Models of autoantibody mediated diseases: Actively nearing the human gold standard

Human autoantibody-mediated diseases associated with a single autoantigenic target provide several valuable opportunities for precision medicine.[1] The implied premise is that mapping of the immune response exclusively against this one antigen is both sufficient and necessary to capture the entirety of the disease neuroimmunobiology. One such autoantigen is the N-methyl-D-aspartate receptor (NMDAR), and autoantibodies directed against the NMDAR are considered causative in patients with NMDAR-antibody encephalitis (NMDAR-Ab-E). This has been shown in passive transfer experiments where infusion of the autoantibodies alone, either using directly patient derived polyclonal or more molecularly precise monoclonal formats, has induced behavioral, molecular, and electrophysiological features which reproduce key aspects of the patient's disease.[2, 3] However, such "passive" autoantibody administration alone fails to capture key elements of the underlying human neuroimmunobiology, including innate and adaptive immune cells, and associated soluble mediators, such as cytokines.

These key components may both assist in higher fidelity disease modelling, whilst also providing platforms to test therapeutics. Indeed, B cell depleting agents, a proven mechanism of drugs which appear efficacious in NMDAR-Ab-E patients, cannot be tested in passive transfer models. To recapitulate pathologically-relevant pathways of the immune system in experimental animals, active immunization models have been developed, using either peptides from the ubiquitously expressed GluN1 subunit of the NMDAR, or GluN1/2B holoreceptors.[4, 5] Actively immunised rodents display neuropsychiatric alterations and CNS lymphocyte infiltrations which mimic distinctive aspects of NMDAR-Ab-E patients. However, to date, therapeutics have not been systematically assessed using these models.

In this issue of *Brain*, Maudes et al[5] test therapeutics on their peptide immunized mouse model of NMDAR-Ab-E and draw increasingly close neuroscience and immunology parallels to the 'gold standard' human model. The sufficiency of systemic peptide administration to reproduce neuropsychiatric features, lower seizure thresholds and – for the first time – a complex movement disorder with stereotypies is consistent with the periphery, not CNS, as the priming site in NMDAR-Ab-E. Immunization with a short peptide (using the $GluN1_{356\text{-}385}$ region) rapidly generated NMDAR-reactive antibodies. In neuroimmunology, we often think of peptide reactive autoantibodies as likely non causative agents, with recognition of the conformationally active, native antigen as a critical feature of pathogenic species. Here, around six weeks post-immunization, the authors show that serum and cerebrospinal fluid IgGs bind the GluN1-2B subunits expressed in HEK293T cells, including those without the $GluN1_{356\text{-}385}$ domain, and those in live primary neuron cultures, strongly suggesting they now recognize conformational epitopes.

This phenomenon of ‘intramolecular’ epitope spread suggests initially restricted T-B cell collaborations later facilitate polyclonal recognition of the NMDAR, a phenomenon which could not be observed with a passive immunization model. Future studies could examine whether the T cell dependent pattern of B cell receptor (BCR) mutations might parallel those observed in humans,[3] suggesting a ‘mimicking’ peptide may in fact initiate human NMDAR-Ab-E. Yet, when considering the two main established triggers of NMDAR-Ab-E, teratomas express NMDARs within neuroglial tissue[6] and herpes simplex encephalitis directly affects the brain.[7] Hence, it may be more likely that the human active immunisation is against the endogenous NMDAR. Further, the intriguing observation that NMDAR-Ab-E patients harbour naïve B cells which target the native GluN1 subunit,[8] suggests a ‘ready to prime’ population may await the NMDAR autoantigen in humans, to initiate the immunization process.

Where might T-B cell interactions take place? The recent rediscovery of human meningeal lymphatics draining to deep cervical lymph nodes (DCLNs), provided Maudes et al with a directed hypothesis to go ‘straight for the jugular’. From peptide immunized mice, they showed GluN1-reactive B cells were generated in DCLNs, but not inguinal lymph nodes, which parallels the detection of GluN1-reactive B cells in the DCLNs, and blood, of humans with NMDAR-Ab-E.[6] This also supports an initial loss of peripheral tolerance and is especially intriguing after a systemic, rather than CNS administered, immunisation. The findings offer a valuable opportunity to consider how B cells might be primed in organs with direct access to CNS antigens.

In the CNS, the authors showed that mice brains had reduced NMDAR density (consistent with a direct effect of the autoantibodies) in addition to B cells and plasma cells (akin to human NMDAR-Ab-E brain tissue), albeit with fewer T cells (perhaps suggesting a limited role for CNS T cells). Additionally, they demonstrated striking microglial activation, another pathological feature associated with NMDAR-Ab-E tissue.[9] In addition, they elegantly showed that these microglia appear to ingest the IgG-NMDAR complex, suggesting their role in phagocytosing the antibody-bound NMDARs may directly contribute to NMDAR-Ab-pathogenesis. These findings raise the possibility of microglia representing a novel therapeutic target in autoantibody-mediated diseases.

Throughout, the authors assessed two therapies with very different modes of action. The first was B cell depletion with a CD20 targeting monoclonal, aiming to directly deplete all B cells, including those which carry NMDAR-reactivities. The second was deployment of a positive allosteric modulator of the NMDAR established to prevent and reverse behavioural features associated with the passive transfer model, which likely acts ‘despite the immune response’, preventing the patient antibodies from modulating NMDARs.[10] Both agents were effective in almost all behavioural, histological and electrophysiological assays, providing credence to the

authors' original concept that their model would be valuable for assessing therapeutics which rely on a variety of mechanisms.

In summary, Maudes et al present a mouse model that faithfully recapitulates key phenotypic, electrophysiolgical and histological characteristics of human NMDAR-Ab-E pathology that can confirm findings first observed in humans, assess the value of multimodal therapeutics, and also generate new concepts which can be subsequently evaluated in humans. While they rightly discuss the inevitably imperfect nature of such animal models, this kind of platform provides a very valuable tool in rare diseases with severely ill patients, where sequential clinical trials to evaluate different drugs and timings may simply never prove feasible. It may also be that a fraction of the millions of dollars spent on such trials will be better spent delivering our growing armamentarium of *in vitro* and *in vivo* models to patient benefit.

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Figure legend. Increasingly accurate mouse modelling of human NMDA receptor antibody encephalitis with active immunisation.

Funding. This was funded in whole or in part by a senior clinical fellowship from the Medical Research Council [MR/V007173/1], Wellcome Trust Fellowship [104079/Z/14/Z] and by the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre (BRC). For the purpose of Open Access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript (AAM) version arising from this submission. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health. SRI has received honoraria/research support from UCB, Immunovant, MedImmun, Roche, Janssen, Cerebral therapeutics, ADC therapeutics, Brain, CSL Behring, and ONO Pharma

Conflicts of interest. SRI receives licensed royalties on patent application WO/2010/046716 entitled 'Neurological Autoimmune Disorders', and has filed two other patents entitled “Diagnostic method and therapy” (WO2019211633 and US app 17/051,930; PCT application WO202189788A1) and “Biomarkers” (WO202189788A1, US App 18/279,624; PCT/GB2022/050614).