Using the Mouse to Model Human Diseases: Cognitive Impairment in Systemic Lupus Erythematosus

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**ABSTRACT.** In this 2020 Dunlop-Dottridge Lecture, the authors discuss cognitive impairment (CI), one of the most prevalent neuropsychiatric syndromes in systemic lupus erythematosus (SLE). Patients often report CI as the most bothersome disease-related manifestation, with a great effect on their quality of life. Nevertheless, studies focusing on CI remain scarce and no effective targeted therapy has been identified. We herein present murine models of CI in SLE with insights into the pathogenesis of this condition as well as the role of the renin angiotensin system in microglial activation. We will discuss the role of neuroimaging as a useful objective assessment tool, describing our experience in previous and ongoing clinical trials of CI in patients with SLE. (J Rheumatol 2020;47:1145–9; doi:10.3899/jrheum.200410)

**Key Indexing Terms:** MURINE MODELS COGNITIVE IMPAIRMENT SYSTEMIC LUPUS ERYTHEMATOSUS

Cognitive impairment (CI) is one of the 19 syndromes that constitute neuropsychiatric systemic lupus erythematosus (NPSLE). The term refers to deficits in any cognitive domain such as learning and executive skills, psychomotor function, attention and memory. The prevalence of CI in SLE is highly variable, ranging from 6.6 to 80%. This wide range can be attributed to the variability in assessments and measures between different reports, the lack of a standardized definition, and most of all the difficulty attributing CI uniquely to SLE.

CI tends to develop insidiously over the course of the disease, independent from other SLE clinical manifestations. It has been shown that patients with CI tend to have higher unemployment rates and decreased quality of life (QOL). During a patient-focused drug development meeting in 2017, individuals with SLE selected forgetfulness and lack of concentration as causing substantial harm to their lives. Most survey respondents referred to these symptoms as “brain fog”.

There are multiple barriers to studying CI in SLE including the lack of a standardized objective assessment and screening tools. Confounding factors that make it difficult to attribute CI exclusively to SLE, such as inclusion of patients with focal NPSLE manifestations for studies. Reconciling these obstacles with the wide prevalence of CI and its major effect on the QOL of patients with SLE is a major unmet need.

**Murine Models of CI in NPSLE**

Mouse models of disease can offer insight into pathogenesis. Most of the SLE mouse models develop antinuclear antibodies (ANA) and immune complex glomerulonephritis. Some murine models of SLE have been assessed for cognitive dysfunction. They provide insights into underlying mechanisms that might be comparable to those in patients with SLE.

**Spontaneous Mouse Models**

The most widely studied spontaneous models of SLE are the New Zealand Black crossed with the New Zealand White mouse (NZB/W), BXSB/Yaa, and MRL/lpr strains.

Female NZB/W mice make ANA and anti-dsDNA autoantibodies and develop glomerulonephritis. The study of this strain is difficult because the time to onset of disease is long and the 2 strains need to be bred together to have offspring for study. Similar to humans with SLE, they exhibit learning impairments and mood-disorder behaviors, and the incidence and severity of SLE is greater in females.

The BXSB/Yaa mouse model differs from others because disease is presented only in male mice. The disease depends on the Y-autoimmune accelerator (Yaa) locus, which is translocated from a region of the X chromosome to the Y chromosome. This region contains 16 genes including Toll-like receptor (TLR)7. TLR7 overexpression leads to activation of the type 1 interferon (IFN) pathway, a critical pathogenic
pathway in SLE\textsuperscript{2}. The affected mice develop an SLE-like disease with ANA, glomerulonephritis, and impairment in spatial memory\textsuperscript{8}.

