Contemporary Clinical Neuroscience

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Neuroimmune Diseases

From Cells to the Living Brain

Springer

Contemporary Clinical Neuroscience

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Contemporary Clinical Neuroscience
ISBN 978-3-030-19514-4 ISBN 978-3-030-19515-1 (eBook)
https://doi.org/10.1007/978-3-030-19515-1

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Bridge between basic and clinical neuroimmunology There are already published reviews and textbooks on basic immunology, clinical diagnosis, and treatment of neuroimmune diseases. Therefore, no one would question the existence of available scientific literature in the field. However, due to the enormous and growing information, beginners, such as students and trainees, find it difficult to capture the large picture of neuroimmune diseases and get access to the essential information, especially during the attempt to extract relevant information for a bedside purpose. Students and even experienced researchers often encounter difficulties in linking fundamental knowledge and the pathomechanisms underlying each disease. Furthermore, clinicians are facing a great challenge: to examine an increasing number of patients in shorter times, with a need to reach the right diagnosis and give the right therapies. Busy clinicians often lack time to explore textbooks when they need quick access to information. There is a clear need for a concise book that explains the relationships between fundamental aspects of neuroimmunology and daily therapeutics, with a pragmatic approach for both junior and experienced clinicians or scientists. Our book aims to create a bridge between basic and clinical neuroimmunology, easily accessible in the daily activities of laboratories or hospitals. In particular, we try to explain the common elemental pathomechanisms underlying a variety of common diseases encountered in daily clinical practice supported by helpful illustrations and tables.

Why is the pathophysiology essential in the field? Historically, new information in basic immunology has expanded the field of neuroimmune diseases and provided novel opportunities for immunotherapies. For example, Cruveilhier (1842) documented the clinical features of multiple sclerosis (MS) [1], and Charcot (1868) subsequently provided the link between clinical features and pathological changes [2]. Furthermore, Guillain-Barré syndrome (GBS) was described as acute ascending weakness by Landry (1859) [3]. However, the infection etiology was proposed for both diseases several decades later. The autoimmune pathogenesis has been confirmed from the pathological and clinical similarities in experimental allergic encephalitis in 1935 for MS [4] and experimental allergic neuritis in 1949 for GBS [5]. These histories highlight the importance of understanding and application of basic

immunological methodologies in the unraveling of the pathogenesis of idiopathic neurological diseases. Many of the novel immunotherapies that were based on advancements in immunology provide promising benefits in autoimmune diseases.

Pathogenic fundamentals between "disorganized immune cells" and "living brain functions" In order to fill the gap between basic and clinical neuroimmunology, the pathogenic behaviors of immune cells will be discussed first. Second, the interactions between immune cells and nerve cells will be considered. The mechanisms of how these "disorganized immune cells" disrupt "living brain functions" will be explained, in order to enhance the understanding of nerve cell dysfunction and its relation to neural deficits in various neuroimmune categories.

Compared with autoimmune diseases in other organs, various autoimmune pathogenic processes are involved in the development of neuroimmune diseases. The apparent differences in clinical manifestations can be attributed to certain fundamental autoimmune processes. These elementary processes include "pathogenic roles of effecter T cells (Th1/17 cells and CD8 T cells) or autoantibodies," "autoimmune triggers by deficits in immune tolerance or molecular mimicry," "pathological permeability of blood-brain (BBB) or blood-nerve barrier (BNB)," and "exacerbation by local neural inflammation." This book aims to elucidate these elementary autoimmune mechanisms shared by divergent neuroimmune categories.

The rapid progress in the field of immunology has uncovered unexpected findings related to these key concepts. For example, identification of various cytokines has multiple actions, including inhibitory regulation of the immune systems, diverse actions on astrocytes and microglia, and secretion of molecular substances that can regulate the BBB or BNB, all of which constitute diverse and sophisticated immune systems. Thus, basic information is being continuously updated, which helps in understanding the pathogenesis of autoimmune diseases of the brain including the roles of novel molecular and cellular substances.

Updated diagnosis and therapeutic strategies In the clinical chapters of this book, we provide the state of the art of diagnosis of each category, based on the background pathogenic process. Based on accumulated clinical evidence, the diagnostic criteria of various neuroimmune diseases have improved recently, allowing early diagnosis and helping in the differential diagnosis. This book will not only contribute to our appraisal of the autoimmune processes but also to appropriate application of immunotherapies. Immunotherapies can be divided into two major types: (1) therapies for the acute phase of the disease, which are designed to stop or halt the autoimmune-mediated destruction of the brain (induction therapies), and (2) disease-modifying therapies for the chronic phase, which serve to prevent relapses (maintenance therapies). The central and peripheral nerve systems have capacities for restoration and compensation, which can be termed the "nervous reserve." The concept of the reserve is based on various intracellular protective apparatuses and synaptic plasticity of the neural circuitry. Thus, early diagnosis and early administration of immunotherapies are important during the disease phase in which the "brain reserve" is still preserved. It is now possible to stop the progression of some diseases using newly developed immunosuppressive drugs.

During our academic practice, we have opportunities to teach residents and trainees, at a time where discovery and progress in neurological domain is constant. This book can be used to bridge the gap between basic and clinical neuroimmunology. It can also be used for board examinations or for the preparation of seminars.

We hope that this book will encourage young students, clinicians, and scientists to join in these exciting intellectual adventures, "from cell to living brain."

We are particularly grateful to our eminent colleagues who have devoted time to deliver excellent contributions. We are also thankful to the whole outstanding editorial team.

