NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS
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NEUROPSYCHIATRIC LUPUS: PATHOGENETIC AND CLINICAL ASPECTS

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DOCTORAL THESIS

NOVEMBER 2022
ATHENS

ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ
ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ
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ΑΤΤΙΚΟΝ ΠΑΝΕΠΙΣΤΗΜΙΑΚΟ ΝΟΣΟΚΟΜΕΙΟ

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ΔΙΕΥΘΥΝΤΗΣ: ΔΗΜΗΤΡΙΟΣ ΜΠΟΥΜΠΑΣ

ΝΕΥΡΟΨΥΧΙΑΤΡΙΚΟΣ ΛΥΚΟΣ: ΠΑΘΟΓΕΝΕΤΙΚΕΣ ΚΑΙ ΚΛΙΝΙΚΕΣ ΠΑΡΑΜΕΤΡΟΙ

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ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

ΝΟΕΜΒΡΙΟΣ 2022
ΑΘΗΝΑ

Ημερομηνία αίτησης: 03-09-2018

Ημερομηνία ορισμού τριμελούς Συμβουλευτικής Επιτροπής: 18-12-2018

Μέλη τριμελούς Συμβουλευτικής Επιτροπής:

Δημήτριος Μπούμπας (Επιβλέπων Καθηγητής)

Γεώργιος Μπερτσιάς

Παναγιώτης Βεργίνης

Ημερομηνία ορισμού θέματος: 18-03-2019

Ημερομηνία καταθέσεως της διδακτορικής διατριβής: 10-10-2022

Πρόεδρος Ιατρικής Σχολής:

Καθηγητής Γεράσιμος Δ. Σιάσος

Επταμελής Εξεταστική Επιτροπή:

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Κλειώ Μαυραγάνη

Πρόδρομος Σιδηρόπουλος

Ευχαριστίες

Γνώρισα πολλούς νέους ανθρώπους με τους οποίους έζησα ξεχωριστές εμπειρίες, οι οποίες με άλλαξαν και με βοήθησαν να γίνω καλύτερος και τους οποίους θα ήθελα να ευχαριστήσω.

Την τριμελή και εφταμελή επιτροπή για τη στήριξη και τη βοήθεια τους με εποικοδομητικά σχόλια.

Σε όλους τους γιατρούς και τους νοσηλευτές της μονάδας Ρευματολογίας και Ανοσολογίας του ΠΓΝ Αττικόν; Μυρτώ Κωστοπούλου, Φάνη Καραγέωργα, Δημήτρη Τσερώνη, Πελαγία Κατσιμπρή, Μιχάλη Αγγελάκο, Βάνα Τζαβάρα, Δήμητρα Κασσάρα, Αντιγόνη Πιέτα, Νεωμίν Καψάλα, Κατερίνα Χαβάτζα, Σοφία Φλούδα, Παναγιώτα Ραψομανίκη, Κωνσταντίνα Τόγια, Αλίκη Ντούρου, Θάλεια Γερογιάννη για όλες τις στιγμές που ζήσαμε και όλες τις γνώσεις που μου μετέφεραν.

Όλους τους φίλους και συναδέλφους του εργαστηρίου του κ. Μπούμπα στο ΙΒΕΑΑ: Πάνο Βεργίνη, Άγγελο Μπανό, Αναστασία Φίλια, Μαρία Γρηγορίου, Κατερίνα Χατζηιωάννου, Νίκο Μαλισσόβα, Παναγιώτη Γκαραντζιώτη, Σταύρο Δούμα, Αλέξανδρο Γρίβα, Μιράντα Παπαδοπούλου, Αθηνά Βαρβέρη, Αθηνά Μπούμπα, Δώρα Τόγια, Μανώλη Γιαλητάκη, Πάνο Πολίτη, Δημήτρη Γκίκα, Τίνα Τσαμπούλα,, Ιωάννη Κοκκινόπουλο, Βίκη Λαμπροπούλου, Ελένη Ζεβροπούλου, Ειρήνη Στεργιώτη και Γιώργο Σέντη. Τους ευχαριστώ για τις ωραίες στιγμές που περάσαμε μαζί, γελάσαμε, συζητήσαμε κοινωνικά και επιστημονικά και συνεργαστήκαμε.

Θα ήθελα να ευχαριστήσω όλα τα μέλη από τα facilities του ΙΒΕΑΑ, που με βοήθησαν να ολοκληρώσω τη διδακτορική μου διατριβή και συγκεκριμένα την Αναστασία Αποστολίδου (FACS), Τάσο Δελή και Ελένη Ριγανά (Confocal), Γιάννη Βατσέλα (Genome center), Βαγγέλη Μπαλάφα, Παύλο Αλεξάκο (animal facility) και Άννα Αγαπάκη (ιστολογία).

Στους συναδέλφους συνειδικευόμενους, επιμελητές και διευθυντή καθηγητή Δ. Βασιλόπουλο της Β’ Πανεπιστημιακής Παθολογικής κλινικής του ΓΝΑ «ΙΠΠΟΚΡΑΤΕΙΟ» για τη εκπαίδευση μου στην εσωτερική παθολογία.

Όλους τους φίλους και την οικογένεια μου για τη στήριξη και την κατανόηση.

Γιασεμή Κουτμάνη και τη Αλεξία Πολισσίδη, για την επιστημονική της συμβολή στην σχεδίαση και υποστήριξη της διδακτορικής μου διατριβής, χωρίς την οποία δεν θα είχε ολοκληρωθεί.

Τους 3 μέντορες μου… Τον Γιώργο Μπερτσιά, για την άψογη συνεργασία που είχαμε όλα αυτά τα χρόνια καθοριστική του συμβολή στην ερευνητική μου πορεία. Τον Αντώνη Φανουριάκη: αν δεν τον είχα γνωρίσει την πρώτη μέρα που επισκέφτηκα το ρευματολογικό ιατρείο, όλα αυτά μπορεί να μην είχαν συμβεί. Στον άνθρωπο που με έκανε να αγαπήσω τη ρευματολογία, το Λύκο και την έρευνα, τον κ.κ. Μπούμπα. Τον ευχαριστώ πολύ για όλα και ελπίζω να τον κάνω περήφανο. Θα είναι πάντα για εμένα ο πιο σημαντικός άνθρωπος στην ιατρική μου πορεία.

Και τέλος την Δώρα, αισθάνομαι πολύ τυχερός που την γνώρισα και μοιραστήκαμε μαζί όλη αυτή την εμπειρία που μόνο θετικές αναμνήσεις μου έχει αφήσει. Ήταν πάντα εκεί και με βοήθησε σε κάθε μου βήμα.

Contents

ABSTRACT

Neuropsychiatric systemic lupus erythematosus (NPSLE) is an emerging frontier in lupus care encompassing a wide spectrum of clinical manifestations. Despite recent progress in the field, understanding of NPSLE in terms of diagnosis, pathogenesis and treatment remains deficient, because of limited access to tissue, diversity and complexity of clinical manifestations, and overlap with non-SLE related neuropsychiatric events.

As part of my thesis, we established the Attikon SLE cohort, one of the largest in the world with close to 800 patients, and utilized it to study NPSLE. In this cohort, neuropsychiatric disease as presenting manifestation was present in 11.5% of patients, while 17.6% of patients exhibited at least one lupus-related neuropsychiatric event until most recent follow-up. Of note, neuropsychiatric involvement at onset, was independently associated with transition from mild/moderate to more severe disease. Demyelinating events were observed in 3.7% of SLE patients, equally distributed between primary SLE-demyelination and overlap SLE-MS. We also identified a significant number of patients with demyelination who did not fulfill criteria for either MS or SLE and these patients exhibit lupus-like autoimmune features and may represent a distinct entity, which we coined ‘demyelination with autoimmune features’.

In reference to NPSLE pathogenesis, I used the NZB/W-F1 mouse model, which develops spontaneous nephritis at 6 months of age, to study neuropsychiatric disease. In this strain, at the pre-nephritic stage (age 3 months), although the blood brain barrier (BBB) remains intact, we found hippocampus-related behavioral deficits resembling human diffuse neuropsychiatric disease, including depression, anxiety, decreased cognition and impaired coordination. This phenotype is mediated by disrupted hippocampal neurogenesis, with hippocampal neural stem cells (hiNSCs) exhibiting increased proliferation combined with decreased differentiation and survival due to excessive apoptosis. This is accompanied by activation of the microglia towards an inflammatory-state with increased secretion of pro-inflammatory cytokines and chemokines. Later in the course of the disease, when mice develop nephritis (6 months) a disrupted BBB allows immune components of peripheral blood, particularly B-cells, to penetrate into the hippocampus, further enhancing inflammation with locally

increased levels of IL-6, IL-12, IL-18 and IL-23. Among these cytokines, IL-6 and IL-18 directly induce apoptosis of adult hiNSCs *ex vivo*, while for the remaining cytokines there are no receptors to exert effects on adult hiNSCs. Of note, in contrast to the pre-nephritic stage, an interferon (IFN)-α signature was present in this stage. We conclude that an intact BBB with microglial activation disrupting the formation of new neurons within the hippocampus represent early neuropsychiatric changes in NPSLE. In the NZB/W-F1 model, neuropsychiatric disease is an early event that occurs prior to generalized lupus immunologic activity. Early intervention targeting activation of microglia/inhibition of IL-6 or IL-18, or protection of neurons may be a reasonable therapeutic strategy, while in later stages IFN targeting may be more effective.

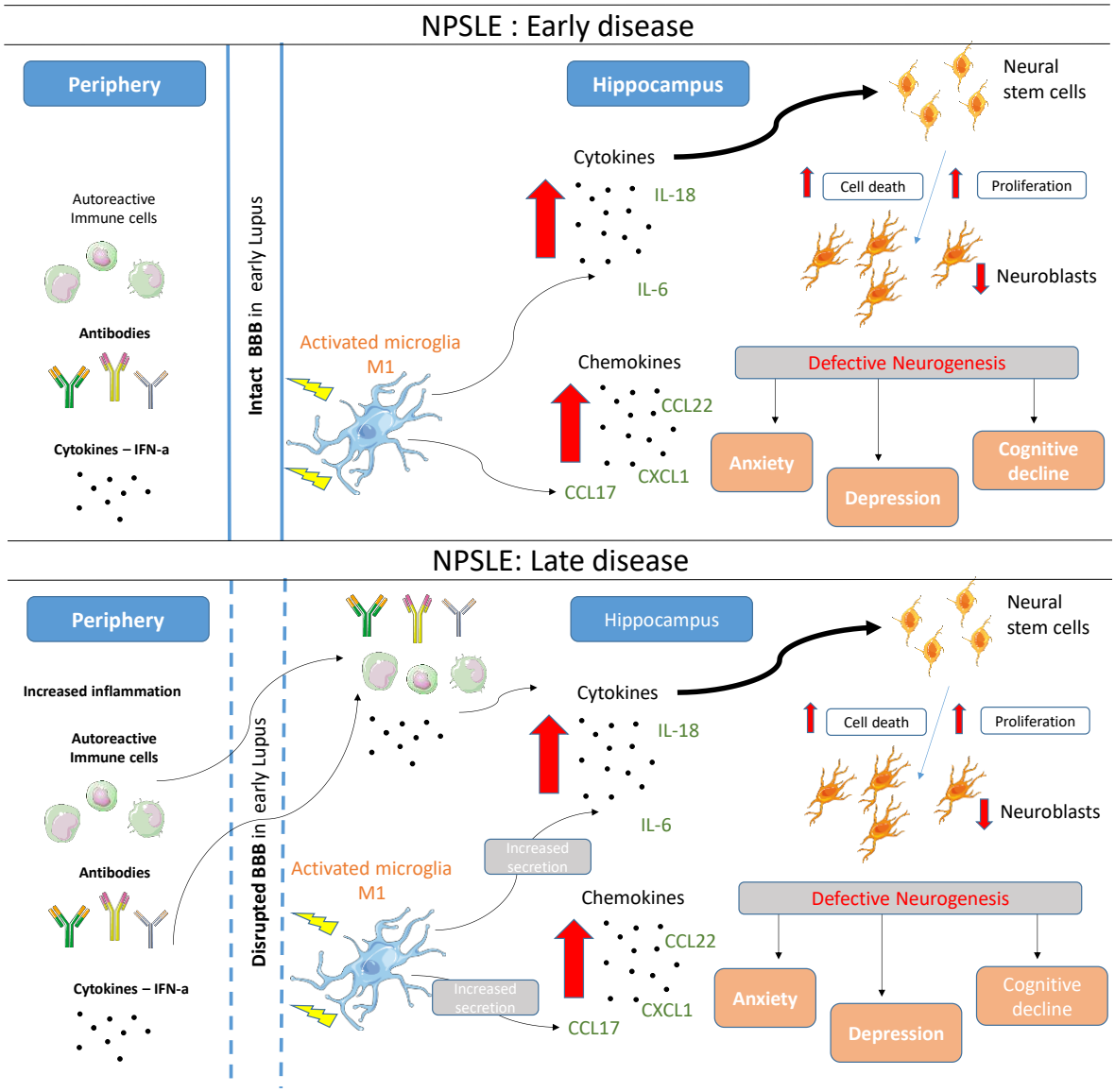
ΠΕΡΙΛΗΨΗ

Ο νευροψυχιατρικός συστηματικός ερυθηματώδης λύκος (ΝΨΣΕΛ) είναι ένα αναδυόμενο πεδίο στη φροντίδα του λύκου περιλαμβάνοντας ένα ευρύ φάσμα κλινικών εκδηλώσεων. Παρά την πρόσφατη πρόοδο, η κατανόησή μας για το NPSLE όσον αφορά τη διάγνωση, την παθογένεια και τη θεραπεία παραμένει ανεπαρκής, λόγω της περιορισμένης πρόσβασης στον εγκεφαλικό ιστό, της ποικιλομορφίας και της πολυπλοκότητας των κλινικών εκδηλώσεων και της αλληλοεπικάλυψης με νευροψυχιατρικά συμβάντα που δεν σχετίζονται με τον ΣΕΛ. Ως μέρος της διατριβής μου, δημιαούργησα την κοόρτη ΣΕΛ «Αττικόν», μια από τις μεγαλύτερες στον κόσμο με σχεδόν 800 ασθενείς και χρησιμοποίησα αυτή την κοόρτη ως ερευνητικό εργαλείο για τη μελέτη του ΝΨΣΕΛ. Σε αυτήν την κοόρτη, η νευροψυχιατρική διαταραχή ως πρωτοεμφανιζόμενη εκδήλωση ήταν παρούσα στο 11,5% των περιπτώσεων, ενώ το 17,6% των ασθενών εμφάνισε τουλάχιστον ένα πρωτογενές νευροψυχιατρικό συμβάν εώς το τέλος της παρακολούθησης. Αξίζει να σημειωθεί ότι η νευροψυχιατρική εμπλοκή κατά την έναρξη, συνδέθηκε ανεξάρτητα με τη μετάβαση από ήπια ή μέτρια νόσο σε σοβαρή νόσο. Απομυελινωτικά επεισόδια παρατηρήθηκαν στο 3,7% των ασθενών με ΣΕΛ, εξίσου κατανεμημένα σε πρωτοπαθή απομυελίνωση και επικαλυψη ΣΕΛ με πολλαπλή σκλήρυνση. Επιπλέον, διαπίστωσα ότι ένας σημαντικός αριθμός ασθενών με απομυελίνωση δεν πληρούν τα κριτήρια ούτε για πολλαπλή σκλήρυνση ούτε για ΣΕΛ και αυτοί οι ασθενείς εμφανίζουν αυτοάνοσα χαρακτηριστικά που συναντάμε στον λύκο και μπορεί να αντιπροσωπεύουν μια ξεχωριστή κλινική οντότητα, «απομυελίνωση με αυτοάνοσα χαρακτηριστικά». Στη συνέχεια, διερεύνησα τις παθογενετικές πτυχές του ΝΨΣΕΛ χρησιμοποιώντας ποντίκια NZB/W-F1 ως μοντέλο του ΝΨΣΕΛ. Αυτό το στέλεχος, στο προνεφριτιδικό στάδιο, αν και ο αιματοεγκεφαλικός φραγμός (ΑΕΦΟ είναι άθικτος, εμφανίζει ελλείμματα συμπεριφοράς που σχετίζονται με τον ιππόκαμπο που ανακεφαλαιώνουν την ανθρώπινη διάχυτη νευροψυχιατρική νόσο. Αυτός ο φαινότυπος διαμεσολαβείται από διαταραγμένη νευρογένεση του ιππόκαμπου με τα αρχέγονα νευρωνικά κύτταρα (ΑΝΚ) να παρουσιάζουν αυξημένο πολλαπλασιασμό σε συνδυασμό με μειωμένη διαφοροποίηση και επιβίωση λόγω υπερβολικής απόπτωσης. Αυτό συνοδεύεται από ενεργοποίηση της μικρογλοίας προς μια φλεγμονώδη κατάσταση με αυξημένη έκκριση προφλεγμονωδών κυτοκινών και χημειοκινών. Αργότερα, στο νεφρικό στάδιο, ο

διαταραγμένος ΑΕΦ επιτρέπει στα ανοσοποιητικά συστατικά του περιφερικού αίματος, ιδιαίτερα στα Β-κύτταρα, να διεισδύσουν στον ιππόκαμπο ενισχύοντας περαιτέρω τη φλεγμονή με τοπικά αυξημένα επίπεδα IL-6, IL-12, IL-18 και IL-23 . Μεταξύ αυτών των κυτοκινών, η IL-6 και η IL-18 επάγουν άμεσα την απόπτωση των ενήλικων ANK ex vivo. Συμπερασματικά, άθικτος BBB με μικρογλοιακή ενεργοποίηση που διαταράσσει το σχηματισμό νέων νευρώνων εντός του ιππόκαμπου μεσολαβεί πρώιμες νευροψυχιατρικές αλλαγές στο NPSLE.

CURRENT STATUS OF PATHOGENESIS OF NPSLE (2022)

Graphical abstract



INTRODUCTION

A DIFFUSE DISEASE OF THE PERIPHERAL CIRCULATION (USUALLY ASSOCIATED WITH LUPUS ERYTHEMATOSUS AND ENDOCARDITIS)

By GEORGE BAEHR, M.D.
AND (By Invitation)
PAUL KLEMPERER AND ARTHUR SCHIFRIN
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CLINICAL PICTURE. The clinical course is characterized by a more or less prolonged, irregular fever with a tendency to remissions of variable duration (weeks, months or even several years), by involvement of synovial and serous membranes (arthritis, pericarditis, pleuritis), by depression of bone-marrow function (leukopenia, thrombopenia, anemia) and by clinical evidences of vascular alterations in the skin, the kidneys and the other viscera. The disease often ends fatally after a period varying from four weeks to five years. One of the most remarkable characteristics of the disease is its sex linkage. Twenty-two of our 23 cases were females, 18 in the second or third decade of life; all were between puberty and the menopause.

Trans Assoc Am Physicians 50:139, 1935

A. Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease with a strong female predominance estimated to affect more than 8,000 individuals in Greece (total population approximately 10 millions)(1). Few, if any, diseases can claim to have a phenotype heterogeneity greater than SLE. Every patient with lupus tends to differ from the other, in a disease that has common, less common, and rare −which can nevertheless be severe−manifestations. At disease onset, multisystem involvement is a different clinical scenario than organ-dominant disease (e.g., kidney, central nervous system or hematological). Moreover, even within the same organ-system, the severity of inflammation may vary from mild to organ-threatening (2). Common manifestations are useful for an early diagnosis and their presence should raise the suspicion of underlying SLE, but they typically lack specificity as they may also occur in the setting of other diseases (3).

Approximately half of lupus patients are diagnosed with mild disease initially with less than 20% having severe disease at onset (4). For those presenting with mild disease in the absence of specific autoantibodies (eg anti-dsDNA) or characteristic lupus manifestations (eg malar rash), definite diagnosis represents a challenge (5). Lupus diagnosis remains clinical, because existing classification criteria for the disease (6,7) fail to classify up to 25% of patients, especially at early stages (8,9). In this regard, "non-criteria" manifestations may aid in an earlier diagnosis of lupus.

The phenotype, clinical course and outcome of lupus differ around the world, depending on the population under study. Caucasians are more likely to have a less severe disease and a mild phenotype is maintained throughout the disease course in 50% of patients (1). In contrast, Afro-Americans and Hispanics exhibit a more aggressive course, with high incidence of lupus nephritis (LN) (10) and neuropsychiatric manifestations (11). Childhood-onset SLE usually displays worse outcomes and more severe disease, as compared to adult-onset patients (12), while patients with late-onset lupus typically have lower disease activity and a milder disease course (13,14).

Several cohort studies around the world have documented the natural history and morbidity of the disease, contributing substantially to increased awareness (15). More

recently, emphasis has been put on the patterns of disease activity and targets of therapy, with remission and low disease activity emerging as new frontiers (16). Moreover, management recommendations have attempted to decrease the heterogeneity in lupus care, by providing evidence- and expert opinion-based guidance (17). However, among patients who present with a certain phenotype, there is a paucity of data regarding potential changes of severity over time, ie. whether the disease will remain mild throughout its course or progress to a more severe form. Such data may have clinical and therapeutic implications for early disease.

Classification criteria for SLE have been developed to ensure the inclusion of homogeneous groups of patients in clinical studies (5). Nonetheless, these criteria are often used in clinical practice to aid diagnosis. In this regard, the Systemic Lupus International Collaborating Clinics (SLICC) 2012 criteria (7) were reported to have increased sensitivity (18,19) and capture more patients at the population level, as compared with the American College of Rheumatology (ACR) 1997 criteria (20). Still, clinical diagnosis may precede classification (1,21), suggesting that especially at early stages, not all individuals with SLE will fulfil the criteria. Moreover, organ-dominant forms may occur imposing further classification challenges. Recently, the European League Against Rheumatism (EULAR) jointly with the ACR have introduced new classification criteria (22), which are based on two novel concepts, namely antinuclear antibodies (ANA) as an entry criterion coupled with variably weighed features (23).

B. Neuropsychiatric Systemic lupus erythematosus

Approximately 30% of all neuropsychiatric manifestations in patients with SLE are attributed to disease itself (so-called "primary" neuropsychiatric lupus). Cerebrovascular events, seizure disorders, acute confusional states and cranial or peripheral neuropathies are the most common primary neuropsychiatric events. Primary neuropsychiatric lupus (NPSLE) is mediated of either microvasculopathy or autoantibodies and inflammatory mediators. The diagnosis of primary NPSLE requires the exclusion of other causes and the clinical evaluation directs the selection of appropriate investigations. These include measurement of autoantibodies, neuroimaging, electrophysiological studies, neuropsychological assessment and analysis of cerebrospinal fluid to assess brain function and structure. Treatment should include the management comorbidities, use of symptomatic therapies and more specific interventions with either immunosuppression or anticoagulation depending on the underlying pathogenetic mechanism. Although the prognosis is variable, recent studies suggest a more favorable outcome of primary neuropsychiatric events compared to neuropsychiatric manifestations attributed to non-SLE causes.

Studies of the prevalence and incidence of NPSLE have been highly variable (21-95%)(1,24–27). Some of the earlier reports are limited due to 1) the lack of standardized criteria and definitions of neuropsychiatric events including the attribution of neuropsychiatric events to lupus and non-lupus causes; as well as 2) failure to use common and validated instruments to quantify significant outcomes such as irreversible damage and quality of life or 3) retrospective study design.

In 1999 the American College of Rheumatology developed a standard nomenclature and definitions of 19 neuropsychiatric syndromes, which can be divided into diffuse and focal, central and peripheral neuropsychiatric subsets (Table 1)(28). Guidelines on investigations and diagnostic criteria for each neuropsychiatric manifestations are provided. Whether using the ACR nomenclature in clinical practice treating a patients or in research setting, it is important to determine the attribution of each neuropsychiatric event to SLE or other non-SLE cause (29). The ACR nomenclature lists potential causes other than SLE for each neuropsychiatric syndrome, responsible in part or entirely for the manifestation. These definitions and guidance of ACR

classification, have been used to develop attribution models for neuropsychiatric event in SLE (30). Depending on the stringency of the attribution decision rules, the percentage of neuropsychiatric event attributed to SLE in new-onset SLE varies from 18% to 39% of neuropsychiatric events in 6% to 14% of patients over the first two years of the diagnosis. Although, mood disorders and headache are the most common neuropsychiatric syndromes in SLE, seizures, strokes and neuropathies are the most common manifestations attributed to SLE. The cumulative occurrence of neuropsychiatric events increased over time, although the proportion of events attributed to lupus and non-lupus causes are critical to optimizing therapies and conducting research.

Regardless of attribution, most, although not all, neuropsychiatric event in lupus patients are linked to significant negative impact on health-related quality of life, even when other factors the influence health-related quality of life such as irreversible organ damage, medications and SLE disease activity are taken into account. Clinically significant changes in health-related quality of life concur with physician determination of deterioration or improvement in neuropsychiatric event over time, indicating that health-related quality of life is a valid clinical outcome in research studies in NPSLE.

Table 1. The American College of Rheumatology nomenclature for neuropsychiatric lupus (NPSLE) syndromes

Focal NPSLE	Diffuse NPSLE	Central NPSLE	Peripheral NPSLE
Seizures	Psychosis	Seizures	Cranial neuropathy
Cerebrovascular disease	Acute confusional state	Cerebrovascular disease	Polyneuropathy
Myelopathy	Aseptic meningitis	Myelopathy	Mononeuropathy
Movement disorder	Demyelinating syndromes	Movement disorder	Myasthenia gravis
Cranial neuropathy	Anxiety disorder	Psychosis	Autonomic neuropathy
Polyneuropathy	Cognitive dysfunction	Acute confusional state	Plexopathy
Mononeuropathy	Mood disorder	Aseptic meningitis	Guillain-Barré syndrome
Myasthenia gravis	Headache	Demyelinating syndromes	
Autonomic neuropathy		Anxiety disorder	
Plexopathy		Cognitive dysfunction	
Guillain-Barré syndrome		Mood disorder	
		Headache	

C. Prognosis of Neuropsychiatric Lupus

Due to the diversity of clinical syndromes and associated neurological deficits (ranging, for instance, from mild numbness to hemiparesis), the outcome of NPSLE is challenging to define. Overall, central and peripheral nervous system involvement is linked to increased morbidity and mortality in SLE (31,32). Thus, in a large international inception cohort, mortality rate was 16% over 10 years of follow-up in patients with NP events attributed to SLE as compared to 6% in patients with no events and 7% in patients with SLE-unrelated NP events (31). Although the majority of SLE-attributed manifestations were resolved, they were related to significantly reduced health-related quality of life. In agreement, studies focusing on specific syndromes such as seizures (33), psychosis (34), and peripheral nerve disease (35) suggest high rates of resolution and low rates of recurrence yet a negative impact on the quality of life. Other manifestations, such as myelopathy (36), cerebrovascular disease (37,38) and demyelinating syndromes (39), can result in overt neurological disability. Notably, SLE patients with stroke exhibit poorer outcomes compared to the general population in terms of recurrence, recovery and mortality (37). On the other hand, common NP events not attributed to SLE, such as mood disorders (40) and headache (41), are associated with lower rates of resolution, with nearly 50% of the patients still suffering even after long-term follow-up.

D. Treatment of Neuropsychiatric lupus erythematosus

Among the plethora of manifestations encountered in patients with systemic lupus erythematosus (SLE), neuropsychiatrics is probably the most challenging in terms of diagnosis and management (42). Neuropsychiatric SLE (NPSLE) encompasses a diversity of syndromes from the peripheral and central nervous system (CNS), ranging from headaches and anxiety disorders to stroke, seizures and psychosis. In about 30% of the cases, NPSLE is due to underlying disease pathophysiology (so-called 'primary' NPSLE), while the remaining cases are related to comorbidities, administered therapies, or other causes. Primary NPSLE, which is the main scope of this review, is increasingly prevalent in contemporary cohorts with an adjusted population incidence rate of 0.5 per 100,000 persons/year (1). Accordingly, it may affect approximately 20–30% of the patients including the younger ones and those with early disease (31,43). NP manifestations incur a significant burden in SLE associated with reduced healthrelated quality of life, increased health-care utilization, disability and irreversible organ damage (37,44–48). Importantly, patients with NPSLE, especially those with damage from the NP domain, are at increased risk for mortality as compared to other SLE patients and the general population (32,49–51). Despite the recent advent of successful randomized controlled studies of novel therapeutic agents in SLE and lupus nephritis (52,53), relevant data are disappointingly absent in NPSLE as these patients are typically excluded from trials. In fact, the rarity of certain neurological and psychiatric manifestations makes recruitment of a sufficient number of patients particularly challenging. Treatment of NPSLE is also perplexed by the obscure and complex pathophysiology that variably involves ischemic/thrombotic and inflammatory mechanisms, as well as the neuronal response to these insults. Notwithstanding these considerations, in this article, we summarize the existing literature with regard to diagnosis, treatment and outcome focusing on studies published in PubMed during the last 5 years and recommendations issued by the European League Against Rheumatism (EULAR) pertaining to the management of SLE patients who manifest NP syndromes (17,54). While acknowledging the paucity of high-level evidence, we attempt to introduce practical rules and guidance to facilitate routine clinical practice. We conclude by discussing future perspectives on various NPSLE aspects based on

encouraging progress in the fields of neuro-immunology, advanced neuroimaging, biomarkers and novel therapeutic approaches.

1. The EULAR recommendations for NPSLE

In response to the need to standardize and improve the care of SLE patients who present with NP events, the EULAR endorsed evidence and eminence-based recommendations for the diagnosis and treatment of NPSLE published in 2010 (54). In this review, following analysis of a large number of articles, cumulative incidence rates of different NPSLE syndromes and major risk factors for SLE-related NP disease were reported. General principles were discussed, particularly that NP manifestations in SLE patients should be evaluated as in the general population, and treated accordingly with symptomatic, anti-inflammatory and/or anti-platelet/thrombotic agents. Furthermore, multiple statements involving the diagnostic work-up, treatment and monitoring of specific syndromes were developed, most of them with excellent agreement among experts. However, due to limited randomized controlled trials in the field, the level of evidence for the most statements was low (54). Recently, the EULAR recommendations for the management of SLE were updated including statements for NPSLE, in particular, underscoring the importance of attribution to SLE – as opposed to non-SLE – related manifestations (17).

Table 2. Practical tips for clinical practice when evaluating a patient with possible NPSLE

Tip	Comment
1. *The ACR nomenclature can miss a patient with primary NPSLE*	NP manifestations like small-fiber neuropathy or posterior reversible encephalopathy syndrome are not included in the ACR nomenclature but may occur in SLE patients (even more frequently than certain included manifestations)
2. *Do not rely exclusively on MRI to diagnose primary NPSLE*	MRI is neither sensitive, nor specific for the diagnosis of NPSLE; ~40% of patients with diagnosed NPSLE have normal conventional MRI
3. *A normal-appearing MR scan does not necessarily indicate a structurally and functionally intact brain*	Advanced neuroimaging techniques have shown microstructural and functional abnormalities in normal-appearing white matter of NPSLE patients
4. *When suspecting primary NPSLE, assess patient for generalized disease activity outside the nervous system*	Active SLE (SLEDAI > 6) is a risk factor for various NP manifestations
5. *Evaluate your patient for the presence of aPL antibodies*	aPL are a risk factor for NPSLE, especially cerebrovascular disease, chorea, seizures, myelopathy and cognitive dysfunction
6. *Symptomatic therapies are recommended for most patients with NP manifestations*	Choice of symptomatic treatment depends on neuropsychiatric manifestation, but is recommended similar to the general population
7. *A single, first episode of seizures in SLE may not warrant anticonvulsants, nor immunosuppressive treatment*	~ 60% of uncomplicated seizures with normal EEG resolve spontaneously and do not need specific treatment
8. *In SLE patients with stroke, keep a low threshold to administer immunosuppressive therapy, especially if aPL are negative*	~40–50% of strokes in SLE occur in the context of generalized disease activity and ~30–40% are in aPL-negative patients
9. *If cognitive impairment is suspected, a screening test for cognitive function is useful*	The Montreal Cognitive Assessment test (more sensitive) or the Mini Mental State Examination (more specific) are easy to screen patients for possible cognitive impairment; a positive test indicates need for thorough neuropsychological assessment
10. *If in doubt regarding attribution of a NP manifestation to SLE, an attribution model can be used*	A score > 7 in the Italian attribution model for NPSLE has a sensitivity of 88% and specificity of 82% for the diagnosis of primary NPSLE
11. *If still in doubt, a trial of glucocorticoids may be justified to monitor the therapeutic criterion*	Clinical response to a therapeutic trial of glucocorticoids at medium-to-high doses (e.g., ≥0.5 mg/kg/day) may justify subsequent use of immunosuppressive agents

ACR, American College of Rheumatology; NP, neuropsychiatric; aPL, anti-phospholipid; SLEDAI, SLE disease activity index; EEG, electroencephalogram

3. Challenges in the clinical management of NPSLE

3.1. Does this patient have primary NPSLE?

What investigations should be undertaken? Attribution of an NP manifestation to SLE (primary NPSLE) is critical to the degree that it will affect subsequent therapeutic decisions, particularly the institution, or not of immunosuppressants. To date, no single biomarker has been found to be specific for NPSLE; therefore, diagnosis relies largely on the judgment of experienced physicians and multidisciplinary groups. To assist physicians in the diagnosis of NPSLE, the American College of Rheumatology (ACR) has proposed a list of case definitions including diagnostic criteria, pertinent exclusions or confounding factors, and methods of ascertainment for a total of 19 NPSLE syndromes (Table 1) (55). 'Major' manifestations, such as seizures, acute confusional state, psychosis and others, are more often attributed by treating physicians to underlying SLE, especially when they occur in the context of generalized disease activity. The challenge is greater when it comes to less specific NP manifestations that are also common in the general population, like headaches, mild cognitive impairment, mood or anxiety disorders. For some of these 'minor' manifestations, there is even doubt that they actually represent bona fide NPSLEs, best exemplified in the case of headaches. Indeed, the sentinel study by Ainiala et al. (56) showing that removal of such manifestations from the ACR definition of NPSLE leads to improved diagnostic specificity, could tempt someone to suggest that only major manifestations should be considered for NPSLE diagnosis. However, this view is contradicted by the observation that animal SLE models experience exactly these types of NP manifestations (anxiety, disturbed behavioral patterns). To aid physicians, significant efforts to develop attribution algorithms for NPSLE have been undertaken. The multicenter Systemic Lupus International Collaborating Clinics (SLICC) group has introduced two models of different stringency which based the attribution of a NP manifestation on three parameters: i) timing of the manifestation in relation to SLE diagnosis (the closer to diagnosis, the more confident the attribution), ii) presence or absence of certain confounding factors for each manifestation, and iii) exclusion of 'common' (minor) manifestations (29). More recently, the Italian study group on NPSLE suggested a numerical score for attribution, which included the: i) temporal relationship of NP manifestations to SLE diagnosis (score ranging 0 to 3), ii) presence of minor or common

NP events (scored 0 if present or 3 if present), iii) presence of confounding factors according to the ACR case definitions (55) (score ranging from 0 if >1 factors to 2 if none), and iv) 'favoring' factors (score ranging from 0 if none to 2 if >1 factors) (57). Accordingly, the total attribution score can range from 0 to 10 and our independent, as well as a multicenter validation of this algorithm showed that a cutoff point of 7 provides the best combination of sensitivity and specificity (87.9% and 82.6%, respectively) for NPSLE (30,58). Attribution models can be helpful for physicians with limited experience in NPSLE, although the need to refer to appendix lists with the confounding and favoring factors can limit their use in daily practice. To this end, some practical tips may be useful when facing a patient with possible NPSLE, although it is understandable that it is hard to give a unifying approach for such diverse clinical syndromes (Table 2). An initial step would be to exclude non-SLE related causes, with a diagnostic workup tailored to the individual manifestation (Table 3), as outlined in the EULAR recommendations (17,59). A relevant example is the recommended work-up for cerebrovascular events, which should include cardiac monitoring for at least 24 hours with automated rhythm detection, imaging of both extracranial and intracranial arteries, and echocardiography (preferably, transesophageal) with imaging of the proximal aortic arch (38). Next, in our practice, we assess the presence of factors that are in favor of attributing an NP manifestation to SLE. In this regard, the presence of generalized (extra-neurological) disease activity cannot be overemphasized. Presence of aPL antibodies or history of previous NPSLE are also pertinent risk factors (59). Brain MRI abnormalities, especially multiple, bihemispheric WMHs, although not specific, could be supportive of primary NPSLE as are abnormal cerebrospinal fluid (CSF) findings (pleocytosis, increased protein concentration). In doubtful cases, we occasionally administer a trial of glucocorticoids (0.5 mg/kg/day prednisone equivalent, then tapered quickly) and monitor the therapeutic criterion(60). If there is improvement, subsequent immunosuppressive therapy can be considered.

Table 3. Diagnostic testing in SLE patients presenting with neuropsychiatric symptoms based on the EULAR recommendations.

Diagnostic work	Clinical syndrome	Comments
Brain MRI (conventional)	SLE patients with CNS syndromes	• Test of choice in imaging of NPSLE • Useful to exclude SLE unrelated pathologies • Recommended protocol: conventional T1/T2, FLAIR, DWI, and Gd-enhanced T1 sequences
Advanced MRI	SLE patients with CNS syndromes	• Limited use in routine clinical practice • Limited diagnostic utility
MRA/brain angiography	Cerebrovascular disease/CNS vasculitis	• Indicated to diagnose CNS vasculitis • Consider in patients with multiple strokes
CSF examination	Febrile patients High suspicion of infection	• Indicated to exclude CNS infection • Mild abnormalities are common in active NPSLE
EEG	Seizures	• Indicated to diagnose underlying seizure disorder • Epileptiform EEG predicts seizure recurrence
Electrodiagnostic testing (NCS/EMG)	Peripheral nerve disease	• Diagnosis of mononeuropathy • Distinguish multiplex mononeuritis from polyneuropathy • Differentiation between axonal and demyelinating neuropathies
Brain Biopsy	-	• Not recommended for NPSLE • Consider excluding other conditions (e.g., CNS lymphoma)
Nerve biopsy	Peripheral nerve disease	• Rarely indicated
Skin biopsy	Small fiber disease	• Indicated to diagnose small-fiber neuropathy • Consider when electrodiagnostic studies are normal
Neuropsychological tests	Cognitive disorder ACS	• Screening/diagnosis of cognitive dysfunction (e.g., MOCA) • Identification of acute confusion (CAM, DRS, MDAS)
Specific antibodies	Psychiatric disorder	• Anti-ribosomal P antibodies: limited utility • Anti-neuronal antibodies: limited utility
Visual evoked potentials	Optic neuritis	• Detection of optic nerve involvement
Heart echocardiography/ECG	Cerebrovascular disease	• Detection of structural heart abnormalities • Consider cardiac monitoring for 24 h

MRI: magnetic resonance imaging, NPSLE: neuropsychiatric lupus, FLAIR: Fluid-attenuated inversion recovery, DWI: Diffusion-weighted imaging, MRA: magnetic resonance angiography. CNS: central nervous system, CSF: Cerebrospinal fluid, EEG: Electroencephalography, NCS: nerve conduction study, EMG: Electromyography, MOCA: Montreal Cognitive Assessment, ACS: Acute confusional state, CAM: Confusion Assessment Method, DRS: the Delirium Rating Scale, MDAS: Memorial Delirium Assessment Scale, ECG: electrocardiogram, 24 h: 24 hours

3.2. Is NPSLE inflammatory or thrombotic?

Following the attribution/diagnosis of NPSLE, the next question pertains to whether the manifestation is mediated by a predominantly inflammatory or thrombotic mechanism, and thus will require treatment with immunosuppressive or antithrombotic agents, respectively. Manifestations typically considered of inflammatory origin include optic neuritis, transverse myelitis, peripheral neuropathy (especially mononeuritis multiplex), recurrent seizures, and diffuse syndromes such as psychosis, acute confusion, and aseptic meningitis. This scenario is further supported by the presence of generalized lupus activity or flare (44). An ischemic/thrombotic pathogenetic mechanism is usually implicated in the presence of aPL antibodies and/or suggestive ischemic lesions in MRI. Apart from their apparent association with cerebrovascular disease, aPL antibodies have been consistently associated with

movement disorders/chorea, seizures, myelopathy and cognitive impairment (59,61,62). Although the aforementioned distinction is generally useful, in certain circumstances it is hard to differentiate between the two mechanisms or it may be that both are in operation, as also suggested by neuroimaging and autopsy data (63,64). Strokes are illustrative examples, in this regard. In our experience, more than half of the stroke episodes in SLE patients occurred in the presence of generalized disease activity (65), a finding confirmed by others (66). Thus, although frank cerebral vasculitis is only rarely documented by neuroimaging studies, one cannot rule out the putative role of endothelial inflammation affecting the brain vasculature (vasculopathy), with obvious therapeutic implications as discussed below.

3.3. Should symptomatic therapies be used in NPSLE?

Following correction of any coexisting aggravating factors such as infections, drug adverse effects, or metabolic disturbances, the EULAR recommends the use of symptomatic treatments such as anticonvulsants, antidepressants, anxiolytics and/or antipsychotics, depending on the specific NP manifestations (17,59). However, there is a paucity of randomized evidence to clearly establish the effectiveness of such treatments in the context of NPSLE, and thus, their use remains empirical and extrapolated from the general population. Specifically, anti-epileptic therapy should not be prescribed by default in all lupus patients with a first episode of convulsions given that most individuals suffer from self-limited seizures without recurrences (67). Anticonvulsants should be considered in cases with recurrent seizures, partial seizure as the presenting seizure, brain structural abnormalities, focal neurological signs, epileptiform discharges in electroencephalograms, serious brain injury, or if disease-related risk factors for recurrence are present. The latter include moderate-tohigh titers of aPL antibodies, high disease activity, renal involvement, and concurrent stroke (54,68). Mood disorders are prevalent in SLE, most often not due to direct immune insult at the CNS, but rather associated with the overall disease burden, comorbid diseases, or other factors (40,43,69). Irrespective of attribution to SLE or not, depressive behavior should be managed with antidepressants which can be administered alone or adjunctively to anti-inflammatory /immunosuppressive

treatment. Biofeedback-assisted cognitive-behavioral and mindfulness-based cognitive therapies also have a favorable impact on depressive symptoms (70). Similar to their use in the general population, antipsychotics are indicated to control psychotic symptoms in patients with SLE. Duration of treatment should be individualized and evaluated on a regular basis considering that the majority of NPSLE patients will experience a single psychotic event (71,72). Notably, in a large SLE cohort, one-fourth of patients with disease-attributed psychosis had never received antidepressants and/or antipsychotic drugs, implying a favorable prognosis and/or response to immunosuppressive agents (72). Dopamine antagonists can be used in the setting of movement disorders or to control agitation in an acute confusional state pending the completion of diagnostic work-up (73). With regard to management of cognitive dysfunction, a randomized controlled trial (RCT) of memantine (serotoninergic receptor and nicotine acetylcholine receptor antagonist) failed to demonstrate improvement in cognitive performance over placebo in SLE patients (74). Coexisting exacerbating factors, particularly anxiety and depression, should be treated appropriately as they may impact on cognitive function. Finally, psychosocial interventions have yielded encouraging results (75,76)although additional trials will be required.

3.4. When is immunosuppressive treatment indicated in NPSLE?

The rationale for immunosuppressive therapy in NPSLE lies in its presumed pathogenesis driven by inflammatory mediators and autoantibodies/immunocomplexes. This is reinforced by the observation that many NP events develop in the setting of generalized lupus activity or flare, and, secondly, that patients with positive anti-Ro and/or anti-Sm autoantibodies are at increased risk for NPSLE (47,77–79). Empirical evidence has demonstrated the efficacy of glucocorticoids and immunosuppressants in a variety of inflammatory NPSLE syndromes. To date, a single RCT published by Barile-Fabris et al. (80), in 2005, has evaluated an induction regimen of 3 g of intravenous methylprednisolone (IV MP) followed by monthly pulses of intravenous cyclophosphamide (IV CY; 0.75 g/m2) versus IV MP bimonthly every 4 months for 1 year and then IV CY or IV MP every 3

months for another year. Enrolled patients had recurrent seizures, optic neuritis, peripheral or cranial neuropathy, coma, brainstem disease, or transverse myelitis, and the trial revealed superiority of the IV CY regimen (clinical response in 18/19 patients as compared to 7/13 counterparts who received IV MP alone). Furthermore, several observational studies have illustrated the effectiveness of IV CY, usually administered in combination with moderate-to-high starting dose of glucocorticoids and/or IV MP, in a wide range of NP manifestations including psychosis, rare forms of peripheral neuropathies and movement disorders (71,81–87). In the majority of reports, CY-treated patients had moderate-to-severe neurological deficit or had failed previous treatment with glucocorticoids alone or in combination with azathioprine. The latter can be considered as first-line regimen in less severe cases or to maintain a good clinical response induced by high-dose glucocorticoids, thus allowing for their gradual tapering. Mycophenolate (2000–3000 mg/day) is increasingly being used to treat active CNS lupus based on non-randomized studies (47,48,82–86) and extrapolation from the lupus nephritis trials, demonstrating comparable efficacy against CY (88). Intravenous immunoglobulin (IVIg) can be an alternative in selected cases of peripheral nervous system involvement or when conventional immunosuppressives are contraindicated (82,84,85). With abovementioned treatments, improvement in NP syndrome occurs in 70–80% with complete resolution of neurological deficit ranging 30–40%. Physicians often face the dilemma of whether to consider immunosuppressive therapy for NP manifestations with a more doubtful inflammatory basis; examples include nonthrombotic cerebrovascular disease in the absence of aPL antibodies, optic neuropathy with normal MR scans, and moderate cognitive impairment. In such cases, treatment decisions should be individualized taking into account the overall assessment of SLE activity, the severity and evolution of the NP manifestation (89) (Table 4). Any surrogates of lupus CNS involvement such as brain MRI abnormalities, especially in young patients with no metabolic risk factors, and intrathecal inflammation (e.g. increased protein levels) should also be considered. In this context, our study in 60 SLE patients with stroke revealed high prevalence of unremitted/active disease (60%) leading to frequent initiation of immunosuppressants, especially with cyclophosphamide or azathioprine (90). Another study assessing functional and structural brain changes in SLE patients found an inverse association of serum inflammatory markers and organ damage with neurocognitive function (91), which

suggests that some cases of lupus cognitive impairment might benefit from antiinflammatory/immunosuppressive treatment. Finally, a low threshold for immunosuppressive treatment is recommended for NP manifestations of the presumed thrombotic (i.e., associated with aPL antibodies) mechanism, which however, are relapsing or deteriorating, although relevant data are very limited. To this end, it can be argued that the existing treatment paradigm in NPSLE remains rather simplistic, as it is likely that different pathogenic mechanisms variably contribute to the same NP manifestations (92).

