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


Summary

- Antibodies against the β3 subunit of GABA_A 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 the 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, some autoantibodies against CNS antigens were shown to present in patients without an underlying, or diagnosed, neoplasm.

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 antigen target, activation of the lytic complement cascade or directly interference with ion channel function (discussed in Vincent et al for 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. 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 al 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 α1 or β3 subunits. Antibodies to the GABA_A α1 and γ2 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 α, β and γ subunits surrounding a central ion-selective chloride channel. Their main function is to depress neuronal excitability.

The paper by Ohkawa et al identified novel autoantibodies to the β3 subunit of the GABA_A 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 GABAA demonstrated that the autoantibodies bound an extracellular epitope on the β3 subunit. The autoantibodies did not bind other GABAA receptor subunits, though evidence of cell surface expression of individual subunits was not provided. However, a β3-subunit-specific knockdown experiment confirmed that the autoantibodies no longer bound the hippocampal neuron surface when the β3 subunit was removed from the channel complex. The autoantibodies downregulated surface GABAA 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 GABAA 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 GABAA receptor subunits 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.6 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 GABAA receptor subunit within the tumour tissue would have been useful. The use of a semi-quantitative approach to measure autoantibody levels with cellsurface 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-β3 GABAA 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 GABAA 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 GABAA 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,7 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 GlyR1 subunit of the glycine (Gly) receptors,8 9 and those against the BI subunit of the GABAA receptors.10 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,11,12 GABAA13 and Gly receptors.14 30-40% of neurons in the CNS use GABA as their neurotransmitter and inhibitory effects are predominantly mediated via the GABAA receptor. Okhawa et al15 demonstrated that autoantibody levels depressed inhibitory currents of surface GABAA receptors but did not have any effects on excitatory currents mediated by AMPA receptors. It is likely that a prolonged exposure (>24 hrs) to 

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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.

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. 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 Okhawa et al extend the clinical spectrum of autoimmune encephalitides 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.

REFERENCES


