors affecting IL-1RA function and production are important in the pathogenesis of FIRES. CXCL10, a chemoattractant for multiple cell types including natural killer (NK) cells, macrophages, T and B cells is elevated in CSF[149, 152] along with increased expression of MMP-9 which is involved in inflammatory cell migration across the blood brain barrier[143, 155]. Other cytokines such as IL-2 important for T cell development[148, 152], IL-12 involved in Th1 pathways[149, 151], IL-4 involved in Th2 pathways[148, 149], IL-17 involved in Th17 pathways[149, 152] and CXCL12 a homeostatic chemokine[152] appear unchanged in CSF. Of note, IL-6 and IL-8 elevation as well as elevation of neopterin, a non-specific marker of immune activation, was also found in the CSF of febrile SE patients at significantly higher levels than in non-inflammatory neurological conditions or chronic epilepsy patients with daily seizures. This indicates that there is an inflammatory milieu in febrile SE[149] and these markers are not specific to FIRES patients. On the other hand, there were differing cytokine profiles amongst FIRES and afebrile SE controls suggesting that the alterations are not solely due to seizure

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activity[149]. In NORSE, cytokine alterations are also seen, but in contrast to FIRES, appears to involve both the innate and the adaptive immune system with elevations in IL-6, TNF-α, IL-2, IL-12, IL-4 and IL-10[128] which may explain the greater response to first-line immunotherapies such as IVIG and PLEX which act mainly via modifying the adaptive immune system[138, 141]. Like the AE studies, ELISA and multiplex bead assays were used to measure cytokine levels in these studies and future studies utilising high sensitivity techniques may reveal differences beyond the current limits of detection and help to advance our understanding of these diseases. The cytokine profiles found in FIRES and NORSE (table 2) supports an immune contribution to their pathogenesis and the significance of this contribution is highlighted by a limited number of successful case reports utilising anti-cytokine therapies. These reports utilise therapies such as anakinra[145, 151], an interleukin-1 receptor antagonist, and tocilizumab an interleukin-6 receptor antagonist[128] in cases refractory to a variety of other immunotherapies such as IVIG, steroids, PLEX and second line therapies such as rituximab. Furthermore, following treatment with anakinra, normalisation in IL-8 and IL-6 levels along with a reduction in seizure frequencies occurred in one case[151]. These are promising results, although reporting bias may affect the generalisability of these findings and more extensive research is required.

The prognostic implications of cytokine alterations in FIRES and NORSE is still unclear, but evidence taken from the febrile SE literature suggests a possible correlation with outcomes. In particular, patients with T2 MRI hippocampal hyperintensity, have higher IL-6 and IL-8 and lower IL-1RA:IL-6 and IL-1RA:IL-8 ratios than febrile SE patients with normal MRI brains[154]. T2 MRI hippocampal hyperintensity is correlated with the development of hippocampal sclerosis and atrophy in febrile SE[156] and the elevated inflammatory cytokines in this group suggests that a greater inflammatory response is associated with increased neuronal injury and may predispose to subsequent epilepsy.

Overall, there is reported overlap between NORSE, FIRES and febrile SE, but also differences quantitatively and qualitatively in cytokine profiles. Further studies need to be undertaken to better characterise these profiles and their association with disease aetiology and subsequent development of epilepsy. This in turn will enable patient selection for trials looking at the anti-epileptogenic properties of anti-cytokine therapies.

Rasmussen's encephalitis

Mostly affecting children, this condition is usually isolated to one cerebral hemisphere and begins with infrequent focal seizures which then evolves into frequent focal aware seizures with EPC in 63% occurring in association with progressive hemiparesis[135]. This is followed by progressive loss of hemispheric function with worsening hemiparesis, hemianopia, cognitive decline and dysphasia associated with unilateral

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hemispheric atrophy on MRI and neuronal loss on histopathology[125, 131, 135]. Eventually, patients stabilise but most are left with permanent neurological deficits and, although seizures decrease in frequency, epilepsy remains in >90% cases[135].