Table 4. Scenarios favoring the use of immunosuppressive versus anti-thrombotic treatment in NPSLE

Factors favoring the use of immunosuppressive treatment
▪ Younger patients
▪ NPSLE occurring close to SLE diagnosis
▪ Increased generalized (non-neurological) lupus disease activity or flare
▪ High score in NPSLE attribution algorithms
▪ NP manifestations of presumed inflammatory mechanism [1]
▪ Cerebrovascular event with negative aPL antibodies and after exclusion of other embolic causes
▪ Evolving or deteriorating NP syndrome, not responding to symptomatic treatments
▪ Relapsing NPSLE
▪ Moderate-to-severe neurological deficits
▪ Inflammatory CSF
▪ Abnormal MRI in the absence of confounding factors [2]
▪ Improvement with trial of glucocorticoids
Factors favoring the use of anti-thrombotic treatment
▪ Positive aPL antibodies, especially at moderate/high titers [3]
▪ Cerebrovascular disease with positive aPL antibodies
▪ Ischemic/thrombotic lesions on MRI in the context of atherosclerotic risks factors or aPL antibodies
▪ High cardiovascular risk [3,4]
▪ NP manifestations of presumed ischemic mechanism not responding to immunosuppressive agents

[1] Especially recurrent seizures, optic neuritis, myelopathy, psychosis, peripheral nerve disease, acute confusional state after excluding other causes

[2] Advanced age, smoking, atherosclerotic risk factors, chronic glucocorticoids use

[3] After balancing individual bleeding risk

[4] Assessed by risk assessment tools (e.g., QRISK3)

NP, neuropsychiatric; MRI, magnetic resonance imaging; CSF, cerebrospinal fluid, aPL, anti-phospholipid

3.5 Are biologics effective in NPSLE?

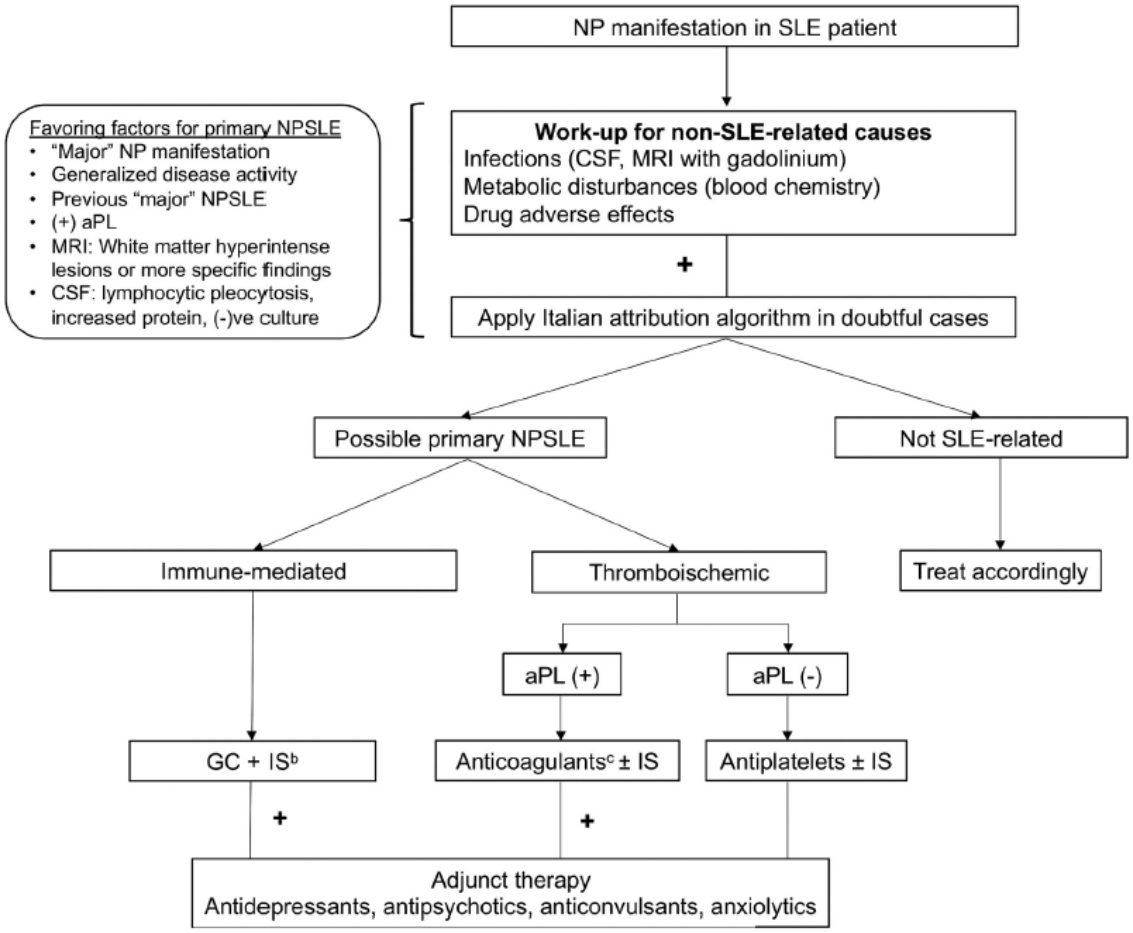
Despite the lack of supporting RCTs, B-cell depleting agents, such as rituximab (anti-CD20 monoclonal antibody), have demonstrated efficacy in observational studies of active SLE, including NPSLE. Most reports evaluated refractory disease (discussed below) with the majority of patients having previously received CY or other immunosuppressants in combination with glucocorticoids (82,83,93–96). In a few cases, rituximab has been administered as a first-line treatment of severe NP manifestations (97,98), although this practice is currently not recommended, unless conventional immunosuppressive agents are contraindicated. Belimumab (anti-BAFF), currently licensed for SLE patients with active disease despite standard-of-care treatment, has not been formally evaluated in NPSLE. Although belimumab inhibits the activation, survival and differentiation of B-cells and thus, might be presumed to improve CNS disease, postmarketing data have triggered cautiousness due to increased – albeit marginally – rates of serious depression (by 0.30%) and suicide or self-injury (by 0.50%) in belimumab- versus placebo treated SLE patients (99). The reason for this effect remains elusive, although it might be related to the local expression of BAFF and its receptors in the CNS (100). Pending additional studies, belimumab is not indicated for the management of active NPSLE.

3.6. When should antiplatelet/coagulant treatment be considered in NPSLE?

Administration of antiplatelet or anticoagulation therapy depends on two factors: i) the presenting NP manifestation, and ii) the presence or not of aPL antibodies. SLE patients with a high-risk aPL profile should be offered antiplatelet therapy for primary thrombo-prophylaxis, including prevention of cerebrovascular disease (17,54,101), irrespective of whether they have experienced an NP event. For aPL-positive patients who develop NP manifestations, the pertinent question relates to whether antiplatelets suffice or oral anticoagulation should be used instead. To this end, a clear indication for anticoagulation exists only in the case of aPL-related ischemic stroke, thus fulfilling the criteria for thrombotic antiphospholipid syndrome (APS). In such cases, the EULAR recommends for a target INR of 2–3 or 3–4, depending on the patient's individual risk

and history of recurrent thrombosis, while the combination of low-dose aspirin to a vitamin K antagonist with a target INR 2–3 is an alternative (101). Use of novel oral anticoagulants should generally be avoided because APS patients with arterial thrombosis (including stroke) are at higher risk for recurrence (101,102). For other NP manifestations that occur in the context of aPL antibodies, there is little evidence to support the generalized use of anticoagulation. A systematic literature review found that adding anticoagulation to background immunosuppressive therapy offered no additional benefit in lupus myelopathy (103). Nonetheless, in manifestations strongly linked to aPL antibodies, especially seizures and chorea, that are not responding to the combination of immunosuppressive and antiplatelet treatment, anticoagulation might be considered a rescue treatment although relevant data are missing. If aPL antibodies are negative, anticoagulation is not typically indicated, with the exception of selected cases of cardioembolic cerebrovascular disease (e.g., due to atrial fibrillation) and following consultation by a stroke specialist. Most stroke cases in aPL-negative patients will nevertheless be managed with low-dose aspirin and other preventative measures, including lipid-lowering agents, as in the general population. For NP manifestations other than cerebrovascular disease, antiplatelets might be considered only in the context of primary prevention and following cardiovascular risk assessment (104). To this end, Figure 1 outlines our approach to the diagnosis and management of NPSLE in daily clinical practice.

Figure 1. Diagnostic and therapeutic approach to suspected neuropsychiatric involvement in SLE.



NP, neuropsychiatric; aPL, antiphospholipid antibodies; MRI, magnetic resonance imaging; CSF, cerebrospinal fluid; IS, immunosuppressive agents; GC, glucocorticoidsa Excluding headache,anxiety disorder, mild mood disorder, mild cognitive impairment, polyneuropathy without electrophysiologic confirmationb Preferred agents: azathioprine (for mild cases), mycophenolate, cyclophosphamide, rituximab. Anticoagulants are indicated mainly for aPL-related stroke; for other aPL-associated manifestations (e.g. chorea), antiplatelet therapy may be sufficient

3.7. How should NPSLE be monitored?

There are no firm data regarding optimal monitoring of patients with NP involvement. This frustrating reality is reflected in most observational studies, which have used a (subjective) 7-point Likert scale (ranging from symptom resolution to patient death) to assess the NPSLE prognosis and response to therapy (93,105). Accordingly, the monitoring approach should be individualized and guided also by the type of manifestation. Repeat imaging could be considered in cases with abnormal baseline MRI findings, to assess response to therapy, as well as if there is clinical evidence for NPSLE relapse. While useful in cases, e.g. of lupus myelopathy or demyelinating episodes, the value of follow-up MRI is less clear in cases with nonspecific WMHs, which have a poor correlation with the clinical syndromes and response to therapy. Timing and frequency of repeat neuroimaging may be as frequent as monthly, in cases of longitudinal myelopathy with severe neurological deficits to assess response to immunosuppression, and to every 6–12 months in other manifestations with abnormal baseline MRI. Similarly, other examinations should be tailored to specific scenarios (for instance, electroencephalogram in seizures, electroneurogram in peripheral nervous system involvement).

3.8. How should refractory or relapsing NPSLE cases be treated?

Although complicated by the abovementioned shortcomings in monitoring and the lack of standardized definitions, about 10–30% of NPSLE patients will demonstrate inadequate clinical response to first-line immunosuppressive treatment including cyclophosphamide. As part of the evaluation of disease refractoriness, physicians should consider – through multidisciplinary approach – the extent of permanent neurological damage that might have accrued, which thus may not be reversible upon treatment. This is particularly relevant for manifestations, such as cerebrovascular disease, myelopathy and peripheral neuropathies, where delays in initiation of treatment may result in irreversible deficits. A large body of non-randomized evidence suggests the effectiveness of rituximab (in combination with glucocorticoids) across a wide range of central and peripheral nervous system manifestations (82,83,93–96). Response

rates are reported to be as high as 90% although this might be an overestimation due to reporting bias. The duration of treatment is decided on an individual basis but some patients will require prolonged cycles of rituximab in order to consolidate and maintain the response. IVIg represents an alternative especially in cases of peripheral nervous system involvement (82,93,94,106). Plasma exchange and immunoadsorption represent third-line treatment options depending on the available expertise (107). In light of the paucity of published data, cases of relapsing NPSLE can be re-induced with the same regimen that was initially used or be treated as a refractory disease, especially in the scenario of multiple relapses.

4. Conclusions

Although the rheumatological community has witnessed significant progress with controlled trials of SLE, including lupus nephritis, there is still weak evidence to guide most therapeutic decisions in SLE patients who develop NP manifestations. Nonetheless, results from well-characterized NPSLE cohorts with long follow-up and advanced neuroimaging studies assessing both the CNS structure, perfusion and function, and basic/translational research elucidating pathogenic mechanisms in neuroinflammation and injury, have all contributed to our better understanding of this complex entity. Importantly, the EULAR recommendations (17,54) provide a useful framework for the treatment of NPSLE through integration of existing evidence and expert opinion. Still, a number of pertinent issues remain ill-answered, such as the precise indications and intensity of immunosuppressive therapy according to different NP manifestations, effectiveness of new biologics in CNS lupus, the role of antithrombotic agents in aPL-negative or positive NPSLE patients who, however, do not fulfill the APS criteria, the definition of clinical (and imaging) response criteria to be implemented in a 'treat-to-target' strategy similar to other SLE manifestations. To address these challenges and consider the low frequency of certain NP manifestations, collaborative and interdisciplinary research efforts should be intensified aiming at a better classification of NPSLE based on the prevailing pathophysiology (inflammatory, ischemic/thrombotic, mixed) and detailed assessment of therapeutic responses and long-term outcomes. We remain optimistic that technological advances in genomics, imaging and big-data analysis will facilitate these endeavors.

Highlights

- Attribution algorithms can help to discriminate SLE-related *versus* -unrelated neuropsychiatric events
- Determining the inflammatory or ischemic/thrombotic basis of NPSLE by considering the type of manifestation, the disease state, serological (e.g., antiphospholipid antibodies) and imaging findings, is critical to guide appropriate treatment
- Cyclophosphamide in combination with glucocorticoids are recommended for active, severe inflammatory NPSLE
- Rituximab can be used in refractory cases, whereas the role of other biologics remains unknown
- Besides novel anti-inflammatory agents, neuroprotective strategies are being explored in NPSLE
- Research in the field of biomarkers and advanced neuroimaging modalities might further assist the diagnosis and treatment of patients with NPSLE

E. Future perspectives in Neuropsychiatric Lupus

1. Clinical tools and endpoints

In addition to its profound heterogeneity, clinical trials in NPSLE are plagued by the lack of objective, clinically meaningful, and 'hard' endpoints to monitor therapeutic response. Thus, existing disease activity indices (e.g., SLE Disease Activity Index, British Isles Lupus Assessment Group index) and definitions of remission, are overly generic and insensitive to changes in NP syndromes (108). To overcome this limitation, better clinical tools need to be developed that can be used both in trials and in practice. To ensure accuracy, in our view, these endpoints will inevitably have to be tailored according to each manifestation. In an observational study on the use of CYC in NPSLE, we used predefined objective criteria to define the complete response, partial response, stabilization, and deterioration/therapy failures for each individual manifestation (93). As an example, the complete response to myelopathy was defined as an Edmus grading scale at last, follow-up better than the baseline, along with a modified Rankin scale (mRS) <1; a mRS <3 indicated partial response. A similar approach to develop formal outcome measures for use in clinical trials and routine practice will require assembly of a Task Force of experts on NPSLE and subsequent validation studies.

2. Advanced neuroimaging tools

Significant progress has been made over the years in the field of neuroimaging modalities, which have yielded promising results (109,110). Various techniques have been used in SLE including quantitative MRI (qMRI) consisting of MR relaxometry, perfusion-weighted MRI, functional MRI (fMRI), proton magnetic resonance spectroscopy (1 H-MRS), magnetization transfer imaging/ratio (MTI/MTR), diffusion weighted/tensor imaging (DWI/DTI) and positron emission tomography (PET)/ single-photon emission computed tomography (SPECT), all demonstrating high sensitivity to detect CNS structural and functional abnormalities. A few notable examples include the quantification of MTR histogram peak heights in the brain WM, which are significantly lower in inflammatory than ischemic NPSLE and nonNPSLE patients

(111). Interestingly, these alterations were reversed following immunosuppressive treatment, thus offering a possible diagnostic and monitoring tool (112). DWI/DTI has revealed microstructural abnormalities in normalappearing WM in NPSLE although further research is required to put these results into clinical context (113). Moreover, 1 H-MRS, which measures the concentration of neuronal metabolites, has detected increased levels of myoinositol and choline (suggestive of glial activation and vasculopathy) and decreased levels of N-acetylaspartate (consistent with neuronal impairment) in SLE (114,115). At the functional level, MR studies have revealed altered brain responses, particularly in default mode network regions and the caudate, correlating with cognitive performance (116), and resting state fMRI has suggested perturbed brain functional connectivity in SLE patients (117). Recently, our group performed perfusion-weighted MRI and observed decreased cerebral blood flow in SLE and NPSLE patients, especially in the semioval center in normal-appearing WM. This finding, coupled with conventional MRI data, could discriminate SLErelated versus - unrelated NP disease with an excellent specificity (118). Finally, 18-FDG PET has demonstrated both hypoand hyper-metabolism in the WM and other brain regions, the latter finding being indicative of the CNS inflammatory response (119,120). Notwithstanding these advances, none of the abovementioned neuroimaging tools has yet been introduced in routine clinical practice due to pending validation and standardization studies and also because protocol registration and data analysis require relevant expertise. Another consideration is that no single method has sufficiently high accuracy for NPSLE. Therefore, a combination of different neuroimaging markers will most likely be needed, similar to other neurological diseases (121,122). To this end, machine learning algorithms could be advantageous in constructing effective diagnostic or prognostic models based on a combination of scrutinized neuroimaging, serological and clinical data (123).

3. Biomarkers

In view of the limitations of existing clinical and neuroimaging tools, research efforts are directed toward the identification of novel biomarkers for diagnosis, pathophysiological classification (i.e., inflammatory versus thrombotic) and monitoring of NPSLE. A number of candidate serum and/or CSF proteins have been evaluated,

most of them displaying insufficient diagnostic accuracy (109). CSF IL-6 levels have been correlated with NPSLE, especially diffuse syndromes; however, clinical utility is limited due to modest specificity against other inflammatory CNS disorders (124,125). Other mediators, such as TNFlike weak inducer of apoptosis (TWEAK), have also been investigated as a putative marker of compromised blood-brain barrier in SLE (126,127). Furthermore, the determination of neuronal cell surface autoantigens via mass spectrometry has been used to differentiate NPSLE from non-NPSLE with high accuracy (128). Hopefully, -omics technologies might further enhance the unbiased biomarker identification through the analysis of easily accessible biological specimens.

4. Novel therapeutic agents

Driven by progress in the fields of immunology and novel drug design, a number of targeted/biological agents are currently being evaluated in SLE. Anifrolumab, a fully human monoclonal antibody that binds to type I interferon receptor (IFNAR) and blocks the activity of all type I interferons, has shown promising results in a large phase III RCT in active SLE (129). Patients with active NP disease were excluded from that trial; therefore, the location of anifrolumab in the treatment of CNS lupus is unknown. Notably, animal studies have yielded conflicting results since adenovirus administered interferon was shown to exacerbate mental disorders, deficits in sociability and cognitive impairments in NZB/W F1 lupus-prone mice (130), but anti-IFNAR treatment failed to reverse NP disease in the MRL/lpr model (131). Other therapeutic approaches currently explored at the preclinical stage include modulators of the sphingosine 1-phosphate receptor (132) and TWEAK/Fn14 (126) pathways, and small molecules, such as Bruton's tyrosine kinase inhibitors (133). Notably, the extent to which the presumed efficacy of these agents is driven by systemic versus CNS-directed effects remains elusive. Figure 2 depicts possible therapeutic agents targeting diverse pathways implicated in the pathogenesis of NPSLE.

5. Neuroprotective strategies

Neuroprotective strategies represent an evolving area in the treatment of neurological disorders, aiming at prevention of brain injury from immunological or other insults, correction of defective neuronal cells, and/or induction of regeneration and recovery of the injured nervous system (134,135). These agents act through multiple mechanisms targeting neurotoxic pathways, excitotoxic cascades, distinct inflammatory responses, free radical toxicity, and neuronal cell death by apoptosis (134). Candidate drugs include calcium channel blockers, sodium channel blockers, gamma-aminobutyric acid (GABA) antagonists, glutamate inhibitors, nitrogen oxide inhibitors, and free radical scavengers. In CNS lupus, a subset of anti-DNA antibodies cross-reacts with the NR2 subunits of NMDAR leading to neural cell apoptosis (136). Accordingly, NMDAR blockage with glutamate inhibitors (selfotel, aptiganel, and eliprodil) might offer neuroprotection. Indeed, anti-NMDAR strategies resulted in a reduced infarct size in stroke mouse models (137). Disappointingly, human trials were terminated prematurely due to side effects and poor benefit induced by these agents (138). A recently characterized neuroprotective pathway in NPSLE involves the angiotensin-converting enzyme (ACE) as part of the kallikrein–kinin/renin-angiotensin system (139,140), which regulates several physiological processes including brain functions and inflammation (140,141). In MRL/lpr mice, administration of captopril, a centrally acting ACE inhibitor, resulted in reduced type I interferon response, microglia activation, and IgG deposits at the CNS, and improved NP performance (140). The putative beneficial effects of captopril were also demonstrated in murine NPSLE induced by anti-DNA antibodies cross-reacting with NMDAR (139). From a mechanistic perspective, ACE degrades bradykinin into inactive peptides, and accordingly, the interferon-suppressive effect of captopril is dependent on the bradykinin receptor (140,142). Accordingly, ACE inhibitors represent candidate neuroprotective agents for further clinical evaluation.

Figure 2.

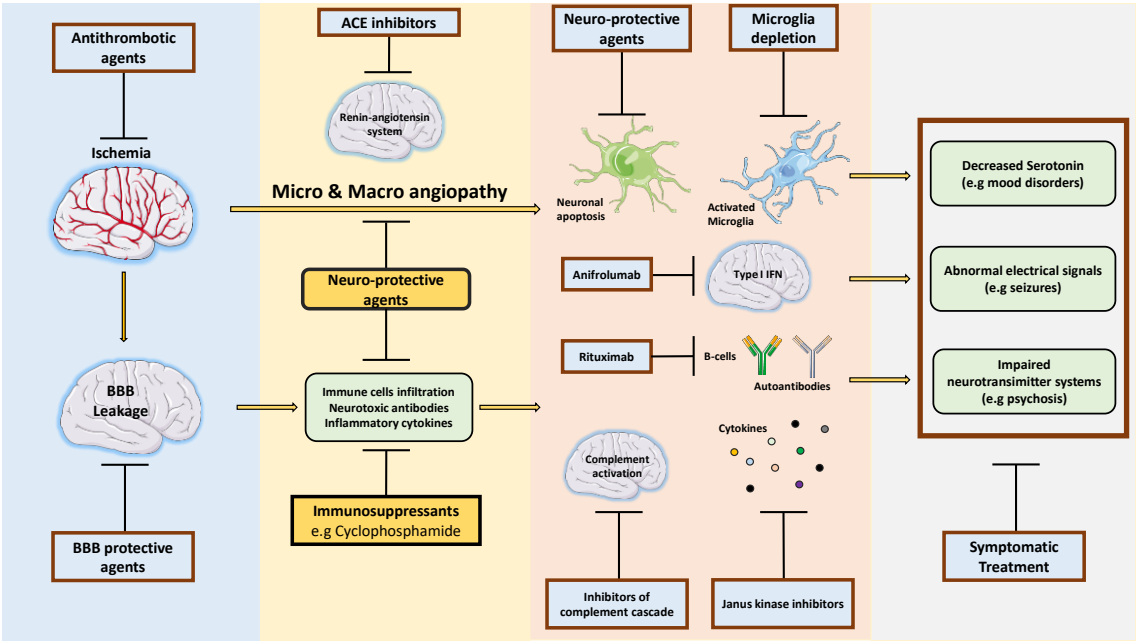


Figure 2 (73). Overview of major mechanisms and effector pathways implicated in CNS lupus pathogenesis presenting as targets of current and future therapies. NPSLE has complex pathogenesis involving blood-brain barrier (BBB) disruption that enables circulating neurotoxic autoantibodies and immune components to reach the brain. Neuronal cell-targeting autoantibodies and B cells are implicated as evidenced by the detection of anti-N-methyl D-aspartate receptor (anti-NMDAR) and anti-ribosomal P antibodies in a fraction of NPSLE patients. Effector molecules and pathways have been demonstrated including TWEAK/Fn14, sphingosine 1-phosphate signaling, Nogo-a/NgR1, renin−angiotensin system, and tyrosine kinases. Animal studies and brain autopsies also underscore an important role for complement activation. Recently, microglia has emerged in CNS disease especially cognitive dysfunction. Observations from neuroimaging and autopsy studies emphasize the role of ischemia in NPSLE. Notably, ischemic pathologies, such as microthrombi and diffuse vasculopathy, tend to coincide with findings suggestive of neuroinflammation, such as vasculitis and complement deposition, thus reiterating the notion that both processes may be involved in SLE CNS injury. ACE: angiotensin-converting enzyme; IFN: interferon

F. Pathogenesis of Neuropsychiatric lupus erythematosus

Systemic lupus erythematosus (SLE) frequently affects the central and peripheral nervous system, a syndrome collectively termed neuropsychiatric SLE (NPSLE)(143). Up to 40% of SLE patients may experience at least one NP event over the course of their disease, with less than half of these manifestations directly attributed to lupus *per se*(144). The underlying pathogenesis remains ill-defined (89), due to limited access to tissue, the diversity and complexity of clinical manifestations, and the overlap with non-SLE related NP events(143).

One of the early key assumptions in NPSLE was that a disrupted blood brain barrier (BBB) allowed autoantibodies and immune components of peripheral blood to penetrate into the central nervous system (CNS), causing inflammation and damage(145). Among autoantibodies, anti-N-Methyl-D-Aspartate receptors (anti-NMDA) and anti-ribosomal P (anti-RP) can become pathogenic upon entering the brain; the role of other autoantibodies remains poorly understood(146)(147). Recently, type I interferon (IFN) and microglial cells have emerged as central players in CNS disease, with recent studies substantiating their role in NPSLE(148)**(149).

Overview and Evolving Concepts in NPSLE

In NPSLE, a "mosaic" of genetic, environmental and neuroendocrine factors culminates in *neuroinflammation and cerebral ischemia*, the two major mechanisms operant(150). Brain autopsies of patients with NPSLE show diffuse vasculopathy, microthrombi, microinfarction, macroinfarction and vasculitis, along with complement deposition(151)*. The presence of "vasculopathy" is supported by the high prevalence of white matter hyperintense lesions on brain MRI, representing microvascular disease, and the strong association of certain NPSLE syndromes with antiphospholipid antibodies (aPL)(89)(61). On the other hand, in the setting of a BBB disruption, the presence of inflammatory mediators and autoantibodies in the cerebrospinal fluid (CSF) of lupus patients highlights the role of an immune response and CNS inflammation(152). In clinical practice, in a given patient it is often hard to distinguish between ischemia and inflammation. When in doubt both immunosuppressive and antithrombotic agents, especially in aPL-related NP events, may be used(17).

Brain barrier disruption: Global vs localized

BBB is a highly selective semi-permeable border of CNS vessels, formed mainly by brain capillaries at the level of endothelial cells with specialized tight junctions(153). The umbrella term ‘’BBB disruption’’ denotes the impairment of any structure of the human CNS that can potentially be distorted, allowing immune and toxic components of the blood to enter(145)(154). Historically, BBB disruption was the first pathophysiological mechanism proposed to play a role in NPSLE pathogenesis. Early studies showed the presence of IgG, albumin, and inflammatory cytokines in the CSF of patients with lupus and in lupus-prone mice(152)(155). Due to the complexity of BBB and inability to fully visualize the loss of integrity in vivo, it remains unclear whether these molecules originate from peripheral blood or are produced intrathecally.

Over the last years, more structures of the brain have been recognized as ‘’barriers’’ of the CNS, including the blood-CSF barrier (BCSFB). The choroid plexus is a plexus of modified ependymal cells located in the ventricles that produces the cerebrospinal fluid. The BCSFB - located at choroid plexus (CP) epithelial cells- is the natural “dam” between the systemic circulation and CSF. Thus, the presence of inflammatory mediators in the CSF of NPSLE patients(152) can also be explained by a disrupted BCSFB rather than global dysfunction of BBB. Accordingly, in recent years studies have focused on BCSFB in MRL/lpr mice, demonstrating that BCSFB is disrupted in the absence of BBB dysfunction(156). A recent study confirmed the presence of infiltrating leukocytes through the BCSFB of MRL/lpr mice and detected CD4+ and CD8+ T-cells at the level of choroid plexus (CP). Of interest, T-cells were predominantly T-follicular helper cells (Tfh) producing IFN-γ and Bcl-6, with an almost complete absence of regulatory, T-cells such as T-follicular regulatory cells and Tregs(157)*. Together, these results suggest that the abnormal BCSFB may represent a central mechanism in NPSLE pathogenesis, although this hypothesis requires further study.

Two interesting anatomical components that potentially regulate the movement of immune mediators from the systemic circulation into the CNS, are the meningeal barrier and glymphatic system(158)(159). The former may represent another route for immune substances to move into CNS. On the other hand, the glymphatic system is a

recently introduced perivascular system, which participates in the clearance of interstitial solutes out of the CNS(160) and allows the exchange of molecules between CSF and interstitial spinal fluid (ISF). In neurodegenerative diseases, such as Parkinson's and Alzheimer's, the glymphatic system inhibits the clearance of proteins, participating in the underlying pathogenesis(161). To date, there are limited studies regarding its role in the pathogenesis of other CNS diseases.

Autoantibodies in NPSLE

In addition to anti-NMDA, aPL and anti-RP, many autoantibodies have also been detected in NPSLE patients, yet they lack sensitivity and specificity(146)(147). From a clinical perspective, B-cell depletion with rituximab may be beneficial in some NPSLE cases(95). Of note, this has not been confirmed in murine studies, as early B-cell and/or antibody depletion did not modify or prevent neuropsychiatric disease in MLR/lpr mice(146). The same group showed that NP manifestations remained unaffected after early bone marrow transplantation, while systemic inflammation, including nephritis, was attenuated(162). Thus, the role of B-cells and antibodies in CNS disease has not been fully elucidated(147)(146).

A subset of anti-ds DNA antibodies (termed DNRAb) recognize an extracellular domain of the NMDA receptor subunits NR2a and NR2b, and thus cross-react with the NMDA receptor, leading to neural cells apoptosis both in human and murine disease(136). Direct injection of DNRAb in mice induced neuronal apoptosis at the level of hippocampus, leading to cognitive impairment. The effect of anti-NMDA antibodies is dose-dependent, since at high concentrations they can induce excitotoxic cell death, whereas at lower concentrations they do not cause neuropsychiatric manifestations(163). Of interest, these abnormalities were detectable even when DNRAbs were no longer present in the hippocampus(164). Anti-NMDA antibodies may damage the BBB *in vitro* and penetrate into the CNS(165). Nevertheless, these antibodies may also be present in SLE patients without NP involvement(166)(167) and thus these data need to be interpreted with caution.

Anti-RP antibodies are highly specific for SLE and have been associated with several NPSLE syndromes, especially psychosis and depression(167)(168). Anti-RP react with

epitopes on the surface of neuronal cells, known as cross-reacting neuronal surface protein P (NSPA)(169). González *et al* demonstrated that NSPA is a ubiquitin ligase which regulates the function of the NMDA receptor at the synaptic region(170). Anti-RP bind to NSPA which is distributed in brain regions involved in memory and emotion leading to neuronal apoptosis via intracellular Ca2+ influx(171). This provides a molecular link between NSPA and the NMDAR - known to be involved in plasticity and synaptic transmission related to memory -, suggesting a possible pathogenic role for anti-RP. Importantly, injection of these antibodies through the limbic system or peripheral circulation leads to cognitive impairment and depression in mice(172)(173).

aPL antibodies are major risk factors for NPSLE, especially for focal syndromes like cerebrovascular disease(61)(174). aPL carriers may also be at increased risk for subclinical atherosclerosis, although this has not been firmly established(175); aPL may also affect the small vessels creating a microthrombotic environment within the CNS and consequent cerebral microangiopathy. This local vascular injury to small vessels may disrupt the BBB(176)(177). Intracerebroventricular injection of aPL induced a hyperactive behavior in mice implying a direct pathogenic role(178).

The role of the activation of microglia in NPSLE pathogenesis

Microglia, the resident macrophage cells of the brain, account for 10–15% of all neuronal cells. They act as the first and main form of active immune defense in CNS, responding to pathogens and injury by changing morphology and migrating to the site of infection/injury, where they destroy pathogens and remove damaged cells(179). As part of their response, they secrete various cytokines, chemokines, prostaglandins and reactive oxygen species.

Accumulating evidence support an active role for *microglial cells* in the pathogenesis of NPSLE. Lupus-prone mice lacking estrogen receptor alpha experienced a significant reduction in memory errors, which correlated with decreased number of activated microglial cells and an accompanying reduction of CNS inflammation(180). Administration of colony stimulating factor-1 receptor (CSF-1R) kinase inhibitor - which crosses the BBB causing microglia depletion(181) - in MRL/lpr mice improved depression(182)*. Microglia are activated by sera of patients with SLE *in vitro*, but the

actual factors responsible for this activation are unknown (183). More recently, robust evidence for the role of microglia in CNS lupus came from a study by Bailas *et al*, who documented an IFN-driven microglia-dependent synapse loss pathway, using the 564Ig mouse model(148)**. In this paper, peripheral type I IFN was found to enter the brain and activate the IFNαR and microglia. The latter then engulfed synaptic material leading to synapse loss and subsequent cognitive impairment. Mice treated with IFNαR blocking antibody (anifrolumab) exhibited attenuation of CNS disease.

Another study(139)**, used the DNRAb+ mouse model (immunization with the DWEYS peptide) to explore the role of microglia in autoantibody-mediated CNS lupus. DNRAb+ mice exhibited increased microglia activation and a decrease in dendritic complexity, which was reversed when microglia was depleted. This decreased spine density and dendritic complexity were dependent on C1q. The latter binds to dendrites using high mobility group box 1 protein as mediator, with C1q serving as a bridge to NMDARs. Importantly, administration of captopril [an angiotensin-converting enzyme (ACE) inhibitor, which crosses the BBB] significantly reversed the activation of microglia and improved the cognitive function of mice(139)**.

In MLR/lpr mice, reactive microglia may be activated through the nuclear factor κB (NF-κB) pathway, highlighting the role of TNF-α as mediator; inhibition of NF-κB led to decreased CD68 expression (activation marker) in microglia(184). In another study, treatment with fingolimod (a modulator of sphingosine-1-phosphate, which sequesters lymphocytes within lymph nodes) attenuated the depressive behavior and cognitive impairment of MLR/lpr mice. RNA-sequencing analysis of fingolimod-treated microglia revealed downregulation of multiple immune-mediated pathways, including NF-κB signaling and IFN response with negative regulation of type I IFN-mediated signaling; this was associated with increased IFNβ expression(185)*. Finally, lipocalin-2 (LCN2), a protein which promotes microglial M1 polarization(186) was detected at increased levels in the serum of NPSLE subjects. Lupus-prone mice with LCN2 deficiency performed better in neuropsychiatric tests exhibiting decreased microglia activation and brain apoptosis. LCN2 directly regulates immune microglia-associated pathways suggesting yet another pathogenic mechanism(187). Overall, these data indicate that microglia cells are central players in CNS lupus and may serve as targets for novel therapies.

Intracellular signaling pathways in NPSLE: A role of kinase inhibitors?

Tumor Necrosis Factor-like Weak inducer of apoptosis (TWEAK), a TNF superfamily member, promotes the activation of NF-κB and mitogen-activated protein kinase via its receptor, fibroblast growth factor-inducible 14 (Fn14)(188). Evidence towards the involvement of the TWEAK/Fn14 pathway in NPSLE is growing. TWEAK displays a dual role in both neuroinflammation and cerebral ischemia(189). Increased expression of TWEAK/Fn14 was detected within the cerebral cortex of MRL/lpr mice; knocking-out Fn14 improved depression and cognitive function(190). Importantly, this finding was accompanied by a reduction of immune infiltrates, fibronectin, IgG deposition and complement activation in brain histology(191). Intracerebroventricular injection of TWEAK in wild-type mice induces cognitive dysfunction and depression-like behavior through increased BBB permeability and accelerated neuronal cell death(191)(192).

Bruton's tyrosine kinase (BTK) is essential for the function of B-cells and macrophages. Inhibition of this pathway by use of a specific inhibitor (BI-BTK-1) in MRL/lpr mice, resulted in decreased accumulation of macrophages, T-cells and B-cells in the CP and improved cognitive function(193)*. In view of the recent promising data of baricitinib in SLE(194), ibrutinib, a selective BTK inhibitor, could potentially prove useful in neuropsychiatric disease. Of interest, evobrutinib, another BTK inhibitor, was evaluated in patients with multiple sclerosis in a Phase 2 trial with promising results(195).

Neurite outgrowth inhibitor-A (Nogo-a) with its respective receptor, NgR1, form a signaling pathway which mediates inhibition of neuron generation. Compared to other autoimmune or neurological diseases, patients with NPSLE overexpress Nogo-A in the CSF(196). Increased levels of Nogo-α/NgR1 were also observed in MLR/lpr mice; administration of Nogo-66(1−40), an antagonist, improved cognitive function, decreased expression of proinflammatory components and reduced axonal degeneration and demyelination(196). (Table 5)

Type I Interferon and NPSLE

SLE is characterized by a robust IFN molecular signature in most patients. A link between NPSLE and IFN has been proposed based on clinical and molecular findings of monogenic interferonopathies, such as Aicardi–Goutières syndrome (AGS). AGS is an inflammatory disorder mainly affecting the skin and brain, characterized by aberrant secretion of type I IFN and lupus-like systemic features(197). Among the responsible mutated genes for AGS, is the three prime repair exonuclease 1 (TREX1)(197), a susceptibility gene for SLE(198) and, more specifically, CNS lupus (199). Brain pathology of patients with AGS shows small vessel disease, including aneurysmal dilation, vasculitis and thrombotic microangiopathy(197) findings also seen in SLE(151)*.

Of note, IFN-α causes endothelial cell damage promoting abnormal angiogenesis in SLE patients, which may also involve CNS vessels(200). Whether IFN *per se* causes cerebrovascular disease, frequently manifest in patients with increased IFN levels, or is merely an epiphenomenon, remains to be defined. Patients with various diseases treated with IFN-α or IFN-β, developed thrombotic microangiopathy suggesting a possible role of IFN on vascular damage(201). Monogenic interferonopathies could serve as a model to study the role of IFN in NPSLE pathogenesis.

Table 5. Therapeutic Targets in NPSLE

Target	Evidence and Rationale	Experimental setting	Potential drugs	References
Type I IFN pathway	Type I IFN activates microglia, which then engulfs synaptic material leading to cognitive impairment. Mice treated with IFNαR blocking antibody, exhibited attenuation of CNS disease.	564Igi lupus-prone mice	Anifrolumab (Type I IFN receptor inhibitor)	7**
Renin-Angiotensin system	Microglia and C1q are essential in neuronal damage process. ACE inhibitors can prevent microglia activation preserving cognitive status and neuronal function.	BALB/c mice immunized with DWEYS peptide, leading to DNRAb+ production	Captopril, other ACE inhibitors	47**
BTK	Treatment with BI-BTK-1 (a novel inhibitor of BTK) significantly attenuated the neuropsychiatric disease along with decreased accumulation of macrophages, T-cells and B-cells within the CNS	MRL/lpr mice	BTK inhibitors (BI-BTK-1, ibrutinib, evobrutinib)	57*
Nogo-a/ NgR1 pathway	Nogo-a/ NgR1 pathway is involved in NPSLE. Treatment with Nogo-66(1−40) antagonist improved cognitive function and myelin repair	MRL/lpr mice	Nogo-66 (1−40), an antagonist of NgR1 receptor	60
S1P signaling pathway	Modulation of the S1P signaling pathway may serve	MRL/lpr mice	Fingolimod, a S1P receptor modulator that sequesters	49*

	as a novel therapeutic target in CNS lupus		lymphocytes within lymph nodes	
LCN-2, a protein which promotes microglial M1 polarization ; a major regulator of innate immunity	Increased levels of LCN-2 were detected in the serum of NPSLE subjects. Cognitive impairment and depression-like behavior were attenuated in lupus-prone mice lacking LCN-2.	Sle1,3 lupus-prone mice	-	51
Activated microglia cells	Lupus-prone mice treated with CSF-1R (microglia depletion) exhibited improvement in the depression-like behavioral deficit	MRL/lpr mice	GW2580, a small CSF-1R kinase inhibitor; depletion of microglia	45*
TWEAK/ Fn14 pathway	TWEAK/Fn14 interactions promote the loss of BBB integrity and increase neuronal damage and the accumulation of inflammatory cells in the choroid plexus	MRL/lpr mice	Monoclonal antibodies (hIgG1) against Fn14	54
Complemen t cascade	Complement deposition was increased in brain tissue of SLE patients suggesting an underlying pathogenic role.	Human brain autopsies	Eculizumab (inhibitor of complement factor C5	10*

IFN, Interferon; IFNaR Interferon-a Receptor; CNS, Central nervous system; ACE, angiotensin converting enzyme; BKT, Bruton's tyrosine kinase; S1P, sphingosine-1-phosphate; LCN-2, Lipocalin-2; CSF-1R, colony stimulating factor-1 receptor; TWEAK/Fn14; Tumor Necrosis Factor-like Weak inducer of apoptosis/ fibroblast growth factor-inducible 14.

Transcriptomic analysis in SLE: Brain as a causal tissue

Transcriptomic analysis of SLE by RNA sequencing has revealed novel molecular signatures for disease susceptibility and severity(202)*. These studies have also shown that brain is not only a target tissue but also a causal tissue in SLE. More specifically, using SLE GWAS signals and eQTLs from 44 tissues, we found that SLE-associated polymorphisms regulated gene expression not only in the blood but also in other tissues,-including the basal ganglia- suggesting that SLE genetic susceptibility may affect multiple tissues including CNS(202)*. These findings provide additional evidence that the brain may also be a causal tissue in SLE corroborating earlier data linking the nervous and the immune system.

Novel brain imaging techniques and clues for NPSLE pathogenesis

Approximately 40% of SLE patients with established neuropsychiatric disease do not show abnormalities on conventional brain imaging. Furthermore, no consistent association exists between any neuroimaging finding and specific neuropsychiatric syndrome or severity. To this end, a number of advanced imaging techniques have been tested in order to increase sensitivity and detect more subtle abnormalities. Indeed, imaging techniques have provided additional evidence for microglial activation. A recent study(203) demonstrated intracellular changes in glia with increased diffusivity of choline and creatine. The authors suggested that this finding could serve as an imaging marker for glial activation in response to inflammation; of note, this correlated also with disease activity. Microglia activation has also been shown in NPSLE by positron emission tomography (PET) and [11C] DPA-713 using a radiopharmaceutical substance that targets mitochondrial translocator protein, a protein upregulated during glial cell activation(204).

Regarding cerebral perfusion, our group examined whether dynamic susceptibility contrast-enhanced perfusion MRI (DSC-MRI), a minimally invasive and widely available method of cerebral perfusion assessment, may assist the diagnosis of NPSLE. We found decreased cerebral blood flow in the semioval center bilaterally in normal-appearing white matter region of NPSLE patients(118)*. Importantly, the combination of DSC-MRI-measured blood flow in the semioval centre with conventional MRI was

found to improve the attribution of neuropsychiatric events to SLE. Another technique, magnetization transfer imaging (MTI), uses the magnetization transfer ratio - histogram peak height (MTR-HPH) as a marker of the integrity of tissue microstructure; the latter was found decreased in individuals with inflammatory NPSLE manifestations compared to patients with presumed ischemic ones(205). Decreased MTR-HPHs values were reversed with immunosuppressive treatment, pointing towards an inflammatory process rather than ischemia. Proton Magnetic resonance spectroscopy (1H-MRS), which measures the concentration of several types of neurometabolites, has also been used in NPSLE. These studies have shown increased levels of myo-inositol and choline(115)(114), consistent with glial activation and vasculopathy, along with decreased N-acetylasparate(115)(114), compatible with neuronal impairment in patients with neuropsychiatric manifestations.

Recently, functional MRI in SLE subjects with cognitive dysfunction revealed structural and functional brain changes and an inflammatory process pointing out the multifactorial nature of NPSLE(116)*. Finally, PET studies in NPSLE have shown both increased (hypermetabolism) and decreased (hypometabolism) FDG uptake, consistent with inflammation and tissue loss, respectively. The most common finding was hypermetabolism in the parieto-occipital grey matter(206), even in the absence of MRI lesions. Collectively, these neuroimaging findings suggest that both inflammation and tissue loss may be operant in NPSLE.

Figure 3.