Tokyo, Japan — Hiroshi Mitoma
Mons, Belgium — Mario Manto

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Contents

Part I
Fundamental Mechanisms and General Principles

Overview of Mechanisms Underlying Neuroimmune Diseases

Sandip Ashok Sonar and Girdhari Lal

Abstract The neuroimmune diseases are caused by autoimmune demyelination, opportunistic and neurotrophic infections, paraneoplastic conditions, neurodegeneration, and neuropsychiatric disorders. These diseases are multifactorial, complex, and heterogeneous with varied clinical and pathological features and often triggered by the interplay of genetics, environmental factors, and dysregulated immune activation. The molecular mimicry of neuronal antigens, generation of onconeural antigens, inflammation-induced neuronal antigen release, and cross-presentation are thought to activate the autoreactive T and B lymphocytes. The activation of several innate immune pathways, generation of effector T cells, production of autoantibodies, inflamed blood-brain barrier, and activated microglia, astrocytes, oligodendrocytes, and neurons are known to contribute to the development of neuronal diseases. The majority of current research is focused on the genetic association, biomarker discovery, differential diagnosis, treatment choices, and identification of immunological and neurological basis of neuroimmune diseases. In this chapter, we discuss the clinical and pathological features of neuroimmune diseases and also present an overview of the current understanding of the immunological and neurological mechanisms. We also highlighted the cellular and molecular interactions in the generation of autoantibodies, inflammatory $CD4^+$ and $CD8^+$ T cells, reactive microglia and astrocytes, and importance of the blood-brain barrier in neuroinflammation and autoimmunity.

Keywords Autoimmune demyelination · Autoantibody · Autoreactive T cells · Blood-brain barrier · Neuroinflammation

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_1

Abbreviations

AChR	acetylcholine receptor
AD	Alzheimer's disease
ADEM	acute disseminated encephalomyelitis
ALS	amyotrophic lateral sclerosis
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANNA-1	anti-neuronal nuclear antibody type 1
AQP4	aquaporin 4
ASD	autism spectrum disorder
BBB	blood-brain barrier
BCSFB	blood-cerebrospinal fluid barrier
BNB	blood-nerve barrier
Bregs	regulatory B cells
Caspr2	anti-contactin-associated protein 2
CNS	central nervous system
CSF	cerebrospinal fluid
DAMPs	damage-associated molecular patterns
EAE	experimental autoimmune encephalomyelitis
GABA	gamma-aminobutyric acid
GAD65	glutamic acid decarboxylase 65
HD	Huntington's disease
HSV	herpes simplex virus
HTT	huntingtin
LGI1	leucine-rich glioma-inactivated-1
MAG	myelin-associated glycoprotein
MBP	myelin basic protein
mGluR	metabotropic glutamate receptor
MOG	myelin oligodendrocyte glycoprotein
MRI	magnetic resonance imaging
MS	multiple sclerosis
NMDA	anti-N-methyl-D-aspartate
NMOSD	neuromyelitis optica spectrum disorders
PCA2	Purkinje cell cytoplasmic antibody 2
PD	Parkinson's disease
PML	progressive multifocal leukoencephalopathy
PP-MS	primary progressive multiple sclerosis
RR-MS	relapsing-remitting multiple sclerosis
SCLC	small-cell lung carcinoma
SLE	systemic lupus erythematosus
SOD1	superoxide dismutase 1
SP-MS	secondary progressive multiple sclerosis
SPS	stiff-person syndrome
Tregs	regulatory $CD4^+$ T cells

TREM2	triggering receptor expressed on myeloid cells 2
Trm	tissue-resident memory T cells
VZV	varicella-zoster virus
WNV	West Nile virus

Introduction

Divergence and Convergence in Neuroimmune Diseases

The neuroimmune diseases comprise a group of heterogeneous disorders that involve the immune system to affect the functions of the central nervous system (CNS), peripheral nervous system (PNS), and autonomous nervous system (ANS). The immune activation against neuronal antigens forms the basis of almost all of the neuroimmune diseases. They are characterized mainly by inflammatory, autoimmune (cell-mediated or humoral), demyelinating, neurodegenerative, parainfectious, paraneoplastic, and traumatized neurological deficits [1]. The neuroinflammation is a prominent feature of the neuroimmune disorders, and various immunotherapeutic interventions show considerable clinical benefits [1, 2]. Based on the selective white or gray matter pathology, neuronal autoimmunities are categorized into acquired demyelinating syndromes or antibody-mediated autoimmune encephalopathies. Based on the pattern of pathological lesions caused in the nervous system, neuroimmune diseases are also stratified as a monofocal or multifocal neurological deficits. Further, depending on the nature of radiological findings, they are typified as lesional (focal areas of hyperintensity), black holes (regional loss of tissue), and atrophy (loss of brain volume). Despite these distinct pathological differences, several neuroimmune diseases share overlapping clinical features, radiological findings, and pathological mechanisms that often make the diagnosis, management of the associated disabilities, and monitoring the clinical progression complicated. The recent technological advances in magnetic resonance imaging (MRI) coupled with the clinical and pathological findings have shown some promise in diagnosing a specific disorder with some precision.

Blood-Brain Barrier and Blood-Nerve Barrier

The nervous system is known to control almost all the vital voluntary and involuntary functions of the body and maintain homeostasis. The dynamic physiological barriers ensure the bare minimum immune reactions at different anatomic sites of the nervous system without hampering the immunosurveillance. The blood-brain barrier (BBB) and the blood-nerve barrier (BNB) actively maintain the homeostasis of the CNS and PNS, respectively. These barriers regulate the access of neuronal tissue to the circulating immune cells and inflammatory mediators [3, 4].

Additionally, blood-cerebrospinal fluid barrier (BCSFB) controls the accumulation of inflammatory mediators and immune cells in the ventricles and cerebrospinal fluid (CSF) draining compartments of the CNS and functions as a neuroprotective barrier [4, 5]. The biochemical and physiological properties of the BCSFB make them an active site for neuroimmune interaction and propagation of neuroinflammation [6]. Therefore, focused studies are needed to precisely understand the association of BCSFB-associated inflammation with the pathology of different neuroimmune disorders. Although these barriers sequester the neuronal antigens from the peripheral immune system, the molecular mimicry between microbial antigens and neuronal antigens represents a significant trigger for neuroinflammation in genetically susceptible individuals. The breakdown of the BBB and BNB and infiltration of effector lymphocytes, macrophages, and neutrophils are the hallmarks of several neuroinflammatory conditions of the CNS and PNS [4, 7].

