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Blood-brain barrier disruption and neuroinflammation as pathophysiological mechanisms of the diffuse manifestations of neuropsychiatric systemic lupus erythematosus

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ABSTRACT

Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease that can involve nervous system commitment known as neuropsychiatric systemic lupus erythematosus (NPSLE). The diagnostic of NPSLE is complex because the symptoms range from focal symptoms (e.g., strokes, thrombotic events) to diffuse disorders affecting cognition, mood and level of consciousness (e.g. acute confusional state, psychosis). Both type of manifestations of NPSLE differ in their pathological mechanisms. The focus of this review will be on the mechanisms that lead to the blood-brain barrier (BBB) disruption and to the neuroinflammation related with the diffuse manifestations of NPSLE.

1. Introduction

Systemic lupus erythematosus is an autoimmune disease with heterogeneous manifestations, in which environmental exposures in a genetically susceptible individual trigger the activation of dysregulated immune responses resulting in loss of tolerance to self-antigens [1]. A hallmark of the disease is the production of a large quantity of autoreactive antibodies that form immune complexes with self-antigens, causing tissue and organ damage [2]. The organs involved in the manifestations of the disease include kidneys, joints, hematopoietic organs and nervous systems [3].

The nervous system is commonly affected in both children and adults with SLE and this is associated with a worse prognosis and more accumulative damage in the patients [4]. Neuropsychiatric systemic lupus erythematosus (NPSLE) generates numerous manifestations encompassing both the central nervous system (CNS) and peripheral nervous system (PNS), with symptoms that range from thrombotic events to disorders affecting cognition, mood and level of consciousness [5].

Nervous system involvement in SLE is the least understood manifestation of the disease and it remains a complex diagnostic entity because of the multiple disease presentations it encompasses [6]. The American College of Rheumatology (ACR) Nomenclature for NPSLE provides case definitions for 19 neuropsychiatric (NP) syndromes seen in SLE, with reporting standards and recommendations for laboratory and imaging tests [7]. The researchers that have used this nomenclature to classify NPSLE reported that the overall prevalence among the populations varies widely, ranging from 37% to 95% [8]. The main reason for this variation has been the inclusion of non-specific minor symptoms of uncertain clinical significance. However this may also be due to factors such as study design, baseline characteristics of the patients and the lack of consistency in the attribution of NP events to SLE [9,10].

In the situation where no causes for the NP syndromes can be found it is considered primary NPSLE, whereas secondary NPSLE is the result of side effects of infections, drugs prescribed or metabolic alterations derived from multiorgan damage [11]. Given the absence of a diagnostic gold standard capable to determine reliably the presence of primary NPSLE, this process is achieved through an exclusion process [12].

In the CNS, diffuse cerebral manifestations (e.g., acute confusional state and psychosis) that are often transient, reversible on therapy, and not consistently associated with brain pathologic abnormalities, have a different pathogenesis from the focal symptoms (e.g., strokes, thrombotic events, others), which are usually acute in onset, permanent even with therapy, and frequently associated with pathologic lesions at autopsy [13]. Tissue injury because of pathogenic antibodies and the effects of various cytokines in a disrupted abnormal blood-brain barrier (BBB) leads predominantly to diffuse symptoms, whereas vascular

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occlusion because of antiphospholipid antibodies (aPL) and complement proteins cause focal disease [14,15]. The focus of this review paper will be on the mechanisms that lead to the BBB disruption and the neuroinflammation associated with the diffuse manifestations of NPSLE.

2. Cytokines and complement influence BBB disruption

The BBB forms an interface between the blood and the brain that protect the brain from the penetration of unwanted compounds and cells [16]. The BBB is composed primarily of endothelial cells (ECs), astrocyte end-feet, pericytes, perivascular macrophages, and a basal membrane. This barrier is the result of the tightly sealed monolayer of ECs with tight junctions (TJs) and adherens junctions (AJs). The BBB allows the free diffusion of certain small essential water-soluble nutrients, while other complex nutrients rely on highly selective transport systems to enter the brain [17].

