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Novel pathogenic antibodies give insight into the role of GABA_A receptors in the central nervous system

Article being reviewed: Ohkawa T, Satake S, Yokoi N, Miyazaki Y, Ohshita T, Sobue G, Takashima H, Watanabe O, Fukata Y, Fukata M. Identification and characterization of GABAA receptor autoantibodies in autoimmune encephalitis. *J Neurosci*. 2014;34:8151-63.

Summary

- Antibodies against the $\beta 3$ subunit of GABAA receptors identified in patients with thymomas.
- Spectrum of autoimmune encephalitides extended with discovery of pathogenic antibodies to inhibitory channel.
- Identification of antibodies involved a comprehensive characterisation of pathogenicity.
- Clinically, improvement is observed but coincides with multiple interventions and does not directly address whether this may be due to a depression in autoantibody titres.
- Binding of antibodies may alter network excitability, as inhibitory neurotransmission is likely to be impaired.

Over the past decades, our understanding of the interactions between the immune system and the brain has been challenged. Research showed that antibodies against central nervous structures can be produced by our own immune system often without an identifiable cause. This can lead to loss of the target antigen and inflammation of brain tissue. These autoantibody-mediated conditions are collectively referred to by the term autoimmune encephalitides. Patients typically present with subacute onset of memory loss, psychiatric disturbance, confusion, seizures, and in some cases abnormal movements. The targets of these pathogenic autoantibodies have been identified as receptors or ion channel-associated proteins expressed in the central nervous system (CNS) – the N-methyl-D-aspartate (NMDA) receptors and the voltage-gated potassium channel (VGKC) complex proteins are the most commonly identified autoantibody targets. Whilst initially considered a purely paraneoplastic phenomenon associated with tumours outside of the CNS,¹ autoantibodies against CNS antigens were shown to be present in patients without an underlying, or diagnosed, neoplasm.²

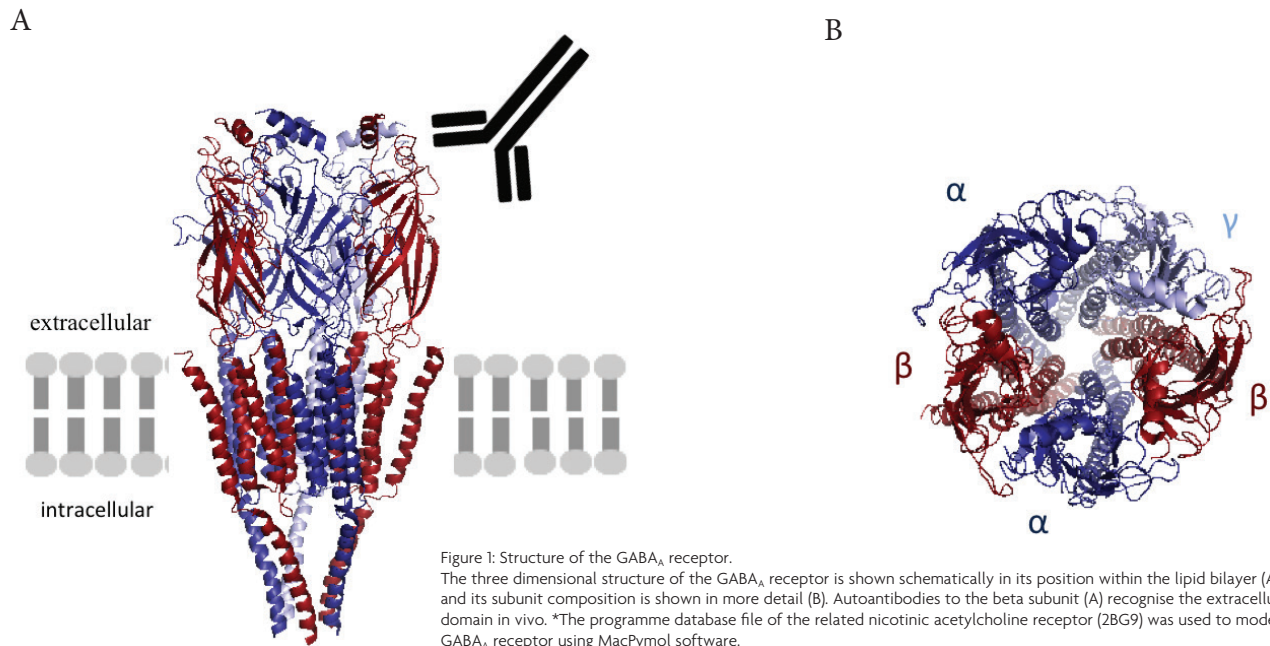
Whether the autoantibodies are pathogenic per se, or whether they are merely a marker coinciding with a separate disease process are questions that