Local Immune Cascades

The local inflammatory cascades in the nervous system induce the neuronal antigen presentation to the infiltrating T and B cells and cause reactivation and differentiation of antigen-reactive T cells and generation of autoantibodies. The resulting neuroinflammation is further fueled by the production of the damage-associated molecular patterns (DAMPs) and infiltration of other inflammatory cells such as monocytes, macrophages, neutrophils, dendritic cells, and antigen-non-specific lymphocytes that trigger the critical events of demyelination and axonal damage. The CNS-resident cells such as astrocytes and microglial cells also actively contribute to the inflammatory response in the local microenvironment [8].

Therapeutic Strategies of Neuroimmune Diseases

Interestingly, the conventional immunotherapeutic interventions, corticosteroids, and immunosuppressors provide a variable amount of clinical benefits in several neuroimmune disorders [9]. Furthermore, the precise targeting of the specific inflammatory pathways and immune cell activation and infiltration in the nervous system has been shown a promise to control the clinical course [10, 11]. Future studies may also highlight the critical role of immune-mediated cross talks and signaling pathways in several neuroimmune diseases. Numerous efforts are ongoing to define the strategies to block precisely the effector immune cells without disturbing the regulatory cells and the neuronal homeostasis, which requires the interdisciplinary efforts of basic and clinical scientists to better understand the cellular and molecular basis of neuroimmune interactions in the health and disease. The highlights of different immunotherapeutic approaches to treat neurological disorders with different etiologies and multiple immunopathological mechanisms are discussed in more detail in chapter "General Principles of Immunotherapy in Neurological Diseases".

Clinical Features of Neuroimmune Diseases

Lesion-Dependent Neurological Symptoms

The neuroimmune disease covers a wide variety of neurological disorders ranging from autoimmune demyelination, neuroinflammatory, neuroinfections, neoplastic, neurodegenerative, neuropsychiatric, and traumatic conditions. Depending on the specific areas affected in the nervous system, neuroimmune diseases exhibit an alteration in the nervous system homeostasis, loss of sensory and motor functions, neurodegeneration, and impaired cognitive and behavioral functions and social skills. Majority of neurological diseases follow a prodrome phase, ranging from few to several weeks, mainly characterized by symptoms such as fever, headache, malaise, anxiety, psychiatric changes, and mild infection. The typical symptoms of the neuroimmune diseases include painful nerve-joint and nerve-muscle connections, muscle weakness, paralysis and alterations in the locomotor functions, loss of coordinated movements, visual impairments, seizures, tremors, convulsions, depression, dementia, cognitive impairment, and social and behavioral changes.

Association of Other Neuroimmune Diseases

A given individual may have one type of neurological disorders or may develop other closely resembling neurological deficits. For example, a patient with optic neuritis, an inflammatory condition that affects the optic nerves may eventually develop demyelinating multiple sclerosis (MS) [12]. Similarly, some of the patients with the neuropsychiatric condition, anti-N-methyl-D-aspartate (NMDA) receptor encephalitis with no signs of demyelination subsequently develop MS and neuromyelitis optica spectrum disorder (NMOSD). The MS and NMOSD are characterized by the presence of autoantibodies such as anti-myelin oligodendrocyte glycoprotein (MOG) and anti-aquaporin 4 (AQP4) in the cerebrospinal fluid (CSF), respectively [13, 14].

Multifocal Lesions

A given neuroimmune disease may selectively restrict to the CNS, like MS pathology targets the brain and spinal cord, or the PNS, such as chronic inflammatory demyelinating polyneuropathy (CIDP) which targets antigens explicitly on peripheral nerves. Notably, some of the clinical cases of neuroimmune disorders such as Guillain-Barre Syndrome (GBS), Bickerstaff brainstem encephalitis, and Miller Fisher /Fisher Syndrome have been shown to involve both CNS and PNS [15]. However, whether inflammation in the CNS and PNS occurs concurrently or

sequentially is unclear. The involvements of distinct, shared, or secondary immune dysfunction mechanisms are also not clearly understood.

Divergent Immune Mechanisms

A given neuroimmune disorder may be a result of one type of immunological insult or may even involve more than one mechanism. Some of the neuroimmune diseases such as MS are multiphasic, constituting primary progressive MS (PP-MS), secondary progressive MS (SP-MS), and relapsing-remitting MS (RR-MS), where patients exhibit relapse (development of new focal lesion) and remission (resolution of active lesion) of neurological symptoms and some also show progressive relapsing MS (PR-MS) [16]. It has been broadly considered that approximately 80–85% of MS patients initially show RR-MS course, and about half of them subsequently develop irrevocable neurological symptoms without clinical relapse or development of new lesions in the CNS white matter and progress into SP-MS [16, 17]. The rest 15–20% of MS patients generally exhibit PP-MS course with progressive neurological deficits beginning with the onset of disease symptoms, and about 5% of MS patients who show clinical relapses during PP-MS course are referred as PR-MS, and this worsens the neurological deterioration [16, 17]. Such heterogeneous nature of immunopathogenesis may dictate the acute versus chronic disease progression and require different immunotherapeutic approaches. Although MS is a major demyelinating disease of the CNS, few other closely resembling diseases with atypical MRI findings and clinical course are often misdiagnosed as MS, which do not respond or instead deteriorate upon MS treatment are known as an atypical inflammatory demyelinating syndrome. This syndrome includes demyelinating conditions such as optic neuritis, neuromyelitis optica spectrum disorder (NMOSD), acute disseminated encephalomyelitis (ADEM), Balo's concentric sclerosis, Marburg's multiple sclerosis, and Schilder's diffuse myelinoclastic sclerosis [18, 19]. With some exceptions, individual neuroimmune diseases are rare. Nevertheless, collectively they pose a significant threat of morbidity, disability, and mortality as well as substantial clinical, social, and economic burden, and therefore, are increasingly recognized as of significant public health importance.