Alteration of the BBB forming cells and/or disruption of the tight junctions can cause increased permeability, resulting in the influx of proinflammatory molecules and cells that upset normal brain function and lead to neuronal injury [18]. It has been shown that excessive amounts of neurotransmitters, cytokines, chemokines and peripheral hormones might influence BBB permeability [16]. Mechanisms for breakdown of the BBB are incompletely understood, but appear to involve direct effects of inflammatory molecules on endothelial regulation of BBB components, as well as indirect effects through cell-mediated injury [18]. There are many observations regarding the effect of cytokines in the BBB and it is often assumed that any elevation in cytokines is accompanied by a deterioration of the BBB [19]. It is important to make clear that the BBB disruption seen in diffuse NPSLE is caused by alterations at the paracellular level, in which the expression of TJ proteins and cell adhesion molecules is decreased leading to "leakiness" resulting in the influx of proinflammatory molecules and cells that upset normal brain function and lead to vascular and neural injury [18]. In this part we will discuss specifically the role of TWEAK as a cytokine that affects the BBB integrity, but we will show that the complement protein fragment C5a also participate in this pathogenic process.

2.1. TWEAK and Fn14 in the BBB

Tumor necrosis factor-like weak inducer of apoptosis (TWEAK), is a member of the tumor necrosis factor (TNF) superfamily of cytokines that is synthesized as a type II transmembrane protein from which a soluble ~17 kDa ligand factor with biological activity can be released (soluble TWEAK, sTWEAK). sTWEAK induces various cellular responses in vitro including cell proliferation, migration, differentiation, angiogenesis, and the expression of pro-inflammatory molecules such as IL-8, MCP-1 (monocyte chemoattractant protein-1), ICAM-1 (Intracellular adhesion molecule-1), and *E*-selectin in human umbilical ECs, and IL-6, IL-8, and ICAM-1 in astrocytes. TWEAK activity is mediated via binding to fibroblast growth factor-inducible 14 (Fn14), a 14 kDa member of the TNF receptor superfamily. Fn14 is expressed in a variety of cells and tissue types including fibroblasts, endothelial and epithelial cells [20].

It has been shown that TWEAK may play an important role in BBB disruption and is an essential component of NPSLE diffuse symptoms pathogenesis [21]. One study demonstrated that Fn14 is up regulated in brains of MRL/lpr mice and that the severe depression-like behavior observed in MRL/lpr Fn14 WT mice is significantly reduced in Fn14 deficient mice. In this study, the BBB permeability was assessed through the calculation of the albumin quotient, and it was increased in the MRL/lpr Fn14WT mice. This BBB breach was related with the fact that the IgG ratio was also increased in this mice population, which means that TWEAK increased the permeability of the BBB and that promoted the diffusion of pathogenic autoantibodies to the brain parenchyma [22].

On the other hand, the study by Stephan and cols evaluated the properties and integrity of the BBB through an in vitro model that used human brain cerebrum microvascular endothelial cells (HBCMEC) cultures. The BBB integrity was estimated in a permeability assay that evaluated the passage of two distinct molecules through the monolayer of HBCMECs and measuring the transepithelial electric resistance (TEER) of the monolayer. The results showed that TWEAK increased significantly the permeability coefficient of the HBCMECs monolayer while decreasing the TEER [23].

2.2. Complement and BBB integrity

Three different pathways and about 40 proteins constitute the complement system, which is a major component of the humoral innate immune system. Complement activation generates fragments such as C3a and C5a that bind to specific G protein coupled receptors, C3aR and C5aR1, respectively [24]. These peptides play a key role in the recruitment of immune cells and initiation of the cytokine cascades. They modulate the generation of cytokines such as IL-1β, TNF-α, IL-6, IL-8, IL-17 and INF-γ by myeloid and T cells, starting neuroinflammatory mechanisms [25].