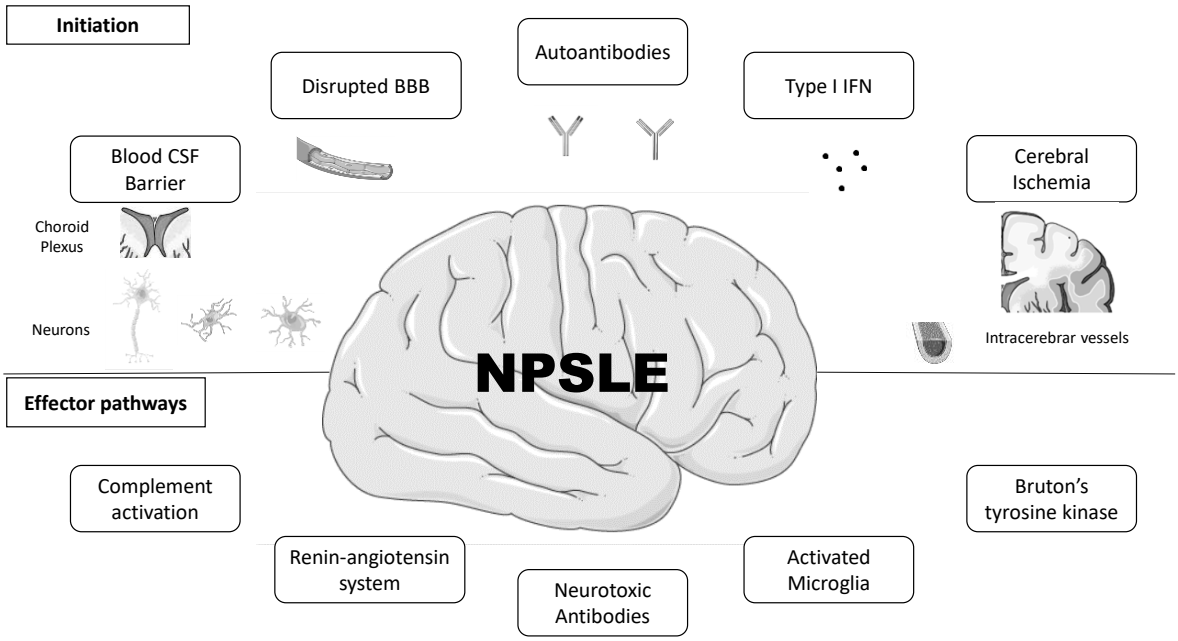


Figure 3. Proposed pathophysiologic mechanisms in NPSLE. Collectively these mechanisms target various components of the CNS including neurons (synapse, myelin sheath), astrocytes, microglia and the cerebral vasculature.

Summary

NPSLE remains only partly understood, both in terms of pathophysiology and management, the latter remaining largely empiric (144). Most evidence derives from studies in animal models, which interestingly do not manifest the full spectrum of human NPSLE (eg. severe manifestations, like seizures or myelopathy are not seen in mice); rather they exhibit more subtle abnormalities and, as such, may not completely model the human disease (207). Notwithstanding this limitation, advances have certainly been made in our understanding of disease pathogenesis (Figure 2). With regards to treatment, recent findings suggest new potential therapeutic opportunities, such as type I IFN blockade, ACE inhibition and kinase inhibitors (148)**,(139)**,(193)*. We anticipate that some of these pathways may serve as targets for the development of new drugs or for repositioning of already existing ones.

Key Points

1. Neuroinflammation and cerebral ischemia are the two major pathogenetic mechanisms in NPSLE
2. Abnormal BCSFB may represent an additional central mechanism in NPSLE pathogenesis
3. Microglia cells emerge as central players in CNS lupus and targets of novel therapies
4. Advanced imaging techniques may dissect the multifactorial nature of CNS lupus

Research Agenda

• Further definition of the molecular signature of NPSLE by transcriptomic analysis including single cell RNA sequencing
• Correlation of molecular subphenotype with clinical subgroups of NPSLE
• Exploration of the brain not only as a target tissue but also as a causal tissue in the pathogenesis of lupus
• Development and testing of molecular markers for neuroinflammation, ischemia and demyelination
• Exploration of the glymphatic system and its role in NPSLE
• Delineation of the relative importance of interferon pathways in intracerebral vascular beds
• Improved biomarkers for disease activity, prognosis and response to therapy
• Repositioning of drugs inhibiting pathways found to be relevant for lupus

G. Clinical challenges in Neuropsychiatric lupus

Due to its multifactorial nature and clinical heterogeneity, neuropsychiatric SLE (NPSLE) cannot be diagnosed on the basis of a single clinical, serological or imaging finding. Rather, an integrative approach is required, reflected also in algorithms that have been developed to aid the attribution of NP events to SLE or not. Abnormalities in the cerebrospinal fluid and/or magnetic resonance imaging are rarely pathognomonic; still, despite their modest sensitivity and specificity, these tests are helpful to exclude other pathologies, particularly infections and malignancies. In certain cases, the final NPSLE diagnosis will rely on experienced physicians and be confirmed with patient follow-up. Manifestations of presumed inflammatory origin, such as optic neuritis, recurrent seizures, and transverse myelitis, are treated with high-dose glucocorticoids and immunosuppressive agents, with cyclophosphamide being recommended for moderate/severe cases. There is also culminating evidence to suggest the effectiveness of mycophenolate, also by extrapolation from its use in lupus nephritis. For certain NP syndromes, however, such as cognitive dysfunction or psychiatric disorders, the decision for immunosuppressive treatment is not always obvious and will depend on multiple factors, including the severity and progression of neuropsychiatric involvement, overall assessment of SLE status, and brain MRI findings, ascertained through a multi-disciplinary approach. Occasionally, a ‘watchful waiting’ strategy or reevaluation after a therapeutic trial with a moderate dose of glucocorticoids can be helpful. In NPSLE patients with positive antiphospholipid antibodies, treatment should consider antiplatelets or anticoagulants, the latter clearly indicated only in cases of stroke; still, physicians should have a low threshold to administer also immunosuppressive therapy because published clinical, imaging, and biopsy data suggest that often, both pathogenic mechanisms (i.e., ischemic/thrombotic and inflammatory) co-exist. The majority of NPSLE patients will improve with treatment, although complete resolution of neurological deficit occurs in about half of them. Physicians should take into account any delays in initiation of immunosuppressive treatment and also, assess the extent of accrued neurological damage, in order to determine the expectations and efficacy of treatment. In the refractory (or relapsing) cases, rituximab (anti-CD20 monoclonal antibody), administered for one or more consecutive 6-monthly cycles, represents the best studied

option. Although many uncertainties still exist in the management of NPSLE, significant research efforts are being directed toward the identification of accurate biomarkers for disease diagnosis and pathophysiological classification, which could prove helpful for personalized treatment decisions. In the same context, various neuroimaging protocols are evaluated in SLE patients, which can provide a detailed portrait of the anatomical and functional defects in the nervous system, further optimizing the selection of treatment and evaluating prognosis. To this end, SLE has entered into the 'era' of biological agents, a couple of them having already been approved or succeeded in phase III trials. However, their efficacy on NP disease remains unknown and thus, are currently not indicated in active NPSLE. In addition, findings from basic/translational research are expected to deliver novel candidate agents that might be therapeutic by acting locally to regulate immune responses in the central nervous system. Finally, intriguing results demonstrating the putative role of the kallikrein–kinin/renin-angiotensin system in neuronal injury opens up the possibility to implement neuroprotective strategies in the prevention and treatment of NPSLE.

i. Clinical aspects

Lupus (2020) 0, 1–9
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LUPUS AROUND THE WORLD

Evolving phenotype of systemic lupus erythematosus in Caucasians: low incidence of lupus nephritis, high burden of neuropsychiatric disease and increased rates of late-onset lupus in the 'Attikon' cohort

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Objective: This study aimed to analyse the phenotype of systemic lupus erythematosus (SLE) at first presentation and during follow-up in a newly established SLE cohort based at 'Attikon' University Hospital. The hospital combines primary, secondary and tertiary care for the region of Western Attica, Greece. **Methods:** This study comprised a mixed prevalent and incident cohort of 555 Caucasian patients diagnosed with SLE according to American College of Rheumatology 1997 criteria and/or the Systemic Lupus Erythematosus International Collaborating Clinics (SLICC) 2012 criteria. Demographic and clinical characteristics, patterns of severity, treatments and SLICC damage index were recorded for each patient at the time of diagnosis and at last evaluation. **Results:** The mean age at lupus diagnosis was 38.3 years (standard deviation = 15.6 years), with a median disease duration at last follow-up of two years (interquartile range 1-11). At initial presentation, the most common 'classification' manifestations were arthritis (73.3%), acute cutaneous lupus (65%) and unexplained fever (25%), while among symptoms not included in any criteria set, Raynaud's phenomenon (33%) was the most common. Kidney and neuropsychiatric involvement as presenting manifestations were present in 10.3% and 11.5% cases, respectively. Irreversible damage accrual was present in 17.8% within six months of disease diagnosis, attributed mainly to thrombotic and neuropsychiatric disease. At last evaluation, 202 (36.4%) patients had developed severe disease, of whom more than half were treated with pulse cyclophosphamide. **Conclusion:** In this cohort of Caucasian patients, lupus nephritis is not as common as in older cohorts, while neuropsychiatric disease is emerging as a major frontier in lupus prevention and care. These data may help to document changes in the natural history and treatment of SLE over time and may have implications for its early recognition and management. *Lupus* (2020) **0,** 1–9.

Key words: Prevalent cohort; incident cohort; lupus criteria; non-lupus criteria; damage

Introduction

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease with a strong female predominance estimated to affect more than 8000 individuals in Greece (total population approximately 10 million).[1] Its clinical presentation encompasses a widely heterogeneous spectrum of phenotypes, ranging from mild or 'organ limited'

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Received 10 November 2019; accepted 4 February 2020

10.1177/0961203320908932

to full-blown life-threatening disease. Mild manifestations, such as skin rashes, inflammatory arthritis, leucopaenia/lymphopaenia, non-scarring alopecia and oral ulcers, are common among patients, while involvement of major organs is less frequent.[2] Common manifestations are useful for an early diagnosis, and their presence should raise the suspicion of underlying SLE, but they typically lack specificity, as they may also occur in other diseases.[3]

Approximately half of lupus patients are diagnosed with mild disease initially, with less than 20% having severe disease at onset.[4] For those presenting with mild disease in the absence of specific autoantibodies (e.g. anti-dsDNA) or characteristic lupus manifestations (e.g. malar rash), definite diagnosis represents a challenge.[5] Lupus diagnosis remains clinical because existing classification criteria for the disease[6,7] fail to classify up to 25% of patients, especially in the early stages.[8,9] In this regard, 'non-criteria' manifestations may aid in an earlier diagnosis of lupus.

The phenotype, clinical course and outcome of lupus differ around the world, depending on the population under study. Caucasians are more likely to have less severe disease, and a mild phenotype is maintained throughout the disease course in 50% of patients.[1] In contrast, Afro-Americans and Hispanics exhibit a more aggressive course, with a high incidence of lupus nephritis (LN)[10] and neuropsychiatric manifestations.[11] Childhood-onset SLE (cSLE) usually displays worse outcomes and more severe disease compared to adult-onset patients,[12] while patients with late-onset lupus typically have lower disease activity and a milder disease course.[13,14]

In this study, we sought to assess and describe the phenotype of lupus systematically at the time of presentation and throughout the disease course in a newly established cohort, the 'Attikon' lupus cohort, consisting exclusively of Caucasians. To this end, we recorded clinical manifestations, treatment, damage accrual and co-morbidities.

Methods

The 'Attikon' cohort

The 'Attikon' University Hospital is the largest tertiary medical centre of Western Attica, responsible for the care of approximately two million local citizens. In 2014, a rheumatology unit was established, serving as a referral centre for patients with lupus. Starting in September 2014, a cohort of patients with SLE was established in the rheumatology unit. The cohort (still ongoing) includes 555 Caucasian patients. It consists of a 'prevalent cohort' and an 'inception' cohort. The 'prevalent cohort' includes 237 patients with a SLE diagnosis prior to the establishment of the 'Attikon' cohort, who continue their regular follow-up in 'Attikon' University Hospital. The 'inception cohort' includes 318 SLE patients who have been diagnosed in 'Attikon' University Hospital and who have been followed ever since. For each patient, the first visit to the unit and registration in the cohort is defined as the 'enrolment' visit. Patient registration for the purpose of the study was completed in June 2019.

Patients and clinical assessment

Diagnosis of SLE was established by the American College of Rheumatology (ACR) 1997[6] and/or the Systemic Lupus Erythematosus International Collaborating Clinics (SLICC) 2012 criteria,[7] either at diagnosis or during the disease course.

We used a standardized form containing the ACR and SLICC classification criteria, as well as an additional list of clinical items not captured in these criteria sets. Patient files were also scrutinized for the following variables: (a) main demographic characteristics, (b) co-morbidities based on the Charlson Comorbidity Index,[15] (c) immunological tests and (d) past and present medications. The timing of the appearance of each clinical item and of serological tests was documented as present either (a) at diagnosis or (b) during the course of the disease. At every patient visit, any new manifestation was added to the database, thus ensuring a continuous recording.

Definitions

Kidney involvement was defined as (a) a kidney biopsy with a diagnosis of LN according to the 2003 International Society of Nephrology/Renal Pathology Society classification[16] or previous histological criteria for LN, and/or (b) by fulfilment of classification criteria for SLE (ACR and SLICC criteria) after exclusion of other causes.[6,7] The latter was mainly the case for patients diagnosed with SLE in the past (1995 or earlier), when kidney biopsy was not performed routinely. Chronic kidney disease (CKD) was defined as a glomerular filtration rate of $<60\,\text{mL/min/1.73}\,\text{m}^2$ for three months or more, and end-stage renal disease (ESRD) as initiation of kidney-replacement therapy.[17] Neuropsychiatric manifestations were classified as either primary neuropsychiatric SLE

(NPSLE, attributed to SLE;[18] using a combination of multidisciplinary physician judgment with attribution models,[19] as previously described) or secondary NPSLE (neuropsychiatric manifestations not attributed to SLE) or manifestations of uncertain attribution. Neuropsychiatric manifestations were classified as 'minor' and 'major', according to the definition by Ainiala et al.[20] Minor manifestations include headache, anxiety disorders, mild mood disorders, mild cognitive impairment and polyneuropathy without electrophysiological confirmation. The SLICC damage index (SDI) was used for the assessment of irreversible organ damage.[21] The revised Sydney classification criteria were used for definite diagnosis of antiphospholipid syndrome (APS).[22] For the definition of cSLE and late-onset SLE, cut-offs of 18 and 50 years, respectively, were used. All patients were categorized as having 'mild', 'moderate' or 'severe' lupus based on physician assessment and the presence of BILAG group A (for severe), group B (for moderate) or groups C/D/E (for mild) manifestations cumulatively during the course of their disease.[23]

Statistical analysis

Descriptive statistics were undertaken for continuous variables, and mean values/standard deviation (*SD*) or median/interquartile range (IQR) were calculated for normally and non-normally distributed variables, respectively. The chi-square test and Student's *t*-test were used to compare categorical and continuous variables, respectively. For all comparisons, a *p*-value of <0.05 was considered statistically significant. All statistical analyses were performed using the IBM SPSS Statistics for Windows v25.0 (IBM Corp., Armonk, NY). Captured data are stored electronically at 'Attikon' University Hospital and are accessible only by rheumatologists in the unit.

Results

Demographics and co-morbidities

The 'Attikon' cohort consists of 555 SLE patients, all of whom are Caucasian. The mean age at lupus diagnosis was 38.3 ($SD = 15.6$) years, and the median disease duration at last follow-up was two years (IQR 10 years). The female-to-male ratio was approximately 9:1, with a less pronounced ratio in patients diagnosed after 50 years of age (late-onset; approximately 4:1). A total of 135 (24.3%) patients were diagnosed with late-onset SLE, while 57 (10.3%) patients were identified as having cSLE. At the time of diagnosis, 294 (53%) subjects had mild disease, while 143 (25.8%) and 118 (21.2%) were diagnosed as having a moderate or severe phenotype, respectively. Irreversible damage accrual was prevalent in 99 (17.8%) patients already within six months from disease diagnosis. The respective items of the SDI are shown in Supplemental Table S1.

The most frequent associated diseases and co-morbidities were thyroid disease (29.1%; mainly Hashimoto's thyroiditis), obesity (22.2%), hypertension (20.2%), dyslipidaemia (15.7%), major depression (11.2%), osteoporosis (8.2%), diabetes mellitus (5.4%) and valvular heart disease (4.5%). The percentage of active smokers at enrolment was as high as 32.8%, which is consistent with the rate of the general population in Greece.[24]

Clinical manifestations and immunological profile

The most common clinical manifestations at disease onset are summarized in Table 1. Of manifestations included in previous sets of classification criteria, inflammatory arthritis (73.3%), acute cutaneous lupus (65%; mainly photosensitive rash (50.8%) and malar rash (39.8%)) and leucopaenia (23.8%) were the most common. A quarter of patients (25%) presented with unexplained fever, an item recently included in the new European League Against Rheumatism (EULAR)/ACR classification criteria. Among 'non-criteria' symptoms, the most frequent at diagnosis were Raynaud's phenomenon (33.0%), while livedo reticularis and lymphadenopathy were observed in 6.8% and 6.7%, respectively. At the time of diagnosis, 6.3% of patients were negative for antinuclear antibodies (ANA), while only 8.5% were positive for anti-Smith, 36.9% for anti-dsDNA and 37.3% for antiphospholipid antibodies (aPL). Serological items at diagnosis and cumulatively are summarized in Figure 1.

Lupus nephritis and neuropsychiatric disease

At the time of disease diagnosis, kidney involvement was present in only 57 (10.3%) patients, while 61 (11%) more patients exhibited LN during follow-up, reaching an overall prevalence of 21.3%. Among patients with biopsy-proven LN, the most common histological patterns were class III/IV (45.3%), class V (23.8%) and a combination of class III/IV and class V (19%). Eight (6.8%) patients reached ESRD, with four already at the time of diagnosis and four over the course.

Table 1 Clinical manifestations at diagnosis and at last follow-up ($N = 555$)

Clinical items	*At diagnosis*	*Cumulatively*
Arthritis, *n* (%)	407 (73.3)	473 (85.2)
Acute cutaneous lupus, *n* (%)	361 (65.0)	393 (70.8)
Malar rash, *n* (%)	221 (39.8)	250 (45.0)
Photosensitivity, *n* (%)	282 (50.8)	297 (53.5)
Chronic cutaneous lupus, *n* (%)	55 (9.9)	62 (11.2)
Oral/nasal ulcers, *n* (%)	98 (17.7)	143 (25.8)
Non-scarring alopecia, *n* (%)	124 (22.3)	175 (31.5)
Lupus nephritis, *n* (%)	57 (10.3)	118 (21.3)
Primary NPSLE, *n* (%)	64 (11.5)	98 (17.6)
Serositis, *n* (%)	64 (11.5)	104 (18.7)
Leucopaenia, *n* (%)	132 (23.8)	196 (35.3)
AIHA, *n* (%)	15 (2.7)	19 (3.4)
Thrombocytopaenia, *n* (%)	68 (12.3)	88 (15.9)
Raynaud's, *n* (%)	183 (33.0)	205 (37.0)
Fever, *n* (%)	138 (25.0)	171 (31.0)
Livedo reticularis, *n* (%)	38 (6.8)	57 (10.2)
Lymphadenopathy, *n* (%)	37 (6.7)	51 (9.2)

NPSLE: neuropsychiatric systemic lupus erythematosus; AIHA: autoimmune haemolytic anaemia.

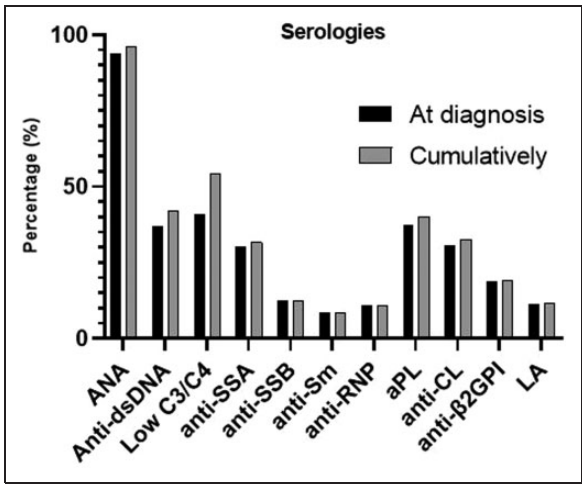
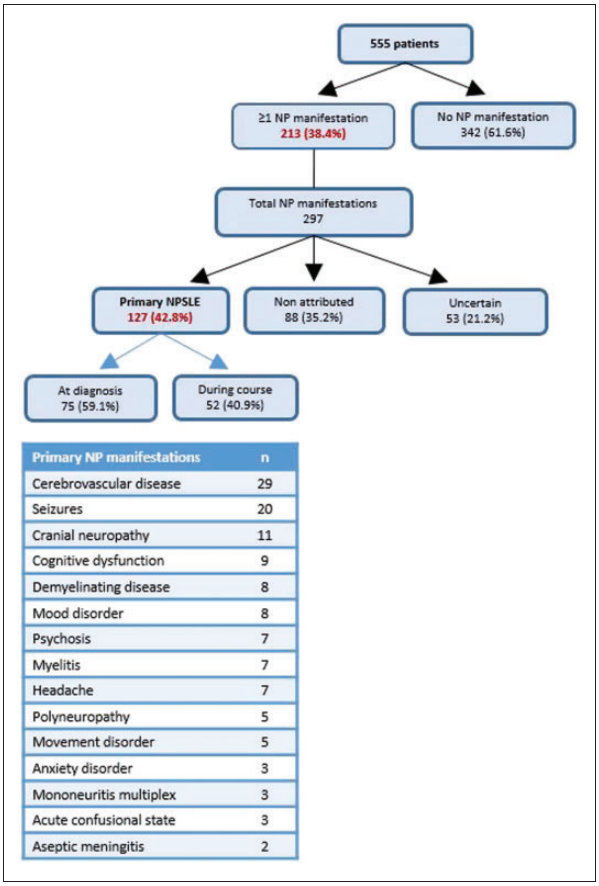


Figure 1 Immunological profile of subjects with SLE in the 'Attikon' cohort at diagnosis and cumulatively. SLE: systemic lupus erythematosus; LA: lupus anticoagulant; aPL: antiphospholipid antibodies.

Fifteen (12.7% of those with kidney involvement) patients developed CKD.

In our cohort, 213 (38.4%) patients developed at least one neuropsychiatric manifestation, while the total number of neuropsychiatric manifestations captured was 297. A total of 129 primary neuropsychiatric manifestations were observed in 98 (17.6% of total cohort population) patients. Approximately two-thirds (64/98) of NPSLE patients had at least one SLE-related neuropsychiatric manifestation at the time of diagnosis, while



Primary NP manifestations	n
Cerebrovascular disease	29
Seizures	20
Cranial neuropathy	11
Cognitive dysfunction	9
Demyelinating disease	8
Mood disorder	8
Psychosis	7
Myelitis	7
Headache	7
Polyneuropathy	5
Movement disorder	5
Anxiety disorder	3
Mononeuritis multiplex	3
Acute confusional state	3
Aseptic meningitis	2

Figure 2 Flow chart of all neuropsychiatric manifestations and types of events of the 'Attikon' cohort. Among 297 manifestations recorded, 127 were attributed to SLE, corresponding to 98 patients (17.6% of the whole cohort).

34 (34.7%) patients manifested NPSLE during follow-up. The most common primary neuropsychiatric manifestations were stroke, seizure disorder and cranial neuropathy (Figure 2).

Rare and severe 'non-criteria manifestations'

The use of classification criteria for diagnosis has raised concerns about the possibility of missing a diagnosis, especially in patients with early and incomplete disease. A high cumulative prevalence of moderate to severe 'non-criteria manifestations' was captured in our cohort. Non-criteria manifestations attributed to SLE were (number of patients at diagnosis/cumulatively): vasculitis (12/22), pulmonary embolism (11/22), pneumonitis (7/15) interstitial lung disease (6/15), autoimmune hepatitis (8/11), ocular involvement including uveitis, episcleritis and retinal vasculitis (4/8), pulmonary arterial hypertension (3/8), myocarditis (3/7), diffuse alveolar haemorrhage (3/6), peritonitis (2/6),

thrombotic thrombocytopenic purpura-like syndrome (3/5), myositis (2/4) and macrophage activation syndrome (2/4). Although these manifestations were individually rare, in sum 67 (12.1%) patients presented with such a manifestation at onset. Also, 108 (19.5%) patients developed one or more 'non-criteria' major organ involvement during the course of their disease, suggesting a high cumulative prevalence of non-typical SLE manifestations.

Secondary APS

Fifty-seven (10.3%) SLE patients (female:male approximately 3:1) were diagnosed with secondary APS. Among them, 51 (89.5%) patients exhibited thrombotic APS, 12 (21%) obstetric APS and six (10.5%) both thrombotic and obstetric APS. Nine (15.8%) of these patients had been diagnosed with APS prior to the diagnosis of SLE (mean years to SLE diagnosis = 7.9 ($SD = 6$ years)), while in 30 (52.6%) patients, the diagnoses of lupus and APS were made simultaneously. Eighteen (31.6%) patients developed APS over the course of the disease (mean disease duration until APS diagnosis = 6.9 years ($SD = 8.5$ years)). The most common thrombotic events were deep-venous thrombosis (47.4%; $n = 27$) and stroke (29.8%; $n = 17$). Among APS patients, lupus anticoagulant positivity was detected in 28 (49.1%), while triple positivity was observed in 14 (24.6%) subjects.

cSLE versus late-onset SLE

The frequency of individual manifestations at the time of diagnosis was not different between cSLE and late-onset SLE (Table 2), with the exception of fever (more prevalent in cSLE: 40.4% vs. 12.6% in late-onset; $p < 0.001$). Over the course of the disease, the cSLE population developed LN, acute cutaneous lupus, oral ulcers and non-scarring alopecia more frequently. Of 57 cSLE patients, 22 (38.6%) and 10 (17.6%) developed LN and NPSLE, respectively. Accordingly, LN and NPSLE were observed in 24 (17.8%) and 23 (17.1%) patients, respectively, among the late-onset group ($n = 135$). Contrary to LN, NPSLE appears to have a steady prevalence, irrespective of age group.

Therapies

All administered immunosuppressive drugs (both current and past medications) are summarized in Figure 3. Azathioprine (AZA) and methotrexate (MTX) were the most commonly used immunosuppressive agents for mild/moderate disease (31.7% and 26.8%, respectively), while calcineurin inhibitors were rarely used. Belimumab was used in 51 (9.2%) patients. Mycophenolate mofetil was administered at a lower rate (17.7%) in moderate/severe cases. Specifically, it was used in 20 patients with moderate disease (11.5%; 21/182) and in 70 with severe lupus (36.8%; 77/209). For life-threatening, refractory or severe SLE ($n = 209$), intravenous cyclophosphamide (CYC) was the main therapeutic option (56.5%; 118/209). Rituximab (RTX) was administered 'off-label' for severe/refractory disease in 39 (20.6% of those with severe disease) patients. A significant percentage of patients (31.5%; 175/555) did not receive glucocorticoids at enrolment. Hydroxychloroquine was discontinued in 44 (7.9%) patients due to side effects, mainly due to allergic reactions and ocular toxicity.

Discussion

There are several well-established lupus cohorts around the globe. As the phenotype of the disease and the available treatments evolve over time, it is also essential to assess relatively 'fresh' cohorts, which may provide a more accurate picture for modern lupus. We present such a SLE cohort, consisting exclusively of Caucasian patients, with approximately two-thirds of patients having an early diagnosis (e.g. disease duration of less than five years). This communication includes a thorough description with regards to clinical manifestations, particularly at the time of diagnosis, autoantibodies, demographics, co-morbidities, severity pattern, damage accrual over time and administered treatments.

A comparison of clinical manifestations at first presentation among our cohort and other cohorts around the world is summarized in Table 3.[3,25–27] In Caucasian populations, musculoskeletal and skin involvement is common at disease onset, while the incidence of LN and positivity for anti-dsDNA or other lupus-specific autoantibodies are lower compared to non-Caucasian races. Neuropsychiatric disease represents an emerging phenotype among Caucasians.[1] In cSLE, kidney and haematological involvement is common, accounting for more than 40% of lupus patients. Importantly, among 'non-criteria manifestations', Raynaud's phenomenon is often present at initial presentation and could alert physicians towards a diagnosis of lupus. Recently, unexplained fever was added in the new EULAR/ACR classification

Table 2 Clinical characteristics of SLE patients with cSLE versus late-onset SLE

	At diagnosis			*Ever*		
Clinical items	*cSLE (N = 57)*	*Late-onset SLE (N = 135)*	p-*Value*	*cSLE (N = 57)*	*Late-onset SLE (N = 135)*	p-*Value*
Arthritis, *n* (%)	35 (61.4)	95 (70.4)	0.22	45 (78.9)	109 (80.8)	0.77
Acute cutaneous lupus, *n* (%)	41 (71.9)	84 (62.2)	0.19	47 (82.4)	87 (64.4)	**0.013**
Chronic cutaneous lupus, *n* (%)	6 (10.5)	15 (11.1)	0.90	6 (10.5)	15 (11.1)	0.90
Oral/nasal ulcers, *n* (%)	12 (21.1)	13 (9.6)	0.055	20 (35.1)	18 (13.3)	**<0.001**
Non-scarring alopecia, *n* (%)	12 (21.2)	24 (17.8)	0.59	22 (38.6)	31 (23.0)	**0.026**
Lupus nephritis, *n* (%)	8 (14.0)	19(14.1)	0.46	22 (38.6)	24 (17.8)	**0.002**
Primary NPSLE, *n* (%)	7 (12.3)	19 (14.1)	0.74	10 (17.6)	23 (17.1)	0.93
Serositis, *n* (%)	9 (15.8)	23 (17.0)	0.83	10 (17.6)	29 (21.4)	0.53
Leucopaenia, *n* (%)	19 (33.3)	31 (23.0)	0.13	26 (45.6)	43 (31.9)	0.07
Thrombocytopaenia, *n* (%)	7 (12.3)	21 (15.6)	0.55	13 (22.8)	24 (17.8)	0.42
Raynaud's, *n* (%)	17 (29.8)	50 (36.3)	0.33	21 (36.8)	51 (37.0)	0.90
Fever, *n* (%)	23 (40.4)	17 (12.6)	**<0.0001**	25 (43.9)	20 (14.8)	**<0.001**

Statistically significant values are shown in bold.
SLE: systemic lupus erythematosus; cSLE: childhood-onset SLE.

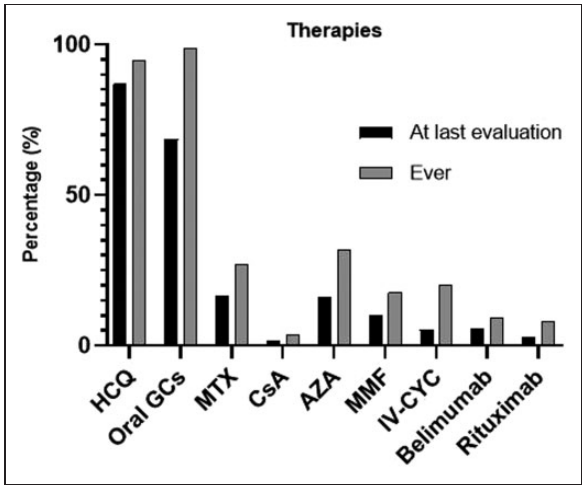


Figure 3 Types of treatment of subjects with SLE at both last evaluation and ever received in the 'Attikon' cohort. GCs: glucocorticoids; IV-MP: intravenous methylprednisolone; HCQ: hydroxychloroquine; IV-CYC: intravenous cyclophosphamide; MMF: mycophenolate; AZA: azathioprine; CsA: cyclosporine; MTX: methotrexate.

criteria[9,28] for SLE, which probably increases the sensitivity for early classification, since fever is prevalent in more than 25% of patients at the time of diagnosis.

The kidney represents the most common major organ involved in SLE and is associated with the worst outcomes.[29] Hispanics and African Americans are more likely to develop LN compared to Caucasians.[10] In a large Asian cohort, LN was also found to be present in 42% of patients at the time of diagnosis.[26] More recently, in the SLICC multi-ethnic cohort, consisting of approximately 2000 lupus individuals, the true incidence of LN was 38%, of whom 80% developed kidney involvement close to lupus diagnosis.[29] In contrast, in our cohort, half of LN cases occurred after diagnosis and during the disease course. Notably, in the SLICC cohort,[29] LN prevalence among Caucasians was approximately 20% (40–50% in Hispanics and African Americans), which is compatible with our results. Thus, LN may represent an overestimated feature in Caucasian lupus patients.

Compared to other large cohort studies, neuropsychiatric disease in our cohort does not seem to have significant differences in terms of risk factors, attribution rates, incidence, epidemiology and timing of NPSLE appearance.[11,19,30–34] In our cohort, 15.1% of lupus subjects developed at least one primary neuropsychiatric manifestation, consistent with findings in other cohorts.[11,30] Only 14 (2.5%) patients developed 'minor' neuropsychiatric manifestations that were considered as attributed to SLE based on the presence of risk factors (e.g. generalized disease activity, aPL positivity and history of primary NPSLE) and multidisciplinary expert physician judgement.[18,19] The incidence of distinct and relatively common neuropsychiatric manifestations, such as seizures, cerebrovascular events, neuropathies and psychosis, are also consistent with currently published large studies,[31–34] with more than half of primary neuropsychiatric manifestations occurring at the time of diagnosis. Notably, our data indicate that neuropsychiatric involvement is more frequent at the time of diagnosis in Caucasians compared to Asians and Hispanics,[25,26] while cSLE seems to have comparable prevalence of primary NPSLE at disease onset compared to our results[27] (Table 3). Thus,

Table 3 Comparison of clinical features of SLE patients at the time of diagnosis from large SLE cohorts around the world

Items *Centre based* *No. patients*	*'Attikon' cohort* *Europe* N = *555*	*Mosca et al.*[3] *Multi-centre* N = *389*	*Pons-Estel et al.*[25] *Latin America* N = *1214*	*Joo et al.*[26] *Asia* N = *996*	*Fiorot et al.*[27] *Latin America (childhood onset)* N = *1312*	*Total* N = *4466*
Malar rash	39.8%	49.5%	23.6%	44%	52.9%	41.1%
Photosensitivity	50.8%	31.6%	24.5%	35%	45.0%	36.8%
Discoid	7.4%	9.3%	5.3%	8%	5.3%	6.5%
Oral ulcers	17.7%	21.6%	10.5%	36%	32.8%	24.6%
Alopecia	22.3%	30.6%	20.3%	–	21.7%	22.3%
Arthritis	73.3%	57.6%	67.3%	65%	68.4%	67.0%
Pericarditis	7.0%	18.8%	2.7%	15%	19.1%	12.2%
Pleuritis	7.6%	22.4%	3.6%	19%	17.6%	13.3%
Renal involvement	10.3%	13.1%	5.3%	42%	40.8%	25.1%
Neuropsychiatric	11.5%	9.2%	4.1%	6%	11.0%	7.9%
Leucopaenia	23.8%	16.2%	5.1%	61%	41.8%	31.6%
Thrombocytopaenia	12.3%	6.6%	5.2%	24%	18.9%	15.5%
AIHA	2.7%	4.6%	2.4%	14%	21.4%	10.8%
Fever	25.0%	34.5%	28.6%	–	–	28.7%
Raynaud's	33.0%	22.1%	10.2%	–	–	18.2%
ANA	93.7%	99.5%	–	100%	93.4%	96.1%
Anti-dsDNA	36.6%	71.7%	–	79%	59.4%	62.1%

ANA: antinuclear antibodies; AIHA; Autoimmune hemolytic anemia; Anti-dsDNA; antidouble-strand DNA.

neuropsychiatric disease represents an increasingly recognized phenotype in lupus patients.

Late-onset lupus represents a distinct phenotype, accounting in most series for up to 10% of lupus patients, generally characterized by a milder disease pattern and lower disease activity over time.[13,14] A quarter of our patients were diagnosed as late-onset SLE, a relatively high percentage not previously reported in the literature. Our results confirm the lower incidence of LN in late-onset disease, while NPSLE seems to have a steady frequency among different age groups. Late-onset patients exhibited a stable clinical course without significant accumulation of additional manifestations over the course of their disease. Moreover, the initial presentation of lupus in terms of clinical manifestations and disease severity did not differ between cSLE and late-onset SLE (Table 2). Only fever at the time of diagnosis was significantly more prevalent in cSLE and adult-onset SLE compared to late-onset SLE. Concerning cSLE, in our experience, initial disease phenotype was not as severe as indicated in the current literature.[2] However, this group ultimately developed more severe disease over the course, with a high incidence of LN.

Our data indicate a more restricted immunological profile due to low rates of positivity of multiple autoantibodies, as was recently reported in a Caucasian cohort.[1] In contrast, the prevalence of multiple autoantibodies is almost double in large cohort consisting of Hispanics, Afro-Americans and Asians.[2,25–27] Approximately 6% of our patients were ANA negative at the time of diagnosis, a finding identical to the multi-centre SLICC lupus cohort.[35]

AZA and MTX remain the main medications for mild/moderate lupus as first-line agents. Despite the progress in the management of severe SLE over the last three decades, cytotoxic therapies such as CYC still represent commonly used drugs for the treatment of severe disease. During the last decades, in addition to cytotoxic therapies, new immunosuppressive and biological agents have been introduced to the armamentarium of SLE treatment.[36] Our data indicate that belimumab and RTX are increasingly used in clinical practice for the management of moderate and severe lupus, respectively.

Our study is limited by the retrospective data collection in approximately one-third of patients in the 'prevalent cohort', which includes approximately one-third of patients diagnosed prior to the establishment of our registry. In this group, we included only lupus subjects with comprehensive medical records and adequate information from their medical history, reflecting the true course of the disease of each patient. The low incidence of some severe manifestations captured in our cohort, such as LN in 21.3%, may be attributed to the skewed distribution of disease duration, since the median disease duration in our cohort is

only two years. Yet, lower rates of LN have also been reported in the 'Leto' lupus cohort in Crete.[1]

In summary, our data confirm the relatively low incidence of LN in Caucasians compared to other racial backgrounds, and describe a more contemporary phenotype of NPSLE with a higher rate of late-onset SLE than previously reported. These data may help to document changes in the natural history and treatment of SLE over time.

Acknowledgements

We thank the staff physicians (Drs M. Aggelakos, K. Thomas, E. Atsali, S. Boiu and L. Fotis) and nurses (A. Ntourou, K. Togia and T. Gerogianni) of the Rheumatology Unit of the 'Attikon' University Hospital of Athens for their referral and care of the patients with SLE.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship and/or publication of this article: This work was funded in part by: The Hellenic Society of Rheumatology; the Foundation for Research in Rheumatology (FOREUM); the Greek General Secretariat of Research and Technology 'Aristeia' action of the Operational Program 'Education and Lifelong Learning' (co-funded by the European Social Fund and National Resources, Aristeia I 2344 to DB); the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement no. 742390); and the SYSCID (A Systems Medicine Approach to Chronic Inflammatory Diseases) under the European Union's Horizon 2020 research and innovation programme (grant agreement no. 733100).

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Supplemental material

Supplemental material for this article is available online.

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Transition to severe phenotype in systemic lupus erythematosus initially presenting with non-severe disease: implications for the management of early disease

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To cite: Nikolopoulos DS, Kostopoulou M, Pieta A, *et al*. Transition to severe phenotype in systemic lupus erythematosus initially presenting with non-severe disease: implications for the management of early disease. *Lupus Science & Medicine* 2020;**7**:e000394. doi:10.1136/lupus-2020-000394

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/lupus-2020-000394).

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Received 12 March 2020
Revised 8 May 2020
Accepted 18 May 2020

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ABSTRACT

Objective Changes in the care of patients with SLE dictate a re-evaluation of its natural history and risk factors for disease deterioration and damage accrual. We sought to decipher factors predictive of a deterioration in phenotype ('transition') in patients initially presenting with non-severe disease.

Methods Patients from the 'Attikon' cohort with disease duration ≥1 year were included. Disease at diagnosis was categorised as mild, moderate or severe, based on the British Isles Lupus Assessment Group manifestations and physician judgement. 'Transition' in severity was defined as an increase in category of severity at any time from diagnosis to last follow-up. Multivariable logistic regression was performed to identify baseline factors associated with this transition.

Results 462 patients were followed for a median (IQR) of 36 (120) months. At diagnosis, more than half (56.5%) had a mild phenotype. During disease course, transition to more severe forms was seen in 44.2%, resulting in comparable distribution among severity patterns at last follow-up (mild 28.4%, moderate 33.1%, severe 38.5%). Neuropsychiatric involvement at onset (OR 6.33, 95% CI 1.22 to 32.67), male sex (OR 4.53, 95% CI 1.23 to 16.60) and longer disease duration (OR 1.09 per 1 year, 95% CI 1.04 to 1.14) were independently associated with transition from mild or moderate to severe disease. Patients with disease duration ≥3 years who progressed to more severe disease had more than 20-fold increased risk to accrue irreversible damage.

Conclusion Almost half of patients with initially non-severe disease progress to more severe forms of SLE, especially men and patients with positive anti-double-stranded DNA or neuropsychiatric involvement at onset. These data may have implications for the management of milder forms of lupus.

INTRODUCTION

SLE is a systemic autoimmune disease with protean clinical manifestations and an unpredictable course.[1] Although prognosis has significantly improved over the years due to earlier diagnosis and more effective treatments, patients with SLE still demonstrate increased mortality and morbidity compared with the general population.[2] Patients' phenotype at disease onset may vary from mild to severe or life-threatening,[3 4] with striking differences among patients from different racial backgrounds. Lupus nephritis (LN) is more common in Hispanics and African–Americans,[5 6] the latter also exhibiting an up to twofold increased risk of neuropsychiatric involvement, compared with Caucasians.[7 8]

Several cohort studies around the world have documented the natural history and morbidity of the disease, contributing substantially to increased awareness.[9] More recently, emphasis has been put on the patterns of disease activity and targets of therapy, with remission and low disease activity emerging as new frontiers.[10] Moreover, management recommendations have attempted to decrease the heterogeneity in lupus care, by providing evidence-based and expert opinion-based guidance.[11] However, among patients who present with a certain phenotype, there is a paucity of data regarding potential changes of severity over time, that is, whether the disease will remain mild throughout its course or progress to a more severe form. Such data may have clinical and therapeutic implications for early disease.

The aim of this study was to describe the severity patterns of a Caucasian SLE cohort in a tertiary SLE referral centre, based at 'Attikon' University Hospital, Athens, Greece. We explored possible baseline prognostic factors related to a 'transition in severity' as well as cumulative damage accrual over the

course of the disease. Our data suggest that, despite significant advances in therapy, transition of disease occurs in a considerable proportion of patients.

PATIENTS AND METHODS

Patients and clinical assessment

'Attikon' University Hospital is a tertiary centre located in a large urban area of Western Attica, responsible for the healthcare of close to two million local residents. An SLE cohort was initiated in January 2014 to include all patients diagnosed with SLE who had a regular follow-up as outpatients. The 'Attikon' lupus cohort consists of a 'prevalent cohort' (patients with an SLE diagnosis prior to the establishment of the patient registry) and an 'inception' cohort (patients followed from diagnosis onwards).[12] A standardised data set, including demographics and clinical and laboratory features of the disease, is completed for each patient at first visit and every follow-up. All immunosuppressive/immunomodulatory drugs administered for the treatment of SLE are also documented, including current treatment (ie, at most recent visit) and past medications. Patient enrolment for the purpose of the study was completed in January 2019.

Patients with SLE fulfilling the American College of Rheumatology (ACR)[13] and/or Systemic Lupus International Collaborating Clinics (SLICC) criteria[14] and who had disease duration ≥1 year were included in this study. LN was defined according to SLE classification criteria and/or kidney biopsy.[13 14] A diagnosis of primary neuropsychiatric SLE (NPSLE) was established according to the ACR definitions,[15] following a combination of expert physician judgement (DTB, AF).[16 17] For patients enrolled in the cohort after the neuropsychiatric manifestation had occurred, attribution to SLE or not was based on patient history and all available data (taking into account a variety of risk factors for NPSLE at the time of neuropsychiatric involvement, ie, antiphospholipid antibodies (aPL), prior neuropsychiatric manifestation, generalised disease activity),[16–18] or was considered as 'uncertain'. For the definition of childhood-onset SLE, a cut-off of 17 years was used,[19] whereas onset after 50 years was defined as late-onset SLE. For the assessment of damage, the SLICC Damage Index (SDI)[20] was captured yearly for each patient.

Definitions of disease severity and 'transition'

For the purpose of this study, the phenotype of SLE was categorised as mild, moderate or severe across two timepoints: diagnosis and most recent follow-up. For patients enrolled in the cohort after the disease had been diagnosed (prevalent cohort), phenotype at diagnosis was based on patient history and all available data on patient file. Medical charts of all patients were scrutinised to detect incident manifestations (at any timepoint across the disease course) from individual organ systems. Stratification of disease during the course of the disease was determined by expert physician (DTB, AF) based on a structured assessment that took into account (1) the presence of disease manifestations graded in severity according to the British Isles Lupus Assessment Group (BILAG) 2004 index glossary[21] and (2) all treatments received by patients. Specifically, *severe* disease was defined as (1) severe SLE manifestation from at least one organ according to the BILAG glossary and/or (2) treatment with cyclophosphamide or rituximab (for any manifestation, other than arthritis) at any time over disease course.[8] *Mild* disease was defined as (1) mild manifestations according to the BILAG glossary, (2) absence of any major organ involvement and (3) maximum treatment with the following: oral glucocorticoids (GC) ≤10 mg/day (prednisone equivalent) or intramuscular GC and/or hydroxychloroquine (HCQ), at any time during disease course. Patients falling between these two definitions were classified as *moderate* disease. Patients were assessed at each visit for possible transition to a more severe form of the disease (ie, from mild to moderate/severe, or from moderate to severe). As this 'transition in severity' was the primary outcome, patients with severe lupus at diagnosis were excluded from this analysis.

Statistical analysis

Descriptive statistics were undertaken for continuous variables, and mean/SD or median/IQR values were calculated for normally and non-normally distributed variables, respectively. χ^2 or Fisher's exact test was used to compare categorical variables, and Student's t-test or non-parametric Mann-Whitney U test was used to compare continuous variables, as appropriate.

