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Mechanisms of neuropsychiatric lupus: The relative roles of the blood-cerebrospinal fluid barrier versus blood-brain barrier

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ABSTRACT

The pathogenesis of neuropsychiatric lupus (NPSLE) is believed to include the entry of circulating neuropathic antibodies to the brain via a pathologically permeable blood-brain barrier (BBB). Nevertheless, direct evidence of BBB pathology or mechanisms underlying BBB dysfunction is missing. Here, we examined BBB integrity in an established NPSLE mouse model (MRL/*fas*$^{lpr/lpr}$). Surprisingly, challenging the barrier with various exogenous tracers demonstrated insignificant changes in BBB permeability. Furthermore, electron microscopy showed no ultrastructure changes supporting hyper-permeability. However, we found that abnormal function of the blood-cerebrospinal fluid barrier (BCSFB) in the choroid plexus underlies brain exposure to neuropathic antibodies. Considerable intrathecal lymphocyte infiltration likely occurs through the BCSFB, accompanied by epithelial hyper-permeability to antibodies. Our results challenge the commonly held view of BBB disruption in NPSLE, supporting a shift in focus to BCSFB dysfunction as a causative factor in the disease.

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1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease that can cause multi-organ damage frequently involving skin, kidney, lung, heart and brain [1–3]. The disease is characterized by loss of tolerance to self-antigens, formation of autoantibodies, and immunological complex deposition followed by leukocyte activation and cytokine production causing systemic inflammation and tissue damage [4,5]. Patients with SLE that manifest one or more of several neuropsychiatric symptoms are classified as 'neuropsychiatric lupus' (NPSLE) [6]. NPSLE is perhaps the least understood but one of the most prevalent manifestation in lupus, which may occur independently of active systemic disease and without serologic activity [4]. There is substantial evidence indicating that NPSLE can be a primary manifestation of brain inflammatory disease, rather than simply an outcome of end-organ dysfunction and/or treatment. Moreover, NPSLE can occur when the systemic disease is absent or stable [6]. The American College of Rheumatology (ACR) defined 19 neuropsychiatric syndromes which may be present in SLE, including focal manifestations (e.g. stroke or seizure) and diffuse syndromes (e.g. depression, anxiety, memory deficits, and general cognitive decline). The focal presentations of NPSLE, which are fairly well understood, are most frequently associated with autoantibodies that cause a hypercoagulable state, such as anti-phospholipid, anti-cardiolipin, and lupus anticoagulant antibodies [7]. At least twenty lupus autoantibodies have been found with correlation to NPSLE especially in the serum and cerebrospinal fluid (CSF) of patients such as anti-nuclear antibody (ANA), anti-*N*-methyl-D-aspartate receptor, and more [8–11]. In addition, a variety of cytokines have been identified as inflammatory mediators which play a pathogenic role and might disrupt the blood-brain barrier (BBB) including IL-1, IL-6, IFNα and TNFα, and TNF-like weak inducer of apoptosis (TWEAK) [5,12,13]. While our understanding of autoimmune dynamics is quite advanced for certain manifestations of SLE, there remain many unanswered questions regarding the mechanisms underlying neuropsychiatric disease in SLE patients (NPSLE).

The discovery of circulating brain reactive lupus autoantibodies in patients and in mouse models highlighted their potential central nervous system (CNS) pathogenicity [8]. Introducing

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anti-*N*-methyl-D-aspartate receptor antibodies (anti-NMDAR) directly into the brains of non-autoimmune mice was found to induce neuronal cell death and impair cognition [9,14], and these have been found in the CSF of SLE patients with CNS disease [8]. Consequently, neuropathic autoantibodies, including anti-NMDAR [4] and anti-ribosomal-P [15], are suggested to be effectors of NPSLE. These studies, however, also demonstrated that circulating neuropathic autoantibodies do not readily access the brain. Only with pharmacological breach of the BBB, triggered by intravenous administration of lipopolysaccharide or epinephrine, may pathogenic autoantibodies access the brain to exert their neurotoxic effects [9].

The identification of such neuropathic autoantibodies in the CSF of SLE patients and in *post mortem* brains strongly supports brain penetrance [9]. While the exact route of antibody entry into the brain and solid evidence for NPSLE-related BBB disruption is missing, the prevalent working hypothesis is that abnormal permeabilization of the BBB is the primary contributor to neuropsychiatric disease in lupus. Nevertheless, abnormal antibody brain penetrance might actually result from a dysfunction in any of the three brain barriers: the BBB, the meningeal barrier, or the blood-CSF barrier (BCSFB). Indeed, the involvement of brain barriers other then the BBB in the pathophysiology of lupus was previously proposed [5,16]. A convincing body of research supports this notion, including the following studies: First, the report of leptomeningeal abnormalities on Gd-DTPA (gadolinium) enhanced magnetic resonance (MRI) imaging that might reflect meningeal barrier abnormalities [17]. Second, elevated levels of albumin and antibodies found in the CSF of SLE patients might reflect BCSFB abnormalities [18,19]. Third, the presence of lymphoid cells in brain ventricles of NPSLE mouse models also led to the hypothesis that immune cells enter into the CSF and induce primary neuronal damage in regions bordering the cerebral ventricle [20]. Finally, CSF from both mice and humans with NPSLE is toxic for neurons and proliferating neural cells [21,22].