There are four progressive pathological stages in RE as described by Pardo et al[131] that are found in a multifocal distribution with differing stages seen within each patient. Stage 0, is normal cortex where no T cell infiltration, microglial activation or neuronal loss is present. Interestingly, a recent study found the presence of small 3-7 cell microglial nodules, some of which stained for HLA-DR (a marker of activation), occurring in cortex that would otherwise be classified as stage 0, suggesting that microglia may be important in setting the stage for this CD8+ T cell mediated encephalitis [157]. Stage 1, or early stage, features nodules of perineuronal and perivascular inflammatory cell infiltrate, mainly lymphocytic, with microglial and astrocytic activation and minimal neuronal injury. Stage 2, or intermediate stage, displays an increase in lymphocytic infiltrate and microglial and astrocytic reactions in a pan-laminar distribution with evidence of neuronal injury and neuronal drop out. Infiltrating lymphocytes are mainly CD8+ more than CD4+ T cells with only infrequent perivascular B cells seen. These CD8+ T cells are clonally expanded with subpopulations being CNS restricted suggesting local organ specific replication in reaction to a local antigen[158]. Furthermore, these CD8+ T cells with granzyme B immunoreactivity are found in apposition to neurons and astrocytes supporting their pathological role in disease[159, 160]. Stage 3, or late stage, is typified by significant neuronal loss and associated cortical atrophy and ongoing astrocytic and microglial activation. Lymphocytic infiltration is less prominent during this stage. Stage 4, or end stage, consists of extensive cortical damage with vacuolation or complete pan-laminar neuronal dropout and degeneration and astrogliosis with minimal residual inflammatory change. White matter and deep cortical structures are also similarly involved (table 1).

It is well known that CD8+ and CD4+ T cells play a central role in RE, with IFN-γ mRNA transcripts being elevated early in RE compared with cortical dysplasia controls likely due to production by these activated cells[161]. However, the importance of microglia must also be recognised as these cells play a key role in driving early inflammation and the inflammasome response[157, 159] (FIG. 2). In one study, cultured human microglia were found to express increased inflammasome associated mRNA transcripts such as *NLRP1*, *NLRP3* and *casp1*. These transcripts were only found at low or minimal levels in cultured astrocytes and neurons implying that microglia are the primary cell responsible for the inflammasome response in CNS disease[159]. *NLRP1* and *NLRP3* code for proteins that assemble to form inflammasomes upon exposure to pathogen associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs) which are detected by pattern recognition receptors (e.g. toll-like receptors)[162]. Inflammasomes make up an important component of the innate immune system and their formation results in activation of caspase-1,

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an enzyme that in turn cleaves pro-IL-1β and pro-interleukin 18 (pro-IL-18) into their proinflammatory active cytokine forms[162]. In terms of RE, surgical brain specimens from patients revealed elevated levels of mRNA transcripts for *NLRP1, NLRP2, casp1*, the gene that codes for caspase-1, *IL-1*β and *IL-18* when compared with multiple sclerosis and mesial temporal sclerosis controls[159]. Another study involving resected tissue from RE patients showed upregulation of toll-like receptor 7 (TLR7) and toll-like receptor 3 (TLR3) expression with corresponding immunohistochemical staining of TLR7 (but not TLR3) on microglia in affected tissues early in disease which was not seen in control tissue from patients with low grade tumours[157]. This corresponded to the immunohistochemical detection, as well as increased upregulation of gene expression, of caspase-1 and the inflammasome products IL-18 and IL-1β within microglial nodules[157]. Furthermore, in the CSF, levels of TNF-α, interferon-γ and granzyme B are elevated along with CD8+ and CD4+ T cells[163] (table 2). TNF-α is a pro-inflammatory cytokine produced predominantly by microglia in the central nervous system[164].