Logistic regression models were used to identify factors that were independently associated with 'transition in severity' and damage accrual. Because patients with initially mild disease may progress to either moderate or severe disease, while those with initially moderate only to severe disease, two different regression analyses were performed, for the identification of baseline risk factors for (1) transition from mild to moderate disease and (2) transition from mild or moderate to severe disease. All variables with a p value <0.20 in univariable analyses qualified for further analysis in age-adjusted multivariable models. P values, ORs and their 95% CI were computed. A stepwise backward selection was performed to eliminate non-significant factors. Model selection and checking were based on tests for linearity, interactions and goodness of fit. For comparisons, statistical significance was indicated as a two-sided p<0.05. All statistical analyses were performed using SPSS V.25.0I.

Information about the study along with the consent form was provided to patients with SLE. All participants signed the informed consent forms.

RESULTS

Patient characteristics

A total of 462 patients, all Caucasians, were included in the study. The mean (SD) age at lupus diagnosis was 37.3

Table 1 Clinical and serological items of SLE at the time of diagnosis and cumulatively

N=462	At diagnosis	Cumulatively
Acute cutaneous lupus*, n (%)	292 (63.9)	324 (70.1)
Malar rash†, n (%)	184 (39.8)	213 (45.1)
Photosensitivity†, n (%)	231 (50.0)	247 (53.3)
Chronic cutaneous lupus*, n (%)	49 (10.6)	56 (12.1)
Arthritis, n (%)	336 (72.7)	398 (86.1)
Alopecia, n (%)	104 (22.5)	155 (33.5)
Oral ulcers, n (%)	78 (16.9)	123 (26.6)
Serositis, n (%)	46 (10.0)	86 (18.7)
Nephritis, n (%)	44 (9.5)	105 (22.7)
NPSLE‡, n (%)	51 (11.0)	86 (18.6)
Leucopenia, n (%)	104 (22.5)	165 (35.8)
AIHA, n (%)	15 (3.2)	19 (4.1)
Thrombocytopaenia, n (%)	52 (11.3)	71 (15.4)
Unexplained fever§, n (%)	109 (23.8)	141 (31.0)
ANA ≥1:80, n (%)	433 (93.7)	443 (95.9)
Low complement, n (%)	156 (39.4)	217 (54.8)
dsDNA, Sm or aPL, n (%)	210 (45.5)	240 (51.9)

*According to SLICC classification criteria.
†According to ACR classification criteria.
‡According to ACR 1999 nomenclature.
§According to EULAR/ACR 2019 criteria.
ACR, American College of Rheumatology; AIHA, autoimmune haemolytic anaemia; aPL, antiphospholipid antibodies; dsDNA, double-stranded DNA; EULAR, European League Against Rheumatism; NPSLE, neuropsychiatric SLE; SLICC, Systemic Lupus International Collaborating Clinics; Sm, Smith.

(15.2) years, with a female to male ratio of ~9:1, and the median (IQR) disease duration to last follow-up was 36 (120) months. Fifty (10.8%) patients were diagnosed with childhood-onset SLE and 98 patients (21.2%) with late-onset SLE.

The most common clinical manifestations at diagnosis were inflammatory arthritis (72.7%), acute cutaneous lupus (63.2%, mainly malar rash and photosensitive rash), leucopenia (22.5%) and non-scarring alopecia (22.5%). LN was manifest at onset in 44 (9.5%) patients, while 61 (13.2%) more patients developed renal involvement during follow-up, reaching an overall prevalence of 22.7%. There were 112 primary neuropsychiatric manifestations observed in 86 patients (18.6% of total population). Approximately 60% of patients with NPSLE (51 of 86) had at least one SLE-related neuropsychiatric manifestation at the time of diagnosis, while 35 (39.7%) patients manifested NPSLE during follow-up. Clinical and serological items are summarised in table 1.

The vast majority of patients in our cohort had received HCQ and oral GC at some point during the course of their disease (95.0% and 98.3%, respectively); at most recent follow-up, the respective percentages were 85.6% and 67.9%. Use of additional immunosuppressive medications is shown in online supplementary figure 1.

Transition of disease severity over time and predictors

The respective distribution of disease severity at diagnosis and over time is depicted in figure 1A. More than half of patients (261 of 462, 56.5%) initially presented with mild disease. Of them, at last assessment, only 131 (50.2%) patients had retained their mild phenotype, while the remaining had evolved to more severe forms: 76 (29.1%) and 54 (20.7%) developed moderate and severe lupus, respectively. Of patients with initially moderate disease (n=109), 32 (29.4%) progressed to severe SLE, while approximately 20% (n=92) of patients had severe manifestations already at diagnosis. This kinetics resulted in an almost equal distribution among the three severity pattern groups (mild, moderate, severe) at last assessment (figure 1A).

Patients diagnosed initially mild disease (n=261) were analysed to identify baseline factors as predictors of disease transition to a moderate phenotype (table 2). In both univariable and multivariable analyses, positive

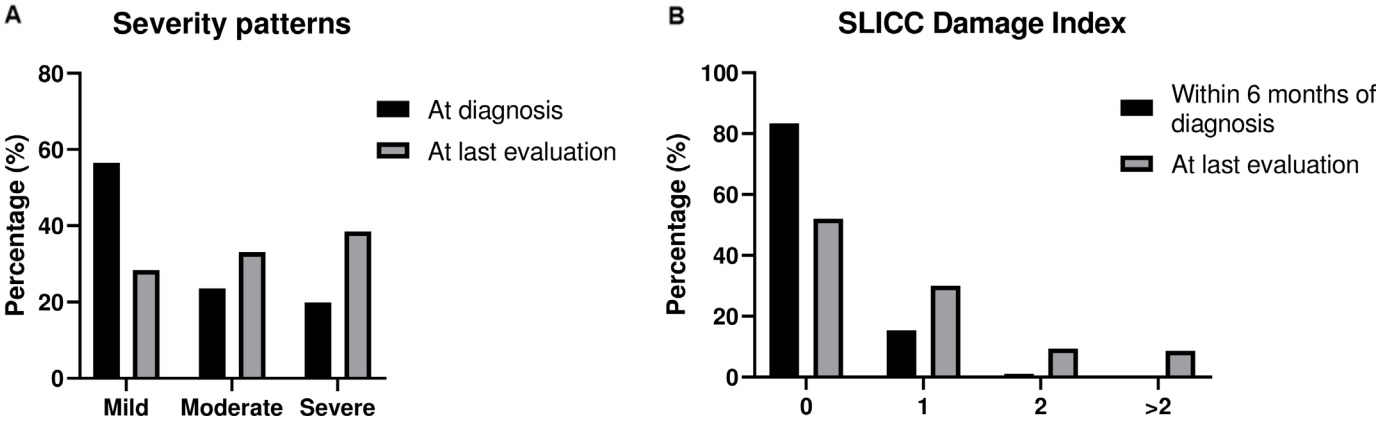

Figure 1 (A) Disease severity patterns of patients with SLE ('Attikon' cohort) at disease onset and at last evaluation. (B) Damage accrual of patients with SLE ('Attikon' cohort) within 6 months after diagnosis and at last evaluation. SLICC, Systemic Lupus International Collaborating Clinics.

Table 2 Baseline features as predictors of phenotype transition from mild to moderate disease

Transition from mild to moderate disease				
Baseline	**Univariable**	**CI**	**Multivariable**	**CI**
SDI (0 vs ≠0)	0.23	0.02 to 1.92		
Age at diagnosis	**0.97**	**0.95 to 0.99**	1.02	0.97 to 1.06
Disease duration	**1.07 (per year)**	**1.03 to 1.11**	**1.05**	**1.00 to 1.11**
Sex (m/f)	0.54	0.14 to 2.08	0.47	0.08 to 2.76
Late-onset SLE	**0.2**	**0.09 to 0.63**	0.36	0.09 to 1.46
Acute cutaneous lupus	0.65	0.36 to 1.24		
Leucopenia	1.02	0.52 to 1.99		
Fever	2.04	0.88 to 4.74		
ANA	4.3	0.51 to 35.67		
dsDNA	**2.72**	**1.44 to 5.15**	**2.39**	**1.07 to 5.32**
Low complement	1.36	0.71 to 2.59		
Anti-Sm	0.49	0.05 to 4.8		

Values in bold represent associations that reached statistical significance (p< 0.05).
anti-Sm, anti-Smith; dsDNA, double-stranded DNA; m/f, male/female; SDI, SLICC Damage Index; SLICC, Systemic Lupus International Collaborating Clinics.

anti-double-stranded DNA (anti-dsDNA) at diagnosis and disease duration were associated with transition to moderate lupus (OR 2.39, 95% CI 1.07 to 5.32 and 1.05, 95% CI 1.00 to 1.11, respectively). For transition to severe disease, we included patients presenting initially with either mild or moderate disease (n=370). First, the two disease states (mild vs moderate) did not differ in their risk of transition to a severe phenotype (table 3). Factors associated with this transition in multivariable analysis were male sex (OR 4.53, 95% CI 1.23 to 16.60), disease duration (OR 1.09, 95% CI 1.04 to 1.14) and especially neuropsychiatric involvement at onset (OR 6.33, 95% CI 1.22 to 32.67); presence of anti-dsDNA marginally did not reach statistical significance (OR 1.89, 95% CI 0.96 to 3.73). For both transitions (ie, from mild to moderate, as well as from mild/moderate to severe), patients with late-onset SLE showed a trend to retain their initial phenotype compared with patients diagnosed before

Table 3 Baseline features as predictors of phenotype transition from mild or moderate to severe disease

Transition from mild/moderate to severe disease				
Baseline	**Univariable**	**CI**	**Multivariable**	**CI**
Severity at diagnosis (moderate to mild)	1.04	0.62 to 1.76		
SDI (0 vs ≠0)	1.14	0.47 to 2.77		
Age at diagnosis	**0.96**	**0.95 to 0.99**	1.00	0.97 to 1.03
Disease duration	**1.10 (per year)**	**1.07 to 1.14**	**1.09**	**1.04 to 1.14**
Sex (m/f)	**3.16**	**1.35 to 7.39**	**4.53**	**1.23 to 16.60**
Late-onset SLE	**0.26**	**0.12 to 0.59**	0.32	0.08 to 1.28
Acute cutaneous lupus	0.97	0.57 to 1.64		
Renal involvement	0.90	0.23 to 3.48		
Neuropsychiatric involvement	**5.28**	**1.54 to 18.07**	**6.33**	**1.22 to 32.67**
Leucopenia	0.71	0.38 to 1.35		
Fever	**2.64**	**1.47 to 4.59**	1.71	0.81 to 3.60
ANA	1.37	0.43 to 4.35		
dsDNA	**2.16**	**1.25 to 3.71**	1.89	0.96 to 3.73
Low complement	**1.94**	**1.10 to 3.39**	1.12	0.55 to 2.26
Anti-Sm	1.85	0.74 to 4.60		

Values in bold represent associations that reached statistical significance (p< 0.05).
anti-Sm, anti-Smith; dsDNA, double-stranded DNA; m/f, male/female; SDI, SLICC Damage Index; SLICC, Systemic Lupus International Collaborating Clinics.

the age of 50, only in univariable analyses (tables 2 and 3). We also examined whether different baseline characteristics could predict transition to moderate versus severe disease in patients diagnosed initially with mild SLE, but the results did not differ significantly (data not shown).

To overcome the potential bias of a shorter disease duration in patients who were less likely to progress to more severe forms (either from mild to moderate, or from mild/moderate to severe), we performed a subgroup analysis in patients with a median disease duration shorter than 3 years; the final age-adjusted and sex-adjusted models remained almost identical in terms of statistical significance (data not shown).

Transition in severity in childhood-onset and late-onset SLE

The childhood-onset SLE population exhibited LN approximately twice more commonly (42% vs 20.6%, p=0.001). Transition to more severe disease at last follow-up was detected in 54.1% of patients with childhood-onset SLE compared with 43.6% in adult-onset patients, a difference not reaching statistical significance. No difference between groups was observed in terms of patterns of severity, SDI and major organ involvement (p>0.05). A higher incidence of moderate/severe disease at diagnosis (combined 56.2% vs 40.3%, p=0.005) and a respective lower incidence of transition to more severe forms (19.7% vs 50.7%, p<0.001) were seen in patients with late-onset disease, as compared with 'non-late-onset' patients. The latter difference remained significant even after adjusting for disease duration.

Baseline predictors for damage accrual during follow-up

Seventy-six (16.5%) patients had already established damage within 6 months of disease diagnosis, mainly due to neuropsychiatric and thrombotic components of the SDI (online supplementary table 1). After a median (IQR) disease duration of 3 (10) years, 241 (52.2%) patients had still not accrued damage (SDI=0). A high damage index (SDI ≥3) was found in 40 subjects (8.6%) (figure 1B).

To identify predictors of damage accrual over time in all patients, we performed univariable and multivariable analyses (n=462) (table 4). Univariable analysis revealed comorbidities including hypertension, dyslipidaemia and obesity as predictors of SDI development. Age at diagnosis, disease duration and severity transition were found to be independent predictors of increased SDI in multivariable analysis. As expected, patients who evolved to more severe forms of lupus and patients with longer disease duration exhibited higher risk of damage development (OR 5.66, 95% CI 2.74 to 11.67 and 1.15, 95% CI 1.09 to 1.22, respectively). When disease duration was examined as a binary variable, subjects with longer disease duration (≥3 years) and transition to more severe forms had a 23-fold risk of damage accrual compared with those with preserved disease state and shorter disease duration (figure 2). The presence of positive aPL also conferred a significant risk of damage accrual in our cohort (OR 2.22, 95% CI 1.09 to 4.53).

Table 4 Baseline features as predictors for damage accrual

Baseline	Univariable*	CI	Multivariable*	CI
Severity at diagnosis (mild vs moderate/severe)	1.26	0.86 to 1.84		
Transition	**6.88**	**4.28 to 11.06**	**5.66**	**2.74 to 11.67**
Age at diagnosis	1.00	0.99 to 1.01	**1.05**	**1.02 to 1.08**
Disease duration	**1.11 (per year)**	**1.08 to 1.14**	**1.15**	**1.09 to 1.22**
Sex (m/f)	1.41	0.75 to 2.65		
Late-onset SLE	0.97	0.61 to 1.55		
cSLE	0.89	0.48 to 1.64		
Fever	**1.62**	**1.05 to 2.51**		
Leucopenia	0.63	0.39 to 1.01		
Obesity	**2.00**	**1.29 to 3.11**		
Hypertension	**2.23**	**1.40 to 3.54**		
Dyslipidaemia	**2.10**	**1.25 to 3.51**		
aPL	1.54	0.95 to 2.50	**2.22**	**1.09 to 4.53**
Anti-dsDNA	1.33	0.89 to 1.99		
Low complement	1.10	0.73 to 1.66		

Values in bold represent associations that reached statistical significance (p< 0.05).
*OR for SLICC Damage Index.
anti-dsDNA, anti-double-stranded DNA; aPL, antiphospholipid antibodies; cSLE, childhood-onset SLE; m/f, male/female; SLICC, Systemic Lupus International Collaborating Clinics.

Figure 2 Relative risk (RR) of damage accrual in subjects with different combinations of disease duration and transition compared with those with short disease duration (<3 years) who never progressed to more severe forms of the disease.

DISCUSSION

The 'Attikon' lupus cohort was established in 2014 with the purpose to study the natural history of SLE in a Caucasian population of the modern era. SLE may often follow an unpredictable course; thus, it would be helpful to predict which patients will ultimately develop severe disease necessitating more aggressive treatment. In this study, we aimed to explore factors which could help identify patients who will eventually develop a severe phenotype, although initially presenting with mild or moderate disease. Importantly, to stratify patients in terms of disease severity, we used a combination of BILAG classification and expert judgement, the former being a validated instrument for SLE activity and severity.[22] We found that, although approximately 60% of patients present with mild disease at onset, almost 50% of them later progress to a moderate and severe phenotype. These data may have important implications for the management of patients with milder forms of the disease, a subset of which may require closer monitoring.

Following the advent of potent immunosuppressive therapies, the phenotype of rheumatic diseases has changed in certain circumstances, with the prevalence of certain severe manifestations having decreased.[23] Most recent cohorts of patients with SLE report rates of LN substantially lower than the ~60% of traditional cohorts, potentially reflecting better disease monitoring and management at the early stages.[6 8 24] In this regard, it was important to find that transition to a more severe phenotype is still a reality for a significant proportion of patients. Few studies have examined the temporal characteristics of different lupus manifestations over time. A study undertaken to inform the recently published ACR/European League Against Rheumatism criteria for SLE described disease manifestations at disease onset, but did not report on subsequent follow-ups.[4] Also, recent updates from the established Hopkins and Toronto lupus cohorts confirmed that the majority of patients with lupus still tend to follow a relapsing-remitting course[25 26]; however, whether flares of disease lead to a more severe disease in terms of new organ manifestations was not specified, although both number and severity of flares are known to contribute to damage in lupus.[27 28]

In a study relevant to our own, Kwon *et al*[29] examined baseline predictors for subsequent development of LN, in patients not presenting initially with renal involvement. Interestingly, anti-dsDNA positivity and younger age at disease onset were independently associated with future LN occurrence, similar to their association with transition to a severe phenotype in our study. These findings strengthen the notion that young, male, anti-dsDNA-positive patients should be under close surveillance for subsequent development of severe disease manifestations. We also found neuropsychiatric involvement at onset to have the strongest association with subsequent transition to severe lupus. Indeed, past neuropsychiatric manifestations have been shown to associate with subsequent occurrence of similar or different neuropsychiatric events and constitute a risk factor for NPSLE.[30 31] This is particularly important, as in our cohort we have found increased prevalence of neuropsychiatric involvement (11.5% of patients at disease onset).

Irreversible damage accrual, measured by the SDI, is a milestone in the natural history of SLE, since it has been directly linked to increased mortality.[32 33] Importantly, at last follow-up, more than 50% (52.4%) of patients in our cohort still had an SDI of 0. Nevertheless, the median disease duration in patients included in the current study was relatively short (3 years), and a significant proportion (16.5%) already had evidence of damage at diagnosis. Not unexpectedly, we found that transition to a more severe phenotype was independently associated with increased risk for damage, especially with increasing disease duration. In a recent work examining damage trajectories in childhood-onset SLE, major organ involvement was also characterised by a more rapid damage accrual.[34] These observations have obvious implications for patients diagnosed at a young age and call for vigilant monitoring and optimal disease control at early disease stages. We also found, in accordance to previous studies, that aPL also contributes independently to damage accrual in SLE.[35 36]

Our study has several limitations. The 'Attikon' lupus cohort consists exclusively of Caucasians; thus, our findings have to be replicated in patient cohorts of different race and ethnicity. Also, in a significant proportion of patients in the prevalent cohort, data regarding history, manifestations and treatments prior to inclusion in the SLE cohort were performed retrospectively. Especially regarding treatments, the specific timing of treatment with each immunosuppressive drug in relation to disease 'transition' was not available in all our patients. One could assume that the higher risk of transition in patients with mild disease may be attributable to undertreatment, rather than the natural history of the disease per se. However, more than 95% of patients in our prevalent cohort have been treated with HCQ and GC, which indicates that patients with mild disease had been prescribed appropriate therapy. Notwithstanding the limitation that

we lack data regarding adherence to treatment, we anticipate that the effect of treatments received would not significantly affect the findings of our study. Lastly, the heterogeneous disease duration in our cohort suggests that use of Cox regression would be more appropriate as it entails time-to-event analyses. The lack of time-to-event data in our prevalent cohort precluded the use of Cox regression; nevertheless, we tried to overcome the potential bias of logistic regression, by performing subgroup analyses in patients with short disease duration.

In summary, despite recent advances, we found that almost 50% of patients with lupus initially presenting with mild disease eventually progress to more severe forms of the disease, highlighting the existence of persistent unmet needs in SLE. Milder forms of lupus may still carry an increased risk to 'convert' over time; thus, increased vigilance and regular monitoring are warranted in patients, irrespective of phenotype at disease onset.

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Acknowledgements We thank the staff physicians (Drs T Karageorgas, D Tseronis, M Aggelakos, D Kassara, K Thomas, E Atsali, S Boiu, L Fotis) and nurses (G Rapsomaniki, A Ntourou, K Togia, T Gerogianni) of the Rheumatology Unit of the 'Attikon' University Hospital of Athens for their referral and care to the patients with SLE. We are also indebted to Dr G Bertsias for his insightful comments.

Collaborators George Bertsias.

Contributors DSN and MK collected data from patient medical charts and also performed the statistical analysis. DSN performed the data entry. AP, SF, KC and AB contributed to maintenance of the Attikon Lupus Registry and assisted in data collection. PK contributed to establishment of the Attikon Lupus Registry. PK and JB assisted in patient recruitment and reviewed the manuscript. DTB and AF conceived and supervised the study. AF performed the statistical analyses. DSN and AF drafted the manuscript.

Funding This work was funded in part by the Hellenic Society of Rheumatology; the Foundation for Research in Rheumatology (FOREUM); the Greek General Secretariat for Research and Technology 'Aristeia' action of the Operational Programme 'Education and Lifelong Learning' (co-funded by the European Social Fund and National Resources, Aristeia I 2344 to DTB); the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement no 742390); and the SYSCID (A Systems Medicine Approach to Chronic Inflammatory Diseases) under the European Union's Horizon 2020 research and innovation programme (grant agreement no 733100).

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval The study was approved by the Ethics Committee of the 'Attikon' University Hospital of Athens (protocol number 103/06-03-2014).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

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BRIEF RESEARCH REPORT
published: 11 May 2022
doi: 10.3389/fneur.2022.889613

Demyelinating Syndromes in Systemic Lupus Erythematosus: Data From the "Attikon" Lupus Cohort

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OPEN ACCESS

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Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: 04 March 2022
Accepted: 19 April 2022
Published: 11 May 2022

Citation:
Nikolopoulos D, Kitsos D, Papathanasiou M, Kapsala N, Garantziotis P, Pieta A, Gioti O, Grivas A, Voumvourakis K, Boumpas D and Fanouriakis A (2022) Demyelinating Syndromes in Systemic Lupus Erythematosus: Data From the "Attikon" Lupus Cohort. Front. Neurol. 13:889613. doi: 10.3389/fneur.2022.889613

Background: The demyelinating syndromes of the central nervous system (CNS) that occur in the context of systemic lupus erythematosus (SLE) may represent a manifestation of neuropsychiatric lupus (NPSLE) or an overlap of SLE and multiple sclerosis (MS). The differential diagnosis between the two entities has important clinical implications because the therapeutic management differs.

Objectives: To characterize CNS demyelinating syndromes in a large SLE cohort as neuropsychiatric SLE (NPSLE) or SLE-MS overlap using a multidisciplinary approach and existing diagnostic (for MS) and classification criteria (for SLE).

Methods: Patients from the "Attikon" lupus cohort (n = 707) were evaluated for demyelinating syndromes. Clinical, laboratory, and neuroimaging data were recorded for each patient. Following multidisciplinary evaluation and application of criteria, the demyelinating syndrome was attributed to either SLE or MS. Patients with transverse myelitis were not included in this study.

Results: We identified 26 patients with demyelinating syndromes (3.7%). Of them, 12 were diagnosed as primary SLE-demyelination (46.2%) and 14 as overlap SLE-MS (53.8%). The two groups did not differ with respect to rheumatologic and neurologic manifestations or autoantibodies. SLE patients with demyelination manifested mild extra-CNS disease mainly involving joints and skin, while severe non-CNS manifestations were rare. However, these patients were less likely to have elevated IgG index (OR 0.055 95% CI: 0.008–0.40) and positive oligoclonal bands (OR 0.09 95% CI: 0.014–0.56), as well as brain lesions in the spinal cord, infratentorial, periventricular, and juxtacortical regions. A single brain region was affected in 9 patients with SLE-demyelination (75%), while all patients with MS-SLE had multiple affected brain regions. MS-SLE overlap was associated with an increased likelihood of neurologic relapses (OR 18.2, 95% CI: 1.76–188), while SLE-demyelination patients were less likely to exhibit neurological deficits (EDSS >0) at the last follow-up visit (50 vs. 78.6% in SLE-MS, respectively).

Conclusions: Demyelination in the context of SLE follows a more benign course compared to a frank SLE-MS overlap. Extension of follow-up will ascertain whether patients with SLE-demyelination evolve to MS, or this is a *bona fide* NPSLE syndrome.

Keywords: systemic lupus erythematosus, multiple sclerosis, demyelination, central nervous system, outcome demyelination in systemic lupus erythematosus

INTRODUCTION

Among the many neuropsychiatric syndromes that constitute neuropsychiatric systemic lupus erythematosus (NPSLE), demyelinating syndrome (SLE-DS), termed lupoid sclerosis in the past (1), is one of the most challenging and less well-studied. Indeed, the definition of SLE-DS, according to the 1999 American College of Rheumatology (ACR) nomenclature, (2) is almost indistinguishable from multiple sclerosis (MS), a prototype organ-specific autoimmune demyelinating disease. Both MS and SLE-DS require objective evidence of central nervous system (CNS) neurological dysfunction, with documentation of dissemination in space and time (multiple episodes and affected areas within the CNS). Moreover, for a diagnosis of MS to be established, old and revised diagnostic criteria mandate prior exclusion of other conditions that can better explain the clinical and paraclinical findings of an individual patient, with SLE being a fundamental alternative diagnosis. This complex reality often creates confusion in physicians who encounter lupus patients with a DS, regarding whether this represents a CNS manifestation of the disease or a mere segregation of two autoimmune diseases (which is far from uncommon in clinical practice) (3). This differential diagnosis affects also the therapeutic management because drugs that used to treat NPSLE and MS, excluding glucocorticoids, are largely different (4).

In a previous work, following a combined rheumatology–neurology longitudinal assessment, we characterized a cohort of patients who presented with DS with atypical features for MS, and who had clinical and/or serological evidence of a systemic autoimmune disease (5). We found that a significant proportion of patients presenting with demyelinating syndrome do not fulfill the criteria for MS after more than 3 years of follow-up, and frequently manifest features of a systemic autoimmune disease (like arthritis or inflammatory rashes), although not formally diagnosed with SLE. We coined the term "demyelination with autoimmune features (DAF)" to describe patients in this "gray area".

As a follow-up to this work, and using the same multidisciplinary approach, we herein attempt to expand on these findings by providing a description of patients with SLE from the established "Attikon" lupus cohort who have experienced a DS without fulfilling the criteria for MS, and comparing them with patients from the same cohort who have been characterized as an overlap of SLE with MS. We undertook this study in an effort to identify similarities and differences, if any, between the two patient subgroups, and potentially identify parameters that may predict which lupus patients with DS will fulfill diagnostic criteria for MS during the course of follow-up.

PATIENTS AND METHODS

Patients and Clinical Assessment

This study is part of a collaborative project initiated in 2016 between the Rheumatology and Clinical Immunology Unit and the Department of Neurology of "Attikon" University Hospital, Athens, aiming to evaluate patients presenting with a DS of immune origin (ie. excluding trauma/compression, ischemia, or demyelination due to metabolic derangements) for the presence of features of an underlying systemic autoimmune disease, mainly SLE. The methodology has been previously described (5); briefly, the two units established a mutual referral algorithm, including i) patients examined in the Department of Neurology with a DS not fulfilling criteria for MS who had features suggestive of a systemic autoimmune disease, and ii) vice versa, patients followed in the Rheumatology and Clinical Immunology Unit for a systemic autoimmune disease who later developed a DS.

The "Attikon" lupus cohort was established in 2015 in the Rheumatology Unit of the "Attikon" University Hospital, Athens, serving as a referral center for patients with lupus, as previously described (6, 7). As of December 2021, it includes 708 Caucasian SLE patients. The present study aimed to characterize CNS demyelinating syndromes within the "Attikon" lupus cohort as neuropsychiatric SLE or SLE-MS overlap, using the same multidisciplinary approach as above, including rheumatologic–neurologic and neuroradiologic evaluation. To this end, we reviewed all patients with SLE for underlying CNS demyelinating disease with respect to clinical and neuroimaging evidence. All patients with possible DS were referred for comprehensive neurological evaluation, including thorough clinical examination and laboratory tests, MRI of the CNS, and cerebrospinal fluid (CSF) analysis, including IgG index and screening for oligoclonal band. Exclusion criteria were a) patients with neuromyelitis optica spectrum disorders (NMOSD) or other primary CNS diseases, b) patients with longitudinal myelopathy spanning three or more vertebral bodies, and c) patients with CNS imaging findings more consistent with microischemic, rather than demyelinating lesions, as judged by an experienced neuroradiologist (MP).

Following inclusion in the study, patients with CNS demyelination were followed at regular visits in both rheumatology and neurology units, with documentation of new clinical, laboratory, and imaging data. At the last follow-up, patients fulfilling the criteria for MS were labeled as "overlap SLE/MS", while DS not fulfilling the criteria for MS were diagnosed as "SLE-DS".

Definitions

Diagnosis of SLE was established by the American College of Rheumatology (ACR) 1997 and/or the Systemic Lupus Erythematosus International Collaborating Clinics (SLICC) 2012 criteria, combined with expert physician judgment (AF, DB). Similarly, the diagnosis of MS was established by 2010 McDonald criteria combined with expert physician judgment (DK, KV) (8). A clinically isolated syndrome (CIS) was defined as a single demyelinating attack without dissemination in time.

Neurological disability and severity at the last follow-up were assessed by the Expanded Disability Status Scale (EDSS) (9). Patients were categorized as having "mild," "moderate," or "severe" neurological disability at the previous visit based on EDSS score. Specifically, mild disability was defined as EDSS $\leq$2, while severe disability was defined as EDSS >4. Patients falling between these two definitions were classified as having moderate disability.

We also used the following definitions regarding response to treatment: (i) no response; neurological symptoms and disability remained stable or worsened during follow-up, (ii) partial response; neurological symptoms and disability improved but did not completely resolve, and (iii) complete response; no neurological symptoms and disability at last visit.

Assessment of MRI

All MRIs were performed on 1.5 or 3 Tesla MR scanners and reviewed by an expert neuroradiologist (MP). Images included a standard clinical protocol for brain imaging with T1 pre- and post-contrast injection, T2 and fluid attenuated inversion recovery (FLAIR) sequences with gadolinium administration. For the most recent MRI of each patient, distribution of demyelinating lesions was divided into 5 regions: a) cortex, b) juxtacortical, c) periventricular, d) infratentorial, and e) spinal cord.

Statistical Analysis

All captured data are stored electronically at "Attikon" Hospital. Descriptive statistics were undertaken for continuous variables, and mean (SD) or median (IQR) values were calculated for normally and non-normally distributed variables, respectively. Chi-square test and Student's *t*-test were used to compare categorical and continuous variables, respectively. Logistic regression was applied to calculate the odds ratio for categorical variables. For all comparisons, a p-value < 0.05 was considered statistically significant. All statistical analyses were performed using the Statistical Package for the Social Science (SPSS; SPSS Inc., Version 25.0, IBM Corp., Armonk, NY, USA). The study was approved by the Ethics Committee of the "Attikon" University Hospital of Athens, and patients provided informed consent for their participation (protocol number 103/06-03-2014).

RESULTS

Demyelinating Syndromes in "Attikon" Lupus Cohort

From a total of 708 SLE patients in the "Attikon" cohort, we identified 26 patients with DS [3.7%, mean age at lupus diagnosis 46.9 (SD 12.3) years]. Median SLE disease duration at last visit was 60 months (IQR 52 months) and median follow-up since the onset of demyelination was 79 months (IQR 118 months). With all data available at the end of follow-up, of 26 patients, 12 were diagnosed as primary SLE-DS (final prevalence 1.7% of the SLE cohort) and 14 as overlap SLE-MS. At the end of follow-up, 5 of the 12 SLE-DS patients were diagnosed as CIS. In the majority of SLE-DS patients, first occurrence of a demyelinating event occurred following the diagnosis of lupus, while the majority of neurologic manifestations in SLE-MS overlap patients preceded SLE diagnosis (**Supplementary Table 1**).

Demyelinating Syndrome in Lupus Is Associated Mild Disease Outside the CNS

Rheumatic clinical features and autoantibodies of the 26 SLE patients with DS are summarized in **Table 1**, in comparison to the remaining SLE cohort. Notably, SLE patients with demyelination tend to exhibit mainly musculoskeletal and mucocutaneous disease features; severe non-CNS manifestations were rarely observed in this patient subgroup. In addition, SLE-DS patients were less likely to be positive for specific lupus autoantibodies, although differences did not reach statistical significance. Additionally, rheumatic clinical manifestations and autoantibodies did not differ between SLE-MS and SLE-DS patients (**Supplementary Table 2**).

Patients With SLE-MS Overlap Display Intrathecal Immunoglobulin Production and a Higher Burden of MRI Lesions in the CNS

Similar to rheumatic clinical manifestations, no significant differences were observed between patients with SLE-DS and SLE-MS in terms of neurologic manifestations (**Supplementary Table 3**). However, CSF and imaging findings differed between the two groups. Notably, patients with SLE-DS were significantly less likely to have an elevated IgG index (OR 0.05 95% CI: 0.008–0.40) and positive oligoclonal bands in the CSF (OR 0.09 95% CI: 0.014–0.56). More specifically, no patient with SLE-DS tested positive for type II oligoclonal bands, indicative of purely intrathecal immunoglobulin production, contrary to SLE-MS overlap patients who were predominantly positive for type II oligoclonal bands (**Table 2**).

Regarding MRI findings, both the brain and spinal cord were more likely to be affected in overlap SLE-MS patients, while optic nerve involvement was similarly affected in the two groups (**Supplementary Table 4**). A detailed anatomical distribution of CNS lesions is shown separately for patients with SLE-DS and SLE-MS in **Figure 1**. As expected, the former were less likely to exhibit brain lesions in the spinal cord, infratentorial, periventricular, and juxtacortical regions. More importantly, only a single brain region was affected in 9/12 patients with SLE-DS (75%), contrary to all SLE-MS patients who had multiple affected brain regions.

TABLE 1 | Clinical features and autoantibodies in SLE patients with and without demyelinating syndromes.

Clinical manifestations	SLE with demyelination ($n = 26$)	SLE ($n = 681$)	*P*-value
Acute cutaneous lupus, *n* (%)	24(92.3)	487(71.5)	**0.02**
Malar rash, *n* (%)	17(65.4)	325(47.7)	0.08
Photosensitivity, *n* (%)	8(30.8)	381(55.9)	**0.01**
Chronic cutaneous lupus *n* (%)	2(7.7)	76(11.2)	ns
Oral ulcers, *n* (%)	6(23)	189(27.8)	ns
Non-scarring alopecia, *n* (%)	6(23)	236(34.7)	ns
Inflammatory arthritis, *n* (%)	24(92.3)	581(85.3)	ns
Serositis, *n* (%)	2(7.7)	128(18.8)	0.15
Lupus nephritis, *n* (%)	1(3.8)	149(21.9)	**0.03**
Neuropsychiatric events*, *n* (%)	6(23)	109(16)	ns
Leukopenia, *n* (%)	4(15.4)	240(35.2)	**0.04**
Thrombocytopenia, *n* (%)	0(0)	116(17)	*ns*
Hemolytic anemia, *n* (%)	0(0)	24(3.5)	*ns*
Fever, *n* (%)	2(7.7)	223(33.6)	**0.007**
Raynaud's, *n* (%)	8(30.8)	260(38.2)	ns
Autoantibodies			
ANA, *n* (%)	24(92.3)	658(96.6)	ns
Anti-dsDNA, *n* (%)	7(26.9)	285(41.6)	ns
Anti-Smith, *n* (%)	2(7.7)	52(7.6)	ns
Low C3 and/or C4, *n* (%)	12(46.2)	329(48.3)	ns
Anti-SSA, *n* (%)	7(26.9)	180(26.4)	ns
Anti-SSB, *n* (%)	3(11.5)	72(10.6)	ns
Anti-phospholipids, *n* (%)	3(11.5)	181(26.6)	0.08
Anti-RNP, *n* (%)	3(11.5)	60(8.8)	ns

**Excluding demyelinating events. Values in bold represent comparisons that reached statistical significance (P < 0.05).*

TABLE 2 | Cerebrospinal fluid findings of patients with SLE-demyelinating syndromes compared to SLE-MS.

	Total ($n = 26$)	SLE-DS ($n = 12$)	SLE-MS ($n = 14$)	*p*-value
IgG index >0.65, *n* (%)	15 (57.7)	3 (25.0)	12 (85.7)	0.002
Positive oligoclonal bands, *n* (%)	24 (53.8)	3 (25.0)	11 (78.6)	0.006
Type II	8	0	8	NA
Type III	4	1	3	0.35
Type IV	2	2	0	NA

NA, Not applicable due to zero values in one of comparators.

Overlap SLE-MS Is Associated With More Relapses and Worse Outcome

At the end of our observational period, overlap SLE-MS was associated with an increased likelihood of relapses (OR 18.2, 95% CI: 1.76–188). Specifically, only 5/12 patients with SLE-DS exhibited a relapse, while 92.9% of SLE-MS patients experienced at least one relapse (p-value = 0.004, **Supplementary Table 5**).

Disease-related outcomes, including response to treatment and neurological disability at the most recent visit, are shown separately for patients with SLE-DS and SLE-MS overlap in **Figure 2**. Importantly, only 3/26 patients (11.5%, 2 with SLE-DS and one with SLE-MS) did not respond to treatment and their neurological symptoms remained unaltered. The majority patients with of SLE-MS (71.4%) showed a partial response of their neurologic symptoms, while complete response was achieved in 3 patients (21.4%). On the contrary, demyelinating episodes in SLE-DS patients resolved completely in 50% (6/12), while 4/12 (33.3%) showed only partial improvement (**Figure 2A**).

Finally, neurological disability, as measured by EDSS, is shown in **Figure 2B**. Patients with SLE-DS were less likely to exhibit neurological deficits (EDSS >0) at the end of follow-up, as compared to patients with SLE-MS (50 vs. 78.6%, respectively, p = ns). Importantly, approximately half of the patients in each group had moderate to severe neurological deficits at the last evaluation.

DISCUSSION

The occurrence of DS in a patient with SLE represents a diagnostic and therapeutic challenge. Whether such a patient has a neurologic manifestation of their systemic autoimmune disease or two different diseases, is not only a question of theoretical value; on the contrary, drugs used to treat SLE and MS differ significantly. Furthermore, some therapies, like interferon-based

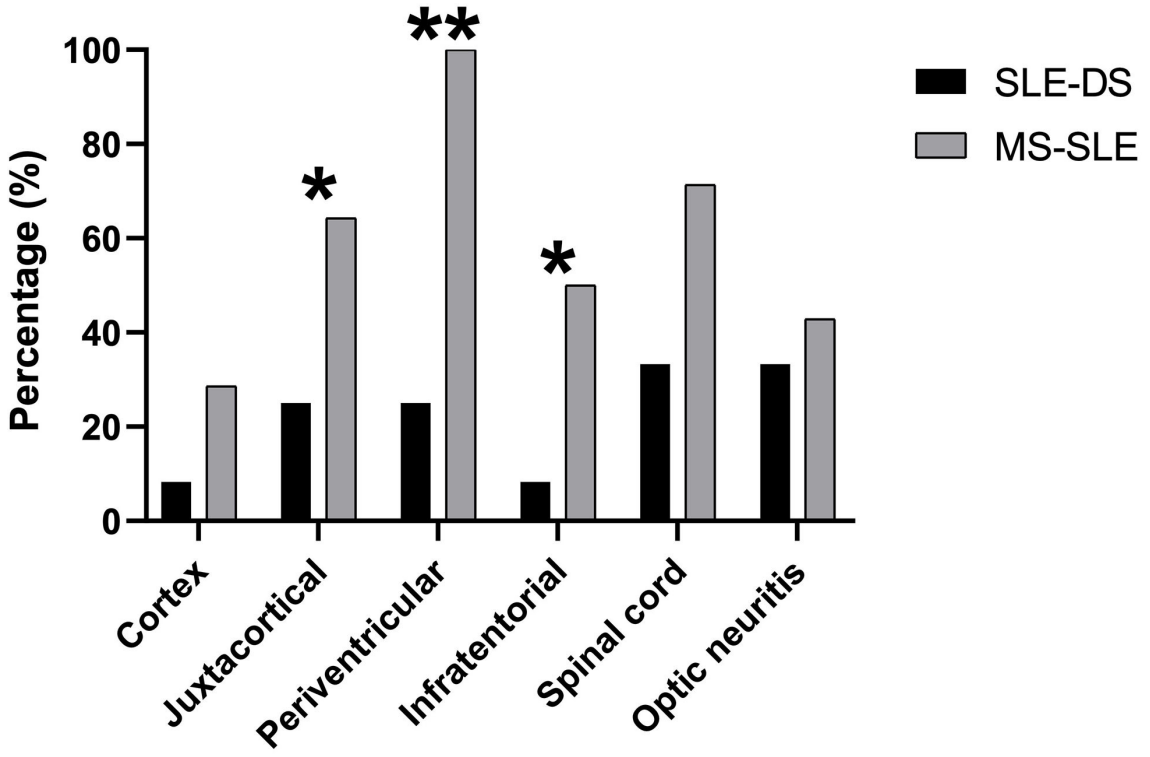

FIGURE 1 | Anatomical distribution of CNS lesions on brain, spinal and orbital MRI, in patients with SLE-demyelinating syndrome and patients with overlap SLE-MS. $^{*}p < 0.05$, $^{**}p < 0.01$

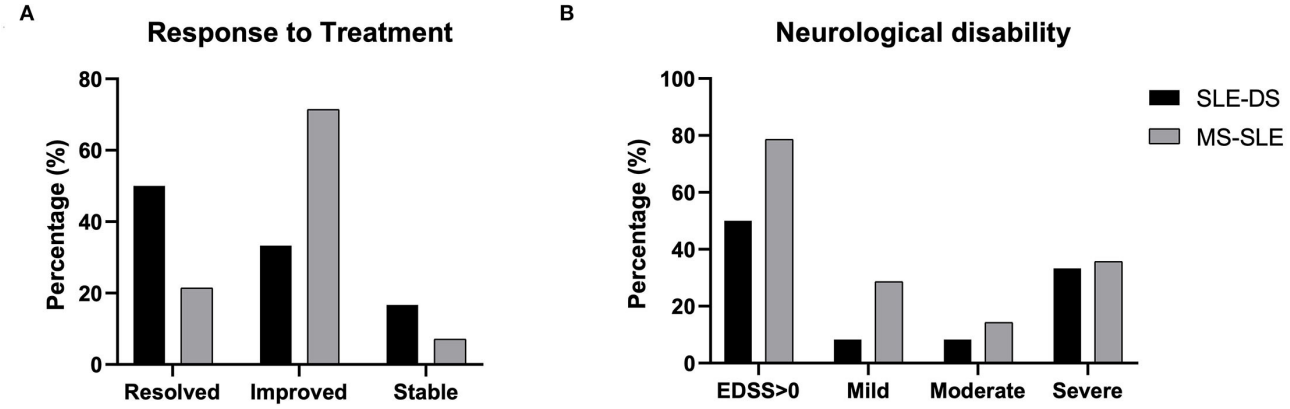

FIGURE 2 | Disease- and treatment-related outcomes at the most recent-follow up visit in patients with SLE-demyelinating syndrome and patients with overlap SLE-MS. **(A)** Response to treatment categorized as i) complete resolution of neurologic symptoms, ii) partial improvement of neurologic symptoms, or iii) no improvement (stable). **(B)** Neurological disability, as measured by the expanded disability status scale. Mild disability: EDSS ≤2; moderate disability: EDSS 2–4; severe disability: EDSS >4. No significant differences were captured between the two groups.

regimens used in MS, may trigger disease flares in patients with lupus, of whom ∼75% exhibit a strong interferon signature (10, 11). To this end, in this study, we aimed to provide a detailed longitudinal evaluation of demyelination presenting in patients with SLE to assess its natural course and identify potential factors that can predict which patients will eventually evolve to frank MS.

Very few studies to date have attempted to describe DS in the context of SLE in detail. Piga et al. (12) performed a systematic literature review, including patients from their own cohort, to identify a total of 104 SLE patients with DS and calculated an estimated prevalence of 1.3%. In this very comprehensive review, the authors opted to include NMO and

NMOSD, which comprised more than 60% of patients, as SLE-related demyelination. Nevertheless, due to the high specificity of anti-aquaporin-4 antibodies for NMOSD, (13, 14), the current consensus argues that when the latter occur in patients with SLE, this most probably represents the coexistence of two autoimmune conditions (15). For this reason, in our study, we excluded patients with NMOSD. We also chose to exclude patients with longitudinal myelopathy attributed to SLE (i.e., anti-aquaporin-4 negative). Although longitudinal myelopathy can be considered a demyelinating condition, it also stands alone as a distinct neuropsychiatric manifestation of SLE. Thus, as the purpose of our study was the differentiation between SLE and MS, we felt that lupus myelopathy does not pose the same diagnostic challenges in patients with a demyelinating syndrome compatible with MS. Finally, in the study by Piga et al. another 27.9% of patients had a CIS. Information on the duration of follow-up was not available, but it would be interesting to know whether at least a proportion of patients with CIS fulfilled criteria for MS in the course of time.

Fourteen patients in our cohort fulfilled the criteria for MS at the most recent-follow up, thus labeled as SLE-MS overlap. Although the segregation of more than one autoimmune disease may occur in the same individual, the coexistence of MS and SLE has only rarely been reported, mainly in case reports. In a previous work from a different SLE cohort (the "Leto" cohort in Crete) (16), we have described another case series of nine patients who fulfilled the criteria for both the diseases (3) We observed similar patient characteristics in both case series. Specifically, overlap patients tended to have a relatively mild SLE phenotype, with no major extra-CNS organ involvement, which did not necessitate intensive immunosuppressive treatment. Contrary, MS tends to follow a relapsing–remitting course, with a variable accumulation of disability, and its severity usually dictates the choice of immunomodulating agents.