have been the focus of study. Antibodies mediate their pathogenicity in several ways but the most common mechanisms are internalisation of their antigenic target, activation of the lytic complement cascade or directly interference with ion channel function (discussed in Vincent et al.³ in more detail). Irrespective of their pathogenic mechanism, the first step always involves binding of the antibody to the antigen: an important paradigm, therefore, is that autoantibodies against cell surface receptors are more likely to be pathogenic than autoantibodies against intracellular antigenic targets.

To determine an autoantibody's pathogenicity, Koch's postulates on infectious diseases were modified to apply to autoimmune conditions.⁴ Autoantibody-mediated pathogenicity can be assumed in cases where (1) an antibody-mediated immune response is present and (2) the antigen has been identified. Furthermore the postulates require that the disease be induced experimentally, both in a (3) passive transfer and (4) an active immunisation model.

Earlier this year, Petit-Pedrol et al⁵ identified autoantibodies to the γ -Aminobutyric acid (GABA)_A receptors in patients with encephalitis who presented with intractable seizures or status epilepticus with no tumours. The antibodies were shown to bind to the $\alpha 1$ or $\beta 3$ subunits. Antibodies to the GABA_A $\alpha 1$ and $\gamma 2$ subunits have also been found in a proportion of patients referred for NMDA receptor antibody testing (Pettingill et al, submitted). GABA_A receptors are postsynaptic GABA-gated pentameric channels made up from 2α , 2β and 1γ subunits surrounding a central ion-selective chloride channel. Their main function is to depress neuronal excitability.^{6,7}

The paper by Ohkawa et al⁸ identified novel autoantibodies to the $\beta 3$ subunit of the GABA receptor in two patients who presented with clinical manifestations of confusion, personality changes, memory loss, and seizures and examined in more detail the possible pathogenic mechanisms. Both patients had invasive cancers of their thymus, which required surgical excision and radiotherapy. They were identified from a cohort of over 100 patients with suspected autoimmune pathology of the CNS by screening patient sera binding to primary hippocampal cultures. The



identity of the antigenic target was examined by using a combination of immunoprecipitation and mass spectrometry. Expression of individual GABA_A demonstrated that the autoantibodies bound an extracellular epitope on the $\beta 3$ subunit. The autoantibodies did not bind other GABA_A receptor subunits, though evidence of cell surface expression of individual subunits was not provided. However, a $\beta 3$ -subunit-specific knockdown experiment confirmed that the autoantibodies no longer bound the hippocampal neuron surface when the $\beta 3$ subunit was removed from the channel complex. The autoantibodies downregulated surface GABA_A receptors over 48 hours in neuronal cultures, consistent with the internalisation mechanisms; the reduction of cell surface ion channels was not mediated by the complement pathway. Additionally, the reduction in cell surface GABA_A receptor levels was also matched by a depression in electrophysiological activity. These autoantibody-mediated effects were specific to patient serum obtained during the manifestation of CNS symptoms; archived serum from one of the patients predating the encephalitis did not affect GABA_A receptor numbers or electrophysiological recordings.