While innate and adaptive cellular mechanisms are involved in the pathogenesis of RE, studies have not revealed a significant role for antibody mediated mechanisms and no disease specific antibodies have been found. For instance, studies of anti-GluR3 antibody positivity in RE have yielded conflicting results and, even where positive, this antibody is not specific for RE making its presence at most a marker of autoimmunity[125]. Screening for other antibodies against VGKCc, LGI1, GAD, NMDAR, glycine receptor, AMPA receptor and GABA receptors have been equally unrewarding[165]. One study, using a cell-based assay, detected antibodies in serum to AMPAR subunits GluA2/3 in two out of 53 patients and found antibody binding to cultured hippocampal and cortical neurons in 10 out of 53 patients. This contrasts with control samples where none had positive results using these methods[166]. However, antibody positivity did not correspond to clinical differences and, in one patient, the anti-AMPAR antibodies appeared only on subsequent serum samples implying that, even when they are found, these antibodies could be an epiphenomenon of the disease rather than pathological[166].

How the interplay between gross neuronal loss leading to structural network changes and the inflammatory milieu with attendant functional changes leads to seizures is not fully elucidated. In RE specimens compared to non-RE specimens, there was increased microglial activation with associated expression of pannexin channels[167]. In the same study patch clamping revealed that RE neurons compared to non- RE neurons had increased cell capacitance and decreased input resistance. This suggests increased inflammation and alterations in pyramidal neuron membrane properties could explain the observed hyperexcitability.

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Given that cell mediated immunity appears to play an important role in RE, medications such as IVIG, steroids, tacrolimus[168], natalizumab[169] have been reported to be effective in improving cognitive and seizure outcomes in some cases. The anti-TNF α therapy Adalimumab has also been reported to substantially reduce seizure burden[170]. Interestingly, response to antibody and B cell targeted therapy such as rituximab and immunoadsorption have also been reported to reduce seizure burden in isolated case reports[171, 172] highlighting the complexity of the immune response in RE. Of note, there are two main goals of therapeutic intervention by which treatment success or failure can be defined: a) seizure reduction and b) limiting functional decline[173]. As always, choice of therapy depends on the mix and severity of symptoms in an individual patient weighed against the risks and benefits of any given intervention. The difficulties in therapeutic decision-making lie where the risk benefit scale is near equilibrium. For instance, hemispherectomy is still the only treatment that can lead to complete seizure control and has been reported to be efficacious in up to 70-80% of cases, making it a useful option for patients with severe epilepsy[168]. However, post-operative functional impairments such as cognitive, language and motor deficits can be significant and will depend on premorbid function, hemispheric dominance and age at surgery – issues that need to be considered and factored into the final treatment recommendation[173]. Currently, immunotherapy is used to prevent functional decline, although the optimal choice and duration of treatment is not clear[173]. Given the complexity of the immune response in RE, it may be that use of combined immunotherapies targeting the cellular and innate immune system may be more successful than monomodal therapy, although this remains theoretical at present.

SUMMARY

This review highlights the various immunological mechanisms that are involved in seizure generation and epileptogenesis in patients with autoimmune encephalitis and acquired epilepsies. The adaptive immune response ranges from the largely reversible antibody mediated seizures of anti-NMDAR AE and anti-LGI1 AE, with minimal risk of subsequent epilepsy, to the florid CD8+ T cell mediated disease of Rasmussen's encephalitis where patients almost universally develop epilepsy. The innate immune response is just as important with the role of microglia in producing various inflammatory mediators and influencing neuronal excitability to the alteration in pro-inflammatory and anti-inflammatory cytokines in FIRES. Often, immunomodulatory therapies targeting the dysfunctional arm of the immune system have been used successfully in these conditions, although the efficacy of the therapy is not always correlated with postulated disease mechanisms exposing gaps in our understanding. Nevertheless, these conditions illustrate the complex interplay between autoimmunity, inflammation, ictogenesis and epileptogenesis, and these principles can aid in therapeutic choice and the development of disease modifying therapies that may also have implications for a wider population of individuals with epilepsy.