A study looking for the role of the complement protein C5a in the brain vasculature suggested that the C5a/C5aR signaling plays a key role in disrupting the BBB integrity. It was first demonstrated a definite loss of BBB integrity in the MRL/lpr mice by IgG infiltration into the brain parenchyma. Then, the bEnd3 immortalized mouse brain endothelial cells were treated with lupus serum and the decrease in the TEER measurements evidenced an increase in the permeability of the monolayer, that was alleviated when the cells received a pretreatment with an antagonist of the C5aR (C5aRant). The results indicate that C5a signaling through C5aR decreases endothelial layer integrity in the mice model [26].

The translational potential of the mice model was demonstrated through the assessment of the role of C5a in human brain endothelial cells and astrocytes, using a novel two-dimensional in vitro set up that emulates the in vivo system. The model consisted on human brain microvascular endothelial cells (HBMVECs) and astroglial cells (normal human astrocytes; NHAs) involved in the formation of the BBB. To evaluate BBB integrity, TEER was measured after treatment with serum obtained from patients with SLE or C5a directly. The TEER values decreased after C5a treatment, which indicate the key role of C5a in endothelial layer integrity through its receptor C5aR1 [27]. The results in this model confirm the findings obtained in the mice model described previously.

2.3. Mechanisms of BBB disruption in diffuse NPSLE

The mechanisms for BBB breakdown in the lupus setting consist in immune mediated attack and complement activation [26]. It has been demonstrated that under pathological conditions, the high expression of cytokines within the brain reduces the expression of TJ proteins and this is associated with an increase in the permeability and transmigration of leukocytes [28]. Data also suggest that these proinflammatory cytokines may also play an important role inducing expression of adhesion molecules ICAM-1 and VCAM-1 in ECs, facilitating the diapedesis of leukocytes to the brain [29]. Another important feature related with the BBB disruption is the fact that matrix metalloprotinase-9 (MMP-9) can also be upregulated by cytokines, so that it can digest proteins present in the vascular basal lamina including collagen, fibronectin and laminin, contributing that way to damage to the vascular integrity [30].

The model proposed by Stephan and cols establishes that TWEAK released during neuroinflammation binds to Fn14 receptors on CNS endothelial cells, resulting in secretion of proinflammatory cytokines and chemokines (CCL-2, IL-8 and IL-6), expression of cell adhesion molecules (ICAM-1) important for leukocyte-endothelium interactions,

activation of the MAPK pathway, and up regulation of MMP-9, with a resulting down-regulation of zonula occludens (ZO-1) proteins that contribute to the disruption of the TJ structure and the subsequent increase in BBB permeability and diapedesis [23].

It was proposed in a mice model that C5a/C5aR signaling has a detrimental effect on BBB stability through the induction of nitric oxide synthase (iNOS) and reactive oxygen species (ROS), that are known to promote the cytoskeletal remodeling shown by the F-actin stress fiber formation in the endothelial cell monolayer [26]. These results were supported and complemented by the in vitro model of human brain endothelial cells and astrocytes, where it was demonstrated that translocation of nuclear factor kappa-light-chain enhancer of activated B cells (NFκB) to the nucleus led to decreased expression of the TJs proteins claudin-5 and ZO-1, and to actin reorganization in the lupus setting. The actin cytoskeleton maintains the structural integrity of the cell, and its reorganization could lead to increased vascular permeability [26], since it causes destabilization of TJs and AJs [24]. It was also shown that C5aR1 is a potentially important therapeutic target for NPSLE, because when C5aR1 was blocked the complement cascades retained their protective functions, and that relieved the symptoms of NPSLE [27].

3. Neuroinflammatory mechanisms

The brain parenchyma may be target of autoantibodies, and there is also evidence showing the pathogenic role of proinflammatory cytokines and chemokines identified in the CSF from NPSLE patients [31]. These agents might directly cause injury, or mediate an inflammatory process through the recruitment CNS-resident cells [32]. Many of the CNS NPSLE syndromes are not permanent, raising the possibility that neuronal injury may not always be lethal and that neural reparative mechanisms are operative [5], indeed it has also been observed that glucocorticoid treatment exerts a reparative effect on the BBB increasing its permeability [16]. The role of cytokines and chemokines, and autoantibodies in generating this injury is discussed below.