Fundamental Pathogenic Factors Determining Clinical Profiles

The neuroimmune diseases are acquired neurological and immunological disorders. The genetic and environmental factors and concurrent infections can influence the development and progression of neuroimmune disorders in a susceptible individual. Nonetheless, autism spectrum disorder (ASD), a neurodevelopmental and neuroinflammatory disorder characterized by impaired cognitive, learning, communication

and social skills, and shows a strong association with the congenital anomalies [20, 21]. However, definitive evidence suggesting the acquired versus inborn nature of childhood autism is still lacking.

Autoantibody- and Cell-Mediated Immune Mechanisms

The autoimmune response to the antigens belonging to the CNS, PNS, and ANS is the major contributing factor for the development of neuroimmune diseases. The involvement of both the autoantibodies and autoreactive T cells have been extensively studied in the various neurological disorders. The autoantibodies may be directed toward the cell surface antigens and cytoplasmic or nuclear antigens. The autoantibodies against the neuronal glutamate receptors and gamma-aminobutyric acid (GABA)-B receptor are associated with limbic encephalitis. Anti-NMDA receptor is linked with encephalitis, and anti-P/Q and N-type calcium channels with encephalopathies and neuropathies. Anti-leucine-rich glioma-inactivated-1 (LGI1), a voltage-gated potassium channel complex, is associated with limbic encephalitis. Anti-muscle and neuronal ganglionic acetylcholine receptors (AChR) are associated with myasthenia gravis and encephalopathies or neuropathies, respectively [22–25]. The antibody against astrocytic water channel, AQP4, is linked with optic neuritis, NMOSD, and ADEM. Immune response against myelin antigens such as MOG, myelin basic protein (MBP), and myelin-associated glycoprotein (MAG) induces MS, NMOSD, and transverse myelitis [26, 27].

Moreover, a strong association of human leukocyte antigen (HLA) polymorphism with disease susceptibility highlights the critical role of T cell-mediated immune responses in neuroimmune disorders. Both $CD4^+$ and $CD8^+$ effector T cells play an important role in the development and progression of MS, Rasmussen's encephalitis, a chronic pediatric neuroinflammatory condition with seizures, unihemispheric atrophy and unilateral locomotor dysfunction, and paraneoplastic syndromes [28, 29]. Additionally, T cell-mediated response also contributes to autoantibody-mediated neuroinflammatory disorders such as ADEM including optic neuritis and NMOSD, anti-NMDAR encephalitis, and stiff-person syndrome, a neurological disorder characterized by epilepsy, cerebellar ataxia, and limbic encephalitis [30].

Neuroinflammatory Cascades

The inflammatory response at the BBB of CNS microvessels is also known to associate with many neuroimmune conditions including CNS vasculitis [31]. The neuroinflammatory conditions are primarily thought to be caused by the autoreactive T cell and humoral response. The other infiltrated immune cells such as monocytes, macrophages, dendritic cells, and neutrophils, and CNS-resident microglia and

astrocytes are also known to induce an inflammatory response in the CNS. These cells recognize the DAMPs, myelin, and other neuronal antigens, secrete inflammatory mediators, and promote the inflammatory response and dictate the progression of clinical symptoms.

Additionally, some of the systemic chronic inflammatory diseases with variable manifestation of the neurological conditions such as systemic lupus erythematosus (SLE) are associated with aseptic meningitis, cerebral venous sinus thrombosis, transverse myelitis, migraine, psychosis, anxiety, seizures, dementia, cognitive dysfunction, neuropathies, and myasthenia gravis [32, 33]. Rheumatoid arthritis (RA) has also been shown to be associated with the cerebral vasculitis, peripheral neuropathies, brainstem and spinal cord compression, and stroke, whereas Sjogren's syndrome and scleroderma are associated with brain and spinal cord lesions, peripheral neuropathies, and trigeminal neuralgia, respectively [34, 35]. The exact pathophysiological mechanisms of these manifestations have not been understood completely. However, some of the studies have highlighted the role of systemic inflammation and altered BBB function allowing the influx of inflammatory molecules, effector innate and adaptive immune cells and the presence of autoantibodies in the CNS and PNS [36, 37].

Triggering of Autoimmunity: Parainfectious and Paraneoplastic Conditions

Infection in CNS Several viral, bacterial, fungal, and parasitic infections induce the CNS inflammation and promote immune-mediated inflammatory damage in the brain and spinal cord. Meningitis, an inflammatory condition affecting the meninges and subarachnoid space, is characterized by neck stiffness, diminished sensory functions, altered mental status, photophobia, and respiratory complications in young and adult individuals [38]. Depending on the nature of infectious cause, the condition is referred to as bacterial meningitis (*Streptococcus pneumoniae*, *Neisseria meningitidis*, *Listeria monocytogenes*, *Haemophilus influenzae*, and *Treponema pallidum*), viral meningitis (enteroviruses, herpes simplex virus-2 (HSV-2), varicella-zoster virus (VZV) and arboviruses), and fungal meningitis (*Cryptococcus neoformans* and *C. gattii*) [39–41]. The encephalitis is an inflammation of brain parenchyma, characterized by the BBB disruption and effector immune cell infiltration in the brain parenchyma. The infection-induced encephalitis possesses a significant threat associated with high morbidity and mortality if failed to diagnose and treat early. The HSV-1 and HSV-2 infection or reactivation represents the major cause and accounts for about 10–15% of cases of encephalitis [42]. The HSV and VZV remain latent in the basal root ganglia in the spinal cord, and their reactivation often leads to the development of myelitis, an inflammation of the spinal cord. Furthermore, the mosquito- and tick-borne viruses such as Japanese encephalitis virus (JEV), West Nile virus (WNV), and tick-borne encephalitis virus are consid-

ered as significant encephalitis-causing agents in a post-monsoon, spring, and autumn season in the endemic regions [43, 44]. The patients with bacterial meningitis often develop encephalitis symptoms, involving cranial neuropathies, hemiataxia, seizures, sensory loss, muscle weakness, and loss of consciousness.