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Figures

Fig 1. Neuroinflammation and Epilepsy Spectrum At one end of the spectrum are the autoimmune epilepsies in which the immune system has a key pathogenic role. The adaptive immune system is predominantly involved and there is good evidence for response to immunotherapy. At the other end of the spectrum, epilepsies with low levels of inflammation, mainly involving the innate immune system are found. In the middle are syndromes such as Rasumussen Encephalitis, NORSE (new onset refractory status epilepticus) and FIRES (febrile infection related epilepsy syndrome) in which neuroinflammation is also vital, but there is a lower response to immunotherapy. In parallel to this, neuronal damage is variable, can be seen across the spectrum and is disease dependent.

Fig 2. Autoimmune and neuroinflammatory mechanisms of ictogenesis. There is a loss of immune tolerance and an inflammatory response that leads to blood brain barrier leak involving matrix metalloproteinase and cytokines with extravasation of inflammatory mediators and cells into the CNS. Subsequent local immune response and intrathecal production of antibodies and cytokines occurs. Antibodies can act via A) internalisation of receptors (anti-NMDAR autoimmune encephalitis), B) disruption of synaptic protein localisation (anti-LGI1 autoimmune encephalitis) or C) acting as neurotransmitter antagonists (anti-$GABA_BR$ autoimmune encephalitis) D) Rasmussen encephalitis involves CD8 T cell cytotoxicity with neuronal damage and astrogliosis with microglial activation and upregulation of inflammasomes E) Coordinating these responses are numerous cytokines that allow communication between cells and in which unique profiles can be discerned depending on the underlying arm of the immune system involved. ADAM22, a disintegrin and metallopeptidase domain 22; ADAM23, a disintegrin and metallopeptidase domain 23; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; GABA, gamma amino butyric acid; GABABR Ab, gamma amino butyric acid B receptor antibody; GrB, granzyme B; LGI-1 ab, leucine-rich glioma-inactivated 1 antibody; MMP, matrix metalloproteinase; NMDAR ab, N-methyl-D-aspartate receptor antibody; VGKC, voltage gated potassium channel.

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Table 1. Human histopathology studies of autoimmune encephalitis, NORSE, FIRES and Rasmussen encephalitis

Condition (N, sample size)	Histopathology				Ref
	Adaptive – Humoral	Adaptive - Cellular	Innate	Neuronal Injury	
Anti-NMDAR AE (N=29)	• IgG deposition in the hippocampus and amygdala, basal forebrain, basal ganglia, spinal cord • perivascular B lymphocytes and plasma cells present	• scant perivascular T lymphocytic cuffing – very few (1%) showing markers of activation (e.g. grB, perforin) • rare parenchymal lymphocytic infiltrate	• microglial proliferation and activation especially in the hippocampus, basal forebrain, basal ganglia, spinal cord (similar distribution to IgG deposition) • no complement deposition	• minimal neuronal loss • less commonly, loss of hippocampal pyramidal cells	18, 33, 62, 63
Anti-LGI1/VGKCc AE (N = 6)	• IgG deposition on neuronal cell surface or diffuse background staining in 1[174] • Perivascular B lymphocytes (mild)	• Minimal focal perivascular T lymphocyte cuffing • Variable, mild, parenchymal infiltration (limbic system – cingulate, hippocampus, amygdala; and midbrain) • GrB positive T cells present; not adjacent to neurons; no evidence of grB release (likely insignificant)	• Variable microglial proliferation • Macrophages in hippocampus and amygdala • Complement deposition on neurons co-localised with markers of neuronal death (TUNEL assay reactivity)	• Significant neuronal cell loss with reactive astrocytes in hippocampus and amygdala	18, 174-176
Anti-$GABA_BR$ AE (N=1)	• Perivascular B lymphocytes	• Parenchymal and perivascular CD4+ and CD8+ T lymphocytic infiltrates	• Activated microglia and astrocytes in the parenchyma	• Hippocampal pyramidal	95