Identification of clinical or laboratory features early in the course of a DS that would help predict which patients will eventually evolve to MS would be very helpful. In terms of clinical presentation, no rheumatic or neurologic manifestation was significantly different between SLE-DS and overlap SLE-MS patients. Contrary, we confirmed the diagnostic value of lumbar puncture and CSF analysis in the work-up of patients with demyelination. Both an elevated IgG index and, especially, the presence of type 2 oligoclonal bands was strongly predictive of a final MS diagnosis since both were significantly more common in these patients compared to SLE-DS. This observation corroborates the most recent update of the diagnostic criteria for MS, wherein the presence of unmatched CSF oligoclonal bands permits the diagnosis of MS, even without proven dissemination in time clinically or on MRI (17). Although the 2017 criteria have been criticized by some for lower specificity, our findings support a low threshold for CSF analysis in patients presenting with DS.

The burden of MRI lesions in the CNS was also significantly different between SLE-DS and overlap SLE-MS patients, both in terms of number and location of lesions. Overlap patients tended to have lesions in locations typical for MS, including the infratentorial region and the spinal cord. By contrast, patients with SLE often had lesions only in a single brain territory. Dissemination of CNS lesions in space is a hallmark of MS, which tends to accrue over time and be associated with progressive neurologic disability (18). Accordingly, overlap SLE-MS patients in our cohort accumulated significantly more neurologic damage until the end of follow-up, as measured by the EDSS.

Our study has several limitations. Firstly, similarly to the criticism of the aforementioned systematic review, the relatively short follow-up (little over 3 years) of our study cannot exclude that lupus patients with a CIS in our cohort will not evolve into definite MS in the future. Also, our study did not aim to address the issue of therapy of demyelination in the context of SLE, either SLE-DS or SLE-MS. In this regard, one cannot exclude that the natural history of the demyelinating syndrome could have been influenced by the administration of immunosuppressive or disease-modifying therapies. Along the same lines, in the era of current biologic therapies, demyelination may occasionally occur as a side-effect of medications (19, 20). Nevertheless, in our series, only seven patients with SLE had received immunosuppressive treatment prior to the first occurrence of a demyelinating event (glucocorticoids, hydroxychloroquine, methotrexate, azathioprine, and belimumab) None of these drugs has been linked to demyelinating episodes as a side-effect.

In conclusion, we present one of the few studies with a detailed description of DS in the context of SLE and, for the first time, a longitudinal assessment of DS occurring in patients with SLE, either prior to or following the diagnosis of lupus. We found that more than 50% of these patients are finally diagnosed with MS, while demyelination in the context of SLE follows a more benign course compared to a frank SLE-MS overlap. More importantly, further extension of follow-up in these patients will ascertain whether the remaining SLE-DS patients evolve to MS, or whether SLE-DS is indeed a *bona fide* syndrome of NPSLE.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Attikon University Hospital Ethics Committe. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DN and DK examined patient files and identified candidate patients for the study and DN drafted the manuscript. MP reviewed all brain imaging data. NK, AP, PG, and AG contributed to patient recruitment and data entry. KV, DB, and AF conceived the study, confirmed final patients'diagnoses,

and critically revised and oversaw the study and the writing. All authors contributed to the article and approved the submitted version.

FUNDING

This work was funded in parts by the Hellenic Society of Rheumatology, the Foundation for Research in Rheumatology (FOREUM), the Greek General Secretariat of Research and Technology 'Aristeia' action of the Operational Program 'Education and Lifelong Learning' (cofounded by the European Social Fund and National Resources, Aristeia I 2344 to D.T.B), the European Research Council (ERC) under the European Union's Horizon 2020 Research and Innovation Programme (grant agreement no. 742390), and the SYSCID (A Systems Medicine Approach to Chronic Inflammatory Diseases) under the European Union's Horizon 2020 Research and Innovation Programme (grant agreement no. 733100).

ACKNOWLEDGMENTS

The authors wish to thank all the staff physicians and nurses of both Departments, for providing care to all patients with SLE or multiple sclerosis, as well as other rheumatologic and neurologic conditions.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.889613/full#supplementary-material

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RHEUMATOLOGY

Rheumatology 2021;00:1–9
doi:10.1093/rheumatology/keaa902

Original article

Demyelination with autoimmune features: a distinct clinical entity? Results from a longitudinal cohort

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Abstract

Objective. CNS demyelinating syndromes occurring in the context of SLE may represent a manifestation of neuropsychiatric lupus, or an overlap of SLE and multiple sclerosis (MS). We evaluated prospectively patients presenting with demyelinating syndrome for clinical and serological evidence of SLE and characterized the evolution of their clinical syndrome to a defined disease.

Methods. Patients with CNS demyelinating syndromes not fulfilling the criteria for MS were evaluated in a rheumatology unit for features of SLE and followed longitudinally (enrolment period 2016–20). Clinical, laboratory and neuroimaging data were recorded at every visit, following multidisciplinary evaluation. At end of follow-up, patients were assessed for their final neurological and rheumatological diagnosis, and classified accordingly.

Results. A total of 79 patients were included in the study [91.1% female, mean (S.D.) age at first demyelinating episode 38.4 (10.3) years, median (interquartile range) observation period 39 (57) months]. At last follow-up, 38 patients (48.1%) had evolved into MS. Of the remaining patients, 7 (17.1%) had SLE, while 34 (82.9%) had features of systemic autoimmunity without fulfilling classification criteria for SLE. The most common rheumatological features of these patients were inflammatory arthritis (73.5%), acute cutaneous lupus (47.1%) and positive ANA (72.1%). Importantly, these patients were less likely to have elevated IgG index (odds ratio 0.11, 95% CI 0.04, 0.32) and positive oligoclonal bands (odds ratio 0.21, 95% CI 0.08, 0.55).

Conclusion. A significant number of patients with demyelination do not fulfill criteria for either MS or SLE at follow-up. These patients exhibit lupus-like autoimmune features and may represent a distinct entity, ‘demyelination with autoimmune features’.

Key words: demyelinating syndromes, multiple sclerosis, systemic lupus erythematosus

Rheumatology key messages

- A significant proportion of patients presenting with multiple sclerosis-like manifestations do not fulfil criteria for multiple sclerosis after 3 years of follow-up.
- These patients frequently manifest lupus-like features and are treated with conventional immunosuppressive drugs.
- Patients with ‘demyelination with autoimmune features’ follow a more benign course compared with multiple sclerosis patients and accrue less neurological disability.

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Submitted 7 October 2020; accepted 23 November 2020

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CLINICAL SCIENCE

Introduction

SLE can affect the CNS encompassing a wide spectrum of neurological and psychiatric features, collectively termed NPSLE [1, 2]. One of the less studied neuropsychiatric manifestations of SLE is the 'demyelinating syndrome', an entity referred to as 'lupoid sclerosis' in the past, due to its striking resemblance to multiple sclerosis (MS). MS is a progressive—potentially disabling—inflammatory disease characterized by multifocal areas of demyelination in the white matter of the brain and the spinal cord [3]. Its diagnosis necessitates objective evidence of central neurological dysfunction with evidence of 'dissemination in space and time' (more than one affected area and more than one episode), provided that other competing diagnoses have been excluded. According to the ACR nomenclature for NPSLE, the diagnostic criteria of 'SLE-demyelinating syndrome' resemble very much those of definite MS, including symptomatic CNS white matter lesions, transverse myelopathy, optic neuropathy, diplopia due to nerve palsies or internuclear ophthalmoplegia, and brain stem disease, each occurring at a different time point [4].

The autoimmune nature of both MS and SLE, the rarity of demyelinating syndrome in published NPSLE cohorts [5–8], and its resemblance to frank MS inevitably pose the question as to whether this particular syndrome represents a bona fide manifestation of NPSLE or a simple concordance of two distinct autoimmune conditions [9]. This differential diagnosis has important practical implications for patient care because, apart from glucocorticoids, the drugs used to treat the two conditions are different. Moreover, IFN-based regimens for MS may carry the potential to trigger flares in patients with lupus, a disease with a prominent IFN signature [10].

Previous studies have attempted to explore the concept of demyelination in SLE [9, 11], but their conclusions are hampered by the inclusion of patients with neuromyelitis optica or longitudinal lupus myelopathy, now considered as distinct demyelinating conditions. Moreover, we have previously described a case series of patients who fulfilled criteria for both SLE and MS [12], wherein we found that MS tended to be the dominant disease and thus to guide immunosuppressive therapy. Since CNS demyelinating syndromes are frequently encountered in clinical practice and pose diagnostic and therapeutic challenges, in this study we sought to explore whether patients who experience a demyelinating syndrome and have 'atypical features' for MS may exhibit features of systemic autoimmunity, either at diagnosis or during follow-up.

Methods

Patients and inclusion criteria

The present study was initiated in 2016 as a collaborative project between the Rheumatology and Clinical Immunology Unit and the Department of Neurology of 'Attikon' University Hospital, a tertiary referral centre of Western Attica, Greece, responsible for the care of approximately 2 million citizens. The aim was to assess patients presenting with a demyelinating disorder of immune origin (i.e. excluding trauma/compression, ischaemia or demyelination due to metabolic derangements) for the presence of features of an underlying systemic autoimmune disease, including—but not limited to—SLE. To this end, the two units established an algorithm of mutual patient referral, which included the following groups of patients: Group 1, patients examined in the Department of Neurology with a demyelinating syndrome not fulfilling criteria for MS who had features suggestive of a systemic autoimmune disease (see below), and Group 2, vice versa, patients followed in the Rheumatology and Clinical Immunology Unit for a systemic autoimmune disease who later developed a demyelinating syndrome. For all patients, the time point of the first demyelinating episode was labelled as T0.

Patients of Group 1 were evaluated through a screening questionnaire for systemic autoimmune features (supplementary Table S1, available at *Rheumatology* online) and immunology laboratory testing [including ANA, C3/C4, anti-dsDNA, antiphospholipid (aPL, aCL IgG/IgM, anti-β2GPI IgG/IgM and LA), anti-ENA and RF]. Patients who had (i) one or more clinical and one or more serological criterion, or (i) two or more clinical or (iii) two or more serological criteria were referred to the Rheumatology Unit of 'Attikon' Hospital for further evaluation by expert rheumatologists (A.F., D.T.B.). A comprehensive dataset including demographics, clinical and laboratory features was completed for each patient at first study visit and every follow-up.

Patients of Group 2 were referred for neurological evaluation by expert neurologists (D.K., K.V.). For all patients, comprehensive neurological evaluation at T0 included (i) thorough clinical examination and laboratory tests, as indicated, (ii) MRI of the CNS (brain ± spinal cord, according to symptoms) and (iii) cerebrospinal fluid (CSF) analysis, including IgG index and oligoclonal band screen (evaluated according to international consensus) [13]. Patients were thereafter followed at regular visits; a diagnosis of MS was established with a combination of the 2010 McDonald criteria and physician judgment [14]. At every visit, progression of disability was also assessed with the Expanded Disability Status Scale (EDSS) [15]. Depending on the available data, patients were classified as either (i) definite MS or (ii) demyelinating disease not fulfilling criteria for MS, both at T0 and at last follow-up.

The following exclusion criteria were applied: (i) patients with neuromyelitis optica spectrum disorders or other primary CNS diseases, (ii) patients with longitudinal myelopathy spanning three or more vertebral bodies (because longitudinal myelopathy represents a condition clinically and radiologically distinct from MS), (iii) patients who experienced a demyelinating syndrome as a consequence of anti-TNF therapy for a rheumatic disease, (iv) patients with CNS imaging findings more consistent with microischemic—rather than demyelinating—

lesions, as is common in SLE (see below, Assessment of MRI) and (v) patients with fewer than 3 visits during follow-up. Patients were also excluded if they did not have evidence of a systemic autoimmune disease, following rheumatological evaluation. Finally, to ensure cohort homogeneity (i.e. patients with 'atypical demyelinating syndromes'), patients who fulfilled criteria for MS at T0 were not included in the study.

The study had a retrospective (patient data prior to study initiation were retrieved from patient files) and a prospective phase, following patient first evaluation by both disciplines. Patient enrolment and follow-up for the purpose of the study was completed in January 2020.

Assessment of MRI

All MRIs were performed on 1.5 or 3 Tesla MR scanners. Images included standard clinical protocol for brain imaging with T1 pre- and post-contrast injection, T2 and FLAIR (Fluid Attenuated Inversion Recovery) sequences. MRIs of brain and spinal cord for all patients were reviewed by an expert neuroradiologist (M.P.). Imaging features suggestive of demyelination included: (i) presence of supra- and infratentorial lesions, (ii) periventricular ovoid lesions lesion (Dawson's fingers), (iii) lesions in the corpus callosum (cross out lesions adjacent to the temporal horns or the corpus callosum), (iv) gadolinium enhancement and (v) hypointense T1 lesions ('black holes'). As specified above, patients with lesions more compatible with non-specific white matter hyperintense lesions (i.e. of possible ischemic/microvascular aetiology, as is common in SLE) were excluded from the study. For the most recent MRI of each patient, the distribution of demyelinating lesions in each patient was further divided in five regions: (i) cortex, (ii) juxtacortical, (iii) periventricular, (iv) infratentorial and (v) spinal cord.

Definitions

We used the following definitions at both T0 and end of follow-up period. (i) MS: fulfilment of 2010 McDonald criteria combined with expert physician judgment (neurology, neuroradiology). (ii) Clinically isolated syndrome: single demyelinating attack without dissemination in time (an attack is defined as patient-reported or objectively observed events typical of an acute inflammatory demyelinating event in the CNS, with duration of at least 24 h, in the absence of fever or infection). (iii) SLE: patients were classified with SLE according to the ACR 1997 criteria [16], (the latter were chosen over more recent criteria sets due to their increased specificity for SLE) [17].

Accordingly, at last follow-up, patients with both definite MS and SLE were labelled as 'overlap MS/SLE'. Demyelinating syndromes not fulfilling criteria for MS in patients with classified SLE were diagnosed as 'primary NPSLE (SLE with demyelinating syndrome)'. Finally, patients with demyelinating syndrome who had features of systemic autoimmunity but did not fulfil criteria for either MS or SLE were labelled as 'demyelination with autoimmune features' (DAF).

Statistical analysis

All captured data are stored electronically at 'Attikon' Hospital. Descriptive statistics were undertaken for continuous variables and mean values (S.D.) or median [interquartile range (IQR)] were calculated, for normally and non-normally distributed variables, respectively. χ^2 test and Student's *t*-test were used to compare categorical and continuous variables, respectively. For all comparisons, a *P*-value <0.05 was considered as statistically significant. All statistical analyses were performed using the Statistical Package for the Social Science (SPSS; SPSS Inc., Version 25.0, IBM Corp., Armonk, NY, USA).

The study was approved by the Ethics Committee of the 'Attikon' University Hospital of Athens and patients provided informed consent for their participation (protocol number 103/06-03-2014).

Results

Demographics and prevalence of MS during follow-up

A total of 79 patients with new-onset atypical demyelinating syndrome were included in the study (female-to-male ratio ~9:1). The mean age at the time of first demyelinating episode (T0) was 37.6 (S.D. 10.8) years and median follow-up duration after T0 was 39 (IQR 57) months. At the end of follow-up, 38 patients (48.1%) were diagnosed with MS; of them, 32 patients (84.2%) were classified as relapsing–remitting MS, 3 (7.9%) as secondary progressive MS and 3 (7.9%) as primary progressive MS. Median time after T0 to reach MS diagnosis was 18 (IQR 15) months. The remaining 41/79 patients (51.9%) did not fulfil criteria for MS at last follow-up, of whom 32 had experienced a clinically isolated syndrome.

Rheumatic and neurological manifestations, autoantibodies and characteristics of cerebrospinal fluid in MS *vs* non-MS patients

Clinical features of SLE and autoantibodies are summarized in Table 1 and supplementary Table S2, available at *Rheumatology* online, separately for patients with MS and non-MS (both at T0 and cumulatively). The most frequent clinical manifestations were inflammatory arthritis, followed by acute cutaneous lupus (photosensitive rash, malar rash or subacute cutaneous lupus). With respect to clinical and serological features, only non-scarring alopecia was associated with non-MS diagnosis [odds ratio (OR) 5.41, 95% CI 1.40, 20.89]. The presence of ANA or other autoantibodies did not differ between the two groups.

Neurological manifestations at T0 and during follow-up for non-MS and MS are summarized in Table 2 and

Table 1 Rheumatological manifestations and autoantibodies[a] of non-MS and MS patients at T0 and cumulatively over the course of follow-up

	T0			Cumulatively		
	Non-MS	MS	*P*-value	Non-MS	MS	*P*-value
Acute cutaneous lupus, *n* (%)	23 (56.1)	18 (47.4)	ns	25 (60.1)	20 (52.6)	ns
Inflammatory arthritis, *n* (%)	30 (73.2)	31 (81.6)	ns	35 (85.4)	33 (86.8)	ns
Oral/nasal ulcers, *n* (%)	5 (12.2)	9 (23.7)	ns	7 (17.1)	11 (28.9)	ns
Non-scarring alopecia, *n* (%)	**13 (31.7)**	**3 (7.9)**	**0.008**	**19 (46.4)**	**8 (21.1)**	**0.02**
Leukopenia, *n* (%)	8 (19.5)	3 (7.9)	ns	10 (24.4)	8 (21.1)	ns
Sicca, *n* (%)	7 (17.1)	6 (15.9)	ns	12 (29.3)	8 (21.1)	ns
Raynaud's, *n* (%)	10 (24.4)	16 (42.1)	ns	10 (24.4)	17 (44.7)	ns
ANA ≥1:80, *n* (%)	28 (68.3)	29 (76.3)	ns	33 (80.5)	31 (81.6)	ns
Low complement, *n* (%)	8 (19.5)	4 (10.6)	ns	11 (26.8)	7 (18.5)	ns
aPL, *n* (%)	8 (19.5)	12 (31.6)	ns	8 (19.5)	13 (34.2)	ns

[a]The rest clinical and serological features are shown in supplementary Table S2, available at *Rheumatology* online. Values in bold represent comparisons that reached statistical significance ($P < 0.05$). T0: time of first demyelinating episode; MS: multiple sclerosis; ns: non-significant.

Table 2 Neurological manifestations[a] and cerebrospinal fluid findings of patients with non-MS and MS, both at T0 and cumulatively

	T0			Cumulatively		
	Non-MS, *N* = 41	MS, *N* = 38	*P*-value	Non-MS, *N* = 41	MS, *N* = 38	*P*-value
Sensory deficits, *n* (%)	**22 (53.7)**	**31 (81.6)**	**0.01**	**24 (58.5)**	**36 (94.7)**	**0.001**
Motor/pyramidal deficits, *n* (%)	11 (26.8)	7 (18.4)	ns	**13 (31.7)**	**22 (57.9)**	**0.02**
Optic neuritis, *n* (%)	10 (24.4)	8 (21.1)	ns	10 (24.4)	9 (23.7)	ns
Diplopia, *n* (%)	5 (12.2)	3 (7.9)	ns	8 (19.5)	5 (13.2)	ns
Lhermitte's sign, *n* (%)	2 (4.9)	2 (5.3)	ns	**2 (4.9)**	**9 (23.7)**	**0.03**
Spastic paraparesis, *n* (%)	0 0	1 (2.6)	ns	2 (4.9)	3 (7.9)	ns
Cerebellar disease, *n* (%)	5 (12.2)	3 (7.9)	ns	7 (17.1)	6 (15.8)	ns
Brainstem disease, *n* (%)	2 (4.9)	2 (5.3)	ns	2 (4.9)	3 (7.9)	ns
		Non-MS		MS		*P*-value
CSF index >0.65, *n* (%)		15 (36.6)		32 (84.2)		<0.0001
Mean CSF index (S.D.)		0.62 (0.19)		0.91 (0.26)		<0.01
Oligoclonal bands (+), *n* (%)		13 (31.7)		26 (68.4)		<0.01
Type II		3		24		<0.001
Type III		8		2		<0.01
Type IV		2		0		ns

[a]The rest neurological manifestations are shown in supplementary Table S2, available at *Rheumatology* online. Values in bold represent comparisons that reached statistical significance ($P < 0.05$). T0: time of first demyelinating episode; MS: multiple sclerosis; CSF: cerebrospinal fluid; ns: non-significant.

supplementary Table S2, available at *Rheumatology* online. Sensory deficits were more common in MS patients at T0 (OR 3.82, 95% CI 1.37, 10.65). No other significant differences were observed between non-MS and MS patients in terms at first presentation (T0). Importantly, MS patients were more likely to have an elevated IgG index (OR 9.24, 95% CI 3.14, 27.19) and positive oligoclonal bands (OR 4.66, 95% CI 1.80, 12.05) (notably type II). Non-type II oligoclonal bands were mainly observed in non-MS patients.

Brain imaging

Anatomical distribution of CNS lesions is shown separately for patients with non-MS and MS in **Fig. 1**. Juxtacortical regions and optic nerve were similarly affected between the two groups. Approximately half of non-MS patients had periventricular lesions, while all patients with MS had at least one lesion in the periventricular area. As expected, non-MS patients were less likely to exhibit brain lesions in the cortex, spinal cord,

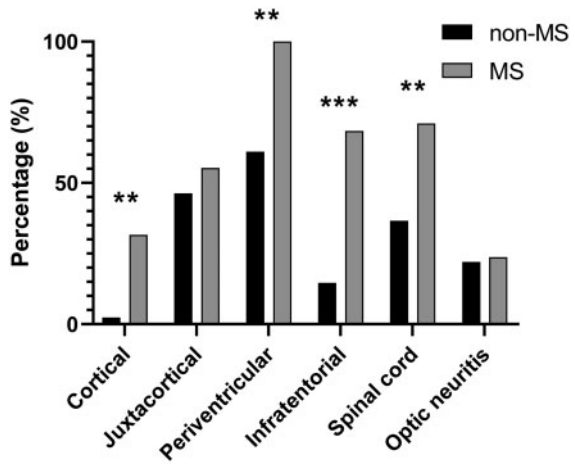
periventricular and infratentorial region. A single brain region was affected in 17 non-MS patients (41.5%) and only 4 MS patients (10.5%) (OR 6.02, 95% CI 1.8, 20.2). Active, gadolinium-enhancing CNS lesions on last brain MRI were evident in three non-MS patients (7.3%) and in 44.8% of MS patients ($n = 17$) (OR 0.09, 95% CI 0.03, 0.37).

Frequency of SLE or other systemic autoimmune disease in patients with demyelinating syndrome

At the end of the observation period, we sought to categorize patients (both those fulfilling criteria for MS and those not), according to a diagnosis of SLE. Among the 38 patients with MS, six patients were also diagnosed with SLE, thus classified as 'overlap MS/SLE'. Similarly, from the 41 'non-MS' patients, seven were diagnosed with SLE; in these patients, the demyelinating syndrome was attributed to SLE *per se*, as a manifestation of primary NPSLE (Fig. 2). Of note, a diagnosis of SLE already at the time of first demyelinating episode (T0) was established in six patients. Regarding other systemic autoimmune diseases, among 21 patients with positive aPL, two met classification criteria for APS (one exclusively with obstetric APS, the other with thrombotic APS and SLE), a disease that has also been implicated in the evaluation of 'atypical MS' in the past [18]. Six patients were positive for anti-Ro (SSA) and/or anti-LA (SSB) antibodies, but none fulfilled classification criteria for SS.

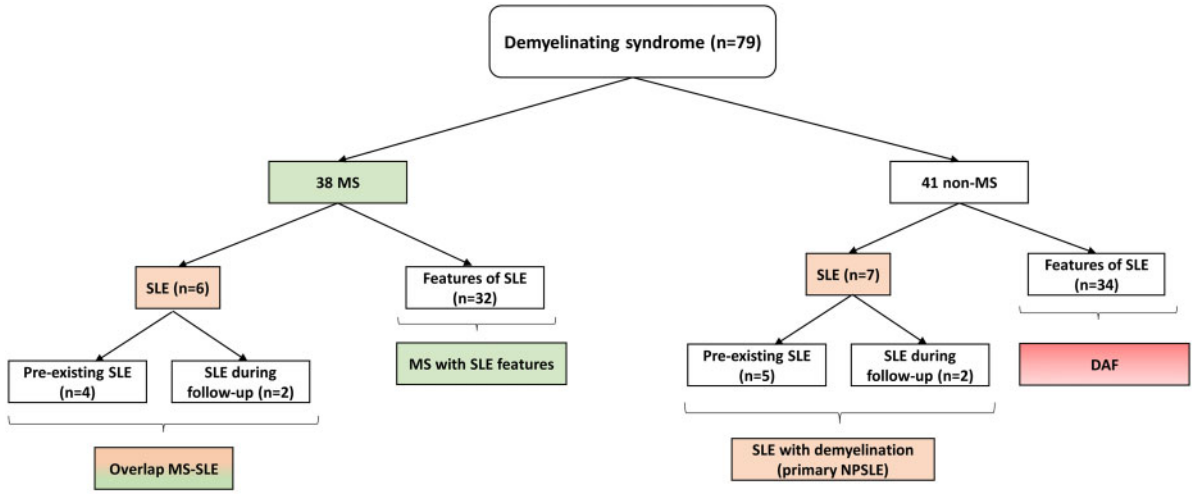
Importantly, a total of 34 patients did not fulfil criteria either for MS or for SLE until the end of follow-up; this patient subset was classified as DAF. The mean age at the time of demyelination (T0) was 37.6 (S.D. 10.9) years and the median disease duration at last follow-up was 44 (IQR 45) months. Half of them ($n = 17$) experienced SLE features at T0, while 14 (41.1%) developed lupus features during follow-up and the remaining 3 were followed in the rheumatology unit for a systemic autoimmune disease prior to T0. This subgroup of patients mainly exhibited musculoskeletal and cutaneous manifestations, such as inflammatory arthritis (70.1%), inflammatory rashes (52.6%), increased hair loss (29.4%), sicca (26.5%) and oral ulcers (20.7%), while they were often positive for ANA (76.5%) and aPL (20.6%). Major organ manifestations such as nephritis, serositis or cytopenias were rarely involved during follow-up. Abnormal CSF findings were infrequent in DAF patients; positive oligoclonal bands were present in 25% and increased IgG index in 32.6%. With respect to neurological

Fig. 1 Anatomic distribution of the MRI lesions identified in the non-MS and MS patients



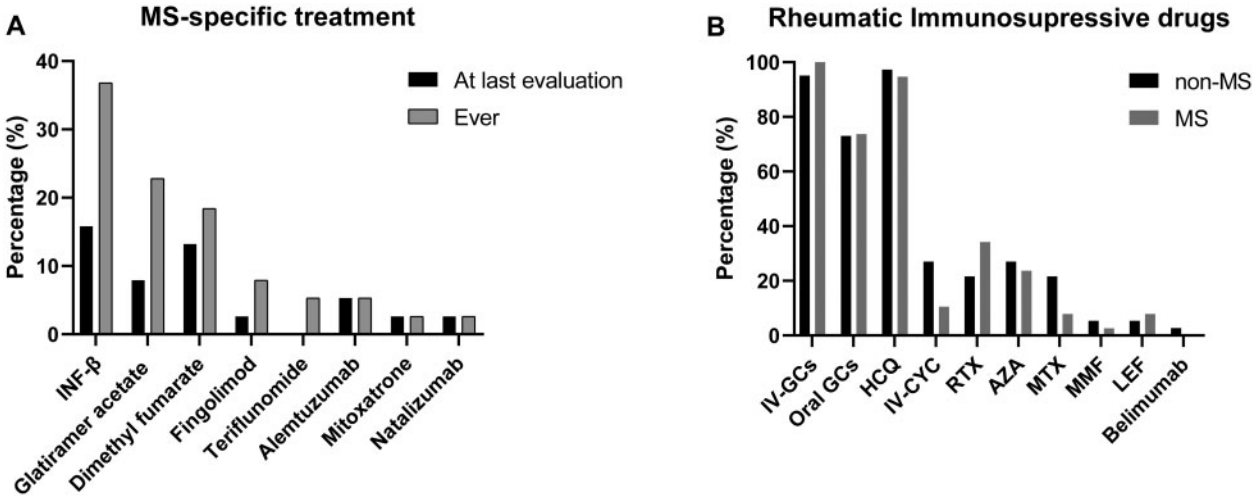
MS: multiple sclerosis. $^{**}P < 0.01$, $^{***}P < 0.001$.

Fig. 2 Flow-chart of patients presenting with atypical demyelinating syndromes ($n = 79$)



MS: multiple sclerosis; DAF: demyelination with autoimmune features.

FIG. 3 Administrated therapies in patients with MS and non-MS



(**A**) Types of MS-specific treatment in subjects with MS, both at last evaluation and ever received. (**B**) All immunosuppressive agents used in patients with non-MS and MS. MS: multiple sclerosis; IV-GCs: i.v. glucocorticoids; GCs; glucocorticoids; IV-CYC: i.v. CYC.

TABLE 3 Demyelination-related outcomes until the end of follow in MS, SLE-demyelination and DAF patients

	MS ($n = 38$)	DAF ($n = 34$)	SLE-demyelination ($n = 7$)
Relapse >0, *n* (%)	34 (89.5)	8 (23.5)	2 (28.5)
Relapses, mean (S.D.)	2.0 (1.7)	0.5 (0.2)	0.3 (0.2)
EDSS > 0, *n* (%)	30 (78.9)	11 (32.3)	5 (71.4)
EDSS, median (IQR)	1 (3)	1.5 (0.9)[b]	3.3 (1.3)[b]
Active brain lesions[a]	17 (44.7)	2 (5.9)	1 (14.3)

[a]Last MRIs. [b]Mean (S.D.). MS: multiple sclerosis; DAF: demyelination with autoimmune features; EDSS: Expanded Disability Status Scale; IQR: interquartile range.

manifestations, sensory deficits were the most common symptom (55.8%), followed by optic neuritis (29.4%); motor disturbances were less frequent in DAF compared with MS patients (OR 0.2, 95% CI 0.07, 0.56).

Administered therapies and outcomes

MS-specific treatment was administered only in patients with definite MS (**Fig. 3A**), the most common drugs being IFN-β (52.6%) and glatiramer acetate (31.6%). On the contrary, patients not fulfilling criteria for MS were treated exclusively with immunosuppressive drugs used to treat rheumatic diseases. MS patients also received immunosuppressive drugs in combination with MS-specific treatment, as shown in **Fig. 3B** for non-MS and MS patients, respectively. Rituximab (RTX) was the main therapeutic option for patients with MS and features of SLE (34.2%). Non-MS patients with severe neurological deficits were mainly treated with i.v. CYC (24.4%) or RTX (19.5%).

Disease-related outcomes including relapses, EDSS at last visit and active brain lesions at last MRI are summarized separately for MS, SLE-demyelination and DAF patients in **Table 3**. MS and SLE-demyelination patients were more likely to exhibit neurological deficits (EDSS >0) at last follow-up visit as compared with DAF patients. As expected, MS patients experienced more relapses requiring i.v. glucocorticoids.

Among DAF patients, eight patients (23.5%) had experienced at least one relapse, mainly corresponding to optic neuritis. At last evaluation, 23 patients (67.6%) had normal neurological examination (EDSS = 0) and only two patients had active brain lesions on last MRI.

Discussion

Patients presenting with a new-onset demyelinating syndrome represent a diagnostic challenge, which is further exaggerated when such a patient has established SLE or features suggestive of the disease. This diagnostic dilemma has important implications for the care of these patients; indeed, while MS and SLE-demyelinating syndrome (as defined in the 1999 ACR nomenclature for

NPSLE) [4] have similar clinical presentations, the drugs used to treat the two conditions differ. Herein, we report the first study designed specifically to assess the natural history of demyelination in the context of systemic autoimmunity. Importantly, in this study, we found that a significant proportion of patients presenting with demyelinating syndrome do not fulfil criteria for MS after >3 years of follow-up, they frequently have features of a systemic autoimmune disease, like arthritis or inflammatory rashes—with some getting a formal diagnosis of SLE—and are often treated with conventional immunosuppressive drugs.

Previous reports and review articles have dealt with the issue of demyelination in SLE [9, 11, 19], yet convincing distinctive findings between SLE-demyelination and MS remain to be established. Consequently, as autoimmune diseases often tend to segregate within families or even within the same individual [20], it is tempting to speculate that demyelination in SLE purely represents an overlap of two distinct conditions. Indeed, we have previously reported a case series of nine patients who fulfilled classification criteria for both MS and SLE, wherein we showed that the neurological disease mostly accounted for patient morbidity and dictated the therapeutic strategy, while lupus tended to have a mild phenotype [12]. In the present study, we identified six additional patients with an overlap of the two diseases and seven patients classified as SLE-demyelination (primary NPSLE). These patients belong to the 'Attikon' Lupus cohort, currently consisting of 627 patients [21, 22]. Thus, the prevalence of SLE-demyelination and the prevalence of MS in SLE approximates 1% for each group. Most importantly, 43% of patients in this case series (34/79) could not be classified as either MS or SLE, and still experienced both demyelinating episodes and rheumatic manifestations.

At present, longer-term follow-up can definitively answer whether a patient with demyelination will ultimately develop MS. Such patients, however, carry the risk of relapses and accrual of neurological disability, if left untreated. Identifying clinical features or biomarkers (serological or imaging) that could help differentiate between MS and non-MS conditions early in the course of the disease is thus of the utmost importance [23]. We found that sensory deficits, motor findings and the Lhermitte's sign (considered typical for MS) were significantly more common in patients ultimately diagnosed with MS, although at T0 only sensory deficits were more common in MS patients. While autoantibodies were of limited diagnostic value, the presence of pure intrathecal oligoclonal bands (type 2) and a markedly elevated CSF IgG index were highly predictive of a final MS diagnosis.

MRI is considered a cornerstone of CNS diagnostics. Not unexpectedly, we found that the involvement of multiple brain regions (OR 12.0), as well as gadolinium enhancement of lesions (OR 10.2), both significantly increased the likelihood of a final MS diagnosis. Patients not finally diagnosed with MS tended to have fewer lesions in restricted locations. Importantly, in our study we excluded patients whose MRI lesions were more compatible with microischaemic lesions (judged by an experienced neuroradiologist), because such lesions are common especially in patients with SLE [24, 25]; it can be argued that even for expert neuroradiologists, the distinction between demyelination and ischaemia is occasionally very difficult and ambiguous cases are frequent in clinical practice. In this regard, the 'central vein' sign (i.e. the presence of a small vein within the white matter lesion) has recently been proposed as a differential diagnostic tool and an imaging biomarker for MS, showing high specificity (although lower sensitivity) for MS *vs* other CNS inflammatory conditions, including SLE [26, 27]. However, demonstration of this sign requires special techniques that are not widely available, and it has not been recommended as part of the standard imaging protocol for clinical purposes. The morphology and thickness of the demyelinating lesions located in the spinal cord may also differentiate SLE from MS, because SLE-related spinal cord lesions tend to be thicker and have a more longitudinal morphology in comparison with MS-related spinal cord lesions. Nevertheless, this would not apply to our study, because we specifically excluded patients with typical longitudinal 'lupus myelopathy', as being a distinct patient subset.

To ensure cohort homogeneity, we chose not to include patients with established MS at T0, in order to explore the association of 'atypical demyelinating syndromes' with systemic autoimmunity, in particular SLE. Nevertheless, patients with established MS and features of systemic autoimmunity are far from uncommon in clinical practice. In our practice, as we have previously described in patients with coexistent SLE and MS, such 'overlap' cases are usually dominated by the neurological clinical picture, which tends to drive therapeutic decisions with MS-specific therapies, while rheumatological manifestations are typically treated with low-potency immunosuppressive drugs (e.g. AZA, MTX or MMF) [12]. Alternatively, B-cell depletion with RTX is an attractive option for cases of MS with features of systemic autoimmune manifestations, as RTX can be used to treat both conditions, but results on its efficacy remain to be seen.

Patients similar to those included in our study currently find themselves in a peculiar 'no man's land' and therapy is often empirical. Recently, in a similar clinical situation, the rheumatology–pulmonology community jointly established the term 'interstitial pneumonitis with autoimmune features', to describe patients with interstitial pneumonitis and clinical or serological features of systemic autoimmunity, who have neither idiopathic pulmonary fibrosis nor a frank systemic autoimmune disease [28]. Akin to this, we propose the term 'demyelination with autoimmune features (DAF)' for patients with demyelination not fulfilling the MS criteria and features of a systemic autoimmune disease.

Our study has limitations that need to be acknowledged. Firstly, the mean duration of follow-up in our

cohort was limited, a little over 3 years. Longer duration of observation would certainly add more information regarding the natural history of the clinical syndromes in our cohort, regarding the diagnosis of both MS and SLE (or another systemic autoimmune disease). Additionally, MRIs of patients were not performed in the same MRI scanner and were not necessarily done at 3 Tesla; however, they all underwent central reading by an expert neuroradiologist. Finally, one could argue that the natural history of both the demyelinating syndrome and systemic autoimmune disease may have been influenced by the administration of immunosuppressive medications prior to establishment of a formal diagnosis; nevertheless, treating patients with a potentially serious condition without waiting for the fulfilment of formal diagnostic or classification criteria is justified and common in clinical practice.

In conclusion, we report for the first time a prospective evaluation of patients presenting with a demyelinating syndrome for the ascertainment of a diagnosis of MS or SLE, through direct examination by specialists from both rheumatology and neurology. A significant proportion of such patients exhibit lupus-like features and are not classified as MS during follow-up. Future progress in the field of serum, CSF or imaging biomarkers will hopefully delineate if these patients have bone fide MS or a neurological manifestation of systemic autoimmune condition. Until then, we propose the umbrella term 'demyelination with autoimmune features' for this challenging subset of patients.

Acknowledgements

We thank the staff physicians and nurses of the Rheumatology Unit and Neurology Department of 'Attikon' University Hospital for their referral and care to the patients with rheumatic diseases.

Funding: This work was funded in part by: The Hellenic Society of Rheumatology; the Foundation for Research in Rheumatology (FOREUM); the Greek General Secretariat of Research and Technology 'Aristeia' action of the Operational Program 'Education and Lifelong Learning' (co-funded by the European Social Fund and National Resources, Aristeia I 2344 to D.T.B.); the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement no. 742390); and the SYSCID (A Systems Medicine Approach to Chronic Inflammatory Diseases) under the European Union's Horizon 2020 research and innovation programme (grant agreement no. 733100).

Disclosure statement: The authors have declared no conflicts of interest.

Data availability statement

All data relevant to the study are included in the article. More detailed data are available upon reasonable request by any qualified researcher who engage in rigorous, independent scientific research.

Supplementary data

Supplementary data are available at *Rheumatology* online.

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ii. Pathogenetic aspects

Update on the pathogenesis of central nervous system lupus

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Propose of review
Neuropsychiatric systemic lupus erythematosus (NPSLE) is an emerging frontier in lupus care encompassing a wide spectrum of clinical manifestations. Its pathogenesis remains poorly understood because of the complexity of pathophysiologic mechanisms involved and limited access to tissue. We highlight recent advances in the pathophysiology of neuropsychiatric lupus.

Recent findings
Disruption of blood–brain barrier (BBB) facilitating entrance of neurotoxic antibodies into the central nervous system (CNS), neuroinflammation and cerebral ischemia are the key mechanisms. Disruption of the BBB may occur not only at the traditional BBB, but also at the blood–cerebrospinal fluid barrier. Certain autoantibodies, such as anti-*N*-methyl-D-aspartate receptors, antiribosomal P and antiphospholipid antibodies may cause injury in subsets of patients with diffuse neuropsychiatric disease. Activation of microglia via autoantibodies, interferon-a or other immune reactants, may amplify the inflammatory response and promote neuronal damage. New inflammatory pathways, such as TWEAK/Fn14, Bruton's tyrosine kinase, Nogo-a and ACE may represent additional potential targets of therapy. Novel neuroimaging techniques suggest alterations in brain perfusion and metabolism, increased concentration of neurometabolites, indicative of glial activation, vasculopathy and neuronal impairment.

Summary
NPSLE encompasses a diverse phenotype with distinct pathogenic mechanisms, which could be targeted by novel therapies or repositioning of existing drugs.

Keywords
autoantibodies, blood–brain barrier, microglia, neuroimaging, neuropsychiatric lupus

INTRODUCTION

Systemic lupus erythematosus (SLE) frequently affects the central and peripheral nervous system, a syndrome collectively termed neuropsychiatric SLE (NPSLE) [1]. Up to 40% of SLE patients may experience at least one neuropsychiatric event over the course of their disease, with less than half of these manifestations directly attributed to lupus *per se* [2]. The underlying pathogenesis remains ill-defined [3], because of limited access to tissue, the diversity and complexity of clinical manifestations, and the overlap with non-SLE related neuropsychiatric events [1].

One of the early key assumptions in NPSLE was that a disrupted blood–brain barrier (BBB) allowed autoantibodies and immune components of peripheral blood to penetrate into the central nervous system (CNS), causing inflammation and damage [4]. Among autoantibodies, anti-*N*-methyl-D-aspartate receptors (anti-NMDA) and antiribosomal P (anti-RP) can become pathogenic upon entering the brain; the role of other autoantibodies remains poorly understood [5,6]. Recently, type I interferon (IFN) and microglial cells have emerged as central players in CNS disease, with recent studies substantiating their role in NPSLE [7▪▪,8].

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Curr Opin Rheumatol 2019, 31:669–677
DOI:10.1097/BOR.0000000000000655

KEY POINTS

- Neuroinflammation and cerebral ischemia are the two major pathogenetic mechanisms in NPSLE.
- Abnormal BCSFB may represent an additional central mechanism in NPSLE pathogenesis.
- Microglia cells emerge as central players in CNS lupus and targets of novel therapies.
- Advanced imaging techniques may dissect the multifactorial nature of CNS lupus.

OVERVIEW AND EVOLVING CONCEPTS IN NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS

In NPSLE, a 'mosaic' of genetic, environmental and neuroendocrine factors culminates in neuroinflammation and cerebral ischemia, the two major mechanisms operant [9]. Brain autopsies of patients with NPSLE show diffuse vasculopathy, microthrombi, microinfarction, macroinfarction and vasculitis, along with complement deposition [10▪]. The presence of 'vasculopathy' is supported by the high prevalence of white matter hyperintense lesions on brain MRI, representing microvascular disease, and the strong association of certain NPSLE syndromes with antiphospholipid antibodies (aPL) [3,11]. On the other hand, in the setting of a BBB disruption, the presence of inflammatory mediators and autoantibodies in the cerebrospinal fluid (CSF) of lupus patients highlights the role of an immune response and CNS inflammation [12]. In clinical practice, in a given patient, it is often hard to distinguish between ischemia and inflammation. When in doubt both immunosuppressive and antithrombotic agents, especially in aPL-related NP events, may be used [13].

BRAIN–BARRIER DISRUPTION: GLOBAL VS. LOCALIZED

BBB is a highly selective semipermeable border of CNS vessels, formed mainly by brain capillaries at the level of endothelial cells with specialized tight junctions [14]. The umbrella term 'BBB disruption' denotes the impairment of any structure of the human CNS that can potentially be distorted, allowing immune and toxic components of the blood to enter [4,15]. Historically, BBB disruption was the first pathophysiological mechanism proposed to play a role in NPSLE pathogenesis. Early studies showed the presence of IgG, albumin and inflammatory cytokines in the CSF of patients with lupus and in lupus-prone mice [12,16]. Due to the complexity of BBB and inability to fully visualize the loss of integrity *in vivo*, it remains unclear whether these molecules originate from peripheral blood or are produced intrathecally.

Over the last years, more structures of the brain have been recognized as 'barriers' of the CNS, including the blood–CSF barrier (BCSFB). The choroid plexus is a plexus of modified ependymal cells located in the ventricles that produces the cerebrospinal fluid. The BCSFB – located at choroid plexus epithelial cells – is the natural 'dam' between the systemic circulation and CSF. Thus, the presence of inflammatory mediators in the CSF of NPSLE patients [12] can also be explained by a disrupted BCSFB rather than global dysfunction of BBB. Accordingly, in recent years, studies have focused on BCSFB in MRL/lpr mice, demonstrating that BCSFB is disrupted in the absence of BBB dysfunction [17]. A recent study confirmed the presence of infiltrating leukocytes through the BCSFB of MRL/lpr mice and detected CD4+ and CD8+ T cells at the level of choroid plexus. Of interest, T cells were predominantly T-follicular helper cells (Tfh) producing IFN-γ and Bcl-6, with an almost complete absence of regulatory, T cells, such as T-follicular regulatory cells and Tregs [18▪]. Together, these results suggest that the abnormal BCSFB may represent a central mechanism in NPSLE pathogenesis, although this hypothesis requires further study.

Two interesting anatomical components that potentially regulate the movement of immune mediators from the systemic circulation into the CNS, are the meningeal barrier and glymphatic system [19,20]. The former may represent another route for immune substances to move into CNS. On the other hand, the glymphatic system is a recently introduced perivascular system, which participates in the clearance of interstitial solutes out of the CNS [21] and allows the exchange of molecules between CSF and interstitial spinal fluid (ISF). In neurodegenerative diseases, such as Parkinson's and Alzheimer's, the glymphatic system inhibits the clearance of proteins, participating in the underlying pathogenesis [22]. To date, there are limited studies regarding its role in the pathogenesis of other CNS diseases.

AUTOANTIBODIES IN NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS: ESTABLISHED PLAYERS AND 'NEW ENTRIES'

In addition to anti-NMDA, aPL and anti-RP, many autoantibodies have also been detected in NPSLE patients, yet they lack sensitivity and specificity [5,6]. From a clinical perspective, B-cell depletion

with rituximab may be beneficial in some NPSLE cases [23]. Of note, this has not been confirmed in murine studies, as early B-cell and/or antibody depletion did not modify or prevent neuropsychiatric disease in MLR/lpr mice [5]. The same group showed that neuropsychiatric manifestations remained unaffected after early bone marrow transplantation, whereas systemic inflammation, including nephritis, was attenuated [24]. Thus, the role of B cells and antibodies in CNS disease has not been fully elucidated [6,5].