3.1. Cytokines

Cytokines are expressed at low levels in the CNS by neurons, astrocytes, microglia and oligodendrocytes. The constitutive expression of genes encoding cytokines and their receptors in the brain suggests that cytokines contribute to normal physiological functions of the CNS [33]. They participate in complex autonomic, neuroendocrine, metabolic and behavioral responses to processes causing brain injury like infection, inflammation and ischemia, but they are also important for neural functions involved in sleep, memory, feeding, ovulation and exercise [34]. Cytokines have an overall incidence in processes related with neuroprotection and neurodegeneration, and they can be regulated by neurotransmitters and hormones. The NPSLE symptoms are the consequence of hyperactivation of the immune system and its associated signaling cascades, resulting in increased levels of proinflammatory cytokines accompanied by glucocorticoid and immune system dysregulation. Cytokines and other immune factors are important in modulation of brain development and also affect adult neuronal plasticity, contributing to cognitive and mood disorders [35].

Passive transport of cytokines across the BBB is not likely, because they are relatively large proteins and their hydrophilic nature does not allow them to cross the BBB. However, four possible ways have been proposed for cytokines to communicate with the brain [36]. Firstly, active transport mechanisms bring specific cytokines into the brain [37]. Secondly, there are specific uptake mechanisms at the luminal surface of the BBB for peptides including IL-1α and IL-1β [38]. In addition, cytokines may enter the brain at specific regions that lack a BBB [39,40]. Thirdly, cytokines may affect the BBB by the induction of adhesion molecules, such as ICAM-1 and VCAM-1 (vascular cell adhesion molecule-1) in the brain endothelium, that promote the migration of lymphocytes across the BBB [41]. Fourthly, cytokines may be able to activate ascendant peripheral nerves. The vagus nerve innervates regions of the body in which immune responses occur (the gut, spleen, thymus, lymph nodes, etc) and provides afferent input to the brain from these regions [42].

Among the cytokines studied, IL-6 has been shown to have the strongest positive association with NPSLE [43–45], furthermore Katsumata and cols found that the intrathecal level of IL-6 in a NPSLE group with acute confusional state (ACS) was significantly higher than in the non-CNS group [46]. Other study demonstrated that IL-6 exerts destructive effects on CNS cells. For example, a study showed a positive correlation between IL-6 levels and a neuronal degradation product denominated neurofilament light chains (NFL), that indicates that IL-6 mediated neuronal destruction might occur during CNS lupus [45].

The TNF family ligands B-cell activating factor of TNF family (BAFF) and a proliferation inducing ligand (APRIL) were evaluated in CSF, and significant differences were found between SLE patients and NPSLE patients regarding levels of APRIL but not of BAFF [47]. Hopia and cols also reported that levels of APRIL in the CSF are elevated in patients with NPSLE compared to controls, and found a positive correlation between CSF APRIL levels and fatigue [48]. Since BAFF, APRIL, and IL-6 are important players in the survival, differentiation, and isotype switching of B cells, they may have an important role in the etiology of diffuse manifestations of NPSLE [47].

Other interesting finding is that the immune complexes formed by CSF antibodies were potent inducers of IFNα, CXCL10, IL-8 and CCL2, all of which have been reported to be elevated in CSF from NPSLE. Therefore, patients with NPSLE have both antigens and autoantibodies to form immune complexes that stimulate immune modulators in the CNS. There are many potential sources of antigens in NPSLE, such as cell damage induced by neurocytotoxic autoantibodies that now include anti-RP (anti-ribosomal phosphoprotein) and anti-NMDAR (anti *N*-methyl-D-aspartate receptor) antibodies. Additionally, injury to ECs by poorly defined mechanisms or ischemic thrombosis associated with anticardiolipin antibodies may release cellular antigens. Regardless of the source of antigen, the presence of CSF autoantibodies that bind to cellular antigens serves as a potentially powerful amplifiers of inflammation in the brain [49].