The MRL/lpr mouse develops an SLE-like disease at a younger age than NZB/W mice. The LPR gene leads to loss of Fas function\textsuperscript{3,14}; however, Fas-deficient humans do not develop an SLE-like phenotype. Similarly, breeding of LPR into B6, Balb/c, AKR, and C3H mice leads to very mild autoimmune disease\textsuperscript{15,16}. The MRL+ mice develop SLE phenotype at 18 months of age; thus they are useful for studying accelerants of the disease\textsuperscript{17,18}. Manifestations of SLE include ANA, glomerulonephritis, and cognitive dysfunction (anxiety, depression, anhedonia, decreased locomotion, and impaired spatial learning)\textsuperscript{8}.

**Induced Mouse Models**

Human SLE is believed to be triggered by environmental factors in genetically susceptible individuals; thus, the study of induced models of SLE is also useful.

Pristane is an isoprenoid alkane abundant in mineral oil. Intraperitoneal injection of pristane to Balb/c mice develops an SLE-like disease with ANA and immune complex-mediated glomerulonephritis with high levels of IFN. It also results in downregulation of the GluN2A subunit of the NMDA receptor in hippocampal neurons and to disrupted learning and memory deficits\textsuperscript{19}. Abnormal levels of IFN-\alpha have been observed in the sera and cerebrospinal fluid (CSF) of patients with mental disorders\textsuperscript{20,21}. Intravenous injection of IFN-\alpha leads to anxiety and depression-like behaviors as well as Cl in female NZB/W mice.

We have developed an induced model of SLE that requires immunization of non-spontaneously autoimmune mice with a peptide mimotope of DNA (DWEYS)\textsuperscript{22}. Immunization with a multimeric form of this sequence results in production of anti-DNA antibodies, immunoglobulin deposition in the kidneys, and cognitive dysfunction in mice in which an antibody can penetrate brain parenchyma. We will describe this model in detail. The anti-DNA antibodies in this model, termed DNRAb, cross-react with the NMDA receptor.

Current evidence suggests that antibodies arise in the CSF of patients with SLE through penetration of the blood brain barrier (BBB); the antibodies in the CSF are polyclonal and there is albumin in the CSF as well. To mimic this clinical scenario, we administered LPS to mice immunized with the multimeric peptide. LPS causes a BBB breach in the hippocampus. Once the antibody penetrates the brain parenchyma, pathology proceeds as a 2-step process. First, the antibody functions as an allosteric modulator of NMDA receptor signaling to cause excitotoxic death of some neurons. This occurs over the course of a week\textsuperscript{23}. High-mobility group box chromosomal protein 1 (HMGB1) is secreted by activated or damaged neurons. Recent studies have demonstrated that HMGB1 binds to the NMDA receptor on surviving neurons where C1q is targeted to the synapses and binds to HMGB1. HMGB1 therefore serves as the bridge between the damaged neurons and C1q, which is detected by microglia and targets the synapses for pruning\textsuperscript{24}.

Binding of DNRAb to NMDA receptors leads to increased free calcium in the cell where it is taken up by mitochondria to buffer. A high level of calcium in the mitochondria promotes cellular respiratory system, thus ROS production. Because of the increased calcium concentration, the mitochondrial membrane potential collapses and the mitochondrial permeability transition pores open, resulting in the release of proapoptotic molecules like Cytc and apoptosis-inducing factor, leading to neuronal death. Concomitantly, calcium activates cytosolic enzymes including phospholipases, proteases, and endonucleases, which promote necrosis\textsuperscript{25}.

In a recent study, DWEYS immunized mice with a forebrain deletion of GluN2B subunits displayed acute neural loss in hippocampal CA1, while GluN2A knockout mice were protected from the DWEYS neuropsychological phenotypes, suggesting the essential mediatory role of GluN2A subunit in SLE cognitive dysfunctions\textsuperscript{26}.