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				neuronal loss and necrosis	
Paraneoplastic limbic encephalitis: anti-hu, anti-ta, anti-ma or antibody negative (N=19)	• No surface IgG deposition • Diffuse cytoplasmic staining of IgG	• T lymphocyte parenchymal and perivascular infiltrate • GrB positive T lymphocytes in apposition to neurons with GrB mediated neuronal cytotoxicity suggested by the presence of CD107a co-staining (marker of GrB release) • High CD8:CD3 ratio (cytotoxic T lymphocytes) compared with surface antigen group	• Microglial proliferation • No complement deposition	• Significant gliosis and neuronal cell loss	18, 36
Cryptogenic NORSE (N=8)	• Not reported	• Mostly no cellular infiltrate • "inflammation" in 2/8[138, 177]	• Microglial proliferation and activation	• Generalised patchy neuronal cell loss and reactive gliosis	138, 146, 147, 177
FIRES/AERRPS (N=22)	• Not reported	• No cellular infiltrate in majority • 1 patient – leptomeningeal infiltrate (unclear cell type)[5] • 1 patient – mild perivascular CD8+ T lymphocyte infiltrate[148]	• 1 patient – pericapillary neutrophilic infiltration; perivascular microglia around areas of spongiosis[143]	• Gliosis and neuronal loss (e.g. bilateral hippocampi)	5, 133, 143, 144, 148

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			• Elevated neopterin (non-specific marker of immune activation) • 1 patient - decreased NK cell activity[148]		
Rasmussen Encephalitis (N=71)	• Meningeal and perivascular cuffing of CD20+ B lymphocytes (not in brain parenchyma)	• Clonally expanded, CNS restricted CD8+ > CD4+ (Th1) lymphocytic parenchyma infiltrate in a multifocal distribution • GrB positive T lymphocyte in apposition to neurons	• Microglial activation and microglial nodule formation • Diffuse staining for IgG, albumin, complement (C3/C4) with absent staining for C9neo antigen suggesting that deposition of IgG and complement is due to BBB leak rather than pathological	• Astrocytic reaction • Extensive cortical, white and deep grey matter neuronal injury and loss restricted to 1 hemisphere	131, 158, 160, 178

Location of pathological changes is biased by selective biopsy/review of certain CNS anatomical regions and small sample sizes. NORSE pathology is likely influenced by selection bias given patients with identified cause (e.g. autoimmune or infectious) or response to therapy are unlikely to undergo biopsy. Abbreviations: AE, autoimmune encephalitis; AERRPS, acute encephalitis with refractory, repetitive partial seizures; BBB, blood brain barrier; GrB, granzyme B; IgG, immunoglobulin G; FIRES, febrile infection related epilepsy syndrome; $GABA_BR$, gamma aminobutyric acid B receptor; LGI1, leucine-rich glioma-inactivated 1; NMDAR, N-methyl-D-aspartate receptor; NORSE, new onset refractory status epilepticus; VGKCc, voltage gated potassium channel complex.

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Table 2: Cytokines and chemokines from studies of autoimmune encephalitis, NORSE, FIRES and Rasmussen Encephalitis

Condition (Control)	Sample – Laboratory Methods (N) references	Cytokine and Chemokine Profiles																								
		Adaptive - Humoral		Adaptive - Cellular													Adaptive – mixed		Innate								
		CXCL 13	BAFF	T cell	Th1		Th2			Th17		Treg			T cell chemok.		B / T cell	Lc chemok.	Anti-inflam.	Pro-inflam.				Neutrophil		Monocyte	NK cell
				IL-2	IFN-γ	IL-12	IL-4	IL-5	IL-13	IL-17	IL-23	IL-10	TGF-β	CXCL 10			IL-7	CXCL 12	IL-1RA	IL-1β	IL-6	TNF-α	*NLRP1/3 & Casp1 & IL-18**	CXCL8	CCL3	CCL2/4	IL-15
Anti-NMDAR AE (NIND)	Serum – ELISA, multiplex bead assay (N = 64) 111, 112	↔	↔	↔	↔	◦	↔	◦	◦	↔	◦	◦	◦	↔			↔	◦	◦	◦	◦	↔	◦	↔	◦	↔	↔
	CSF – ELISA, multiplex bead assay (N= 314) 111, 112, 117	↑	↔	↔	↑	◦	↔	◦	◦	↑	◦	◦	◦	↑			↑	◦	◦	◦	↑	↑	◦	↔	◦	↔	↑