Infection in PNS Several infections are known to cause the development of the inflammatory dysfunction of PNS. The hepatitis C virus (HCV)-induced inflammation is the cause of infectious polyneuropathies with demyelinating and axonal pathology [45]. The Zika virus, a mosquito-borne arbovirus, manifests acute demyelinating polyradiculoneuropathy characterized by ascending paralysis, sensory loss, and respiratory failure [46]. The reactivation of VZV in peripheral nerve ganglia leads to the development of radiculitis with the painful vesicular rashes [47]. The tick-borne *Borrelia burgdorferi*, which causes Lyme disease, is also known to involve in neuroinflammation with clinical meningitis and radiculoneuritis [48].

HIV The human immunodeficiency virus 1 (HIV-1) itself causes the neurological complications in the infected individuals and also promotes opportunistic neurological infections. It is widely considered that HIV-1 uses Trojan horse mechanism to cross the BBB and infiltrates into the CNS through infected monocytes/macrophages, where it further acquires the ability to infect microglia and astrocytes [49, 50]. The HIV-1-associated CNS disorders involve a range of neurologic conditions such as meningoencephalitis, peripheral neuropathy, cranial neuropathy, dementia, and neurocognitive disorders and represent early manifestations of approximately 10–20% of symptomatic patients, while it goes more than 50% in patients with advanced cases [51]. Furthermore, HIV-1-associated immunodeficiency promotes several opportunistic infections having ability to cause neurological diseases such as progressive multifocal leukoencephalopathy (PML; John Cunningham (JC) virus, targets oligodendrocytes), CMV encephalitis, retinitis, and polyradiculitis (cytomegalovirus (CMV), targets almost all the components of CNS and PNS), cerebral toxoplasmosis (*Toxoplasma gondii*, targets CNS), cryptococcal meningitis (*C. neoformans*, targets meninges and subarachnoid space), and tuberculous meningitis (*Mycobacterium tuberculosis*, affects meninges) [52, 53].

Autoimmunity triggered by infections Although the exact trigger of autoimmune reactions to CNS antigens is not known, increasing evidence supports the hypothesis that infectious agents may provide an initial trigger to generate CNS autoimmune response. The data regarding the role of bacterial pathogens as a trigger for CNS autoimmunity is controversial. However, two murine viruses, Theiler's murine encephalomyelitis virus (TMEV) and mouse hepatitis virus (MHV), are capable of inducing demyelination, which resembles to MS [54]. Various pathogen-derived molecules show molecular mimicry to the myelin and other CNS antigens. The L2 protein of human papillomavirus 7, DNA polymerase of EBV and HSV, and hemagglutinin of influenza virus mimic human $MBP_{85–99}$ epitope [55], while DNA

polymerase of HBV mimics human $MBP_{66–75}$ [56]. Interestingly, human CMV capsid protein UL86 981–1003 mimics the rat $MOG_{35–55}$, and a drug transporter, ABC-transporter of *Clostridium perfringens*. Mimic $AQP4_{61–80}$, and there are the two immunodominant epitopes that initiate autoimmune demyelination in MS and neuromyelitis optica, respectively [57, 58]. Similarly, GBS patients show the presence of autoantibodies against neuronal components, and are produced due to the molecular mimicry of various bacterial (*Campylobactor jejuni*, *Mycoplasma pneumoniae*, and *Haemophilus influenzae*) and viral (CMV, HSV-1, HSV-2, VZV, and EBV) pathogens [59]. Other than the usual molecular mimicry, the superantigens and cryptic antigens, apoptotic and necrotic host cell death, and infection-induced bystander immune activation and collateral damage have also been shown to play a critical role in the initiation of the CNS autoimmune inflammation. These fundamental pathogenic factors in infection-related autoimmunity, molecular mimicry, epitope spreading, and bystander activation are shown in Fig. 1.

Autoimmunity triggered by neoplasms The paraneoplastic neurological disorders are rare neurological condition where tumor cells express onconeural antigens that closely resemble the neuronal antigens. The immune response directed against such onconeural antigens contributes to the neuronal tissue damage leading to the development of neuroimmune conditions. These tumors may be systemic or intrathecal, and primarily characterized by the autoantibody production against the neuronal antigens, and may also involve onconeural-specific $CD4^+$ and $CD8^+$ T cell responses [60, 61]. Depending upon the autoantibody produced against the various components of the neural cells, the disease manifests to CNS, PNS, or autonomous nervous system and exhibits cortical/limbic/brainstem encephalitis, cerebral ataxia, encephalopathies, peripheral neuropathies, myelitis, cerebellar degeneration, dermatomyositis, and chronic gastrointestinal pseudo-obstruction. The autoantibodies, anti-neuronal nuclear antibody type 1 (ANNA-1) and ANNA-2 are directed against the neuronal intracellular antigens Hu and Ri, respectively, and known to be associated with small-cell lung carcinoma (SCLC), neuroblastoma, thymoma, lung carcinoma, and breast carcinoma [62, 63]. The SCLC and thymoma are also associated with the presence of anti-AChR (muscle, ganglionic), anti-voltage-gated potassium and calcium channel proteins, and Purkinje cell cytoplasmic antibody 2 (PCA-2) in the CSF. There are increasing cases of ovarian and lung cancer linked with anti-NMDA receptor and anti-glycine receptor antibodies, respectively [64]. The patients with paraneoplastic CNS disorders often show CSF abnormalities such as pleocytosis, increased protein levels, and oligoclonal bands. The other characterized onconeural autoantibodies involved in the pathology of paraneoplastic neurological disorders include anti-Yo (ovarian and breast cancer), anti-CV2 (SCLC, thymoma), anti-Ma2 (SCLC and testis cancer), anti-amphiphysin (SCLC and breast cancer), anti-glial nuclear antibody 1 (SCLC), anti-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor (SCLC, ovarian and testes cancer), and anti-LGI1 (SCLC) and anti-$GABA_B$ receptor (SCLC) [65–67].