Clinically, the distinction between paraneoplastic and non-paraneoplastic autoantibodies may aid the treatment decision: a sustained immune response raised against the neoplasm can be limited by excision of the tumour, whereas non-paraneoplastic autoantibodies can only be targeted by immunosuppressive therapy. Steroids, plasma exchange and intravenous immunoglobulins are often used as first step immunosuppressants, and more aggressive treatment approaches have been used for resistant or relapsing patients.⁹ No large studies have

been performed to date to compare treatment strategies.

Clinical improvement of one of the patients was seen after administration of immunosuppressive therapy (corticosteroids and intravenous immunoglobulins) combined with anti-epileptic drugs. The patient became seizure-free, though cognitive and psychological symptoms persisted. Autoantibody levels were quantified using a cell-based enzyme-linked immunosorbent assay (ELISA) prior to immunotherapy only and it remains unclear whether a depression in autoantibody titres following therapy may have coincided with the alleviation of symptoms. Patient two was treated with chemotherapy alone, and whether the improvement was due to a treatment-related immunosuppression or a reduction in tumour load affecting (paraneoplastic) autoantibody levels remains also unclear. As both patients had invasive thymomas, a paraneoplastic phenomenon may have been likely. Therefore, histological analysis showing the potential expression of GABA_A receptor subunit within the tumour tissue would have been useful. The use of a semi-quantitative approach to measure autoantibody levels with cell-surface ELISA or similar methods, would have also allowed the investigation of the temporal relationship between clinical status and autoantibody levels more closely.

The presence of VGKC-complex autoantibodies in both patient sera further complicates the conclusion as to whether anti- $\beta 3$ GABA_A receptor autoantibodies are specifically responsible for the clinical features. It is also possible that the full spectrum of anti-VGKC-complex associated antibodies has not been identified as yet. Screening of larger patient cohorts with similar CNS features might be helpful in future to address

whether GABA_A receptor autoantibodies are solely linked to invasive thymomas and whether the co-existence of VGKC-complex autoantibodies is typical for this patient group. This detailed characterisation of the GABA_A receptor autoantibody emphasises the importance for the continued screening for novel CNS antigens in patients with encephalitis-like symptoms.

Antibody-mediated pathology was once thought to be rare but since the discovery of autoantibodies against the NMDA receptors,¹ at least thirteen types of autoimmune encephalitis have been described in a rapidly expanding clinical field. Pathogenic antibodies against subunits of inhibitory receptors described to date have included those against the Gly α R1 subunit of the glycine (Gly) receptors,¹⁰ and those against the B1 subunit of the GABA_B receptors.¹¹

Pathogenic antibody binding to synaptic cell surface or structural proteins of inhibitory channels is likely to interfere with inhibitory neurotransmission in the CNS. This would be supported by the cessation of seizures, a possible surrogate of hyperexcitability, when immunotherapy suppresses autoantibody titres. A close study of correlation of autoantibody levels in serum and cerebrospinal fluid and their temporal relationship to symptoms is thus important. Autoantibodies have been linked with hyperexcitability in the case of the VGKC-complexes,^{12,13} GABA_B,¹¹ and Gly receptors.¹⁰ 30-40% of neurons in the CNS use GABA as their neurotransmitter and inhibitory effects are predominantly mediated via the GABA_A receptors. Okhawa et al⁸ demonstrated that autoantibody levels depressed inhibitory currents of surface GABA_A receptors but did not have any effects on excitatory currents mediated by AMPA receptors. It is likely that a prolonged exposure (>24 hrs) to

the antibodies may have altered excitatory neurotransmission as this could mimic better in vivo conditions. Local pathological inflammation may also contribute to excitability in vivo. A localised immune response with the subsequent release of cytokines and a possible element of complement activation might further impact onto local neuronal signalling pathways. Activated microglia and reactive astrocytes may alter the balance between excitation and inhibition in the milieu and may affect neuronal wiring through the formation of a glial scar.¹⁴