It has been suggested that the production of many of these cytokines occurs intrathecally and that the damage seen in NPSLE does not require the involvement of factors derived from blood [44]. For example, it was demonstrated a tenfold elevation of IL-6, IL-8 and IL-10 in CSF of patients with CNS lupus. In contrast, no significant differences in serum IL-6 and IL-8 levels were found between the patients with CNS involvement compared to patients without overt CNS involvement. This difference between the levels of the cytokines in CSF versus serum argues against the hypothesis of a primary systemic production of IL-6 and IL-8 with subsequent passage to CSF. The cellular origin of IL-6 production in the brain remains unknown, but macrophages and ECs as well as brain derived microglia and astrocytes, have the capacity to synthesize these cytokines [50].

Despite the previous findings, Jönsen and cols found a heterogeneous cytokine profile in both serum and CSF, and no apparent correlation to either NP manifestations or disease severity. These results reflect that the diversity of the clinical manifestations and pathogenic pathways are against a common cytokine profile in NPSLE. This is in contrast with findings in selected series in which the impression has been that cytokine determinations in serum or CSF may be of great clinical value in NPSLE [51].

3.2. Autoantibody-mediated tissue injury

A humoral immune response directed against neuronal antigens, ribosomes and phospholipid associated proteins has been implicated in the pathogenesis of NPSLE [12]. In NPSLE tissue injury is initiated by autoantibodies, which may act through the following mechanisms:

turning into receptor agonists (mimicking ligand binding) or antagonists (performing like an allosteric modulator), causing antigenic modulation (for example altering the density of the target antigen through internalization), or mediating their effects by interaction with components of the immune system (activating the complement system or engaging with Fc receptors on inflammatory cells) [52].

Regarding the origin of these autoantibodies, it has been proposed that under pathological circumstances, B cells can enter the CNS where they clonally expand in response to an antigen, and differentiate into plasma cells that secrete antibody directly into the extracellular fluid of the CNS [53]. A murine model where a T-dependent protein antigen was infused into a brain with intact BBB permeability, resulted in intrathecal production of antibodies that was demonstrated by: an elevated antibody index (IAb) which reflects the excess of antibodies in the CSF, and the immunoblotting showing antibodies in brain tissues. These antibodies are produced by cells that morphologically fill the criteria of activated B cells and plasma cells [54].

There are autoantibodies predominantly related to diffuse symptoms of NPSLE [55]. Attention has been particularly focused on anti-NMDAR antibodies as a novel system responsible for some of the complexity found in NPSLE [12]. Anti-NMDAR antibodies became important upon the observation that some anti-DNA (deoxyribonucleic acid) antibodies might cross-react with the NMDAR on neurons [56]. NMDARs are receptors for the neurotransmitter glutamate, which is the major excitatory neurotransmitter in the brain and important for many brain functions [57]. The NMDAR permits influx of calcium initiating a downstream signaling cascade ending in neuronal death. The interest in potential antibody reactivity with NMDARs was enhanced because NMDARs expressed on neurons in the hippocampus are known to be critical in learning and memory. In contrast, NMDARs expressed on neurons in the amygdala are known to modulate behavior in fear conditioning paradigms in rodents. Thus, the binding of antibody to NMDARs might provide insight into two common damages of neuropsychiatric lupus: memory impairment and mood disturbance [56].

The reactivity of autoantibodies to the NMDAR was discovered due to a study performed on the mice anti-DNA monoclonal antibody R4A. To discover this cross-reactive antigen a decapeptide library was probed against the antibody R4A and it was identified the consensus sequence DWEYS. A search of protein databases revealed the consensus peptide to be present in the NR2A and NR2B subunits of the NMDAR of mouse, rat, and human NMDAR. ELISAs performed on the extracellular domains of NR2A and NR2B showed that the R4A antibody indeed bind these antigens in a dose dependent manner [56].