The second phase of pathology starts at 4 weeks after LPS administration, at a time when antibody is no longer detectable in the brain, and is characterized by microglial activation and dendritic pruning of the surviving neurons. This pathology persists for as long as we have observed the mice and is dependent on the presence of microglia and Clq. It manifests with impaired spatial memory. To understand the basis for this impairment, we performed electrophysiologic studies. The pyramidal neurons in CA1 region of the hippocampus represent place cell activity. A neuron fires intensely within a given area and becomes silent in other parts. The area of neuronal firing is known as a place field. Place fields from spatial maps rely on NMDA receptors\textsuperscript{27}. The place fields are expanded in Balb/C mice with DNRAb penetration into the hippocampus, indicating a disrupted CA1 place cell system as a key neural substrate for DNRAb-mediated pathology\textsuperscript{28}.

We also performed 18F-fluorodeoxyglucose micro positron emission tomography (FDG-microPET) in these mice to plot changes in brain metabolism. An inverse relationship was observed between neuronal number and regional metabolism compared to the positive correlation seen in control mice.

Because we found that depletion of microglia can prevent the loss of dendritic arborization or can reverse established injury, we asked whether suppressing microglial activation might also be effective. Treatment of mice with an angiotensin-converting enzyme inhibitor (ACEi) that penetrates the BBB and suppresses microglial activation spares cognitive function when given early or late after LPS administration\textsuperscript{29}. This observation is of great importance as it suggests that there is sufficient neuronal plasticity to recover from the DNRAb-mediated insult.
Neuroimaging

Neuroimaging techniques constitute a promising method to objectively assess cognitive dysfunction in SLE and a potentially useful metric in clinical trials.

Several anatomical and functional neuroimaging modalities have been used in NPSLE, including diffusion tensor imaging (DTI), functional magnetic resonance imaging (fMRI), and PET scans.

DTI is a noninvasive, refined MRI technique that detects the diffusion of water in brain tissue, allowing study of the brain structure and assessment of white matter (WM) integrity measured by fractional anisotropy (FA). Several studies showed various WM correlates of NPSLE in SLE patients with and without acute NPSLE manifestations.

The advantage of fMRI is its ability to detect variations in the deoxyhemoglobin levels in neurons as a measure of neuronal activity, serving as an indirect assessment of cerebral functions such as working memory, executive function, and attention. Different studies demonstrated abnormalities in the hippocampal/parahippocampal regions of patients with SLE both at rest and during a memory task.

The FDG-PET measures the uptake of glucose by the brain, serving as a measure of brain metabolic activity. The glucose metabolism in the brain can be affected by any inflammatory state as well as changes in neuronal density and activity. Therefore, FDG-PET provides a highly sensitive assessment tool for brain pathology.

Bridging Mouse Models to Human Disease

To understand whether the mouse model can inform us about patients, we performed FDG-PET scans of patients with SLE who had no prior evidence of neuropsychiatric disease. We observed a higher resting metabolism in several areas of the brain. These areas of hypermetabolism correlated with the serum DNRAb titers and memory impairment.

To validate these findings, we also assessed FDG-PET images in a larger cohort of patients with SLE. A total of 20 patients with SLE underwent FDG-PET and DTI imaging at baseline and at 15 months. This study was able to reproduce the initial finding of resting hypermetabolism in the hippocampus, orbitofrontal cortex, and basal ganglia as well as identify 3 new regions: sensorimotor cortex, and occipital and temporal lobes. Hypermetabolism in 5 of these regions correlated with poor performance on a memory test. Further, we showed a significant correlation between serum DNRAb, the performance on a spatial navigation task, and resting glucose metabolism in the anterior putamen and frontal cortex. DTI images revealed the presence of regions of decreased microstructural integrity (measured by FA) structurally linked to the hypermetabolic regions. Tractography revealed that connecting tracts in the region of the hippocampus are substantially reduced in patients with SLE compared to healthy controls.

The structural and functional changes remained stable during the followup time of 15 months, suggesting that they possibly represent an “SLE-specific” pathology irrespective of disease activity and other confounders such as medications or prior central nervous system events. Taken together, the hippocampal hypermetabolism, decreased structural integrity of para-hippocampal regions, higher serum DNRAb, and poor performance on spatial memory testing suggest the possibility that the primary event takes place in the grey matter, leading to damage to WM tracts and spatial memory loss.