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Anti-LGI1 AE (NIND)	Serum – ELISA (N=10) [110]	↑	↔	↔	◦	◦	◦	◦	◦	↔	◦	↔	◦	◦	◦	↔	◦	◦	↔	◦	◦	◦	◦	◦	◦
	CSF – ELISA (N = 16) [110]	↑	↔	↔	◦	◦	◦	◦	◦	↔	◦	↔	◦	◦	◦	↔	◦	◦	↔	◦	◦	◦	◦	◦	◦
Mixed AE – anti-Hu, anti-Yo, anti-NMDAR AE, anti-LGI1 AE (HC, NMOSD)	Serum – ELISA (N=19) [113]	◦	◦	◦	↑	↑	↔	◦	◦	↑#	↑#	↔	↔	◦	◦	◦	◦	◦	◦	◦	◦	◦	◦	◦	◦	◦
NORSE (NIND)	Serum (N=7) [128]	◦	◦	↑	◦	↑	↔	↑	◦	◦	◦	↔	◦	◦	◦	◦	◦	↑	↑↑	↑	◦	◦	◦	◦	◦
	CSF (N=7) [128]	◦	◦	↑	◦	↔	↑	↑	◦	◦	◦	↑	◦	◦	◦	◦	◦	↔	↑↑	↑	◦	◦	◦	◦	◦
FIRES/ AERRPS (NIND, HC, IND)	Serum – ELISA, multiplex bead/suspension assay (N=23) [148, 150-152]	◦	◦	↑/↔	↑	↔	↔	◦	◦	↔	◦	↑/↔	◦	↑	◦	↔	↑	↑/↔	↑	↑	◦	↑	◦	◦	◦

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	CSF - ELISA, multiplex bead assay (N = 43) [143, 148-152]	◦	◦	↔	↑/↔	↔	↔	◦	◦	↔	◦	↑/↔	◦	↑	◦	↔	↑↑	↑/↔	↑↑	↑/↔	◦	↑	↑	↑	◦
Rasmussen Encephalitis (NND [179])	Homogenised cortex from surgical specimen – ELISA (N=1) [179]	◦	◦	◦	◦	◦	◦	◦	◦	◦	◦	◦	◦	◦	◦	◦	◦	◦	↑	◦	◦	◦	◦	↑	◦
	Microglia, lc, astrocytes isolated from tissue – expanded and stimulated – RT-PCR*/ ELISA (N=7) [159, 178]	◦	◦	◦	↑	◦	◦	↑/↔	↑/↔	↔	◦	◦	◦	◦	◦	◦	◦	↑*	◦	↑*	↑*	◦	◦	◦	◦

While cytokines are organised into groups based on the main arm of the immune system they act within, this is an oversimplification and cytokine actions often extend beyond one arm of the immune system. A notable limitation to this data is the generally small sample sizes as well as the variability in the techniques used between studies and the selection of the cytokines examined. Key: *RT-PCR used to quantify mRNA transcripts for these molecules; ↑ elevated compared to control; ↑↑ greatly elevated compared to control; ↔ same as control; ↑/↔ variably elevated relative to controls depending on the study; ◦ no data available; # elevated in anti-NMDAR AE and anti-LGI1 AE compared with AE with intracellular antigens and controls. Abbreviations: AE, autoimmune encephalitis; BAFF, B cell activating factor; chemok, chemokine; CCL, C-C motif chemokine ligand; CSF, cerebrospinal fluid; CXCL, C-X-C motif chemokine ligand; ELISA, enzyme linked immunosorbent assay; HC, healthy controls; IFN-γ, interferon gamma; IL, interleukin; IL-1RA, interleukin 1 receptor antagonist; IND, inflammatory neurological disease; lc, lymphocyte; LGI-1, leucine-rich glioma-inactivated 1; NIND, non-inflammatory neurological disease; NK, natural killer cell; NMDAR, N-methyl-D-aspartate receptor; NMOSD, neuromyelitis optica spectrum disorder; NND, non-neurological disease; RT-PCR, real-time polymerase chain reaction; TGF-β, transforming growth factor beta; TNF-α, tumour necrosis factor alpha.