It was demonstrated that the subset of anti-DNA antibodies R4A that cross-reacts with NMDAR can signal neuronal death through an excitotoxic mechanism that activates cellular signaling pathways ending in cell death [58]. The effect of autoantibody binding on hippocampal neurons was evaluated, and it was found that NMDAR-reactive autoantibodies increase the open-state duration of the NMDAR through its union to neurons with activated synapses. At low concentration the autoantibodies induce electrophysiological changes that alter synaptic transmission, but at high titers mitochondria undergoes an irreversible collapse marked by an increase of the inner membrane permeability and swelling, that causes apoptosis and permanent neuronal tissue damage [59].

Another mechanism in which anti-NR2 autoantibodies are implicated, explores the possibility of the union between this antibodies and ECs, since the last ones were recently reported to express the NMDAR subunits NR2a and NR2b [60]. It was shown that purified IgG anti-NR2 glutamate receptor antibodies from patients with SLE bound to ECs from human umbilical veins and activated them through stimulation of the NFκB signaling pathway, which up-regulates adhesion molecule expression and increases proinflammatory cytokines and chemokines release from ECs. It is suggested that this inflammatory state of the endothelium might promote the diffusion of IgG anti-NR2 antibodies from the serum to the CSF [61].

In addition, a study described a correlation between anti NMDAR antibodies and cognitive impairment through the evaluation of neuronal damage in mice treated with the serum of SLE patients [62]. Concurrently, another study found that mice immunized with the DNA peptide mimotope arrayed as an octamer on a poly L-lysine backbone (MAP-peptide), developed cross-reactive anti-dsDNA and anti-NR2 antibodies and displayed a selective impairment in learning and memory function [63]. Both studies demonstrated that there needs to be an insult that compromises the BBB allowing the antibodies access to the brain [62]. Thus, serum titers of the NR2 cross-reactive antibody alone cannot be expected to correlate with antibody-mediated brain damage [63]. To reassert the previous findings, Arinuma and cols demonstrated that CSF anti-NR2 antibody levels, but not serum anti-NR2 antibody levels, were significantly higher in patients with diffuse NPSLE than in patients with focal NPSLE [64].

Antibodies targeting the ubiquitous ribosomal phosphorylated proteins have been reported in association with NPSLE. Anti RP-antibodies are directed towards the three ribosomal P proteins (P0, P1, P2) located at the stalk of the large ribosomal subunit 60s. The immunodominant epitope is localized to the carboxy terminal domain, shared by the three P proteins [65]. It was first demonstrated that the anti-RP antibody was predominantly found in patients with SLE and was not detected in a control population [66]. Then, it was shown that in most of the patients with SLE psychosis, circulating anti-RP antibodies were detectable by both western blotting and radioimmunoassay [67]. Despite the previous findings, the clinical studies that evaluated whether serum anti-RP antibodies correlated with psychosis, have yielded conflicting results. On the other hand, a recent meta-analysis of 14 published studies concluded that serum anti-RP measurements were not sensitive in diagnosing NPSLE and did not discriminate between NPSLE subsets [57].

To determine whether anti-RP autoantibody can induce behavioral changes by interaction with the brain, mice received an intracerebroventricular injection of anti-RP. It resulted that the injection of the anti-RP into mice induced depression-like behavior. Furthermore, it was shown that the areas of the brain delineated by the anti-RP staining pattern include the limbic system (piriform cortex, hippocampus, cingulate cortex), which has been implicated in the pathogenesis of depression [68]. The most likely mechanism that was shown repeatedly in cell culture, would involve the following steps: binding of the antibodies to the surface P0 [69], followed by its internalization and inhibition of protein synthesis [70,71].

This mechanism and the source of the autoantigen was further studied by Matus and cols, who found that the neuronal target for the anti-RP antibodies corresponds to a new integral membrane protein of high molecular mass (331 kDa), that was termed Neuronal Surface P Antigen (NSPA). First, affinity-purified anti-RP antibodies used in biotinylation and immunocapture assays detected NSPA as the only cell surface target, both in a neuroblastoma cell line and in cortex neurons in primary culture. Second, affinity purified anti-RP antibodies from two NPSLE patients with psychosis and rabbit anti-NSPA antibodies both triggered calcium influx into neurons in primary culture showing specific target dependency. Third, anti-RP antibodies elicited caspase-3 activation and neuronal death both in vitro and in the rat brain in vivo. They also showed that anti-RP antibodies from SLE patients without NP disease not only recognize NSPA but also induce calcium influx and apoptosis in neurons, indicating that they possess an equivalent pathogenic potential as psychiatrically associated anti-RP antibodies. Therefore, besides the production of anti-RP antibodies, additional risk factors are certainly required for eliciting psychiatric manifestations [72].

