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SCIENTIFIC COMMENTARIES

'Moonlighting' surface antigens: a paradigm for autoantibody pathogenicity in neurology?

This scientific commentary refers to 'Human autoantibodies to amphiphysin induce defective presynaptic vesicle dynamics and composition' by Werner *et al.* (doi:10.1093/awv324).

The field of CNS autoantibody-mediated diseases has provided a new source of potentially treatable neurological conditions, with pathophysiology that is of increasing interest to neurologists, immunologists and neuroscientists (Irani *et al.*, 2014). These syndromes can feature seizures, cognitive impairment, movement disorders, dysautonomia, rigidity or startle. Most of the more recently discovered autoantibodies target the extracellular domain of natively-expressed neuronal surface proteins, and have the potential to be pathogenic if they gain access to their antigens *in vivo*. These syndromes are often treatable and only a minority are associated with malignant tumours (Leypoldt *et al.*, 2015; Varley *et al.*, 2015). By contrast, the more established paraneoplastic antigens, such as Hu, Yo and Ma2, are associated with malignant tumours and a poor response to immunotherapies. These antibodies are directed to intracellular proteins and are not thought to be pathogenic. Concordantly, antibody passive transfer experiments have yielded negative results (Tanaka *et al.*, 1995).

This is one reason why the pathogenesis of the autoantibody-associated disease stiff-person syndrome (SPS) remains enigmatic. The most common antibodies in SPS target the intracellular enzyme glutamic acid decarboxylase (GAD). However, the possible role of GAD antibodies in disease causation has been somewhat superseded by the discovery that GAD antibodies coexist with neuronal surface antibodies with causative potential (Lancaster *et al.*, 2010; Chang *et al.*, 2013). Antibodies against amphiphysin are found in $<10\%$ of patients with SPS; these individuals are usually female and often have a breast or small-cell lung carcinoma. Unlike many forms of paraneoplastic disease associated with malignant tumours, a few reports suggest this form of SPS responds to immunotherapies, including plasma exchange, with the response correlating well with amphiphysin-antibody levels (Wessig *et al.*, 2003; Sommer *et al.*, 2005).

The SH3-domain of amphiphysin appears to be the immunodominant region and amphiphysin-antibodies bind well on western blots, suggesting a linearized rather than a native conformationally-dependent epitope. Although there are few histopathology reports on this rare disease, the available data do not lend support to a B-cell or antibody-mediated disorder but have shown a marked predominance of CD8 and CD4 T-cells (Wessig *et al.*, 2003). Therefore, evidence argues both for and against the pathogenicity of amphiphysin-antibodies.

A series of elegant experiments from Geis, Sommer and colleagues in previous publications appeared to clinch the issue. Contrary to conventional wisdom, they showed that amphiphysin-antibodies fulfil Witebsky's postulates for antibody causality. They showed that an amphiphysin-SH3 domain-specific population of immunoglobulin G (IgG) was internalized by rat spinal motor neuron–interneuron co-cultures. The antigen specificity was confirmed by the absence of this effect in amphiphysin-knockout mice. GABAergic synapses appeared especially vulnerable (Geis *et al.*, 2010). Furthermore, systemic and intrathecal injection of amphiphysin-antibody SPS IgG into experimental rodents produced a phenotype that closely modelled symptoms in patients: mice developed truncal and hindlimb cramps, an exaggerated lordosis, and bursts of EMG activity (Sommer *et al.*, 2005; Geis *et al.*, 2010). Remarkably, the spasms began only 15–30 min after systemic high-titre amphiphysin IgG injections. The rat spinal cords showed human IgG deposits, consistent with the likely site of pathology. In a separate experiment, IgG from one patient with SPS was shown to bind to the amygdala and attenuate exploratory behaviours, an effect akin to the anxiety seen in SPS (Geis *et al.*, 2012). While behavioural and histological analyses were consistent with amphiphysin-antibody pathogenicity, the molecular basis of these observations remained unclear.

Amphiphysin is known to regulate clathrin-coated vesicle-associated endocytosis, a major mechanism by which synaptic vesicles are recycled

Glossary

Amphiphysin: Amphiphysin belongs to the BAR (Bin-Amphiphysin-Rvsp) family of proteins and is associated with the cytoplasmic surface of endocytotic vesicles. Amphiphysin is involved in clathrin-mediated endocytosis via its interaction with cytoskeletal proteins such as dynamin.
Witebsky's postulates for antibody causality: Ernest Witebsky proposed criteria to determine whether a disease could be termed autoimmune. These include the detection of the antibodies in cases, recognition of the autoantigen, and the demonstration that an experimental animal develops similar changes to affected humans. More recently, the criteria have been slightly modified by others to include circumstantial clinical clues (such as tight correlations between antibody levels and clinical features).

after depolarization-mediated fusion. In this issue of *Brain*, Werner and co-workers report the results of detailed experiments examining the effects of amphiphysin-antibodies on the composition of presynaptic vesicular pools and the implications for neurotransmission (Werner *et al.*, 2016).

Ultrastructural analyses revealed that *in vivo* supramaximal sciatic nerve stimulation caused increases in synaptic density, vesicle pool size, and clathrin-coated vesicle numbers in spinal cord boutons. This may serve to sustain responses to prolonged high-frequency stimulation. Chronic intrathecal application of amphiphysin-specific IgG reversed these effects in rats, which also developed clinical features of SPS. The most marked reduction in vesicles was observed in the presynapses with the highest GABA density. However, the effect was dependent on which vesicle population was under scrutiny; there was a decrease in the resting pool (characterized by synaptobrevin 7) and an increase in the readily releasable pool (expressing synaptobrevin 2). This suggests both depletion of resting vesicles, and impaired endocytosis of other vesicles at the plasma membrane after fusion. In addition, there were alterations in the distribution of endophilin, a protein known to interact with amphiphysin.