Blood-brain barrier integrity could be affected through cytokine-activated receptors on endothelial cells, leading to a further recruitment of immune cells to the CNS. Thus, the effects mediated by pathogenic antibodies against inhibitory channels calls for the need to develop comprehensive in vivo human studies and animal models to determine autoantibody-mediated pathogenicity on a molecular, network and more global level. Understanding how autoantibodies can cause specific symptoms would help us understand not only disease but also brain function.² Clinicians should be guided by the neuropsychiatric symptoms to identify whether an autoimmune cause should be ruled out mainly because the immunotherapy provides clinical improvements. The findings of Ohkawa et al⁸ extend the clinical spectrum of autoimmune encephalitis to include the GABA_A receptors and strongly suggest that future research should focus on further screening of larger patient cohorts to elucidate the downstream effects of autoantibody binding to postsynaptic receptors.

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Strategic Clinical Network for Mental Health, Dementia, Neurological Conditions, Learning Disability and Autism (MHDNL) Strategic Clinical Network (SCN) East of England Epilepsy Event

Conference details: 26 June, 2014; Stansted, UK. **Report by:** Juliet Ashton, Sapphire Nurse Consultant - Epilepsy Commissioning, Epilepsy Society and Victoria Doyle, Quality Improvement lead for Neurological Conditions, Coproduction and Dementia, East of England Strategic Clinical Network, NHS England.

Each of the twelve Strategic Clinical Networks (SCNs) have been tasked to improve services for people with neurological conditions. Services for people with epilepsy has been identified as a priority by all of the SCNs which is reinforced by the decision of the Royal College of GPs to highlight epilepsy as one of its clinical priorities for 2013 – 16.

The East of England SCN certainly lived up to its shared values of “Creating a shared purpose for transformational change” and “Putting patients, clinicians and carers at the heart of decision making” at the Epilepsy Event on June 26th. Over 100 delegates attended the event made up of service users, families, carers, Voluntary sector organisations (Epilepsy Action and Epilepsy Society), The National Development Team for Inclusion, Epilepsy Specialist nurses, Learning Disability nurses, Health Facilitator teams, Neurologists, Commissioners, Social care staff and GPs, to name but a few. The aim was to gather information from all the delegates across the network, sharing examples of good practice, coming together in workshops to identify what people with epilepsy want from their services and what matters most to them. This is the first event I have attended where there has been such a strong voice from the people with epilepsy: I believe the points raised will get listened to by the SCN and taken forward into positive actions.

The day commenced with an introduction and welcome from the morning chair, Dr Max Damian, SCN Clinical Lead, explaining one of the rationale for SCNs was ‘To improve quality and outcomes through connecting services and efficiently using resources’. Dr Damian described the objectives for the day as: bringing together key interest groups involved in epilepsy care and highlighting their perspectives; to outline the elements of a pathway towards a better, more equitable service across the East of England and to identify how we can ensure strong collaboration between those involved in improving services.

This was followed by Dr David Bateman, National Clinical Director for Neurology, who discussed commissioning a better epilepsy service and highlighted the head line figures for the East,

- 16000 people with epilepsy in the East of England 1000 people of which admitted with an unplanned admission (6.25%)
- £400,000 cost minimum?
- 17% are managed by neurologist
- Total bed days per year 3760

What is needed to address these figures are local services, an accurate initial diagnosis, good initial advice and support and appropriate long term care. Dr Bateman, demonstrated the new Public Health England Neurology Intelligence Network <http://www.yhpho.org.uk/mhdnl>, which provides indicators about risk factors, prevalence, access to services, outcomes and finance, and includes profiling tools in particular for epilepsy.

Dr Tejal Mitchell, Neurologist in Peterborough, gave an East of England and National perspective of the second National Audit