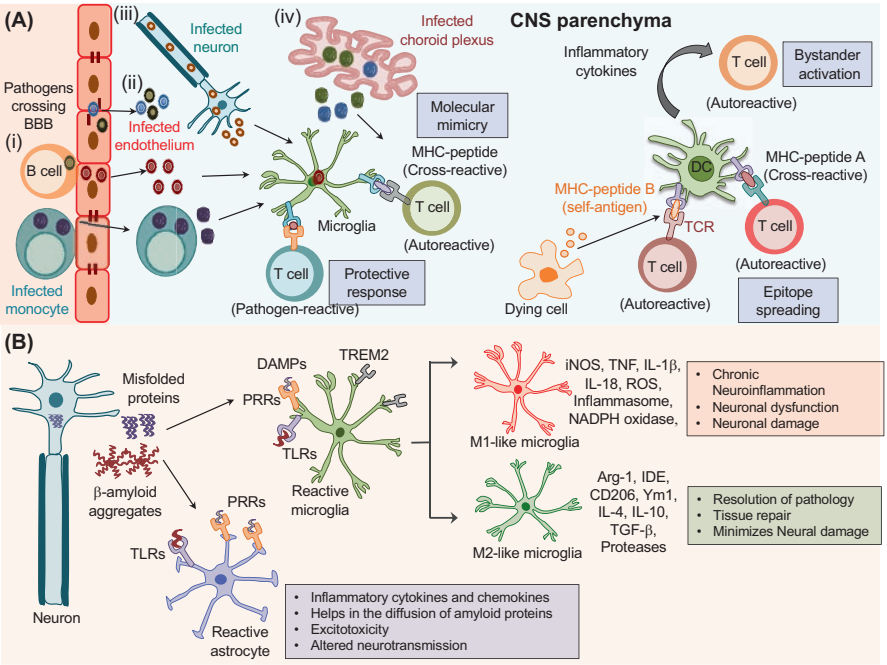

Fig. 1 Mechanisms of neuroimmune diseases associated with neurotrophic infections and neurodegenerative processes. (*A*) Several neurotropic infectious agents enter into the CNS and induce autoimmune response leading to neuronal tissue damage. The infectious agent uses one or more of the four main entry ports to the CNS. (i) *Trojan horse mechanism*. Infected leukocytes directly cross the BBB and provide the gateway to the CNS parenchyma. The EBV-infected B cells, and HIV-1- or *L. monocytogenes*-infected monocytes are classic examples of Trojan horse mechanism. (ii) *Infection to the BBB endothelium*. Several pathogens such as JEV, EBV, JCV, HCMV, and HTLV-1 infect the brain endothelium, whereas some directly cross endothelium via paracellular (WNV, *H. influenzae*, and *N. meningitidis*) or transcellular (*N. meningitidis*, *S. pneumoniae*, *L. monocytogenes*, and *M. tuberculosis*) routes without infecting endothelium. In both cases, pathogens are released into the CSF-draining subarachnoid spaces in the CNS. (iii) *Axonal transport*. Various pathogens infect peripheral sensory or motor nerves and use either anterograde (HSV, VZV, and chikungunya virus) or retrograde (WNV, rabies virus, poliovirus, and *L. monocytogenes*) axonal transport mechanism to enter into the CNS. (iv) *Dissemination through choroid plexus*. Pathogens such as HIV-1, HTLV-1, *H. influenzae*, and *N. meningitidis* enter to the CNS parenchyma via infecting the choroid plexus epithelium and disseminating directly into the CSF through choroid plexus ependymal cells. (*B*) The microglial cells sense the pathogen via different pattern recognition receptors (PRRs) and toll-like receptors (TLRs), process and present pathogen-derived antigens to the sentinel T cells and induce a protective immune response. However, some of these antigens mimic the neuronal antigens and activate neuronal antigen-reactive effector T cells and promote molecular mimicry-induced autoimmunity. The CNS recruited professional antigen-presenting cells, such as dendritic cells, further present antigens to the T cells; these antigens are either cross-reactive pathogen-derived ones or may be obtained from CNS-resident cells (e.g., dying oligodendrocyte or neuron). This leads to the activation of autoreactive effector T cells specifically recognizing broad arrays of CNS autoantigens, a mechanism known as epitope spreading. Additionally, the inflammatory mediators and cytokines produced by antigen-presenting cells may activate a repertoire of T cells, which does not possess TCR specific to CNS antigens; instead, these bystander-activated cells fuel the neuroinflammation and promote tissue damage

Cross Talk Between the Immune System and Degenerative or Psychiatric Diseases

Several studies have suggested a strong correlation between neuroinflammation and neurodegenerative diseases. Some of these diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD) and frontotemporal lobar dementia (FTLD) are associated with reactive phenotype of microglia and astrocytes along with the presence of inflammatory mediators in the CNS [68]. However, substantial evidence suggesting the primary involvement of neuroinflammation in the neurodegenerative processes is still lacking. The signs of neuroinflammation are mainly attributed to the secondary damage to the neurons. It has been recognized that expression of mutant superoxide dismutase 1 (SOD1) in the neurons is associated with the development of ALS [69], whereas mutant α-synuclein in astrocyte is linked with the PD [70]. The mutation of triggering receptor expressed on myeloid cells 2 (TREM2) gene in microglia is associated with AD, PD, and a rare Nasu-Hakola disease [71] and suggests that alterations in the glial cell homeostasis may contribute to the development of neurodegeneration. Nonetheless, further clinical studies and basic mechanistic studies using genetic-deficient mouse models are required to understand the precise role of peripheral and CNS inflammation in the development and progression of neurodegenerative diseases.