In our study we directly investigated the function of brain barriers in an established NPSLE mouse model, the MRL/MPJ-Fas^{lpr}/J mouse (hereafter MRL/lpr). Several spontaneous models of SLE with CNS manifestations exist, including NZB/W-F1, BXSB, and MRL/lpr mice [23,24]. Of these, the MRL/lpr mouse has proven to be a very useful spontaneous model of both SLE and NPSLE, for several reasons. Besides a strong female bias (similar to the human SLE 9:1 female to male ratio), the MRL/lpr mouse has a very similar overall disease pattern to human SLE including renal and cutaneous manifestations [25,26], as well as a neuropsychiatric profile consistent with the diffuse manifestations of human NPSLE including depression-like behavior and memory deficits [27,28]. Importantly, brain reactive lupus autoantibodies were found in both NPSLE patients and in this mouse model [8,29]. There is a significant and growing body of research into manifestations of NPSLE in MRL/lpr mice, including extensive behavioral characterization and brain tissue evaluation [28,29]. Therefore, here we utilized the MRL/lpr mouse strain to thoroughly investigate BBB integrity. Contrary to the commonly held view, exogenous tracer challenges (introducing different tracers into the blood circulation) with confocal and electron microscopy imaging showed no direct evidence of changes in BBB permeability. However, we report an unexpected mechanism of pathology, which likely represents the primary route of antibody entry into the brain through a perturbed BCSFB, a mechanism aligned with previous reports of choroid plexus and CSF antibody enrichment in NPSLE patients [18,19,30]. Based on our findings, we suggest a need to develop new avenues of research by shifting attention from BBB to BCSFB dysfunction.

2. Materials and methods

2.1. Mice

8-week old female MRL/MPJ-*Fas*^{lpr}/J (stock #00485, (hereafter MRL/lpr)) and MRL/MPJ (stock #00486, hereafter MRL/+) mice were purchased from the Jackson Laboratories (Bar Harbor, ME) to establish a local colony. Pups were weaned at 3 weeks of age and raised until 5 or 16 weeks of age, as indicated in each experiment. All mice were bred and maintained in the animal facility of the Hebrew University under specific pathogen-free conditions. All animals were treated according to institutional guidelines approved by the Institutional Animal Care and Use Committee (IACUC) at Hebrew University.

2.2. Disease state evaluation

The MRL/lpr disease phenotype was scored in order to evaluate systemic disease progression as previously described [31]. In brief, phenotype evaluation included scoring of proteinuria (examined by CYBOW 10AC reagent strips for urinalysis) [32] and titers of IgG anti-double stranded (ds)DNA antibodies (examined by enzyme-linked immunosorbent assay (ELISA) [32].

2.3. Tissue preparation

After dissection, brains were placed in 4% paraformaldehyde (PFA, Sigma Aldrich) at 4 °C overnight, cryopreserved in 30% sucrose and frozen in TissueTek OCT (Sakura). Frozen brains were cut to either 30 μm (for vessel profiling) or 10 μm slices for immunofluorescence staining (CM1950, Leica) to produce sagittal brain sections. In all experiments either cortex or choroid plexus (CP) were examined and analyzed [33,34].

2.4. Immunofluorescence

10 μm thick cryo-sections were washed with phosphate buffered saline (PBS) for 15 min at room temperature (RT) and then incubated for 1 h at RT with blocking solution (10% bovine serum albumin (BSA), 10% normal horse serum (NHS), 0.05% triton X-100 in PBS). Slides were incubated with primary antibodies (diluted in 2.5% BSA, 2.5% NHS, 0.05% triton X-100 in PBS) at 4 °C overnight. Slides were then washed with PBS, incubated with secondary antibodies for 1 h at RT, washed and mounted with 4′,6-diamidino-2-phenylindole (DAPI) Fluoromount-G (Southern Biotech).

Primary antibodies used were: hamster anti-mouse CD31 (1:100, Bio-Rad cat No. MCA1370Z), and horseradish peroxidase (HRP)-donkey anti-mouse IgG (1:200 Southern Biotech cat No. 641005) and rabbit anti-albumin (1:200, Sigma-Aldrich cat No.A0433).

Secondary antibodies used were: Cy3 donkey anti-mouse IgG (1:500, Jackson cat No.715165151), goat anti-Armenian hamster (1:500, Jackson cat No.127605160) and Alexa 488 donkey anti-mouse IgG (1:200, Jackson cat No.715545151).

2.5. Leakage analysis

In all experiments, a leakage incident was defined when a tracer was localized outside the endothelial area. At least four non-sequential sagittal sections per animal (of similar cortical locations) were scanned under the microscope and analyzed by a person blind to the animal genotype.

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