4. Concluding remarks

The diffuse manifestations of NPSLE are the product of a humoral immune response directed mainly to neuronal and ribosomal antigens, whose outcome is the altered synaptic transmission of neurons or their

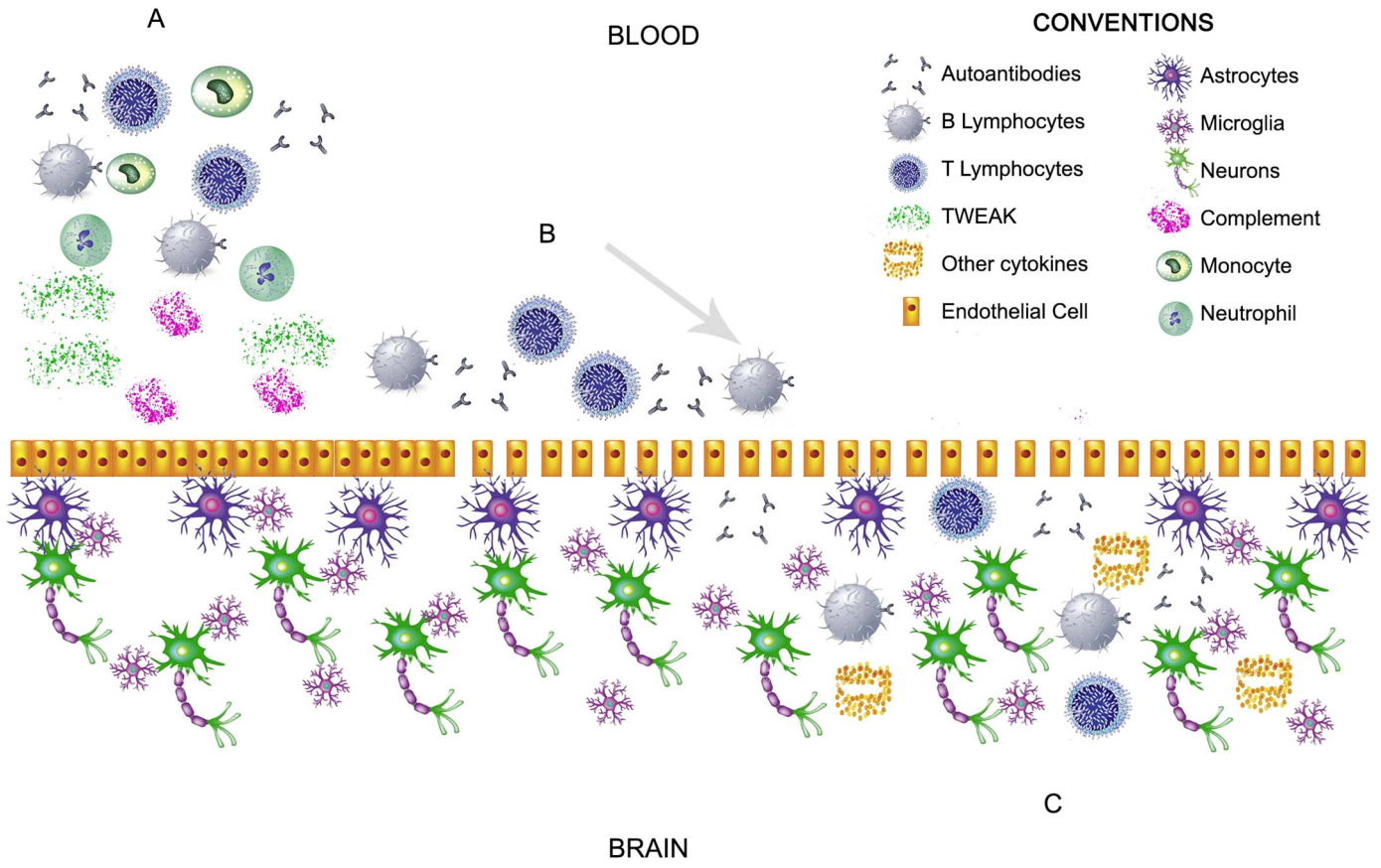

Fig. 1. Patophysiological mechanisms of the diffuse manifestations of neuropsychiatric systemic lupus erythematosus. A) The ECs from the BBB maintain within blood circulation the inflammatory molecules and cells produced in the lupus setting, so that they are isolated from the brain environment. It has been proved that signaling pathways involving the cytokine TWEAK and the complement protein C5a promote the disruption of the BBB through the induction of inflammatory cytokines, modulating the levels of cell adhesion molecules and promoting cytoskeletal alterations that destabilize TJs and AJs. B) The inflammatory molecules and cells gain entry to the brain parenchyma after the breakdown of the BBB. C) The lymphocytes are inflammatory cells that enter to the brain and produce cytokines (like IL-6) and autoantibodies (anti-NMDAR and anti-RP) that are responsible of neuronal damage through the induction of signaling pathways that amplify inflammation or the initiation of calcium influx leading to apoptosis.

excitotoxic cell death. Cytokines in the systemic circulation like TWEAK and complement components like C5a play a clear role in the disruption of the BBB through the induction of ROS, alterations in the cytoskeleton, the modulation of TJs and AJs components, overexpression of adhesion molecules and the enhanced production of proinflammatory cytokines.

As the CNS is an immune specialized site where the immune cell trafficking is tightly regulated, is in the setting of a disrupted BBB where the diffuse manifestations of NPSLE develop, because there is an infiltration of lymphocytes in the brain parenchyma. Once lymphocytes are infiltrated there is intrathecal production of cytokines, although it has been found that ECs, astrocytes and microglia are also capable of producing them. Among the cytokines studies, IL-6 has a reported incidence