Whether inflammation induces neuropsychiatric conditions such as mood and sleep disorders, depression, and schizophrenia is not well understood. However, the compelling data suggest the bi-directional association of sterile inflammation with various neuropsychiatric disorders [72]. The chronic psychological stress is linked with physiological imbalance with an increased level of DAMPs, such as heat shock proteins, extracellular ATP, oxidative radicles, circulating uric acid, and high mobility group box 1. Under chronic psychological stress, immune cells show the increased expression of surface Toll-like receptors (TLRs), intracellular NOD-like receptor 3 (NLRP3), and inflammasome activation with the concomitant increased secretion of tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, IL-6, and IL-10 [73, 74]. The patients with bipolar disorder, depressive disorders, and schizophrenia also show the involvement of CNS inflammation and microglia activation with increased levels of pro-inflammatory cytokines, cytokine receptors, C-reactive protein, chemokines, and soluble cell adhesion molecules in the CSF and frontal cortex. Additionally, various clinical studies have suggested that early-life stress (childhood maltreatment) increases the risk of developing neuropsychiatric disorders in adult life. For example, children with maltreatment are more likely to develop a mood disorder, depressive disorder, bipolar disorder, anxiety, and substance abuse [75–77]. The autoimmune diseases such as SLE and RA are also known to manifest wide varieties of neuropsychological symptoms of the CNS and PNS including mood and anxiety disorders, cognitive dysfunction, seizures, myelopathy, autonomic and cranial neuropathy [78]. Overall various inflammatory mediators

and pathways, effector innate and adaptive immune cells and CNS-resident cells, reactive astrocytes, and microglia orchestrate the pathology of a given neuroimmune condition (see Fig. 1 for the basic mechanisms of neuroimmune diseases associated with neurodegenerative processes).

Mechanisms of Neuroimmune Diseases

Neuroinflammation is induced by the mounting of the immune response to neuronal antigens, alterations in the tissue homeostasis as in the case of neurodegenerative diseases, pathogen-induced immune response, and the oxidative stress and tissue damage during the traumatic conditions. The complex interaction of innate and adaptive immune cells, vascular inflammation, and CNS-resident astrocytes and microglia orchestrate the pathology of neuroimmune diseases.

Mechanisms of T Cell-Mediated Neuroinflammation and Autoimmunity

The cell-mediated autoimmune response is a hallmark of the majority of neuronal autoimmunity. The variations in the several genes associated with the adaptive immunity are linked with susceptibility to develop several CNS autoimmune diseases. Both $CD4^+$ and $CD8^+$ T lymphocytes trigger the initiation and propagation of white and gray matter pathology and, in some cases, also promote the autoantibody response against neuronal antigens.

Breakdown of immune tolerance The negative selection mechanism of thymus eliminates the majority of the autoreactive T lymphocytes. However, some low-affinity self-reactive T cells escape from the thymic central tolerance and mobilize to the peripheral lymphoid organs. The activation of these autoreactive T cells in response to the CNS insult is thought to induce neuronal T cell response. Since genetics and environmental factors also regulate the development of the neuronal autoimmunity, the molecular similarities between the antigens of the pathogenic microorganisms and the neuronal antigens may serve as an initial trigger to activate autoreactive $CD4^+$ and $CD8^+$ T cells [79]. Epstein-Barr virus (EBV) infection or its reactivation is strongly associated with the risk of MS. It has been speculated that EBV shares homology with some of the CNS antigens [80]. This hypothesis also supports that initial priming and the activation of the autoreactive T cells occur outside of the CNS and local reactivation of these cells in the CNS augments the autoreactive T cell response during MS and EAE [81]. This highlights the role of immune tolerance breakdown in the pathogenesis of autoimmune demyelination, and the mechanisms that affect the function of regulatory $CD4^+$ T cells and the

peripheral tolerance in CNS disorders are discussed in more detail in chapter "Immune Tolerance in Autoimmune Central Nervous System Disorders".

Disruption of BBB/BNB The germ-free animal studies have revealed that functional gut microbiome is required for the development and maintenance of BBB integrity [82]. The loss of BBB integrity is a crucial event that occurs at early stages of several CNS autoimmune diseases such as MS, optic neuritis, NMOSD, and EAE [4]. The consequences of BBB breakdown and pathological basis of cell-mediated responses and humoral responses during CNS autoimmunity and paraneoplastic CNS diseases are shown in Fig. 2 and Fig. 3. In the MS, bone marrow-derived circulating monocytes breach the BBB and enter into the CNS parenchyma and serve as a source of mononuclear phagocytes in the inflamed CNS [83]. The cellular and molecular mechanisms of BBB dysfunction during neuroinflammation and autoimmunity are discussed in more detail in chapter "Disruption of the Blood-Brain Barrier During Neuroinflammatory and Neuroinfectious Diseases".