An 18-kDa translocator protein referred to as TSPO is upregulated during microglial activation and therefore serves as a correlate of brain injury and inflammation. Consequently, it has been gaining interest as a target in neuroimaging of various neurodegenerative diseases. Several second-generation radioligands, such as PBR28, have been developed for use in PET imaging providing a higher affinity for the TSPO. One study demonstrated a significant decrease in TSPO distribution in the hippocampus of patients with SLE compared to healthy controls and that these changes were more pronounced in SLE patients with CI.

Therapeutic Interventions

The treatment of CI in SLE is exceedingly challenging, as is the case with NPSLE in general. The clinician is faced with several challenges starting with the attribution of CI to SLE-specific immune mechanisms as opposed to confounders or mimickers (medication side effects, infections, etc.) and objectively assessing the level of impairment. At this time, there is no clinically proven treatment that effectively targets CI in SLE.

As described above, there is significant evidence suggesting that microglia play a central role in the inflammatory cascade leading to CI in SLE. Therefore, a strategy that targets microglial activation, without inducing immunosuppression, is very attractive. The renin angiotensin system is a key player in neuroinflammation and is implicated in microglial activation. Targeting this pathway using ACEi successfully inhibited microglial activation and neuronal damage in various neurodegenerative diseases. A small study in older patients with Alzheimer disease (AD) showed that those patients receiving ACEi experienced a slower rate of cognitive decline. Several observational studies showed similar benefit of ACEi in patients with AD.

Based on the promising results from the DNRAb-mediated mouse model of CI and in keeping with the commitment to find non-immunosuppressive therapy, we have designed a trial of ACEi in SLE patients with CI. The study is a phase II double-blinded randomized multicentral trial comparing the efficacy of lisinopril, an ACEi known to cross the BBB (or non-centrally acting) to benazepril, an ACEi that does not cross the BBB (or non-centrally acting). A major benefit of the trial is the use of objective assessment tools as endpoints. Patients will undergo FDG-PET imaging to...
evaluate resting metabolism at baseline and at 12 months. Secondary endpoints will include changes on a battery of cognitive testing (spatial memory, spatial navigation, automated neuropsychological assessment metrics), patient-reported outcomes, and disease activity measures. Further, the use of PBR28 tracer will allow for the study of microglial activation.

This trial would be the first study in humans, to our knowledge, to investigate the effectiveness of ACEi, a commonly used antihypertensive, in improving CI in patients with SLE, relying on advanced imaging techniques and objective assessment tools.

**Future Perspective**

Activation of microglia is implicated in the pathogenesis of CI in SLE, an NPSLE manifestation characterized by longterm progressive nature of neural degeneration. Detecting and validation of biomarkers based on neuroimaging studies is a fundamental step in the future clinical trials toward treatment of cognitive dysfunction in SLE.

Further research dealing with human and murine models of CI will promote a better understanding of the pathophysiological events that trigger and sustain this strong form of SLE disease and allow the possibility of finding therapeutic targets and an evidence-based approach for further treatment of the disease. We believe ACE inhibition is a rational potential therapeutic target in clinical trials to benefit CI.

Despite its major effect on the QoL of patients with SLE, CI in SLE remains widely understudied and poorly understood, and no targeted treatments are available. One of the major challenges of studying CI in SLE is the lack of objective assessment measures. We described several neuroimaging techniques that can be effectively used as metrics in clinical trials. We presented our experience with previous and ongoing clinical trials relying on sophisticated PET and MRI techniques to assess CI in SLE.

The study of the DNRAb-mediated mouse model of CI allowed us to identify the central role that microglial activation plays in this condition as well as to test promising therapeutic targets. The use of centrally acting ACEi appears to be an encouraging approach because it is mechanistically plausible, the drug has a well-established safety profile, and most of all, it is non-immunosuppressive.

**REFERENCES**