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Conflict of interest

Piero Perucca has received support from the National Health and Medical Research Council (APP1163708), the Epilepsy Foundation, the Royal Australasian College of Physicians, and Melbourne Health. His institution has received speaker honoraria or consultancy fees from Eisai, UCB Pharma, Sun Pharma, Novartis, and Supernus. Terence J O'Brien has received support from the National Health and Medical Research Council, The National Instute of Neurological Disorders and Stroke, and Monash University. He has been supported by research grants and consulancies to his institution from Eisai, UCB Pharma, Praxis Precision Medicines, BioGen, and Supernus. Patrick Kwan has received support from the Medical Research Future Fund Fellowship (MRF1136427). His institution has received research grants from Biscayne Pharmaceuticals, Eisai, GW Pharmaceuticals, LivaNova, Novartis, UCB Pharma, and Zynerba outside the submitted work; he has received speaker fees from Eisai, LivaNova, and UCB Pharma outside the submitted. Mastura Monif has received funding from National Medical Research Council Medical Research Future Fund (MRFF). Her institution also has received funding from Brain Foundation, Charles and Sylvia Viertel Foundation, and Merck Industry funding. The remaining author has no conflicts of interest.
We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Author contributions

THT searched the literature and drafted the manuscript, tables and figures. MM, PP, TO and PK commented on and edited the text, including suggestions for additional sections and references.

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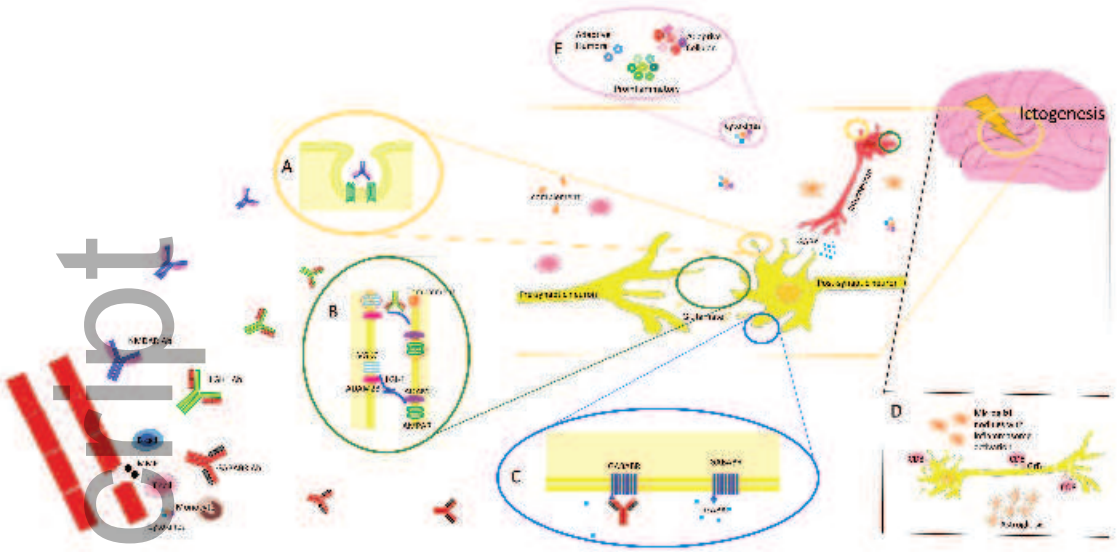

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