in diffuse manifestations and neuronal damage. The autoantibodies present in the circulation also gain entry, but there is also a role of the autoantibodies being produced intrathecally. The effect of anti-NMDAR and anti-RP autoantibodies in neuronal damage has been described as a mechanism that provokes calcium influx and apoptosis. In the Fig. 1. the proposed mechanisms leading to BBB disruption and neuroinflammation are depicted.

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Declaration of interest

The authors declare no commercial or financial conflict of interest.

Submission declaration

The manuscript has not been published or submitted elsewhere. It has been read and approved by all the authors.

Take-home messages

- The disruption of the BBB is a pathogenic event that promotes the infiltration of proinflammatory molecules and cells to the brain, and that results in neuronal injury. It has been demonstrated that signaling cascades involved with the cytokine TWEAK and the complement fragment C5a, increased the permeability of the BBB through the induction of inflammatory cytokines, modulating the levels of cell adhesion molecules and promoting cytoskeletal

alterations that destabilize TJs and AJs.

- The NPSLE diffuse manifestations result in increased level of cytokines. Autoantibodies of CSF are indeed potent induces of cytokines. There are studies which demonstrate the involvement of cytokines like IL-6 with diffuse symptoms and reported neuronal damage. It has been found that the cytokines IL-6, IL-8 and IL-10 are elevated in CSF of NPSLE patients but not in serum, demonstrating that they might be produced intrathecally rather than systemically.
- There are two autoantibodies related with diffuse symptoms of NPSLE: anti-NMDAR and anti- RP. The anti NMDAR antibodies are in fact anti-DNA antibodies that cross-react with the NMDAR causing neuronal death through an excitotoxic mechanism. On the other hand, anti-RP antibodies might be internalized by brain cells, generating inhibition of protein synthesis and therefore apoptosis.

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