A subset of antids DNA antibodies (termed DNRAb) recognize an extracellular domain of the NMDA receptor subunits NR2a and NR2b, and thus cross-react with the NMDA receptor, leading to neural cell apoptosis both in human and murine disease [25]. Direct injection of DNRAb in mice induced neuronal apoptosis at the level of hippocampus, leading to cognitive impairment. The effect of anti-NMDA antibodies is dose-dependent, as at high concentrations, they can induce excitotoxic cell death, whereas at lower concentrations, they do not cause neuropsychiatric manifestations [26]. Of interest, these abnormalities were detectable even when DNRAbs were no longer present in the hippocampus [27]. Anti-NMDA antibodies may damage the BBB *in vitro* and penetrate into the CNS [28]. Nevertheless, these antibodies may also be present in SLE patients without neuropsychiatric involvement [29,30], and thus these data need to be interpreted with caution.

Anti-RP antibodies are highly specific for SLE and have been associated with several NPSLE syndromes, especially psychosis and depression [30,31]. Anti-RP react with epitopes on the surface of neuronal cells, known as cross-reacting neuronal surface protein P (NSPA) [32]. González *et al*. demonstrated that NSPA is a ubiquitin ligase, which regulates the function of the NMDA receptor at the synaptic region [33]. Anti-RP bind to NSPA, which is distributed in brain regions involved in memory and emotion leading to neuronal apoptosis via intracellular Ca^{2+} influx [34]. This provides a molecular link between NSPA and the NMDA receptor (NMDAR) – known to be involved in plasticity and synaptic transmission related to memory, suggesting a possible pathogenic role for anti-RP. Importantly, injection of these antibodies through the limbic system or peripheral circulation leads to cognitive impairment and depression in mice [35,36].

aPL antibodies are major risk factors for NPSLE, especially for focal syndromes like cerebrovascular disease [11,37]. aPL carriers may also be at increased risk for subclinical atherosclerosis, although this has not been firmly established [38]; aPL may also affect the small vessels creating a microthrombotic environment within the CNS and consequent cerebral microangiopathy. This local vascular injury to small vessels may disrupt the BBB [39,40]. Intracerebroventricular injection of aPL induced a hyperactive behavior in mice implying a direct pathogenic role [41].

THE ROLE OF THE ACTIVATION OF MICROGLIA IN NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS PATHOGENESIS

Microglia, the resident macrophage cells of the brain, account for 10–15% of all neuronal cells. They act as the first and main form of active immune defense in CNS, responding to pathogens and injury by changing morphology and migrating to the site of infection/injury, where they destroy pathogens and remove damaged cells [42]. As part of their response, they secrete various cytokines, chemokines, prostaglandins and reactive oxygen species.

Accumulating evidence support an active role for microglial cells in the pathogenesis of NPSLE. Lupus-prone mice lacking estrogen receptor alpha experienced a significant reduction in memory errors, which correlated with decreased number of activated microglial cells and an accompanying reduction of CNS inflammation [43]. Administration of colony stimulating factor-1 receptor (CSF-1R) kinase inhibitor – which crosses the BBB causing microglia depletion [44] – in MRL/lpr mice improved depression [45▪]. Microglia are activated by sera of patients with SLE *in vitro*, but the actual factors responsible for this activation are unknown [46]. More recently, robust evidence for the role of microglia in CNS lupus came from a study by Bailas *et al*. who documented an IFN-driven microglia-dependent synapse loss pathway, using the 564Ig mouse model [7▪▪]. In this article, peripheral type I IFN was found to enter the brain and activate the IFNαR and microglia. The latter then engulfed synaptic material leading to synapse loss and subsequent cognitive impairment. Mice treated with IFNαR blocking antibody (anifrolumab) exhibited attenuation of CNS disease.

Another study [47▪▪], used the DNRAb+ mouse model (immunization with the DWEYS peptide) to explore the role of microglia in autoantibody-mediated CNS lupus. DNRAb+ mice exhibited increased microglia activation and a decrease in dendritic complexity, which was reversed when microglia was depleted. This decreased spine density and dendritic complexity were dependent on C1q. The latter binds to dendrites using high mobility group box 1 protein as mediator, with C1q serving as a bridge to NMDARs. Importantly, administration of captopril

[an angiotensin-converting enzyme (ACE) inhibitor, which crosses the BBB] significantly reversed the activation of microglia and improved the cognitive function of mice [47▪▪].

In MLR/lpr mice, reactive microglia may be activated through the nuclear factor κB (NF-κB) pathway, highlighting the role of TNF-α as mediator; inhibition of NF-κB led to decreased CD68 expression (activation marker) in microglia [48]. In another study, treatment with fingolimod (a modulator of sphingosine-1-phosphate, which sequesters lymphocytes within lymph nodes) attenuated the depressive behavior and cognitive impairment of MLR/lpr mice. RNA-sequencing analysis of fingolimod-treated microglia revealed downregulation of multiple immune-mediated pathways, including NF-κB signaling and IFN response with negative regulation of type I IFN-mediated signaling; this was associated with increased IFNβ expression [49▪]. Finally, lipocalin-2 (LCN2), a protein, which promotes microglial M1 polarization [50] was detected at increased levels in the serum of NPSLE patients. Lupus-prone mice with LCN2 deficiency performed better in neuropsychiatric tests exhibiting decreased microglia activation and brain apoptosis. LCN2 directly regulates immune microglia-associated pathways suggesting yet another pathogenic mechanism [51]. Overall, these data indicate that microglia cells are central players in CNS lupus and may serve as targets for novel therapies.

INTRACELLULAR SIGNALING PATHWAYS IN NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS: A ROLE OF KINASE INHIBITORS?

Tumor Necrosis Factor-like Weak inducer of apoptosis (TWEAK), a TNF superfamily member, promotes the activation of NF-κB and mitogen-activated protein kinase via its receptor, fibroblast growth factor-inducible 14 (Fn14) [52]. Evidence towards the involvement of the TWEAK/Fn14 pathway in NPSLE is growing. TWEAK displays a dual role in both neuroinflammation and cerebral ischemia [53]. Increased expression of TWEAK/Fn14 was detected within the cerebral cortex of MRL/lpr mice; knocking-out Fn14 improved depression and cognitive function [54]. Importantly, this finding was accompanied by a reduction of immune infiltrates, fibronectin, IgG deposition and complement activation in brain histology [55]. Intracerebroventricular injection of TWEAK in wild-type mice induces cognitive dysfunction and depression-like behavior through increased BBB permeability and accelerated neuronal cell death [55,56].

Bruton's tyrosine kinase (BTK) is essential for the function of B cells and macrophages. Inhibition of this pathway by use of a specific inhibitor (BI-BTK-1) in MRL/lpr mice, resulted in decreased accumulation of macrophages, T cells and B cells in the choroid plexus and improved cognitive function [57▪]. In view of the recent promising data of baricitinib in SLE [58], ibrutinib, a selective BTK inhibitor, could potentially prove useful in neuropsychiatric disease. Of interest, evobrutinib, another BTK inhibitor, was evaluated in patients with multiple sclerosis in a phase 2 trial with promising results [59].

Neurite outgrowth inhibitor-A (Nogo-a) with its respective receptor, NgR1, form a signaling pathway, which mediates inhibition of neuron generation. Compared with other autoimmune or neurological diseases, patients with NPSLE overexpress Nogo-A in the CSF [60]. Increased levels of Nogo-α/NgR1 were also observed in MLR/lpr mice; administration of Nogo-66(1-40), an antagonist, improved cognitive function, decreased expression of proinflammatory components and reduced axonal degeneration and demyelination [60] (Table 1).

TYPE I INTERFERON AND NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS

SLE is characterized by a robust IFN molecular signature in most patients. A link between NPSLE and IFN has been proposed based on clinical and molecular findings of monogenic interferonopathies, such as Aicardi–Goutières syndrome (AGS). AGS is an inflammatory disorder mainly affecting the skin and brain, characterized by aberrant secretion of type I IFN and lupus-like systemic features [61]. Among the responsible mutated genes for AGS, is the three prime repair exonuclease 1 (*TREX1*) [61], a susceptibility gene for SLE [62] and, more specifically, CNS lupus [63]. Brain pathology of patients with AGS shows small vessel disease, including aneurysmal dilation, vasculitis and thrombotic microangiopathy [61] findings also seen in SLE [10▪].

Of note, IFN-α causes endothelial cell damage promoting abnormal angiogenesis in SLE patients, which may also involve CNS vessels [64]. Whether IFN *per se* causes cerebrovascular disease, frequently manifest in patients with increased IFN levels, or is merely an epiphenomenon, remains to be defined. Patients with various diseases treated with IFN-α or IFN-β, developed thrombotic microangiopathy suggesting a possible role of IFN on vascular damage [65]. Monogenic interferonopathies could serve as a model to study the role of IFN in NPSLE pathogenesis.

Table 1. Therapeutic targets in neuropsychiatric systemic lupus erythematosus

Target	Evidence and rationale	Experimental setting	Potential drugs	References
Type I IFN pathway	Type I IFN activates microglia, which then engulfs synaptic material leading to cognitive impairment. Mice treated with IFNαR blocking antibody, exhibited attenuation of CNS disease	564Igi lupus-prone mice	Anifrolumab (Type I IFN receptor inhibitor)	[7▪▪]
ACE	Microglia and C1q are essential in neuronal damage process. ACE inhibitors can prevent microglia activation preserving cognitive status and neuronal function	BALB/c mice immunized with DWEYS peptide, leading to DNRAb+ production	Captopril, other ACE inhibitors	[47▪▪]
BTK	Treatment with BI-BTK-1 (a novel inhibitor of BTK) significantly attenuated the neuropsychiatric disease along with decreased accumulation of macrophages, T cells and B cells within the CNS	MRL/lpr mice	BTK inhibitors (BI-BTK-1, ibrutinib, evobrutinib)	[57▪]
Nogo-a/ NgR1 pathway	Nogo-a/ NgR1 pathway is involved in NPSLE. Treatment with Nogo-66(1–40) antagonist improved cognitive function and myelin repair	MRL/lpr mice	Nogo-66 (1–40), an antagonist of NgR1 receptor	[60]
S1P signaling pathway	Modulation of the S1P signaling pathway may serve as a novel therapeutic target in CNS lupus	MRL/lpr mice	Fingolimod, a S1P receptor modulator that sequesters lymphocytes within lymph nodes	[49▪]
LCN-2, a protein, which promotes microglial M1 polarization; a major regulator of innate immunity	Increased levels of LCN-2 were detected in the serum of NPSLE patients. Cognitive impairment and depression-like behavior were attenuated in lupus-prone mice lacking LCN-2	Sle1,3 lupus-prone mice	-	[51]
Activated microglia cells	Lupus-prone mice treated with CSF-1R (microglia depletion) exhibited improvement in the depression-like behavioral deficit	MRL/lpr mice	GW2580, a small CSF-1R kinase inhibitor; depletion of microglia	[45▪]
TWEAK/Fn14 pathway	TWEAK/Fn14 interactions promote the loss of BBB integrity and increase neuronal damage and the accumulation of inflammatory cells in the choroid plexus	MRL/lpr mice	Monoclonal antibodies (hIgG1) against Fn14	[54]
Complement cascade	Complement deposition was increased in brain tissue of SLE patients suggesting an underlying pathogenic role	Human brain autopsies	Eculizumab (inhibitor of complement factor C5	[10▪]

ACE, angiotensin-converting enzyme; BKT, Bruton's tyrosine kinase; CNS, central nervous system; CSF-1R, colony stimulating factor-1 receptor; IFN, interferon; IFNaR, interferon-a receptor; LCN-2, lipocalin-2; NPSLE, neuropsychiatric systemic lupus erythematosus; S1P, sphingosine-1-phosphate; TWEAK/Fn14, Tumor Necrosis Factor-like Weak inducer of apoptosis/fibroblast growth factor-inducible 14.

TRANSCRIPTOMIC ANALYSIS IN SYSTEMIC LUPUS ERYTHEMATOSUS: BRAIN AS A CAUSAL TISSUE

Transcriptomic analysis of SLE by RNA sequencing has revealed novel molecular signatures for disease susceptibility and severity [66▪]. These studies have also shown that brain is not only a target tissue but also a causal tissue in SLE. More specifically, using SLE GWAS signals and eQTLs from 44 tissues, we found that SLE-associated polymorphisms regulated gene expression not only in the blood but also in other tissues, including the basal ganglia – suggesting that SLE genetic susceptibility may affect multiple tissues including CNS [66▪]. These findings

provide additional evidence that the brain may also be a causal tissue in SLE corroborating earlier data linking the nervous and the immune system.

NOVEL BRAIN IMAGING TECHNIQUES AND CLUES FOR NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS PATHOGENESIS

Approximately 40% of SLE patients with established neuropsychiatric disease do not show abnormalities on conventional brain imaging. Furthermore, no consistent association exists between any neuroimaging finding and specific neuropsychiatric syndrome or severity. To this end, a number of advanced imaging techniques have been tested in order to increase sensitivity and detect more subtle abnormalities. Indeed, imaging techniques have provided additional evidence for microglial activation. A recent study [67] demonstrated intracellular changes in glia with increased diffusivity of choline and creatine. The authors suggested that this finding could serve as an imaging marker for glial activation in response to inflammation; of note, this correlated also with disease activity. Microglia activation has also been shown in NPSLE by PET and [11C] DPA-713 using a radiopharmaceutical substance that targets mitochondrial translocator protein, a protein upregulated during glial cell activation [68].

Regarding cerebral perfusion, our group examined whether dynamic susceptibility contrast-enhanced perfusion MRI (DSC-MRI), a minimally invasive and widely available method of cerebral perfusion assessment, may assist the diagnosis of NPSLE. We found decreased cerebral blood flow in the semioval center bilaterally in normal-appearing white matter region of NPSLE patients [69▪]. Importantly, the combination of DSC-MRI-measured blood flow in the semioval centre with conventional MRI was found to improve the attribution of neuropsychiatric events to SLE. Another technique, magnetization transfer imaging (MTI), uses the magnetization transfer ratio – histogram peak height (MTR-HPH) as a marker of the integrity of tissue microstructure; the latter was found decreased in individuals with inflammatory NPSLE manifestations compared with patients with presumed ischemic ones [70]. Decreased MTR-HPHs values were reversed with immunosuppressive treatment, pointing towards an inflammatory process rather than ischemia. Proton magnetic resonance spectroscopy (1H-MRS), which measures the concentration of several types of neurometabolites, has also been used in NPSLE. These studies have shown increased levels of myoinositol and choline [71,72], consistent

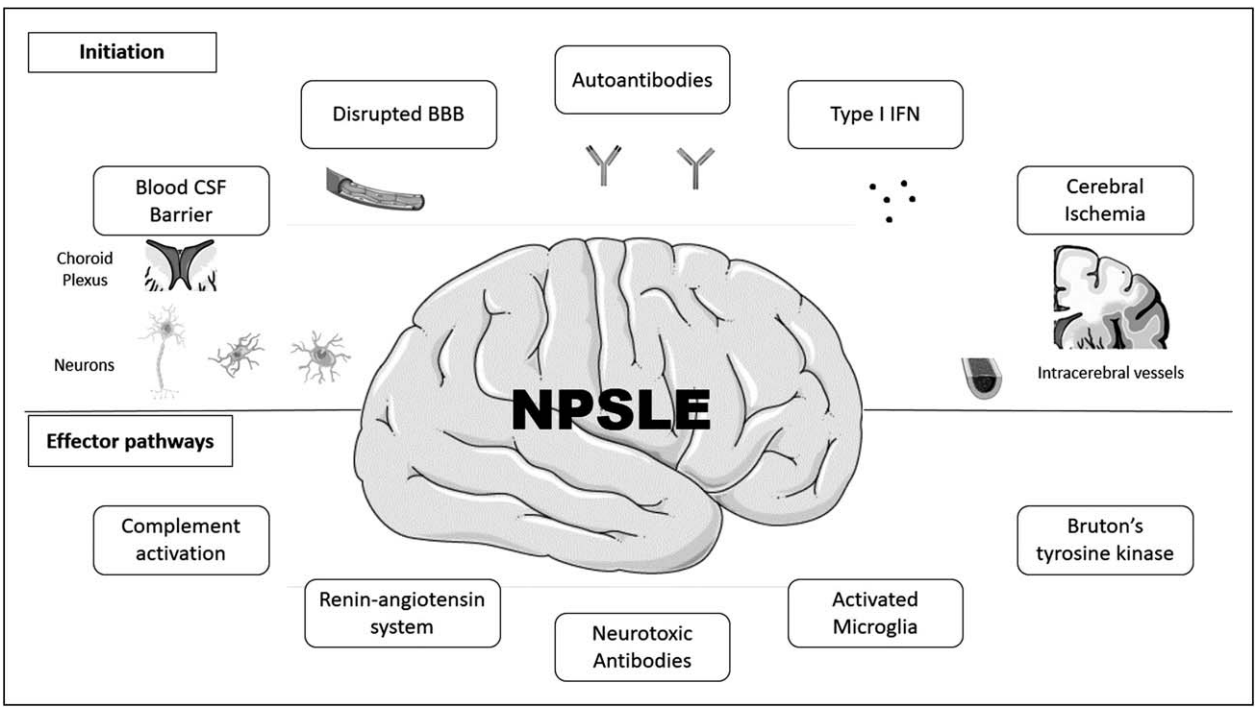

FIGURE 1. Pathogenesis of central nervous system lupus. Proposed pathophysiologic mechanisms in NPSLE. Collectively these mechanisms target various components of the CNS including neurons (synapse, myelin sheath), astrocytes, microglia and the cerebral vasculature. CNS, central nervous system; NPSLE, neuropsychiatric systemic lupus erythematosus.

Table 2. Research agenda

Further definition of the molecular signature of NPSLE by transcriptomic analysis including single-cell RNA sequencing
Correlation of molecular subphenotype with clinical subgroups of NPSLE
Exploration of the brain not only as a target tissue but also as a causal tissue in the pathogenesis of lupus
Development and testing of molecular markers for neuroinflammation, ischemia and demyelination
Exploration of the glymphatic system and its role in NPSLE
Delineation of the relative importance of interferon pathways in intracerebral vascular beds
Improved biomarkers for disease activity, prognosis and response to therapy
Repositioning of drugs inhibiting pathways found to be relevant for lupus

NPSLE, neuropsychiatric systemic lupus erythematosus.

with glial activation and vasculopathy, along with decreased *N*-acetylasparate [71,72], compatible with neuronal impairment in patients with neuropsychiatric manifestations.

Recently, functional MRI in SLE patients with cognitive dysfunction revealed structural and functional brain changes and an inflammatory process pointing out the multifactorial nature of NPSLE [73▪]. Finally, PET studies in NPSLE have shown both increased (hypermetabolism) and decreased (hypometabolism) FDG uptake, consistent with inflammation and tissue loss, respectively. The most common finding was hypermetabolism in the parieto-occipital grey matter [74], even in the absence of MRI lesions. Collectively, these neuroimaging findings suggest that both inflammation and tissue loss may be operant in NPSLE.

CONCLUSION

NPSLE remains only partly understood, both in terms of pathophysiology and management, the latter remaining largely empiric [2]. Most evidence derives from studies in animal models, which interestingly do not manifest the full spectrum of human NPSLE (e.g. severe manifestations, like seizures or myelopathy are not seen in mice); rather they exhibit more subtle abnormalities, and as such, may not completely model the human disease [75]. Notwithstanding this limitation, advances have certainly been made in our understanding of disease pathogenesis (Fig. 1). With regards to treatment, recent findings suggest new potential therapeutic opportunities, such as type I IFN blockade, ACE inhibition and kinase inhibitors [7▪▪,47▪▪,57▪] (Table 2). We anticipate that some of these pathways may serve as targets for the development of new drugs or for repositioning of already existing ones.

Acknowledgements

We are indebted to Drs G. Bertsias, P. Verginis and A. Banos for insightful comments and https://smart.servier.com/ for captures of images in Fig. 1.

Financial support and sponsorship

This work was funded by the Foundation for Research in Rheumatology (FOREUM) and also in part by the Greek General Secretariat of Research and Technology 'Aristeia' action of the Operational Program 'Education and Lifelong Learning' (co-funded by the European Social Fund and National Resources, Aristeia I 2344 to D.B.), the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement no. 742390) and the SYSCID (A Systems Medicine Approach to Chronic Inflammatory Diseases) under the European Union's Horizon 2020 research and innovation programme (grant agreement no. 733100).

Conflicts of interest

There are no conflicts of interest.

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Microglia activation with increased production of IL-6 and IL-18 disrupt hippocampal neurogenesis mediating neuropsychiatric changes during the early stages of the disease

Introduction

Cognitive dysfunction, mood disorders and anxiety, collectively termed diffuse neuropsychiatric SLE (NPSLE) occurs in up to 40 percent of patients with systemic lupus erythematosus (SLE) representing an emerging frontier in lupus care(43). Due to high prevalence of such manifestations in the general population, their attribution to disease itself (so called “primary NPSLE”) relies on the judgment of experienced physicians using a multidisciplinary approach in a process which poses considerable challenges. From a clinical standpoint, identifying the involvement of inflammatory or micro-vasculopathic mechanisms in each neuropsychiatric (NP) event is critical prior to institution of immunosuppression(208).

A crucial brain region implicated in NPSLE is the hippocampus, a complex structure that is associated with cognitive functions such as memory and mood regulation, while its dysfunction may be involved in a variety of neuropsychiatric disorders including Alzheimer's disease, schizophrenia, cognitive ageing, post-traumatic stress disorder, and depressive and anxiety disorders(209,210). More specifically, lupus antibodies display a spectrum of pathologies in the hippocampus including aberrant excitatory signaling, neuronal apoptosis and dendritic pruning(136,139,163,211). Neuroimaging studies have shown hippocampal hypermetabolism in lupus, which is associated with impaired memory performance and mood alterations(212). Hippocampal atrophy is also evident in lupus patients with longstanding disease and cognitive dysfunction(213), while patients with SLE frequently suffer from impaired cognition, depression and anxiety(208). Nonetheless, the molecular mechanisms that link hippocampal function to the development of neuropsychiatric events in lupus remain elusive.

A characteristic feature of the adult hippocampus is its neurogenic activity. Hippocampal neurogenesis is conserved in humans and likely contributes significantly to hippocampal plasticity(214–216). The formation of new neurons in the adult

hippocampus is the result of the physiological neurogenic activity of adult Neural Stem Cells (NSCs) that reside exclusively in a specific area of the Dentate Gyrus (DG) called the subgranular zone (SGZ)(217). Within this region the hippocampal NSCs (hiNSCs) population is either quiescent (non-proliferating) or activated (proliferating). Following their activation, they become fast proliferating neuroblasts and they progressively commit to the neuronal lineage, exiting the cell cycle and migrating into their final position in the DG where they integrate into the pre-existing neuronal network. Hippocampal neurogenesis is functionally associated with mood regulation and cognitive functions including learning and memory(218). Accordingly, dysregulation of hippocampal neurogenesis results in behavioral deficits such as impaired cognition, depressive-like behavior and increased rates of anxiety(219). A plethora of local cues in the neurogenic niche of DG in relation to NSCs homeostasis have been described(220,221). Hormones and neuropeptides are considered crucial mediators of hippocampal neurogenesis while more recently peripheral inflammatory response and neuroinflammation –especially via the secretion of pro-inflammatory cytokines- have also emerged as key regulators(222). Importantly, increasing evidence supports a pivotal role of microglia in the regulation of adult neurogenesis(223).

In view of the role of hippocampus in the neuropsychiatric manifestations (ie. cognitive dysfunction, depression, anxiety) and the involvement of immune responses in NPSLE, we sought to investigate the interplay between hippocampal neurogenesis and neuro-inflammation in a spontaneous NZB/W-F1 lupus-prone murine model. First, we assessed the behavioral phenotype of NZB/W-F1 mice and demonstrated that they exhibit a hippocampus-linked behavioral perturbations recapitulating human disease, suggesting that this polygenic murine model is suitable for investigating the autoimmunity-mediated CNS disease. Next, we elucidated the hippocampal neurogenesis with regards to proliferation, survival and differentiation of hiNSCs. Finally, we pursued a systematic investigation of the inflammatory response in the lupus hippocampus with respect to infiltrating immune cells, microglial cells and local inflammatory molecules. Our data suggest that IL-6 and IL-18 negatively impact hiNSC activity and could serve as therapeutic targets in NPSLE.

Methods

Animals

All procedures in mice were in compliance with the Greek National Law 161/91 for Use of Laboratory Animals and institutional guidelines. All procedures were reviewed and approved by the Greek Federal Veterinary Office (License No.1044/01-03-19, Athens, Greece). New Zealand black♀ x New Zealand white♂ F1 mice (i.e.,NZB/W-F1) spontaneously develop an autoimmune syndrome resembling human SLE(224). NZB/OlaHsd and NZW/OlaHsd mice were purchased from Envigo(225). Wild-type (WT) mice (C57BL/6) were used as controls. All animals were maintained in the BRFAA animal facility. Only female mice were used in this study. Mice were housed 6-10 per cage in a temperature-(21-23^{o}C) and humidity-controlled colony room, maintained on a 12-hour light/dark cycle (07:00-19:00 light-on), with standard food (4RF21, Mucedola Srl,Italy) and water provided ad libitum. All experiments were performed in age-matched female mice at two different time-points. Specifically, experiments were performed in 3 month-old mice (pre-nephritic stage; before the onset of lupus nephritis) and 6 month-old mice (nephritic stage; after the onset of lupus nephritis). At each time-point, age-matched NZB/W-F1 and WT were used. All comparisons were performed between age-matched NZB/W-F1 and WT.

Tissue collection and preparation

All experiments were performed in hippocampi. Mice were deeply anesthetized with a lethal dose of isoflurane. Then, they were perfused transcardially with PBS (1 ml/g) followed by 4% PFA where indicated (i.e., sections for immunohistochemistry). Brains were carefully removed and the hippocampi were dissected. Dissected hippocampi were either snap-frozen in liquid nitrogen and kept at -80^{o}C for RNA extraction or cytokine quantification or remained in PBS on ice and used immediately for flow cytometry experiments or BBB permeability assays.

Behavioral assays

Mice were tested in a comprehensive behavioral test battery to assess possible effects on general depressive-like disorders, anxiety, cognitive function and motor performance/coordination. The following tests were conducted in the same order for all subjects (all females, NZB/W-F1, n=13 and WT, n=14) at 3 and 6 months of age: novel object recognition (NOR), novel object location (NOL) tasks (221), Elevated plus maze (EPM) (226), rotarod (227), Tail suspension test (TST) as previously described(228), Prepulse inhibition (PPI) (229), Sociability (social novelty and social preference) (230) and Sucrose preference test (SPT) (231) as previously described(232). All testing was performed during the light phase between 9:00 and 17:00 with at least 24h between each behavioral assay. Mice were habituated to the testing room 30 min prior to testing and the apparatus were cleaned with 70% ethanol in between animals to minimize odor cues. The testing room was maintained at a temperature of 22±2°C and relative humidity 55±10%. Each mouse was handled for 5 min daily for one week prior to behavioral testing. All parameters were analyzed with an overhead camera, using Ethovision XT9.0 specialized video tracking software.

Novel object recognition/location (NOR/NOL)

NOR and NOL tasks(221) were used to assess recognition and spatial memory. The tests were performed in a Plexiglas chamber (40×40×35 cm) covered with white cardboard paper. The floor was covered with clean bedding material and extra bedding material from each animal's home cage was added on top before the animal's testing. Each task was conducted over four consecutive days. Day 1 consisted of two 10 min habituation trials per animal. During each trial, the animal was habituated to the open field arena without objects present. The inter-trial interval was 60 min, during which time the animal remained in its home cage. Distance traveled (cm) and time spent in the center of the arena were measured. These data were used to assess changes in locomotor activity over trials. Day 2 consisted of two 10 min object familiarization trials per animal. During each trial, the animal was placed in the center of the open field arena facing the opposite wall to the objects. During both trials, the animal was exposed to two identical odourless objects located at a specified distance from each other (10 cm from each adjacent wall) and allowed to freely explore. Days 3 and 4 consisted of a

first trial (pre-test) where the animal was exposed to the two identical odourless objects following a 60 min inter-trial interval for assessment of short-term memory, the second trial (test). Time spent sniffing each object (sec), defined as time spent with the head oriented towards and within 2 cm of the object, was analyzed for the first trial, in order to ensure that there was not a side preference bias for either of the two object positions, or a tendency for neophobia (<20 sec exploration time of objects). In the second trial (test), the animal was returned to the arena and exposed to two objects. In the NOR task (day 3), the one object was the same as the one used in the training trial (familiar object), while the other object was replaced with an odourless novel one (novel object) of similar size. In the NOL task, the two objects were identical to those presented in the pre-test with the position of one object being changed. The positions of the familiar and novel/displaced objects were counterbalanced between animals. Time spent sniffing each object was analyzed and the discrimination index (DI) was calculated for each animal [DI= 100 x (time spent sniffing novel/displaced object - time spent sniffing familiar object) / (time spent sniffing novel/displaced object + time spent sniffing familiar object). All parameters were analyzed with Ethovision XT 9.0 and exploration times were scored manually.

Elevated plus maze

A plus-shaped maze with two open and two closed arms (arms length: 65 cm; arms width: 65 cm) with an elevation of 50 cm above the floor was used in the EPM test. Briefly, each mouse was kept into the central region of the apparatus before the initiation of experiment. The total time spent in the open/closed arms and the number of entries to both open and closed arms were measured for the subsequent 5 min. % open arm time [Time spent on open arms/(Time spent on open arms + time spent on closed arms)] was calculated to interpret the data.

Rotarod

The animals were assessed for motor coordination and balance on an accelerating Rotarod (UGO BASILE)(227). Mice were placed on the rotating rod with a diameter of 7 cm and they were trained to maintain a forward walking pace in order not to fall from the rod. Each animal was given a habituation session and three trials to complete the test. During the 1 min habituation session, the animals were accustomed to walking on the rotating rod at a constant speed of 4 rpm (revolutions per minute). If the animal fell off during this session, it was placed back on the rod. Following the habituation session, the animals were given three 5 min trials with 45 min intervals between them. During each trial, the rotational speed of the rod progressively accelerated from 4 rpm to a maximum of 40 rpm across the 5 minutes. Latency to fall was measured. The latency value from all three trials is averaged for each animal.

Tail suspension test (TST)

TST was performed by hanging the mice at 40 cm above the floor with the help of adhesive tape on a tail tip for 360 sec. First 60 sec was considered as an acclimatization period and for the subsequent 300 sec, immobility time was recorded for each mouse(228).

Prepulse inhibition

Sensorimotor gating was assessed with prepulse inhibition- a cross-species phenomenon in which the startle response is reduced when the startling stimulus is preceded by a low-intensity prepulse (Tsoupri)(229). On the first day, acoustic startle was measured to obviate possible phenotypic confounds. Each animal was placed in a plexiglass restrainer and habituated to a startle chamber for 5 min with white noise (Startle and Fear Interface LE118-8, Panlab, Harvard Apparatus). The test consisted of a series of pseudorandom presentations of sound pulses of varying amplitude (70, 80, 90, 100, 110, 120 dB; 1 s, 20s ITI; 5 times each) and average startle response of the 5 trials was recorded (acoustic startle response). The following day, a PPI protocol was administered consisting of 5 min habituation (background white noise), 10 repeat pulse (115 dB) alone trials, prepulse (80 dB)-pulse trials, presented 10 times in a

pseudorandom order (1s apart; 20s ITI) and 10 no stimulus (white noise) trials. % Prepulse inhibition was calculated as a percentage score: %PPI = [100-(prepulse-pulse)/pulse*100].

Sociability (Social Novelty and Social Preference)

The sociability test was performed in a three chambered apparatus, as previously described (230). The apparatus consisted of a wooden box with partitions separating it into three chambers with dimensions (length/width/height in cm) 60/30/20, being X cm length to the central chamber and X cm each side. Time spent in each chamber and the time spent exploring the stranger mouse or an object in the chamber, was measured. The object was an empty identical cage used to enclose the stranger mouse. Animals used as "strangers" were B67BL/6J females 11-15 months-old and no previous contact with the test mice. Stranger were individually acclimated for 5 min 3 times daily for 3 consecutive days into the cage before the experiment. The sociability test was designed to be concluded in three phases with no resting period among them. Firstly, mice were allowed to explore the three chambers for 10 min along with 2 objects (empty cages) in the side champers. In phase 2, a stranger mouse (stranger 1) was randomly placed in one of the two cages (object). The mouse was then allowed to explore the three champers for 10 min. In phase 3, the original stranger mouse (stranger 1) remained in its wire cage on one side of the apparatus and a new unfamiliar mouse (stranger 2) was placed in the wire cage on the opposite side, which was previously empty during the sociability test. Subsequenlty, the mouse was allowed to explore the three chambers for 10 min. The score was evaluated by the time spent in each chamber and by the time spent sniffing each wire cage.

Sucrose preference test

The SPT protocol was designed to be concluded in 6 days as previously described. In total, 13 female NZB/W and 14 female B57BL/6J mice were used in these experiments. Due to animal facility regulations, a maximum of 9 mice were used on each session, requiring for 3 back-to-back runs of the SPT protocol. Therefore, the whole experiment took place over 18 consecutive days. Initially, all mice of the same strain were placed in the same cage for 48 hours with chow diet and 2 identical bottles, with one containing

drinking water and one with 1% sucrose solution (habituation phase). The mice were then separated into single cages and remained in similar conditions for 24 hours. After that period, the food was removed for 12 hours and after that time the first measurement was taken (water consumption for each bottle). After this step, the food was placed in the cages for 12 hours. Then, both food and water were removed for 24 hours. Finally, the two bottles were placed for 12 hours without food. At the end of the 12-hour period, the final measurement (water consumption) was recorded. Bottle/food removal and replacement were performed at 8:00 and 20:00.

Immunohistochemistry

Sections & staining

Following transcardial perfusion with 4% PFA (1 ml/g), brains were post-fixed in 4% PFA for 4-hours at 4^{o}C and then cryoprotected in 30% sucrose for 2 days, frozen, and cut in 20 μm-thick sagittal sections on a cryostat. Sections were incubated overnight at 4^{o}C with the primary antibody diluted in blocking solution of 5% bovine serum albumin (ITW Reagents, Cat#A1391,0050), 0.3% Triton X-100 in PBS. Sections were washed 3 times in PBS and incubated at room temperature for 90 min with the secondary antibodies in blocking solution(221). For visualization of the nuclei DAPI was used. Finally, sections were mounted with ProLong™ Diamond Antifade Mountant (Cat.P36961,TFS). When indicated, antigen retrieval was used (eBioscience, Cat#00-4955-58) according to manufacturer's instructions.

Image analysis and Quantification

Stereological analysis of the number of the cells was performed with inverted confocal imaging system Leica SP5. Confocal image z-stacks were obtained through the entire slice thickness at 1.5μm (20xlens)(233). For quantification of DCX, SOX2, GFAP and IBA1, every 6th brain section of all series were stained (~80 sections/subject) and positive cells were counted throughout the rostro-caudal extent of the granule cell layer as previously described(221). Morphological criteria were applied to distinguish the horizontal astrocytes from radial glia-like cells (GFAP^{+} cells) as previously described(234). Morphological criteria were applied to distinguish early from late

neuronal progenitors. More specifically, DCX^+ cells without axons were labeled as early progenitors, while DCX^+ with axons were labeled as late neuronal progenitors. Quantification was performed on confocal images using ImageJ software. The final estimated number of positive cells for indicated marker was obtained by multiplying the resulting number by 6 as previously described(221,233).

Flow Cytometry

Single cell suspensions from hippocampi were generated by passing them through a 70 μm cell strainer. Specifically, both hippocampi from each subject were placed in RPMI medium in the presence of DNAse I (0.25 mg/ml, Sigma) and collagenase D (1 mg/ml, Roche) at 37^{o}C for 1-hour before passing through 70μm cell-strainer. When indicated, in order to remove excessive myelin, single cell suspension were resuspended in 30% Percoll and centrifuged at 600 g for 30min with minimum deceleration as previously described(235). For staining of extracellular markers, single cell suspensions were incubated with antibodies for 20 min at 4°C. For the staining of extracellular markers, cells were fixed and stained using the Foxp3 Staining Set (eBioscience, Cat#00-5523-00), as described by manufacturer. All experiments were analyzed with FACS ARIA III (BD, bioscience). All data were analyzed with FlowJo 8.7 and vX0.7 software. All antibodies that were used in flow cytometry experiments are shown in Table 7.

RNA-sequencing analysis

Total RNA from bulk hippocampal tissue was isolated as described by manufacturer (NucleoSpin®RNA). RNA purity and concentration were measured with Nanodrop (IMPLEN) and RNA integrity was assessed on a Bioanalyzer (Agilent). 200 ng total RNA (RNA Integrity Number≥8) was used for library preparation using the TruSeq RNA Sample Preparation Kit (Illumina) following the manufacturer's protocol. Single-end 75-bp or 100-bp mRNA sequencing was performed on IlluminaNextSeq 500 at the Greek Genome Centre, BRFAA, Greece.

Quality of raw sequencing data was assessed using FastQC(236). Raw reads were trimmed for low quality bases (Q<30) and adapter sequences using cutadapt v.3.3 (237). Alignment was performed using the STAR 2.6 algorithm(238) against the human

reference genome (hg38 version) and gene quantification was performed using HTSeq using Gencode annotation file version M19(239). Differential expression analysis was conducted using DESeq2(240). Genes with a p-value≤0.05 and an absolute fold change of >=1.5 were considered statistically significantly deregulated. Heatmaps were created using ggplot2 R package. Pathway and gene ontology (GO) enrichment and network analyses were performed using gProfiler(241). Gene Set Enrichment Analysis (GSEA) was applied in order to reveal enriched signatures in our gene sets(242). As a reference gene set we used the Molecular Signatures Database (MSigDBv7.5). All expressed genes were ranked by descending value of the product of –log10(P-value) and FC. Highly upregulated genes were at the top and highly downregulated genes were at the bottom of the ranked list. GSEA pre-ranked analysis was then performed using the default settings. Enrichment was considered significant when FDR (q-value)<0.25.

RNA-seq data have been deposited to GEO (awaiting accession number).

Cell culture, TUNEL and BrdU assay

Hippocampi of 2.5-month-old female WT mice were removed. The DG was dissected from the hippocampus, digested with a papain-based solution and mechanically dissociated as previously described(221). The dissociated cells were placed in 24-well plates with B27 medium enriched with EGF/bFGF (20 ng/ml). After 12-14 days in culture, the floating neurospheres were trypsin-dissociated following reformation for at least two times before experiments.

Then, neurospheres were dissociated into single cells and were seeded onto poly-l-lysine coated coverslips in 24-well plate at a density of $5x10^4$/well. Experiments were designed to assess the direct effect of IL-6 and IL-18 on NSCs apoptosis and proliferation. TUNEL assay and Brdu/PH3 staining were used for apoptosis and proliferation studies, respectively. For both assays, single cells remained for 24-hours on coverslips and were given the following treatments: i) DMEM/F12 medium (in the presence or absence of EGF/bFGF, for the proliferation and apoptosis assay respectively), ii) recombinant murine IL-6 (100 ng/ml, immunotools) or iii) recombinant murine IL-18 (100 ng/ml, biolegend). For the proliferation study, BrdU

(10 mM) was added 2-hours before fixation. After 24-hours, cells were fixed with 4% PFA in PBS for 10 min. TUNEL assay was performed as described by the manufacturer (Promega-CatNo#G3250). For proliferation assays, cells permeabilized with 0.25% Triton X-100 in PBS followed by blocking with 10% normal goat serum (NGS) in PBS. Incubations with the primary and secondary antibodies were performed for a 6-hour period at 4^{o}C and for 1-hour at room temperature respectively, in blocking solution. DAPI (1 mg/ml) was used for nuclei staining. For detection of BrdU, cells were treated with 2M HCl at 37^{o}C for 10 min following equilibration in borate buffer (0.1 M, pH 8.5) for 10 min prior to primary antibody incubation. Primary and secondary antibodies are shown in Table 7.

Quantitative PCR Analysis (real-time RT-qPCR)

Cells were lysed in RA1 Buffer (Macherey-Nagel). RNA was extracted using a NucleoSpin®RNA-XS isolation kit as described by manufacturer. First-strand cDNA synthesis was performed using PrimeScript™ RT-PCR Kit (Cat.RR037A, Takara). QPCR was carried out using the Kapa Sybr Fast Universal kit (Cat. KK4602,Kapa Biosystems)(243). Relative expression of target genes was calculated by comparing them to the expression of the housekeeping genes *hprt*. Primers that were used for real-time RT-qPCR are presented in the Table 6.

Table 6. List of primers

Gene	Orientation	Sequence
Ccl17	Forward	CGAGAGTGCTGCCTGGATTACT
	Reverse	GGTCTGCACAGATGAGCTTGCC
Ccl22	Forward	AAGACAGTATCTGCTGCCAGG
	Reverse	GATCGGCACAGATATCTCGG
Cxcl1	Forward	ATCCAGAGCTTGAAGGTGTTG
	Reverse	GTCTGTCTTCTTTCTCCGTTACTT
Hprt	Forward	GTGAAACTGGAAAAGCCAAA
	Reverse	GGACGCAGCAACTGACAT

Measurement of Cytokines

Cytokines were measured with LEGENDplex™ Mouse Macrophage/Microglia Panel (13-plex). Snap-frozen hippocampi were diluted in a concentration of 50 mg of tissue/min 1 ml of sterile HBSS containing protease inhibitors (Sigma-Aldrich) following homogenization. The homogenates were centrifuged at 400 g for 15 min at 4°C and the supernatants were collected and used for cytokine quantification with LEGENDplex™ according to the manufacturer's instructions.

Blood brain barrier permeability assays

Blood brain barrier permeability was assessed with Blue Evans assay (EB). Mice were intravenously injected with EB dissolved in PBS in a concentration of 25 mg/kg. After 4 hours, the hippocampus was dissected from each mouse. Lung tissue from 2 mice/group were used as positive control. Dissected hippocampi were incubated with 5 ml formamide/mg tissue overnight at 60^{o}C. Elisa reader at a wavelength of 620 nm was used to quantify the levels of EB. EB concentrations were calculate with the help of a

calibration curve created from adsorption values of seven samples with predefined Evans Blue concentrations as previously described(244).

Statistical analysis

Statistical analysis was performed taking into consideration the experimental setup using unpaired or paired Student's t-test, one-way or two-way ANOVA in GraphPad-Prism v8 software. All P-values, number of samples and independent experiments are reported in the figure legends. Data are presented as means±S.D, P-value<0.05 was considered statistical significance. Samples that were compared, were collected and analyzed under the same conditions.

Table 7. Antibodies used for flow cytometric analysis and immunostaing

Target	Fluorochrome	Dilution	Vendor	Cat.	Application
CD11b	BV510	1/200	Biolegend	101263	FC
CD45	APC	1/200	Biolegend	103212	FC
Ly6G	PE	1/200	Biolegend	127608	FC
Ly6C	BV421	1/200	Biolegend	128032	FC
MHC-II	PerCP/Cy5.5	1/200	Biolegend	107626	FC
B220	FITC	1/200	Biolegend	103205	FC
CD4	PE	1/200	Biolegend	100407	FC
CD8a	PeCy7	1/200	Biolegend	100721	FC
CD80	PE	1/200	Biolegend	104707	FC
CD86	FITC	1/200	Biolegend	105005	FC
iNOS	PeCy7	1:50	eBiosience	25-5920-82	FC
Arginase-1	FITC	1:50	R&D systems	IC5868F	FC
Cleaved-caspase 3	PE	1:50	Cell Signaling	9978	FC
Iba1	FITC	1:50	Abcam	15691	FC
rabbit anti-PH3	unconjugated	1/500	Abcam	5176	IF
Rat anti-Brdu	unconjugated	1/400	Abcam	6326	IF
mouse anti-DCX (doublecortin)	unconjugated	1/100	Santa Cruz	8066	IF
rabbit anti-Sox2	unconjugated	1/100	Cell Signaling	23064	IF
mouse anti-GFAP	unconjugated	1/400	Cell Signaling	3670	IF
mouse IgG	Alexa fluor 555	1/500	Invitrogen	A-21425	IF
rabbit IgG	Alexa fuor 488	1/500	Invitrogen	A-11008	IF
rabbit IgG	CF® 555	1/1500	Biotium	20033	IF
mouse IgG	Alexa Fluor 647	1/500	Invitrogen	A-28181	IF
rat IgG	Alexa Fluor 488	1/400	Invitrogen	A-48262	IF

Results

NZB/W-F1 lupus-prone mice exhibit hippocampus-linked behavior alterations

To investigate early mechanisms involved in neuropsychiatric lupus, we used the New Zealand Black/New Zealand white-F1 (NZB/W-F1) lupus-prone mouse strain, a spontaneous lupus-prone model characterized by systemic autoimmunity. To date, the NZB/W-F1 model has been rarely used to explore NPSLE pathogenesis since its behavioral phenotype has not been fully characterized. Compared to other lupus-prone models, in NZB/W-F1 mice, lupus nephritis (LN) progresses slowly therefore neuropsychiatric disease is not affected by systemic complications of LN (onset at 6-9 months). In order to fully phenotype and characterize the neuropsychiatric disease in NZB/W-F1 strain, we designed a comprehensive behavioral test battery to assess possible effects related to depressive-like behavior, anxiety, cognitive function, motor performance/coordination and sociability. All behavioral tests were conducted in the same order for all subjects (females, NZB/W-F1 n=13 and WT n=14) at two different time-points; 3month-old (pre-nephritic stage) and 6month-old (nephritic-stage) (**Figure 4A**). We found that lupus mice exhibit memory deficits at 3 months of age based on the novel object recognition task, and spatial memory dysfunction at 6 months of age compared to WT mice as indicated by the novel object location task (**Figure 4B, C**). In addition, lupus mice at 3 and 6 months of age exhibited an anxiety-like phenotype in the elevated plus maze (**Figure 4D**) and depressive-like behavior as revealed by enhanced immobility in the tail suspension test and decreased sucrose preference (**Figure 4E, F**). Furthermore, rotarod performance showed impaired motor coordination in 3 and 6 month-old lupus mice compared to WT mice while prepulse inhibition (PPI) revealed a tendency of decreased sensorimotor gating in 3 month-old lupus mice (**Figure 4G, H**). Due to age-related hearing loss in WT mice at 6 months of age, it was not possible to correctly interpret PPI at this age (**Figure 5A, B**). Although no significant differences were observed in the social novelty test, lupus mice exhibited impaired social recognition both at 3 and 6 months compared to WT mice in the social preference test (**Figure 4I, H**). When we compared the behavioral performance of lupus

mice between the two ages, we observed that lupus mice became more anxious over time (**Figure 5B-G**).