Taken together, these findings provide a cogent potential molecular mechanism for the pathogenesis of amphiphysin-antibody mediated SPS. Amphiphysin antibodies appear to disrupt vesicular recycling leading to slower endocytosis, more rapid synaptic exhaustion and a failure of exocytotic synaptic transmission. This mechanism has a predilection for GABAergic interneurons, partly because these neurons often show a high turnover of vesicles. In turn, this may lead to increased motor unit firing with consequent stiffness and spasms, and may account for the recognized therapeutic benzodiazepine response.

This well-designed series of experiments shows several potential limitations. First, the number of patient samples was very small. This calls into question the generalizability of these findings to other patients with amphiphysin antibodies. Second, some patients with amphiphysin antibodies have been reported to have ataxia, dysautonomia and cerebellar features, findings not accounted for by these observations (Pittock *et al.*, 2005). Finally, the presence of amphiphysin antibodies in up to 2% of healthy and disease controls suggests the antibody alone may be insufficient for symptom causation in many adults (Dahm *et al.*, 2014).

Nevertheless, in the samples studied it is intriguing to consider how and where the precise antibody–antigen interaction might take place. It may be that presynaptic boutons have a relatively indiscriminate mechanism of antibody uptake, and any antibodies that survive an intracellular environment can then access their targets. Alternatively, amphiphysin may gain access to the extracellular environment upon fusion of vesicles with the presynaptic membrane. This may give the autoantibodies a brief window of opportunity in which to interact specifically with their transiently-exposed antigenic target. Indeed, the site of antibody–antigen interaction would benefit from more detailed visualization in future experiments and could be a site for therapeutic interventions. While the concept of a transiently-exposed antigen is less likely to translate to the RNA-binding proteins Hu, Yo and Ri, it may be a mechanism by which GAD-antibodies mediate a similar antigen-specific effect (Chang *et al.*, 2013; Hansen *et al.*, 2013). Indeed, 'transient moonlighting' of such antigens to the surface of the synaptic cleft may be a paradigm that allows other intracellular antigens to be targeted by autoantibodies.

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A fatty acid in the MCT ketogenic diet for epilepsy treatment blocks AMPA receptors

This scientific commentary refers to 'Seizure control by decanoic acid through direct AMPA receptor inhibition', by Chang *et al.* (doi:10.1093/brain/awv325).

Dietary therapies can provide control of seizures in patients with drug-refractory epilepsy. There are several types of dietary therapies, all of which are high in fat, restrict carbohydrates to some extent, and are associated with ketosis. In the classical ketogenic diet introduced into clinical practice in the 1920s, the fat component is provided by long-chain triglycerides (LCTs). Recognizing that medium chain triglycerides (MCTs) are more ketogenic than LCTs, Huttenlocher *et al.* (1971) created the MCT oil diet, which permits greater amounts of carbohydrate and protein, and therefore allows a more flexible meal plan. The efficacy of the MCT oil ketogenic diet is equivalent to that of the classic LCT ketogenic diet, and the tolerability is also comparable (Neal *et al.*, 2009). MCTs, which are abundant in coconut and palm kernel oil, have a glycerol backbone and three fatty acid esters with 6 to 12 carbons arranged in a straight chain. The major fatty acids in MCT oil are *n*-octanoic acid (C8; caprylic acid; 50–80%) and *n*-decanoic acid (C10; capric acid; 20–50%). Following ingestion, MCTs are rapidly absorbed into the portal circulation to the liver, in contrast to LCTs which are absorbed by chylomicrons in the lymph. It was previously believed that medium chain fatty acids derived from MCTs are immediately oxidized in the liver to form ketone bodies, but it is now known that appreciable amounts appear in the circulation of patients receiving the MCT diet (Sills *et al.*, 1986*a*). For example, children on the MCT diet had plasma concentrations of decanoic acid in the range of 0.1–0.2 mM. Decanoic acid readily crosses the blood–brain barrier, probably by a combination of diffusion and saturable carrier-mediated transport via a medium-chain fatty acid transporter that also mediates brain uptake of the antiseizure drug valproic acid, itself a medium chain fatty acid (Adkison and Shen, 1996). In this issue of *Brain*, Chang *et al.* show for the first time that decanoic acid is an antagonist of AMPA receptors, providing a possible basis for the antiseizure activity of the MCT diet (Chang *et al.*, 2016).

A number of theories have been advanced to explain the antiseizure activity of high fat ketogenic diets but none has as yet received broad acceptance (Hartman *et al.*, 2007). Early on, it was proposed that ketone bodies are responsible for seizure protection and indeed all of the ketone bodies (acetone, β-hydroxybutyrate and acetoacetate) have been reported to have antiseizure effects in one or another animal model. However, as confirmed by Chang *et al.* the ketones are not generally active in *in vitro* seizure models. Moreover, the activity profile of ketogenic diets in animal models does not correspond with that of the ketones and the degree of ketosis does not correlate with the extent of seizure protection. Other investigators have concluded that the diets enhance GABA synthesis, correcting deficits in inhibitory neurotransmission. More recent studies have implicated increased production of brain bioenergetic substrates such as ATP, creatine and phosphocreatine and increased mitochondrial energy metabolism, which is postulated to