Taken together, these data suggest that NZB/W-F1 mice exhibit hippocampus-linked behavioral deficits early in the course of the disease before the onset of LN, supporting that this model is suitable for investigating autoimmunity-mediated hippocampal neuroinflammation in SLE.

Figure 4.

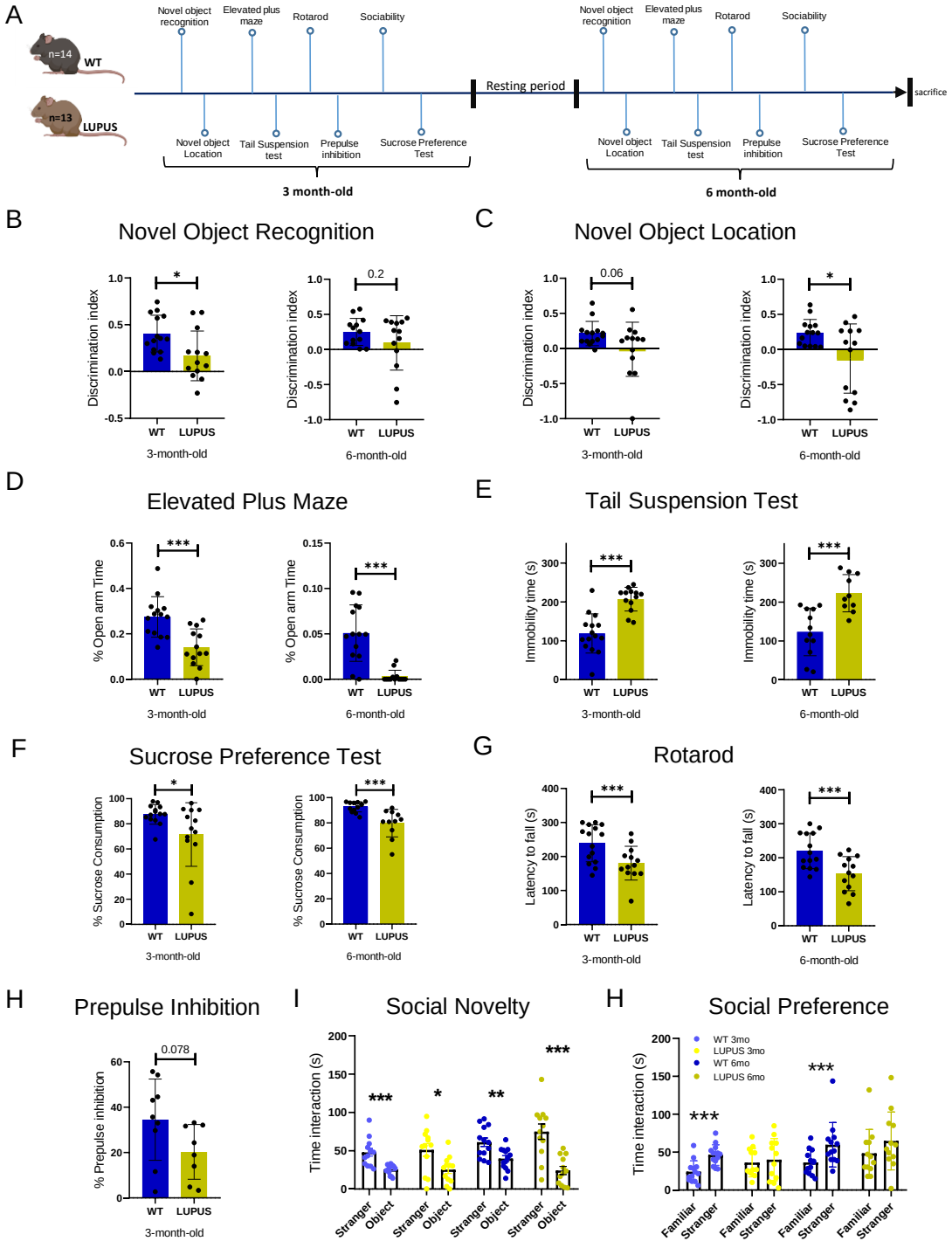

A
n=14
WT
n=13
LUPUS
Novel object recognition
Elevated plus maze
Rotarod
Sociability
Resting period
sacrifice
Novel object Location
Tail Suspension test
Prepulse inhibition
Sucrose Preference Test
3 month-old
6 month-old
B
Novel Object Recognition
Discrimination index
WT
LUPUS
3-month-old
6-month-old
C
Novel Object Location
D
Elevated Plus Maze
% Open arm Time
E
Tail Suspension Test
Immobility time (s)
F
Sucrose Preference Test
% Sucrose Consumption
G
Rotarod
Latency to fall (s)
H
Prepulse Inhibition
% Prepulse inhibition
I
Social Novelty
Time interaction (s)
Stranger
Object
H
Social Preference
WT 3mo
LUPUS 3mo
WT 6mo
LUPUS 6mo
Familiar
Stranger

Figure 4. Hippocampus-linked behavioral alterations including impaired cognition, depressive-like behavior and increased rates of anxiety in female NZB/W-F1 lupus mice at the pre-nephritic and nephritic stages of the disease. (A) Schematic representation of experimental design to assess behavioral phenotype; the same female Lupus (n=13) and WT (n=14) underwent a comprehensive behavioral test battery at 3 and 6 months of age. (B) Novel object recognition evaluates visual recognition memory; expressed as discrimination index (time spent sniffing novel object-time spent sniffing familiar object/total time spent sniffing) and (C) novel object location evaluates spatial recognition memory; expressed as discrimination index (time spent sniffing object in novel location-time spent sniffing object in familiar location/total time spent sniffing). (D) Elevated plus maze evaluates anxiety-like phenotype; expressed as time spent (%) in the open arms. (E) Tail suspension test and (F) sucrose preference test evaluate depressive-like behavior. (G) Rotarod assesses motor performance/coordination. (H) Prepulse inhibition evaluates sensorimotor gating (n=7-8/group). (I) Social novelty and (H) Social preference evaluated with the sociability test. Lupus, NZB/W-F1 stain; WT, C57BL/6; S, seconds; Bars, mean±SD. Data were analyzed: B-H: Student's t test, I,H: two-way ANOVA, Bonferroni post hoc test; *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 5.

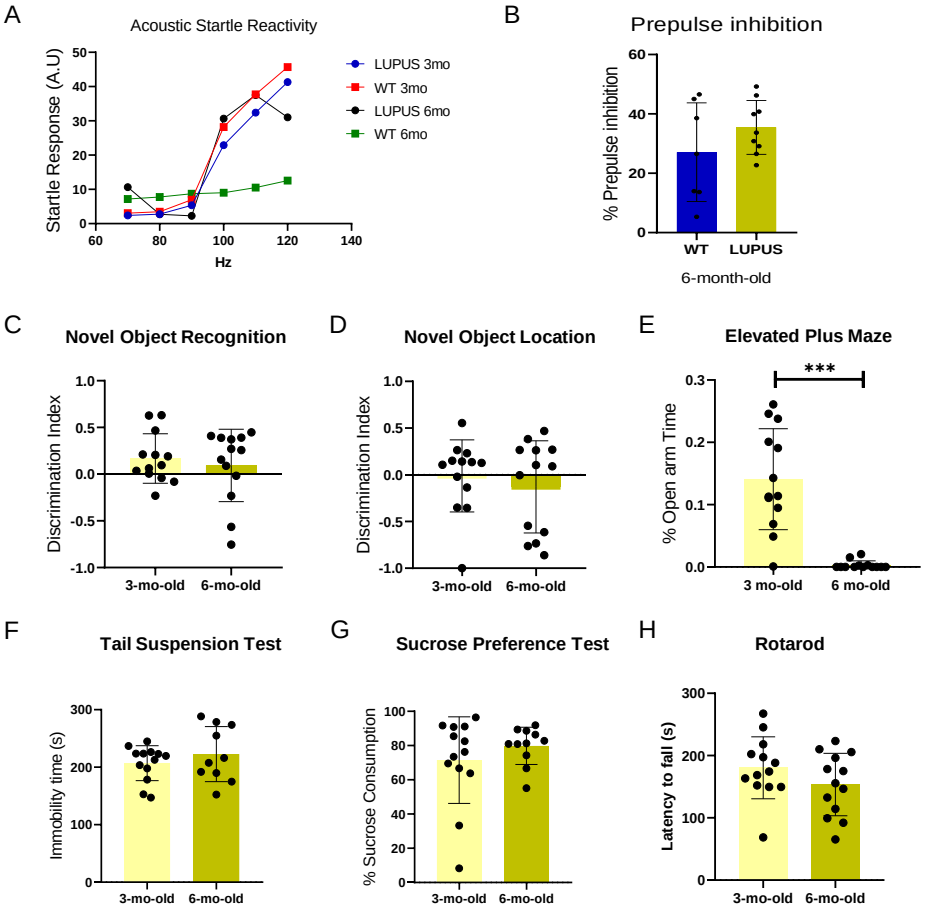


Figure 5. Behavioral profile of female NZB/W-F1 lupus mice at the pre-nephritic and nephritic stages of the disease. (A) Acoustic startle reactivity revealed that 6 month-old WT exhibit defective hearing and thus, (B) prepulse inhibition at 6 month-old mice is not reliable (n=7-8/group). (C-H) Comparison of behavioral phenotype between 3 month-old and 6 month-old female Lupus mice; (C) Novel object recognition evaluates visual recognition memory; expressed as discrimination index (time spent sniffing novel object-time spent sniffing familiar object/total time spent sniffing) and (D) novel object location evaluates spatial recognition memory; expressed as discrimination index (time spent sniffing object in novel location-time spent sniffing object in familiar location/total time spent sniffing). (E) Elevated plus maze evaluates anxiety-like phenotype; expressed as time spent (%) in the open arms. (F) Tail suspension test and (G) Sucrose preference test evaluate depressive-like behavior. (H) Rotarod assesses motor performance/coordination. n=10-13/group, Lupus; NZB/W-F1 stain, WT; wild-type, C57BL/6. Data were analyzed: A: two-way ANOVA, Bonferroni post hoc test; B-H: Student's t-test, *P < 0.05, **P < 0.01, ***P < 0.001.

Hippocampal neurogenesis is disrupted in NZB/W-F1 lupus

As behavioral deficits that are linked to hippocampus are also tightly related to defective hippocampal neurogenesis, we sought to investigate the hippocampal neurogenic activity and adult neural stem cell (NSC) properties in NZB/W-F1 lupus. To this end, we applied biochemical and morphological criteria to distinguish the different cell types involved in the multi-step process of proliferation and differentiation during adult hippocampal neurogenesis (**Figure 6A**). We first examined the neurogenic activity of hiNSCs by quantifying the cells expressing the neuronal progenitor marker doublecortin (DCX). Since the dorsal and ventral areas of DG are differentially involved in cognitive and emotional processes respectively, we analyzed the expression of DCX in the two regions separately(245). Notably, we found profound disruption (~2-fold) of hippocampal neurogenesis in 3-month-old lupus compared to WT mice, which is preserved in 6-month-old mice (**Figure 6B-C**). Disrupted neurogenesis was also evident in the ventral area of the DG both at 3 and 6 months of age compared to WT mice (**Figure 7A, B**). By applying morphological criteria, we differentiated DCX+ neural progenitors into early undifferentiating progenitors (ie. without long processes) and late progenitors (ie. with long processes) and then we estimated the differentiating rate of the neural progenitor cells as the ratio of the early undifferentiating DCX+ cells to the total number of DCX+ cells (**Figure 6D**). Importantly, we observed a decreased rate of differentiation at both ages suggesting that the NZB/W-F1 strain exhibits an impaired neuronal differentiation capacity (**Figure 6E, Figure 7C**).

Defective hippocampal neurogenesis may be caused by a decreased number of radial glia-like cells (RGLs; neuronal precursors), decreased proliferation or increased apoptosis of the neural progenitors in the hippocampal neurogenic area. To investigate the cause of the decreased neurogenesis observed in the NZB/W-F1 mice, we examined the number and activity of RGCs as well as the proliferation capacity and the apoptotic activity of the hiNSCs. At 3 months of age, we did not observe any difference in the number of RGCs as revealed by detection of the GFAP marker combined with morphological criteria suggesting that the decreased neurogenesis observed in the NZB/W-F1 mice is not due to an endogenous reduction of the adult neural stem cells pool of the hippocampus (**Figure 6G**). However, at this age the proliferation of NSCs was increased as revealed by the increased number of SOX2+ cells (**Figure 6H**).

Furthermore, at 3-months we detected a decreased number of quiescent RGLs and an increased number of proliferating RGLs as revealed by the increased number of SOX2+/GFAP+ RGL cells (**Figure 7D-E**). To estimate the proliferation rate of RGLs, we calculated the ratio of Sox2+/GFAP+ RGL cells to the total number of GFAP+ RGLs and we identified an increased proliferation rate of RGLs in the 3-month-old lupus mice (**Figure 6H-I**, **Figure 7F**). These findings indicate an increased proliferative activity of the hiNSCs in 3-month-old lupus mice compared to WT mice. In view of the finding of comparable numbers of neuronal precursors (RGLs) and increased proliferation at the early stages between Lupus and WT mice, we reasoned that the decrease of neurogenesis may be caused by increased apoptosis. Indeed, we detected increased levels of cleaved caspase-3 in the DG-isolated cells of 3-month-old lupus mice (**Figure 7G-H**).

Next, we analyzed the neurogenic activity at the late stage of the disease in the 6-month-old mice compared to WT mice. Similarly to the pre-nephritic stage, the DCX+ cells remained decreased both in dorsal and ventral regions (**Figure 6C, Figure 7B**). Interestingly, at this stage a paradox increase of both quiescent and activated RGLs was detected as well as increased number of proliferating SOX2+ cells in 6-month-old lupus mice (**Figure 6G-H, Figure 7D-F**). However, we observed no differences in proliferating rate of RGLs between lupus and WT mice (**Figure 6I**). These data suggest that the increased number of RGLs at nephritic stage may be explained by the increased activation of RGLs early in the course that leads to increased self-renewal of neural precursors (**Figure 6I**), but ultimately it is not able to overcome the neurogenic deficiency observed in young lupus mice possibly due to increased apoptosis of NSCs (**Figure 7G-H**).

Moreover, over the course of the disease the neurogenesis –albeit disrupted- declines with lower rate as compared to WT mice in accordance with the low exhaustion rate of RGL neuronal precursors, while the production rate of Sox2+ neural progenitors remained steady (**Figure 7I-K**). This observation is supportive to the increased number of RGLs detected in the late stage of the disease.

Figure 6.

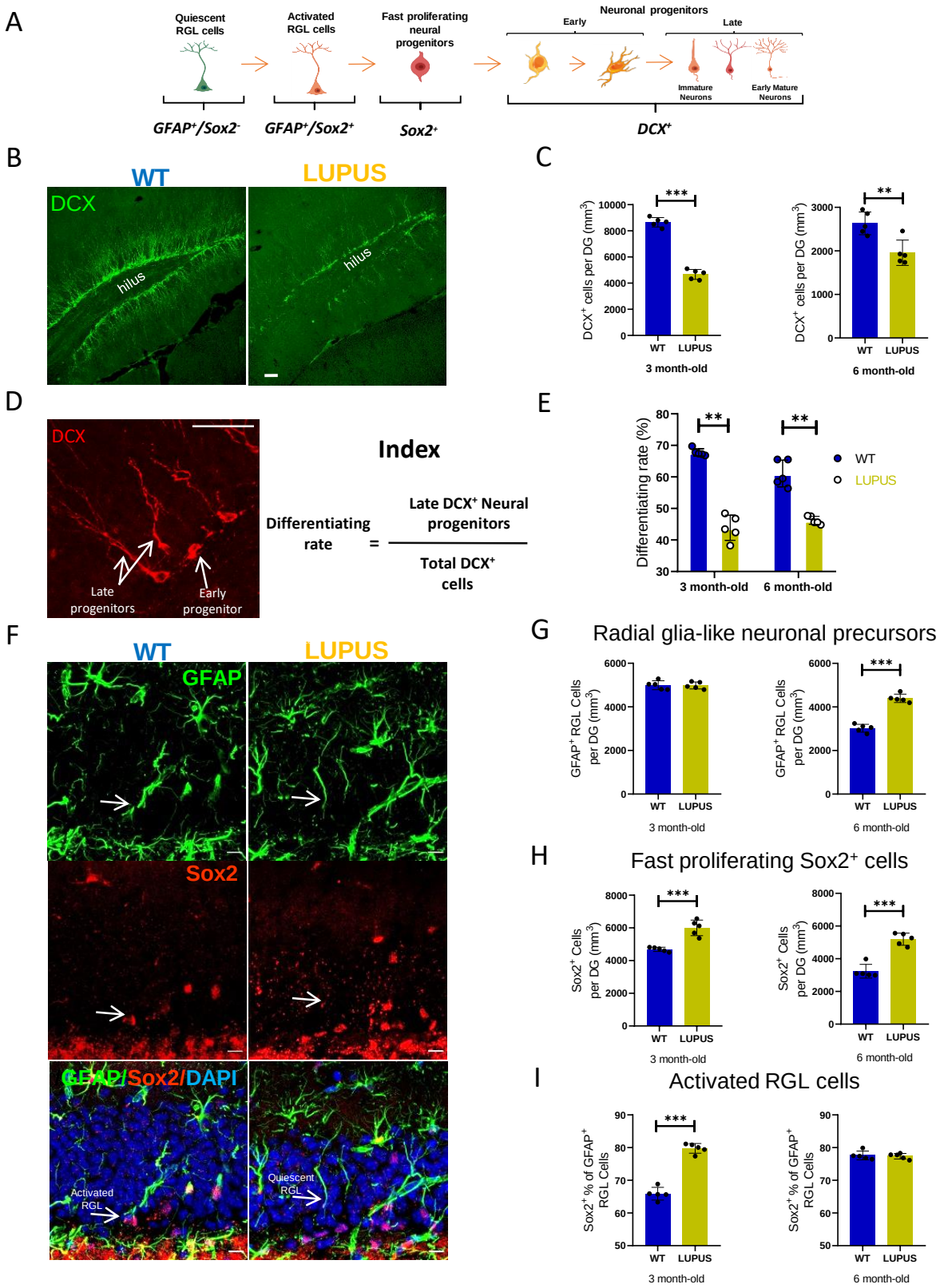

A
Quiescent RGL cells
Activated RGL cells
Fast proliferating neural progenitors
Neuronal progenitors
Early
Late
Immature Neurons
Early Mature Neurons
GFAP+/Sox2-
GFAP+/Sox2+
Sox2+
DCX+
B
WT
LUPUS
DCX
hilus
hilus
C
DCX+ cells per DG (mm3)
WT
LUPUS
3 month-old
6 month-old
D
DCX
Late progenitors
Early progenitor
Index
Differentiating rate = Late DCX+ Neural progenitors / Total DCX+ cells
E
Differentiating rate (%)
WT
LUPUS
3 month-old
6 month-old
F
WT
LUPUS
GFAP
Sox2
GFAP/Sox2/DAPI
Activated RGL
Quiescent RGL
G
Radial glia-like neuronal precursors
GFAP+ RGL Cells per DG (mm3)
3 month-old
6 month-old
H
Fast proliferating Sox2+ cells
Sox2+ Cells per DG (mm3)
3 month-old
6 month-old
I
Activated RGL cells
Sox2+ % of GFAP+ RGL Cells
3 month-old
6 month-old

Figure 6. Hippocampal neurogenesis in NZB/W-F1 lupus mice. (A) Schematic illustration of adult neurogenesis in mice. Radial glia-like (RGL) cells express GFAP and not Sox2 under quiescent state. Upon activation, GFAP+ RGL cells express Sox2. Fast proliferating neural progenitors at early stages of neurogenesis express Sox2. Neuronal progenitors express doublecortin (DCX) and are divided into early and late progenitors based on their morphology. (B) Representative images of immunohistochemical detection of DCX+ neuronal progenitors in the DG of 3 month-old mice; Scale bar: 100mm. (C) Quantification of DCX+ cells in the DG of WT and Lupus mice at 3 and 6 months of age (n=5/group). (D) Representative image of DCX+ early and late neuronal progenitors and the index used to indicate differentiating rate of neuronal progenitors. Scale bar: 10mm. (E) Quantification of differentiating rate of DCX+ neuronal progenitors in the DG of WT and Lupus mice at 3 and 6 months of age (n=5/group). (F) Representative images of immunohistochemical detection of GFAP+ RGL and Sox2+ cells in the DG of 3 month-old mice; Scale bar: 10mm. (G-I) Quantification of (G) GFAP+ RGL neuronal precursors, (H) Sox2+ fast proliferating progenitors and (I) the proliferation rate of RGL neuronal precursors; expressed as the percentage of Sox2+/GFAP+ activated RGL of total RGL neuronal precursors in the DG of WT and Lupus mice at 3 and 6 months of age (n=5/group); DAPI stains nuclei. Lupus, NZB/W-F1 stain; WT, C57BL/6; Bars, mean±SD. Data were analyzed: C,G-I: Student's t test, E: two-way ANOVA, Bonferroni post hoc test; $^{*}P < 0.05$,$^{**}P < 0.01$,$^{***}P < 0.001$.

Figure 7.

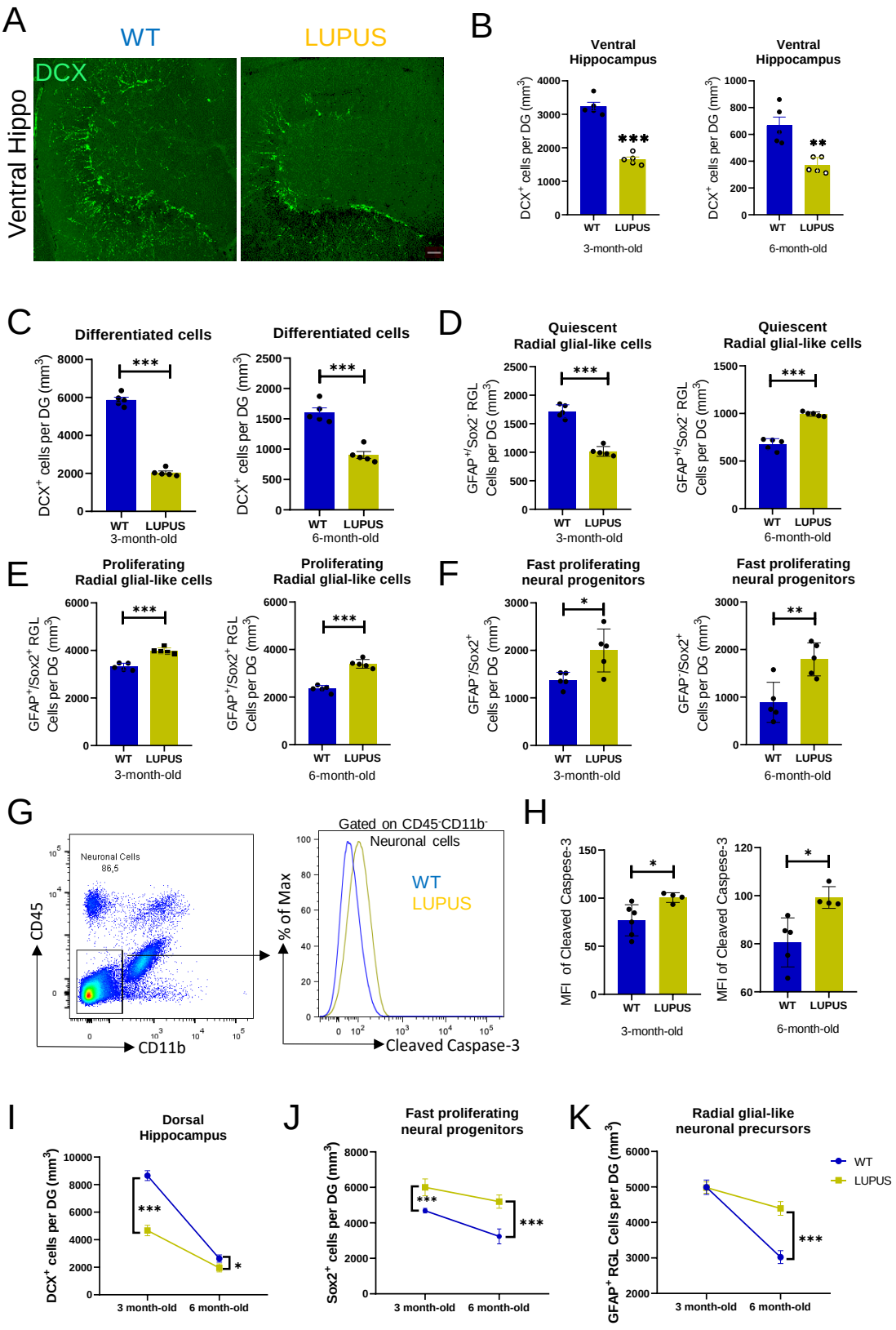

A
WT
LUPUS
DCX
Ventral Hippo
B
Ventral Hippocampus
DCX+ cells per DG (mm3)
WT
LUPUS
3-month-old
6-month-old
C
Differentiated cells
D
Quiescent Radial glial-like cells
GFAP+/Sox2- RGL Cells per DG (mm3)
E
Proliferating Radial glial-like cells
F
Fast proliferating neural progenitors
GFAP-/Sox2+ Cells per DG (mm3)
G
Neuronal Cells
86,5
CD45
CD11b
Gated on CD45-CD11b-
Neuronal cells
% of Max
Cleaved Caspase-3
H
MFI of Cleaved Caspese-3
I
Dorsal Hippocampus
3 month-old
6 month-old
J
Fast proliferating neural progenitors
Sox2+ cells per DG (mm3)
K
Radial glial-like neuronal precursors
GFAP+ RGL Cells per DG (mm3)

Figure 7. Hippocampal neurogenesis in NZB/W-F1 lupus. (A) Representative images of immunohistochemical detection of DCX+ neuronal progenitors in the ventral hippocampus of 3 month-old mice; Scale bar: 100mm. (B) Quantification of DCX+ cells in the ventral hippocampus of WT and Lupus mice at 3 and 6 months of age (n=5/group). (C-F) Quantification of (C) late DCX+ neuronal progenitors (D) Quiescent GFAP+/Sox2- RGL neuronal precursors (E) proliferating GFAP+/Sox2+ RGL neuronal precursors and (F) fast proliferating GFAP-/Sox2+ neural progenitors in the DG of WT and Lupus mice at 3 and 6 months of age (n=5/group). (G) Representative FACS plots of gating strategy, representative histogram and (H) MFI of cleaved caspase-3 in CD11b-CD45- neuronal cells. (I-K) Quantification of (I) DCX+ cells, (J) Sox2+ fast proliferating neural progenitors and (K) the proliferation rate of RGL neuronal precursors; expressed as the percentage of Sox2+/GFAP+ activated RGL of total RGL neuronal precursors in the DG of WT and Lupus mice at 3 and 6 months of age (n=5/group). Lupus, NZB/W-F1 stain; WT (Wild-type), C57BL/6; Bars, mean±SD. Data were analyzed with Student's t test; *P < 0.05,**P < 0.01,***P < 0.001.

Blood brain barrier disruption and Immune cell trafficking orchestrate inflammatory response in lupus hippocampus

To investigate the molecular mechanisms involved in the deregulation of hiNSCs response, we performed RNA-sequencing in the hippocampal tissue of NZB/W-F1 and WT mice at both 3 and 6 months of age. RNA-seq analysis uncovered 578 and 721 differentially expressed genes (DEGs) in 3-month-old and 6-month-old comparisons (eg. NZB/W-F1 vs. WT), respectively (**Figure 9A, B**). Gene ontology (GO) enrichment analysis in 3-month-old mice revealed multiple enriched terms including neutrophil/myeloid leukocyte migration, leukocyte chemotaxis and regulation MAPK cascade in lupus (**Figure 9C**). Accordingly, GO analysis in 6-month-old hippocampi as compared to WT mice revealed a plethora of enriched terms related to inflammatory processes such as innate immune response, immune cell infiltration, cytokines/chemokines production, increased antigen-presentation among others (**Figure 9D**). Importantly, we observed a clear distinction between lupus and WT at 3 months of age and 6 months of age based on specific "inflammatory-associated" genes (**Figure 8A, B**). When comparing RNA-seq data from 6-month-old to 3-month-old lupus hippocampus, we detected only 11 DEGs suggesting that the hippocampal transcriptomic signature remained almost intact during disease progression (**Figure 9E**). These data further support our findings that hippocampus-linked neuropsychiatric disease in NZB/W-F1 lupus is first evident at the pre-nephritic stage. Gene set enrichment analysis (GSEA) of the expression data between 6-month-old and 3-month-old lupus revealed a more profound inflammatory response in the hippocampus of 6-month-old mice, which is mainly mediated from peripheral immune components (**Figure 9F**).

Taking into account the inflammatory signature, we next asked whether the blood brain barrier (BBB) is disrupted in NZB/W-F1 lupus. To this end, we assessed BBB permeability with blue Evans whole brain dye. We did not observe any difference in the 3-month-old mice, yet, BBB was found disrupted in the NZB/W-F1 model at nephritic stage both in the brain and hippocampus (**Figure 8C, Figure 10A**). Thus, the BBB seems to be disrupted in the NZB/W-F1 model of SLE only at 6 months of age.

Next, we examined immune cell trafficking from the periphery in lupus hippocampus via flow cytometry (**Figure 10B**). We detected an increased proportion of $CD45^+$ myeloid population in NZB/W-F1 hippocampus, both at 3 and 6 months of age (**Figure 8D, Figure 10C**). The $CD11b^+CD45^+$ CNS myeloid population comprises infiltrating monocytes, granulocytes, macrophages and microglia(246). Therefore, to further study this population, we performed analysis with additional markers. Inparticular, we observed increased proportion of $CD11b^+CD45^+LyG6^-LyC6^+$ infiltrating monocytes in NZB/W-F1 hippocampus at 6 months of age (**Figure 8E, Figure 10D**). On the contrary, we observed an increased frequency of $CD11b^+CD45^+LyG6^+$ granulocytes in hippocampus of the 3-month-old lupus-prone mice, but not in 6-month-old (**Figure 10E**). Thus, we evaluated the activation of $CD11b^+CD45^+LyG6^-$ non-granolocytic myeloid cells, which were found to be activated only at the nephritic stage of the disease (**Figure 10F**). Taken together, these data indicate a predominant myeloid response in NZB/W-F1 lupus hippocampus both at early and late stages of the disease compared to WT mice.

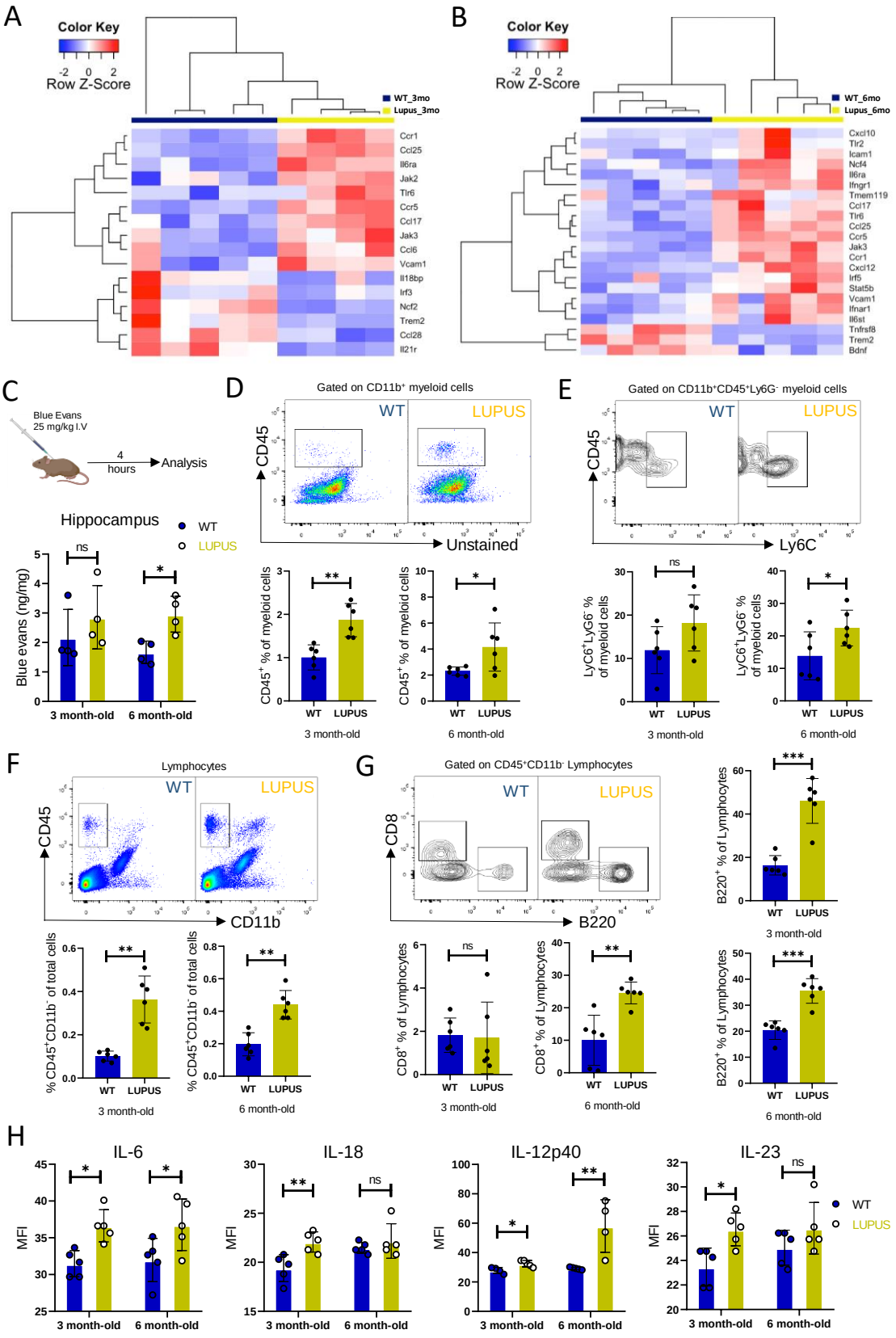
After this, we investigated the infiltrating hippocampal lymphocytes with respect to T-cells and B-cells. We found increased proportion of infiltrating $CD11b^-CD45^+$ lymphocytes in lupus hippocampus both at 3 and 6 months of age compared to WT mice (**Figure 8F**). Importantly, $B220^+$ B-cells were found to be the predominant subpopulation within lymphocytes. Specifically, we observed increased frequency and number of B-cells in NZB/W-F1 hippocampus both at pre-nephritic and nephritic stages (**Figure 8G, Figure 10G**). Concerning T-cells, $CD8^+$ were found to be elevated only in hippocampus of 6-month-old lupus-prone mice, while $CD4^+$ were not detected at increased levels in NZB/W-F1 hippocampal lupus (**Figure 8G, Figure 10H**). These results suggest a robust lymphocytic cell infiltration -particularly B-cells- in the NZB/W-F1 lupus hippocampus at 3 and 6 months of age.

Following this, we asked whether immune cell infiltration and inflammation in NZB/W-F1 hippocampus resulted in increased levels of pro-inflammatory cytokines. Interestingly, we detected increased levels of IL-6, IL-18, IL-12p40, IL-12p70 and IL-23 in the hippocampus of 3-month-old NZB/W-F1 lupus. However, only IL-6, IL-12p40 and IL-12p70 were found to be elevated in the hippocampus of 6-month-old lupus mice (**Figure 8H, Figure 11A**). Accordingly, we did not observe differences

concerning IL-1β, TNF-a, IL-10, TGF-b and G-CSF hippocampal levels between lupus and WT mice (**Figure 11A**).

Together, these findings indicate a pronounced immune cell trafficking in the hippocampus of lupus-prone mice with a myeloid predominant response both at early and late stages of the disease, resulting in increased hippocampal levels of specific pro-inflammatory cytokines.

Figure 8.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Color Key
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Row Z-Score
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Lupus_3mo
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Ccl25
Il6ra
Jak2
Tlr6
Ccr5
Ccl17
Jak3
Ccl6
Vcam1
Il18bp
Irf3
Ncf2
Trem2
Ccl28
Il21r
B
Color Key
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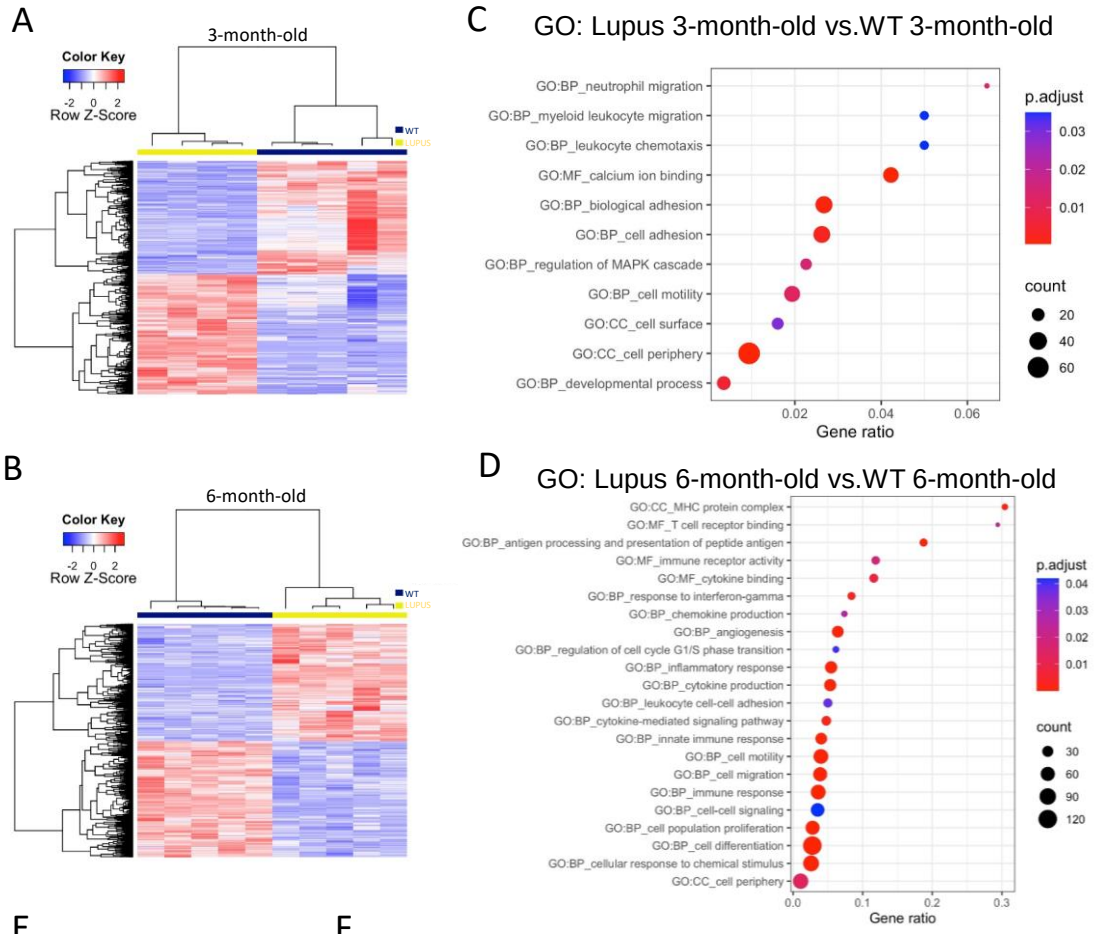
Lupus_6mo
Cxcl10
Tlr2
Icam1
Ncf4
Il6ra
Ifngr1
Tmem119
Ccl17
Tlr6
Ccl25
Ccr5
Jak3
Ccr1
Cxcl12
Irf5
Stat5b
Vcam1
Ifnar1
Il6st
Tnfrsf8
Trem2
Bdnf
C
Blue Evans
25 mg/kg I.V
4
hours
Analysis
Hippocampus
WT
LUPUS
Blue evans (ng/mg)
ns
*
3 month-old
6 month-old
D
Gated on CD11b+ myeloid cells
WT
LUPUS
CD45
Unstained
CD45+ % of myeloid cells
**
*
WT
LUPUS
3 month-old
6 month-old
E
Gated on CD11b+CD45+Ly6G- myeloid cells
WT
LUPUS
CD45
Ly6C
Ly6C+Ly6G- % of myeloid cells
ns
*
WT
LUPUS
3 month-old
6 month-old
F
Lymphocytes
WT
LUPUS
CD45
CD11b
% CD45+CD11b- of total cells
% CD45+CD11b+ of total cells
**
WT
LUPUS
3 month-old
6 month-old
G
Gated on CD45+CD11b- Lymphocytes
WT
LUPUS
CD8
B220
CD8+ % of Lymphocytes
B220+ % of Lymphocytes
ns
**
***
WT
LUPUS
3 month-old
6 month-old
H
IL-6
IL-18
IL-12p40
IL-23
MFI
*
**
ns
WT
LUPUS
3 month-old
6 month-old

Figure 8. Increased immune cell trafficking and inflammatory response in hippocampus of NZB/W-F1 lupus mice. (A-B) Heatmaps of the expression of inflammatory associated genes in hippocampal tissue of WT and Lupus mice at (A) 3 and (B) 6 months of age (n=4-5/group). (C) Evans blue dye was intravenously injected followed by quantification of Evans blue in hippocampus. (D) Flow cytometry analysis; representative FACS plots and frequency of CD11b+CD45+ cells in myeloid (CD11b+) cells. (E) Flow cytometry analysis; representative FACS plots and frequency of CD11b+CD45+Ly6G-Ly6C+ infiltrating monocytes in myeloid (CD11b+) cells. (F) Flow cytometry analysis; representative FACS plots and frequency of CD45+CD11b- lymphocytes in hippocampal cells. (G) Flow cytometry analysis; representative FACS plots and frequency of CD8+ T-cells and B220+ B-cells in lymphocytes (CD45+CD11b-). (H) Quantification of IL-6, IL-18, IL-12p40 and IL-23 in hippocampal tissue. All experiments (C-H) were performed in Lupus and WT mice at 3 and 6 months of age (n=4-6/group) and obtained from 2 independent experiments. Lupus, NZB/W-F1 stain; WT, C57BL/6; Bars, mean±SD. Data were analyzed with Student's t-test, *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 9.



E

DEGS
Lupus 6-month-old
vs.
Lupus 3-month-old

Gene	FDR
Itih3	0,025873
Etnppl	0,034395
Vegfa	0,014108
Zc3hav1	0,022927
Rbm3	0,034395
Spag6	0,000518
Ctss	0,034395
Frem3	0,000999
Trim30d	0,034395
C4b	0,00538
Cyp4f15	0,00778

F

GSEA: Lupus 6-month-old vs. Lupus 3-month-old

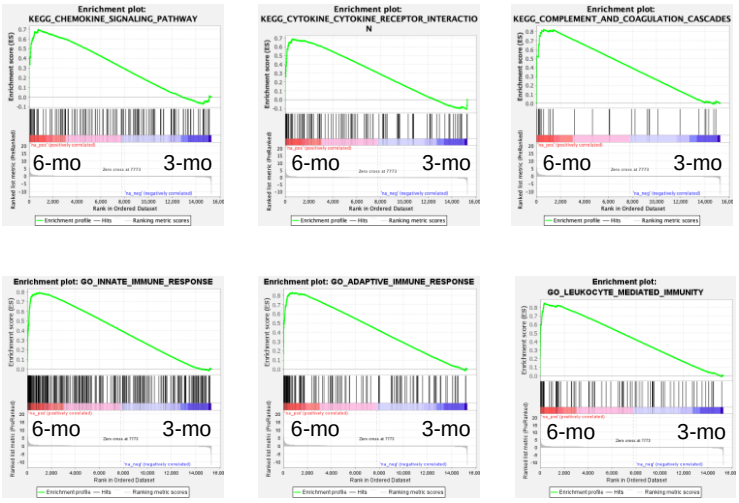


Figure 9. Transcriptomic signature in hippocampal tissue of NZB/W-F1 lupus. (A-B) Heatmap of DEGs (|FC|>1.5, p-value<0.05) in hippocampal tissue between WT and Lupus at (A) 3 and (B) 6 months of age (n=4-5/group). (C-D) GO enrichment analysis; enriched terms in Lupus versus WT mice at (C) 3 and (D) 6 months of age. (E) DEGs (|FC|>1.5, FDR<0.05) in hippocampal tissue between 3 month-old and 6 month-old lupus mice. (F) GSEA plots showing enriched terms of 6 month-old versus 3 month-old lupus mice comparison. Lupus, NZB/W-F1 stain; WT (Wild-type), C57BL/6; DEG, differentially expressed gene; FC, fold change; FDR, false discovery rate; GO, gene ontology; GSEA, gene set enrichment analysis.

Figure 10.

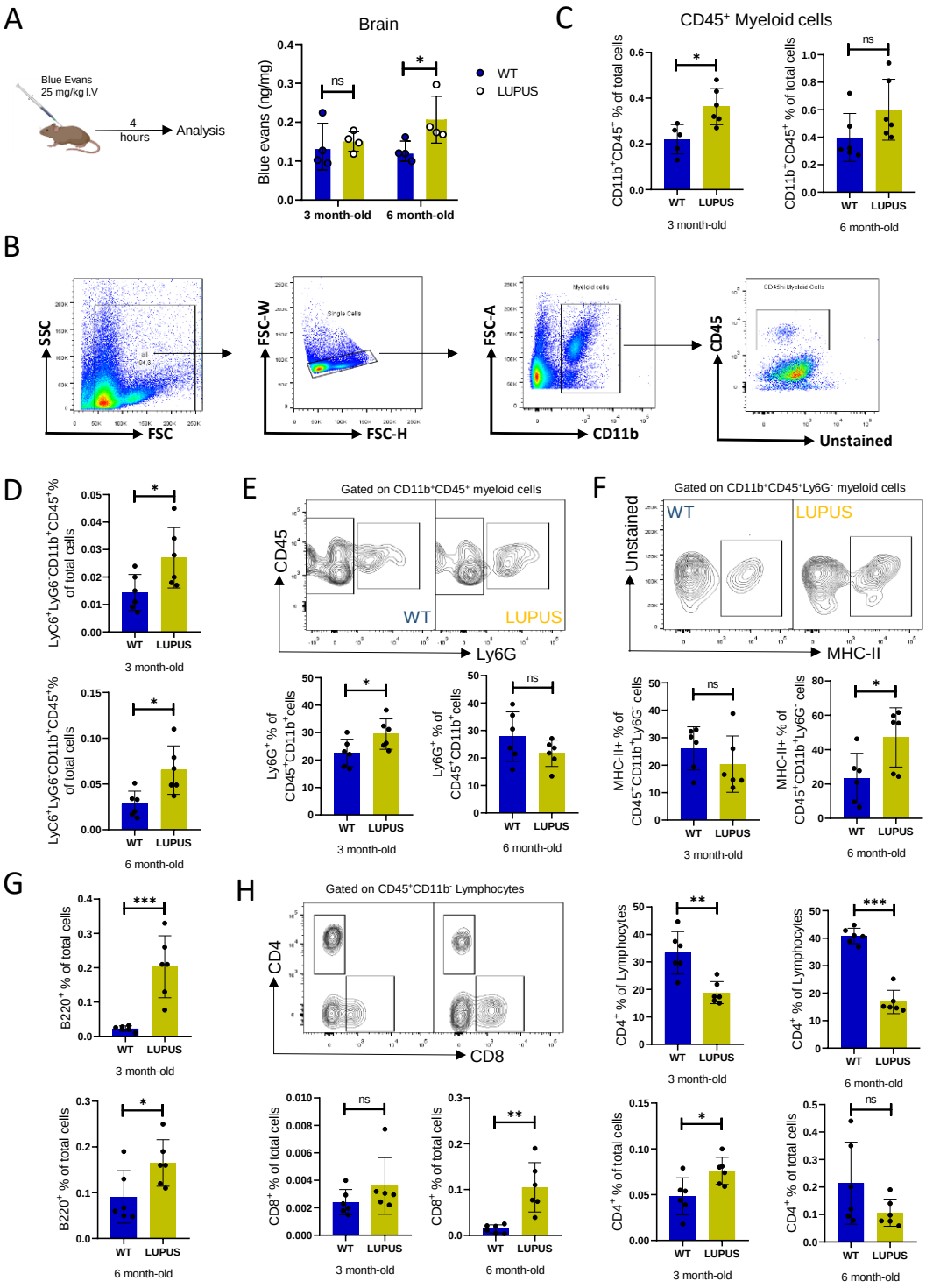

A
Blue Evans
25 mg/kg I.V
4
hours
Analysis
Brain
Blue evans (ng/mg)
ns
*
WT
LUPUS
3 month-old
6 month-old
C
CD45⁺ Myeloid cells
CD11b⁺CD45⁺ % of total cells
*
ns
WT
LUPUS
3 month-old
6 month-old
B
SSC
FSC
FSC-W
FSC-H
FSC-A
CD11b
CD45
Unstained
D
Ly6C⁺Ly6G⁻CD11b⁺CD45⁺% of total cells
3 month-old
6 month-old
E
Gated on CD11b⁺CD45⁺ myeloid cells
CD45
Ly6G
Ly6G⁺ % of CD45⁺CD11b⁺cells
Ly6G⁺ % of CD45⁺CD11b⁺ cells
F
Gated on CD11b⁺CD45⁺Ly6G⁻ myeloid cells
Unstained
MHC-II
MHC-II+ % of CD45⁺CD11b⁺Ly6G⁻ cells
G
B220⁺ % of total cells
H
Gated on CD45⁺CD11b⁻ Lymphocytes
CD4
CD8
CD8⁺ % of total cells
CD4⁺ % of Lymphocytes
CD4⁺ % of total cells

Figure 10. Blood brain barrier permeability and immune cells populations in the NZB/W-F1 lupus hippocampus. (A) Evans blue dye were intravenously injected in WT and Lupus mice at 3 and 6 months of age (n=4/group) following quantification of Evans blue in whole brain. (B) Flow cytometry gating strategy. (C) Flow cytometry analysis; frequency of CD11b+CD45+ cells in total cells. (D) Flow cytometry analysis; frequency of CD11b+CD45+Ly6G-Ly6C+ infiltrating monocytes in total cells. (E) Flow cytometry analysis; representative FACS plots and frequency of granulocytes (Ly6G+) in myeloid (CD11b+CD45+) cells. (F) Flow cytometry analysis; representative FACS plots and frequency of MHC-II+ cells in CD11b+CD45+Ly6G- myeloid cells. (G) Flow cytometry analysis; frequency of B-cells (B220+) in total cells. (H) Flow cytometry analysis; representative FACS plots and frequency of CD4+ T-cells in lymphocytes and the frequency of CD4+ and CD8+ T-cells in total cells. All experiments were performed in Lupus and WT mice at 3 and 6 months of age (n=4-6/group) and obtained from 2 independent experiments. Lupus, NZB/W-F1 stain; WT, C57BL/6; Bars, mean±SD. Data were analyzed with Student's t-test, *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 11.

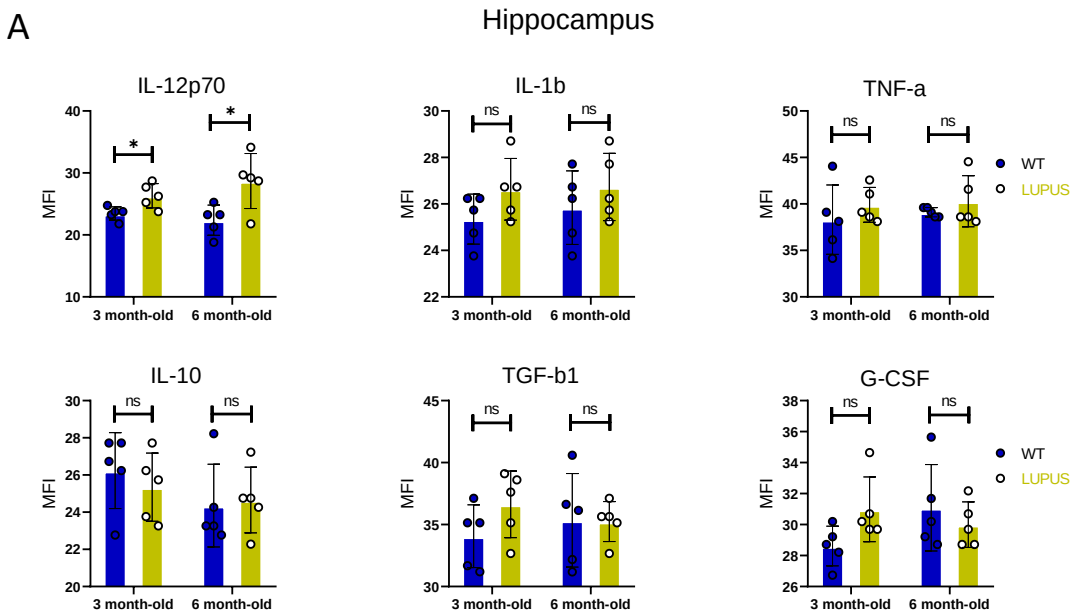


Figure 11. Cytokine expression in lupus hippocampus. (A) Quantification of IL-12p70, IL-1b, TNF-a, IL-10, TGF-b1 and G-CSF in hippocampal tissue. All experiments were performed in Lupus and WT mice at 3 and 6 months of age (n=4-5/group) and obtained from 2 independent experiments. Lupus, NZB/W-F1 stain; WT, C57BL/6; Bars, mean±SD. Data were analyzed with Student's t-test, *P < 0.05.

Hippocampal microglia are activated towards a pro-inflammatory state and contribute to the local inflammatory burden

Although we revealed a clear periphery-mediated immune response in the hippocampus of lupus-prone mice, the BBB remained intact in 3-month-old mice with a less profound immune cell infiltration. Thus, we asked whether the CNS resident cells contribute to the hippocampal inflammatory burden, especially at early stages of the disease. As described above, we have found an increased proportion of $CD45^+$ cells within the myeloid population (**Figure 8D**). Moreover, Suß et al.(244) detected $CD45^+$ activated microglia within the $CD11b^+$ CNS population by performing single cell RNA sequencing. Thus, we anticipated that microglia may be activated and play a pathogenetic role in the disease. Notably, we observed an increased expression of CD45 in hippocampal $CD11b^+$ myeloid cells, which is suggestive of microglia activation (**Figure 13A, B**). To further investigate the role of microglia, we used flow cytometry techniques (**Figure 13C**). To better document the activated status of microglia, we performed immunohistological detection of the Iba1 marker; a pan-microglial marker whose expression increases with microglial activation(247). Indeed, we observed increased expression of Iba1 in whole lupus hippocampus (**Figure 12A, B**) and particularly in the areas of DG, CA1 and CA3 (**Figure 14A-D**), both at 3 and 6 months of age. Finally, we verified that microglia are activated in the subgranular zone, granule cell layer and hilus; subregions of the neurogenic DG area (**Figure 12C, D**).

Next, we sought to elucidate the pathogenetic role of microglia. Initially, we revealed that hippocampal microglia expressed increased levels of iNOS suggesting that microglia are activated towards the M1 stage in lupus (**Figure 12E**). M1-like microglia are characterized as inflammatory microglia with increased release of pro-inflammatory cytokines(248). On the contrary, we did not detect increased microglial expression of arginase-1 (**Figure 12F**) that indicates, along with stable hippocampal levels of IL-10 (M2-microglia produce IL-10; (**Figure 11A**) indicating that microglia are not activated towards the M2 phenotype (eg. anti-inflammatory microglia) in the NZB/W-F1 model of SLE. Taken together, these data suggest that microglia cells in lupus are activated towards a pro-inflammatory cytokine-producing phenotype.

Currently, microglia cells have emerged as antigen-presenting cells of the CNS(249). Having detected a transcriptomic signature that suggests increased antigen presentation in the hippocampus of lupus-prone mice (**Figure 9D**), we investigated whether microglia contribute to this signature. Indeed, hippocampal microglia from NZB/W-F1 lupus express increased levels of markers suggestive of antigen presentation. Specifically, hippocampal microglia of 3-month-old lupus-prone mice exhibit elevated levels of CD86 and MHC-II, while 6-month-old mice have increased microglial levels of CD86, MHC-II and CD80 in their hippocampi (**Figure 12G-I**). These data indicate that lupus microglia may enhance local immune responses via antigen presentation.

In addition to cytokines, microglia are capable producers of chemokines(250) that attract immune cells from the periphery to target organs (eg. Hippocampus). To further explore this, first, we measured the levels of selected chemokines in hippocampus revealing that lupus mice carry increased amounts of CCL17, CCL22 and CXCL1 (**Figure 15A**). To evaluate whether microglia are the main producing cells of chemokines, we measured the mRNA levels of the aforementioned chemokines from sorted lupus microglia. Of note, lupus microglia express increased mRNA levels of CCL17, CCL22 and CXCL1 compared to WT mice (**Figure 12J, Figure 15B**), which is correlated –at least in part- with the levels in the hippocampal tissue. These results suggest that microglia cells in lupus release chemokines attracting immune cells to further inflame the hippocampal tissue.

Taken together, lupus microglial cells in the hippocampus including the DG are activated and exhibit a gamut of pathogenetic properties including increased release of cytokines and chemokines and enhance antigen presentation to other immune cells. These data suggest that microglia are key orchestrators of CNS lupus pathogenesis. To this end, microglia-mediated neuroinflammation may directly impact the hNSCs response via the release of immune reactants.

Figure 12.

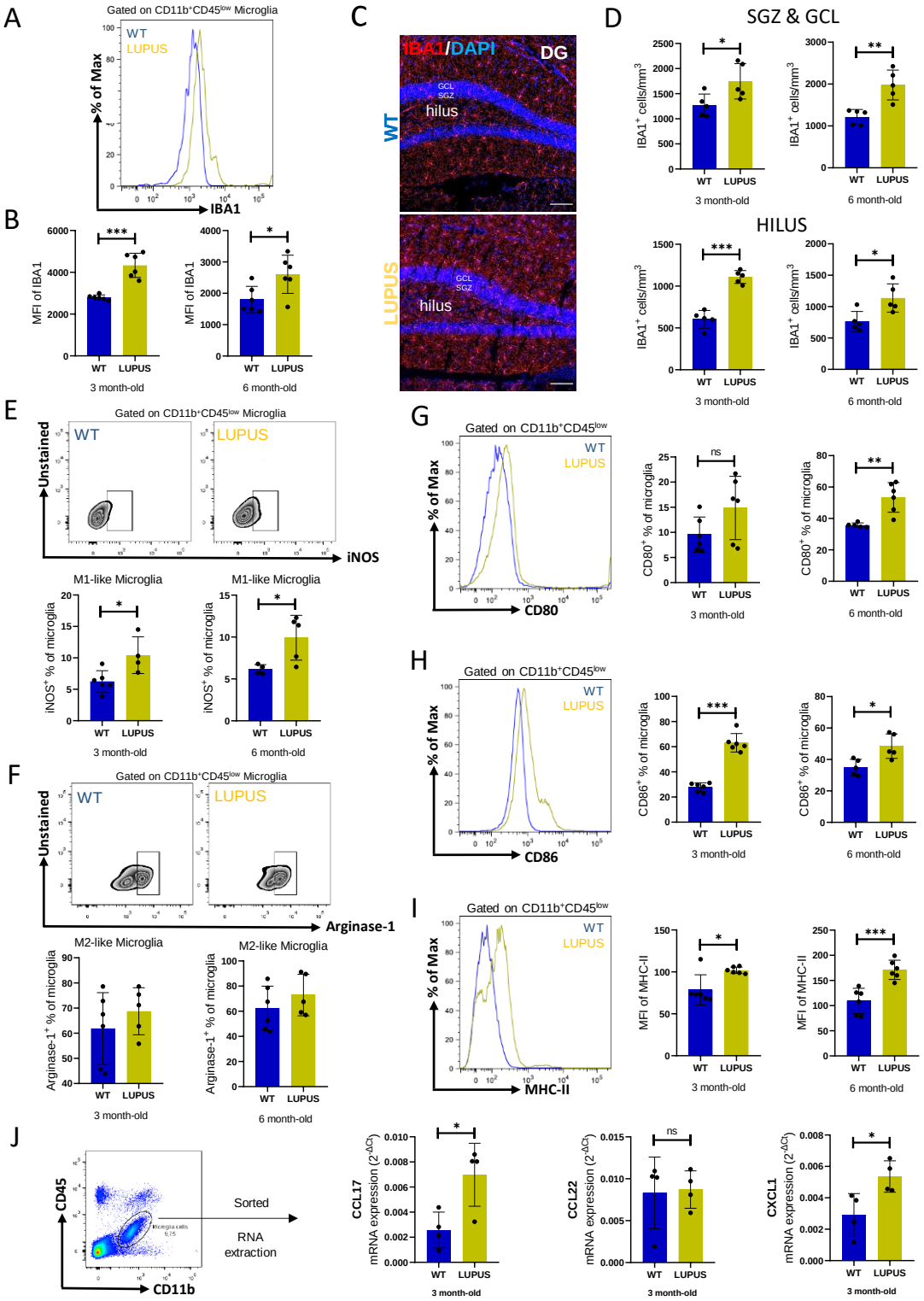

A
Gated on CD11b+CD45low Microglia
WT
LUPUS
% of Max
IBA1
B
MFI of IBA1
3 month-old
6 month-old
C
IBA1/DAPI
DG
GCL
SGZ
hilus
WT
LUPUS
D
SGZ & GCL
IBA1+ cells/mm3
3 month-old
6 month-old
HILUS
E
Gated on CD11b+CD45low Microglia
Unstained
iNOS
M1-like Microglia
iNOS+ % of microglia
F
Arginase-1
M2-like Microglia
Arginase-1+ % of microglia
G
CD80
CD80+ % of microglia
H
CD86
CD86+ % of microglia
I
MHC-II
MFI of MHC-II
J
CD45
CD11b
Sorted
RNA extraction
CCL17
CCL22
CXCL1
mRNA expression (2-ΔCt)

Figure 12. Activation of hippocampal microglia towards a pro-inflammatory state in NZB/W-F1 lupus mice. (A) Flow cytometry analysis; representative histogram and mean fluorescence intensity (MFI) of IBA1 in microglia cells (CD11b+CD45low). (B) Representative images of immunohistochemical detection of IBA1+ microglia cells in the DG of 3 month-old mice; Scale bar: 50mm. (C) Quantification of IBA1+ microglia cells in the subgranular zone (SGZ)/Granule Cell Layer (GCL) and hilus of WT and Lupus mice at 3 and 6 months of age (n=5/group). (D) Flow cytometry analysis; representative FACS plots and frequency of iNOS+ pro-inflammatory microglia cells (CD11b+CD45low). (E) Flow cytometry analysis; representative FACS plots and frequency of Arginase1+ anti-inflammatory microglia cells (CD11b+CD45low). (F) Flow cytometry analysis; representative histogram and frequency of CD80+ cells in microglia cells (CD11b+CD45low). (G) Flow cytometry analysis; representative histogram and frequency of CD86+ cells in microglia cells (CD11b+CD45low). (H) Flow cytometry analysis; representative histogram and MFI of MHC-II+ cells in microglia cells (CD11b+CD45low). (I) Quantification of CCL17, CCL22 and CXCL1 mRNA levels of sorted microglia with real time RT-qPCR in 3 month-old mice. All experiments were performed in Lupus and WT mice at 3 and 6 months of age (n=4-6/group) and obtained from 2 independent experiments. Lupus, NZB/W-F1 stain; WT, C57BL/6; Bars, mean±SD. Data were analyzed with Student's t-test, *$P < 0.05$,**$P < 0.01$,***$P < 0.001$.

Figure 13.

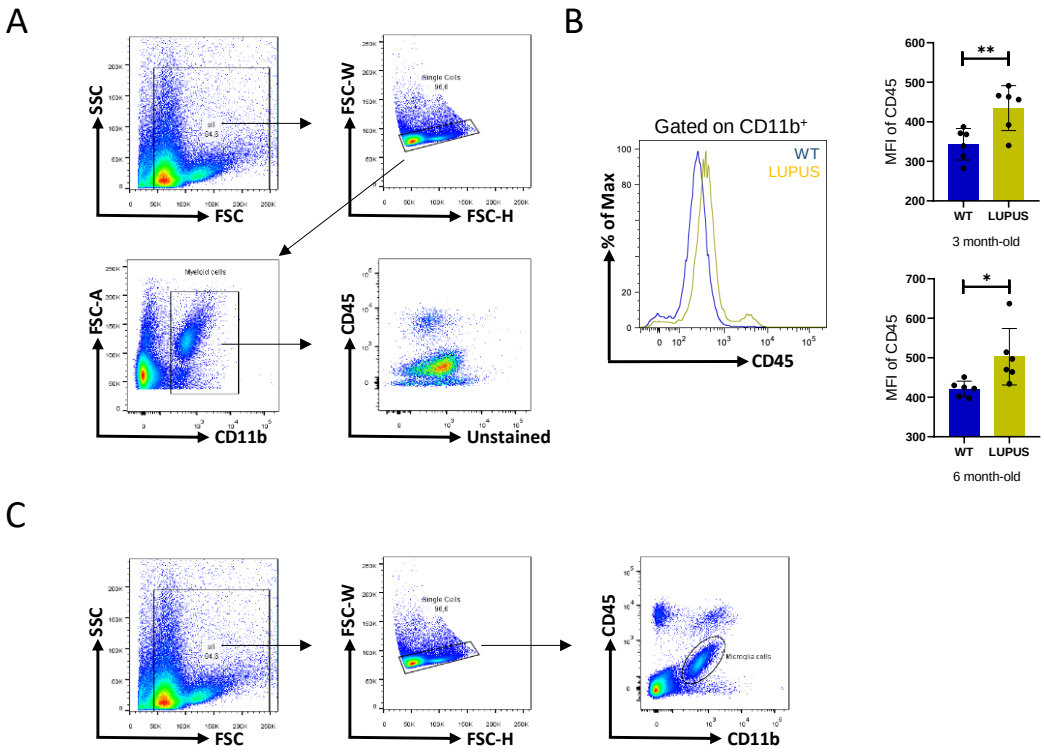


Figure 13. Increased CD45 expression in myeloid population in lupus hippocampus. (A) Representative FACS plots of gating strategy for myeloid cells in hippocampal tissue. (B) Flow cytometry analysis; representative histogram and mean fluorescence intensity (MFI) of CD45 in myeloid cells (CD11b+). (C) Representative FACS plots of gating strategy for myeloid cells in hippocampal tissue. All experiments were performed in Lupus and WT mice at 3 and 6 months of age (n= 5-6/group) and obtained from 3 independent experiments. Lupus, NZB/W-F1 stain; WT, C57BL/6; Bars, mean±SD. Data were analyzed with Student's t-test, *P < 0.05.

Figure 14.

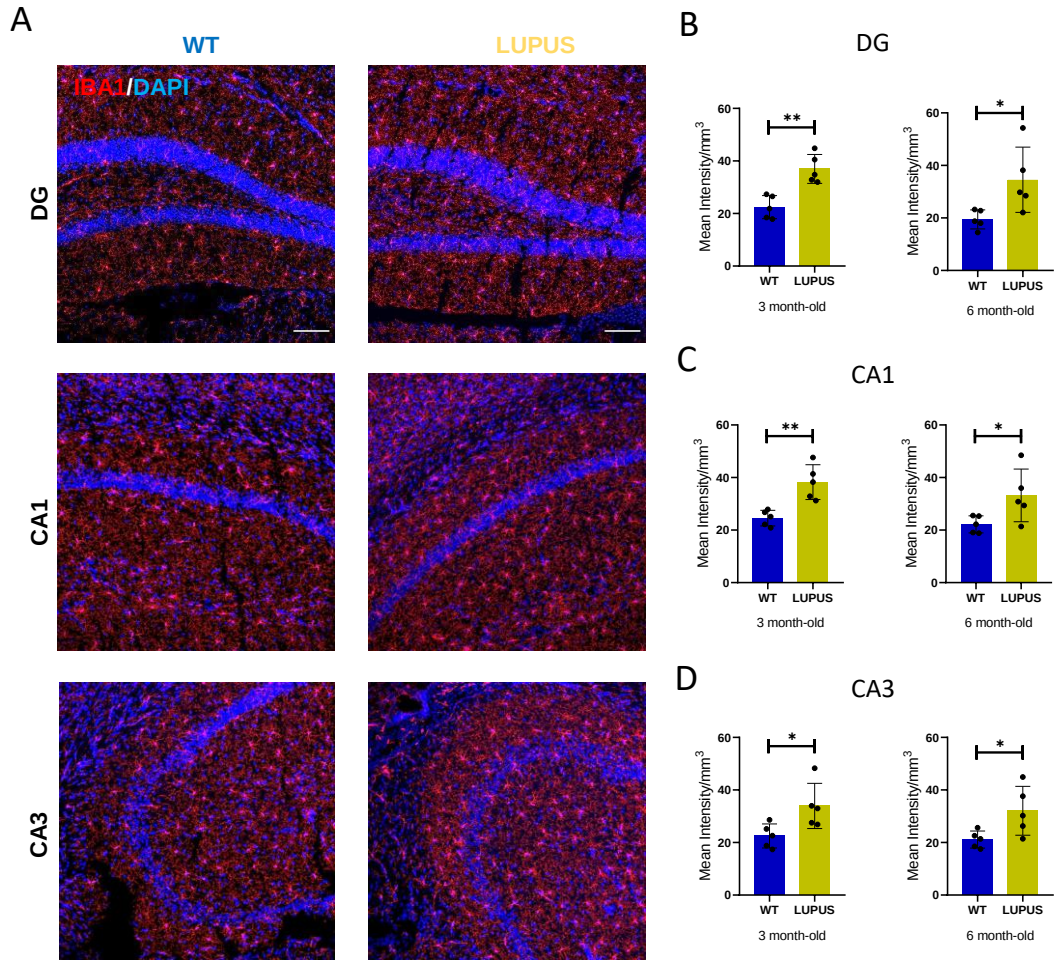


Figure 14. Microglia are activated in distinct regions in lupus hippocampus. (A) Representative images of immunohistochemical detection of IBA1+ microglia cells in the DG, CA1 and CA3 of 3 month-old mice; Scale bar: 50mm. (B) Quantification of mean fluorescence intensity (MFI) of IBA1+ in the DG, CA1 and CA3 of WT and Lupus mice at 3 and 6 months of age (n=5/group). All experiments were performed in Lupus and WT mice at 3 and 6 months of age (n=5/group) and obtained from 3 independent experiments. Lupus, NZB/W-F1 stain; WT, C57BL/6; Bars, mean±SD. Data were analyzed with Student's t-test, *P < 0.05.

Figure 15.

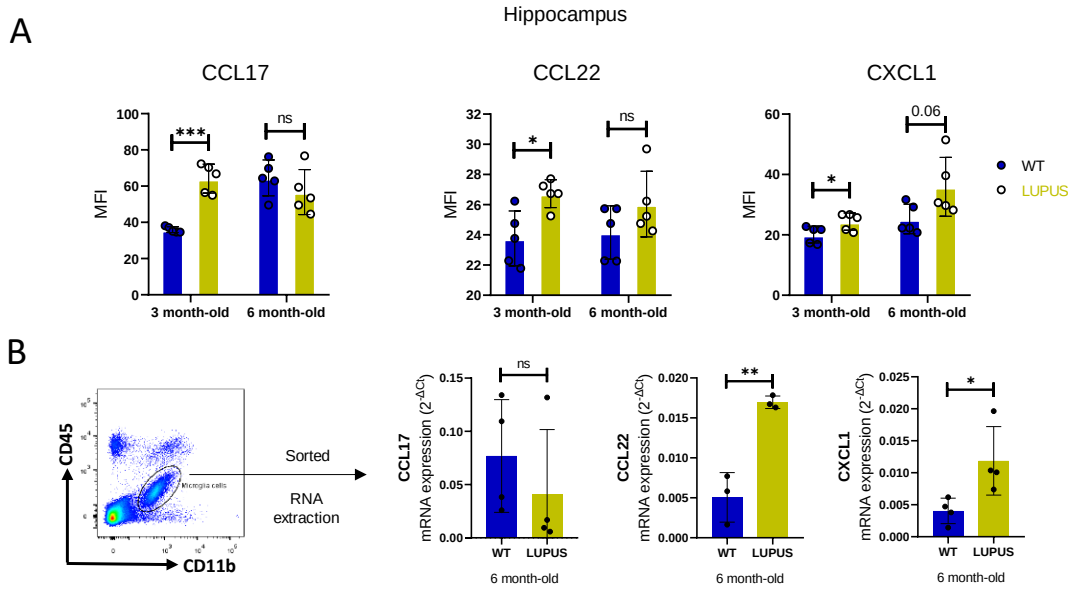


Figure 15. Lupus microglia secrete increased levels of chemokines in hippocampus. (A) Quantification of CCL17, CCL22 and CXCL1 in hippocampal tissue. (B) Quantification of CCL17, CCL22 and CXCL1 mRNA levels of sorted microglia with real time RT-qPCR in 6 month-old mice. All experiments were performed in Lupus and WT mice at 3 and 6 months of age (n=4-6/group) and obtained from 2 independent experiments. Lupus, NZB/W-F1 stain; WT, C57BL/6; Bars, mean±SD. Data were analyzed with Student's t-test, *P < 0.05,**P < 0.01,***P < 0.001.

IL-6 and IL-18 directly promote proliferation and apoptosis of hippocampal neural stem cells

Finally, we sought to elucidate whether the hippocampal inflammatory response via cytokines *per se* are potential regulators of hNSCs response. First, we went through published available RNA-seq data by Koutmani et al.(221) to check whether adult hNSCs express receptors for cytokines that are found to be elevated in NZB/W-F1 hippocampi (ie. IL-6, IL-12, IL-18, IL-23). Of note, hNSCs express receptors for IL-6 and IL-18, but not for IL-12/IL-23. Therefore, we examined the effects of IL-6 and IL-18 on adult hNSCs *ex vivo* with respect to proliferation and apoptosis. We isolated the DGs of 2-month-old WT mice and cultured the NSCs with growth factors until neurospheres are formed (14 days). Then, we exposed the hNSCs to either IL-6 or IL-18 for 24 hours before assessing proliferation and apoptosis via Brdu/pH3 and TUNEL assay, respectively (**Figure 16A**). Interestingly, IL-6 and IL-18 have similar effects on adult hNSCs by promoting proliferation (**Figure 16B-E**) and inducing apoptosis (**Figure 16F, G**). Yet, IL-18 seems to induce a more profound increase in proliferation and apoptosis as compared to IL-6. More importantly, the effects of IL-6 and IL-18 on adult hNSCs are consistent with the hNSCs response *in vivo*.

Together, these data suggest that IL-6 and IL-18 are direct orchestrators of hippocampal neurogenesis in NZB/W-F1 lupus, which is characterized by increased proliferation and decreased survival of neural progenitors resulting in disrupted neurogenesis.

Figure 16.

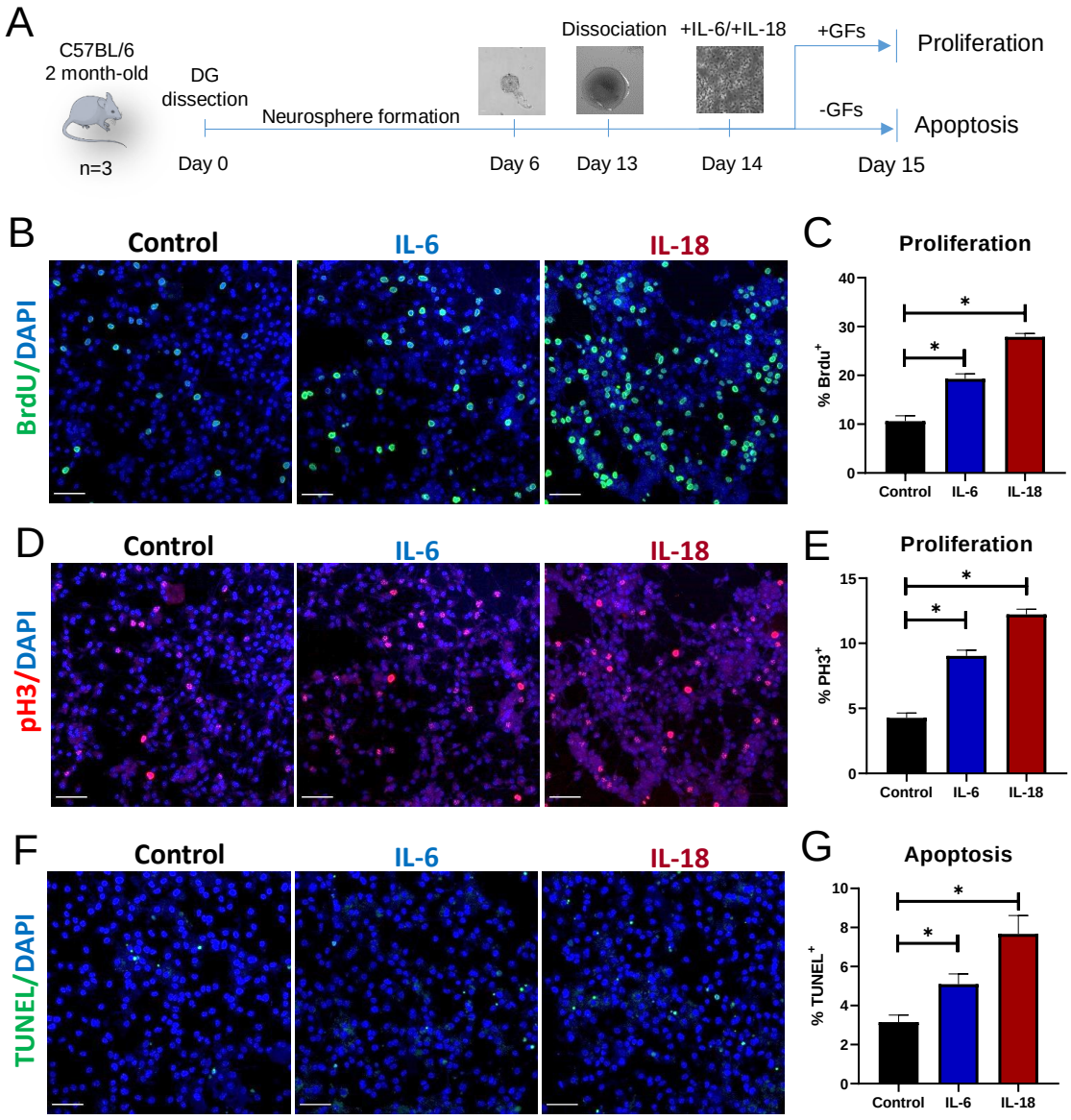


Figure 16. Interleukin-6 and Interleukin-18 directly promote proliferation and increase apoptosis of adult hiNSCs *in vitro*. (A) Experimental design for the assessment of direct effects of Interleukin-6 and Interleukin-18 on proliferation and survival of adult hiNSCs. (B, D, and F) Representative images of adult hiNSCs in culture stained against (B) BrdU and (D) PH3 or (F) subject to apoptosis with TUNEL assay after exposure to IL-6 or IL-18. (C,E,F) Quantification of the proliferative (C, E) and apoptotic (F) effects of IL-6 and IL-18 on adult hiNSCs. Data are representative of three independent experiments. DAPI stains nuclei; Scale bar: 10mm; Bars, mean±SEM; IL-6, interleukin-6; IL-18, interleukin-18. Data were analyzed with Mann-Whitney U test, *P < 0.05, **P < 0.01.

DISCUSSION

Cognitive dysfunction, mood disorders and anxiety are common in NPSLE, particularly attributed to hippocampal changes. The attribution of each manifestation to lupus has important diagnostic and therapeutic implications since involvement of inflammatory mechanisms require the institution of immunosuppressive therapy (208,251). In this study, we showed that NZW/B-F1 spontaneous lupus mouse recapitulates the diffuse human NPSLE and more importantly, their neuropsychiatric phenotype is associated with defective hippocampal neurogenesis. We also demonstrated that blood brain barrier disruption, immune cell trafficking and microglia activation orchestrate the inflammatory response in lupus hippocampus resulting in increased levels of pro-inflammatory cytokines. Among such cytokines, we identified IL-6 and IL-18 as direct inducers of apoptosis of adult hiNSCs *ex vivo* and we propose that inflammatory mediators induce neuropsychiatric changes in lupus via direct disruption of hippocampal neurogenesis.

The pathogenesis of CNS lupus remains obscure due to restricted access to human tissue and limited animal models that mimic the human disease(251,252). To date, most data on NPSLE pathophysiology derive from studies in the MRL/lpr strain which develops an SLE-like disease including neuropsychiatric manifestation due to loss of Fas function although Fas-deficiency in humans does not lead to a lupus-like phenotype(253). Another model of lupus-albeit not spontaneous but induced-commonly used to study cognitive impairment in SLE, requires immunization of mice with a peptide mimetope of DNA (DWEYS)(254). This model produces anti-DNA antibodies, termed DNRAb, that cross-react with the NMDA receptor causing cognitive dysfunction(136). Although this model has provided useful mechanistic insights in the pathogenesis of antibody-mediated CNS lupus, the NPSLE pathophysiology is complex involving multiple inflammatory mechanisms mediated by a diverse set of cytokines, infiltrating immune cells and activated microglia.

Herein, we performed a comprehensive analysis of the neuropsychiatric phenotype in the NZB/W-F1 strain, the classical spontaneous lupus model(255). To date a comprehensive characterization of neuropsychiatric behavior of lupus is lacking and the animal models that are used to mimic the disease are far from the actual pathological

condition(252,253). In this study, we demonstrated that this model exhibits hippocampus-linked behavioral deficits including anxiety, depressive-like behavior and impaired cognition recapitulating human diffuse neuropsychiatric lupus. Importantly, these changes are detected in early stages of the disease (onset at 3 months), therefore NPSLE development is unaffected by disease complications such as lupus nephritis (onset at 6-9 months) and the ensuing uremia which may affect cognitive function. These findings substantiate the use of this animal model in NPSLE research.

Various lines of research suggest that NPSLE has complex pathogenesis involving immune cell infiltration and BBB disruption that enables circulating neurotoxic autoantibodies and immune components to reach the brain(251,256). Based on advanced neuroimaging techniques, several brain regions seem to be affected in NPSLE with hippocampus being a prominent target of immune system resulting in neuropsychiatric alterations(119,212). Accordingly, RNA-sequencing of NZB/W-F1 hippocampal tissue revealed a profound inflammatory response with a myeloid predominant response and lymphocytic infiltration, particularly B-cells. Herein, we showed for the first time that lupus hippocampus is infiltrated -early in the course of the disease- by a unique immune cell profile orchestrating neuroinflammation.

Recent studies highlight the role of the microglia cells in NPSLE in addition to peripheral-mediated immune response in the CNS. Microglia cells, as part of their physiological response, secrete cytokines, chemokines, prostaglandins and reactive oxygen species(257). Current evidence indicate that microglia are activated in lupus resulting in synaptic pruning and cognitive dysfunction(258). Angiotensin-converting enzyme and sTREM-1 seem to initiate microglia activation, while complement components and PI3K-AKT signaling pathway are key downstream mediators of microglia-mediated neuronal damage in NPSLE(139,259). In addition, activated microglia during sustained inflammation, phagocytose astrocytic end-feet and disrupt BBB permeability(260). We demonstrated that before the onset of the disease, microglia are activated towards an inflammatory phenotype (M1-like) contributing to local inflammatory response via secretion of pro-inflammatory cytokines. Moreover, lupus microglia produce increased amounts of chemokines attracting immune cells from the periphery into the brain. To this end, increased expression of CD80, CD86 and MHC-II of hippocampal microglia may contribute to underlying inflammation via

increased antigen presentation; further studies are needed to expand these findings. Our study contributes to further substantiating of the pathogenetic properties of microglia in NPSLE.

The finding of an intrinsic microglial stimulation as an early event in NPSLE is intriguing. Activated microglial cells initially migrate to the BBB and protect its integrity. However, subsequently they are transformed into a reactive phenotype that phagocytose BBB components to initiate leakage of systemic substances into the parenchyma and cause widespread neuroinflammation(260). To this end activation of microglia may be a key initiating event in NPSLE. Of interest, in a recent combined genomic-genetic analysis we combined eQTL analysis from the Genotype Tissue Expression (GTEx) project and SLE-associated genetic polymorphisms and found that lupus susceptibility variants may regulate gene expression in the blood but also in other tissues including brain. From these analyses blood and brain emerged as the primary causal tissues in SLE(261) suggesting that brain is not only a target organ in SLE but also an initiator of autoimmunity.

Inflammation of the central nervous system has been long recognized to lead to remodeling of the physiological activity of NSCs(262). The effect of inflammation on hippocampal neurogenesis has been studied *in vivo* in several murine models of autoimmune diseases with contradictory results. In experimental models of multiple sclerosis and inflammatory bowel disease, hippocampal neurogenesis is enhanced during the acute phase of inflammation, while progressively depleted over the course of the disease(233,263,264). Defective hippocampal neurogenesis has been observed in models of induced-arthritis and BAFF-transgenic mouse; a model of systemic autoimmunity(265–267). These data underscore that distinct inflammatory milieus affect NSCs in several ways.

To date, thorough assessment of hippocampal neurogenesis has not been conducted in MLR/lpr lupus-prone mouse or DNRAb-induced lupus; only one study demonstrated enhanced proliferation of hiNSC at disease onset in MLR/lpr lupus model(268). In this direction, we characterized the hippocampal NSCs response step-by-step in NZB/W-F1 lupus-prone mice and identified the molecules that orchestrate local inflammation at early and late stages of the disease. Hippocampal neurogenesis is disrupted in

ZNB/W-F1 lupus as a result of increased apoptosis. In addition, increased proliferation and self-renewal of neuronal precursors was evident in lupus that may be explained –at least in part- by excessive apoptosis. The lupus-prone hippocampus exhibits increased amounts of IL-6, IL-12, IL-18 and IL-23, yet NSCs express receptors only for IL-6 and IL-18. These cytokines induce apoptosis *ex vivo* in hiNSCs and appear to be key players of NSCs response in SLE. Of note, human NPSLE is associated with increased levels of IL-6 in CSF, while increased levels of serum IL-18 are linked to increased probability of neuropsychiatric involvement(269,270). Moreover, the defective neurogenesis was captured early in the course before the onset of full-blown disease, suggesting that low-grade hippocampal neuroinflammation is an early event in lupus. From a clinical perspective, the neuropsychiatric involvement occurs close to diagnosis of SLE implying that disrupted neurogenesis may precede these symptoms in human SLE.

Cytokines have been long recognized to alter the neurogenic activity of NSCs, yet most data derived from *in vivo* studies and *ex vivo* experiments only on embryonic NSCs(262). This is the first study which assess *ex vivo* the direct effect of either IL-6 or IL-18 on adult hiNSCs with regards to survival and proliferation. Previous reports indicate that IL-6 promote proliferation *in vivo* and may increase apoptosis of adult NSCs (271,272). These data are in line with our findings further supporting the crucial role of IL-6 on adult neurogenesis. Experiments on embryonic neural progenitors' culture showed that IL-18 induces neuronal cell death, while its effect on proliferation remains elusive (273). Our study unraveled the direct effects of IL-18 on adult hiNSCs shedding light on the biological impact of IL-18 on adult neurogenesis.

Our study highlights microglia and IL-6/IL-18 as potential therapeutic targets in NPSLE. CSF-1R inhibitor, which cross the BBB and cause microglia depletion has been administrated in murine lupus with favorable CNS-related outcomes (139). However, this drug depletes not only microglia, but also macrophages raising safety concerns (181). Targeted therapies with monoclonal antibodies against IL-6 or IL-18 may be beneficial, yet do not cross BBB due to their size. Alternatively, JAK inhibitors can be used to target downstream cytokine-mediated pathways as effectively penetrates the BBB. Type-I interferon (IFN) has emerged as central player in CNS disease causing endothelial cell damage (200) and M1-microglia activation (274). Of note, lupus sera can induce M1 activation of brain microglia (275). Because NZB/W-F1 is characterized

by IFN-a signature at early stage (276), we reason that early CNS IFN-response could initiate microglia activation and BBB disruption making anifrolumab a promising therapeutic target for diffuse NPSLE. However, in the NZB/NZW animal model we did not detect a clear IFN transcriptomic signature within hippocampus at the pre-nephritic stage but later during full-blown disease. Whether this is a feature of other animal models of NPSLE or a unique feature of our model remains to be determined.

In conclusion, our findings support the validity of the NZW/B-F1 lupus as a model of human NPSLE with these mice exhibiting cognitive dysfunction, depression and anxiety both at early and later stages of the disease. We show that inflammation orchestrates hippocampal response in lupus mediated by both periphery and intrathecally-derived immune mediators. IL-6 and IL-18, which are elevated in lupus hippocampus, directly induce apoptosis in hiNSCs resulting in defective neurogenesis and increased self-renewal of neuronal precursors. These findings underlie the behavioral phenotype of NZB/W-F1 lupus suggesting that disruption of hippocampal neurogenesis is an early event of NPSLE. Since neuropsychiatric events-especially cognitive dysfunction- in SLE may be initially subtle, inhibiting microglial activation via molecules like ACE-inhibitors or other compounds may be helpful (140,277). At later stages, therapeutic strategies such as inhibition of interferon or complement components may be more helpful. We propose that low-grade inflammation mediated by activated microglia preceding the diagnosis of SLE negatively impacts the hiNSCs response resulting in impaired cognition, anxiety and depression. Inhibition of microglia activation and IL-6 and IL-18 may represent early therapeutic targets in diffuse NPSLE.

KYE FINDINGS

- In Attikon Lupus Cohort, lupus nephritis is not as common as in older cohorts, while neuropsychiatric disease is emerging as a major frontier in lupus prevention and care.

- Almost half of patients with initially non-severe disease progress to more severe forms of SLE, especially patients with neuropsychiatric involvement at onset.

- Demyelination in the context of SLE follows a more benign course compared to a frank SLE-MS overlap.

- A significant number of patients with demyelination do not fulfill criteria for either MS or SLE at follow-up. These patients exhibit lupus-like autoimmune features and may represent a distinct entity, ‘demyelination with autoimmune features’

- The NZB/W-F1 lupus strain exhibit hippocampal-linked behavioral perturbations recapitulating human NPSLE validating it as an animal model of diffuse neuropsychiatric SLE.

- Despite the widespread assumption that disturbance of the BBB is a prerequisite for NPSLE, our data suggest that intrinsic mechanisms within the brain may initiate the disease.

- Microglia activation towards an inflammatory state (M1-like) is an early event in neuropsychiatric lupus resulting in hippocampal inflammatory response mediated by pro-inflammatory cytokines.

- Interleukin-6 and interleukin-18 induce hippocampal-linked behavioral defects in lupus via the disruption of hippocampal neurogenesis and decreased number of neuronal cells.

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