Amplification by the microglial cells and astrocytes The phagocytes and microglial cells are abundantly localized to the CNS lesions of the RR-MS, PP-MS, and SP-MS patients and induce the myelin and axonal damage possibly via secretion of oxidative and inflammatory molecules [84]. In EAE, an animal model of MS, the activation of microglial cells is often associated with the disruption of the BBB, subsequent infiltration of effector immune cells, and induction of several of inflammatory pathways, leading to demyelination and neuronal damage [4, 85]. It has been particularly noted that glia limitans, a basement membrane formed by astrocytic end-feet, also serves as a barrier at the face of the damaged BBB [85]. The activated astrocytes and microglia or macrophage-derived matrix metalloproteinase-2 (MMP2) and MMP9 help transit of T cells through this barrier. Astrocytes are the most abundant glial cell type in the CNS and contribute to the various inflammatory and regulatory functions. There are several immunopathological mechanisms that target astrocyte and promote neuroinflammation. The effector and regulatory roles of astrocytes during various T cell-mediated and antibody-dependent CNS autoimmune diseases are discussed in more detail in chapter "Autoimmune Astrocytopathy".

Role of the meningeal tertiary lymphoid structures In addition to the role of the peripheral inflammation in the activation and effector function of autoreactive T cells, the meningeal inflammation and contribution from the inflammatory meningeal stromal cells and the tertiary lymphoid structures have been shown to play a crucial role in the induction of CNS autoimmunity [86]. The meningeal tertiary lymphoid structures represent the local sites of the immune activation and facilitate the cell-to-cell interactions and reactivate the autoreactive T cells and B cells during EAE and MS [87, 88]. Interestingly, IFN-γ through STAT1 signaling promotes the BBB disruption and allows the directional migration of the $CD4^+$ T cells from the luminal to the abluminal side of the brain endothelium [89]. Additionally, several important TNF receptor superfamily molecules have shown to increase the BBB permeability and promote neuroinflammation in CNS autoimmunity [90].

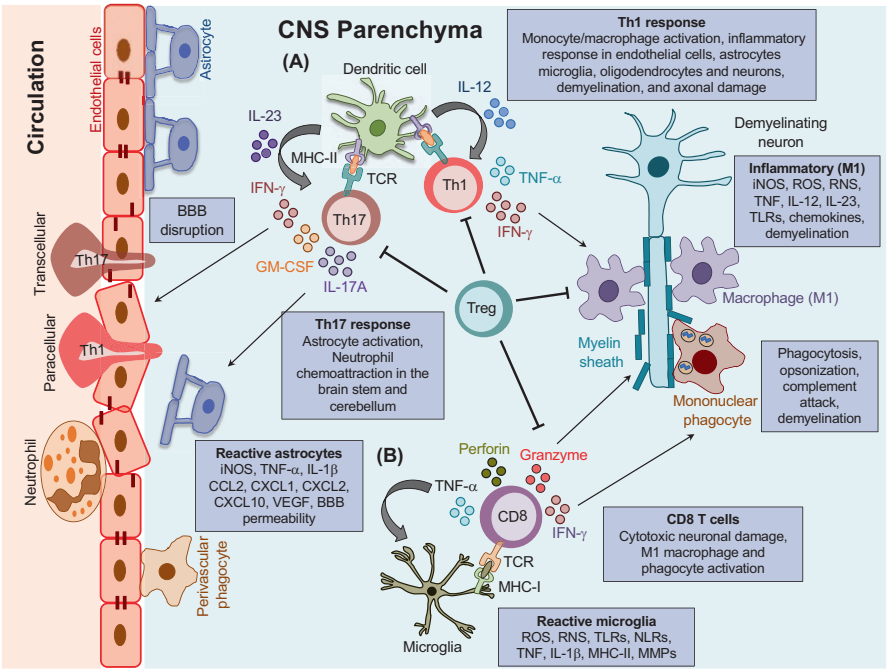

Fig. 2 Consequences of the blood-brain barrier breakdown – T cell-mediated immune response. The BBB breakdown is one of the critical events that lead to the development of autoimmune demyelination during MS and EAE. Myelin-reactive effector CD4⁺ T cells and inflammatory cytokines produced by these cells are key drivers in damaging the BBB. The altered BBB allows the entry of myelin-reactive effector CD4⁺ T cells (Th1 and Th17) through a series of rolling, adherence, crawling, capture, and diapedesis steps. The transmigration of Th1 and Th17 cells occurs through either paracellular (through endothelial cell-cell junctions) or transcellular route (through endothelial cell body). In the inflamed CNS parenchyma, antigen-presenting cells such as dendritic cells, perivascular macrophages, and microglia present myelin or CNS antigens to the effector CD4⁺ and CD8⁺ T cells and reactivate them. (*A*) Under the influence of IL-12 or IFN-γ, CD4⁺ T cells acquire Th1 phenotype (T-bet, IFN-γ and TNF-α). Alternatively, IL-23 stabilizes the Th17 phenotype (RORγt, IL-17A, IL-17F, IL-21, and IL-22). The IL-23 stimulation of myelin-reactive Th17 cells induces a Th1-like signature (T-bet, IFN-γ, and GM-CSF) in Th17 cells, which are highly encephalitogenic. The Th1 cytokines also influence the activation of monocytes, mononuclear phagocytes, M1-like macrophages, and M1-like microglia. The M1-like inflammatory cell types and other phagocytes induce neuronal and oligodendrocyte damage via phagocytosis, opsonization, and complement activation. Contrarily, Th17 response induces neutrophil infiltration predominantly in the brainstem and cerebellum. Neutrophils secrete various pro-inflammatory cytokines and cytotoxic molecules that drive demyelination and neuronal damage. Both Th1 and Th17 inflammatory cytokines involve directly or indirectly (via reactive astrocytes) in the process of BBB breakdown, which drives the infiltration of various lymphocytes and myeloid cells in the inflamed CNS. (*B*) CD8⁺ T cells reactivated in the inflamed CNS produce inflammatory cytokines, perforin, and granzyme that directly cause neuronal and oligodendrocyte damage and also activate mononuclear phagocytes and M1-like macrophages and microglial cells. The scavenger microglia and mononuclear phagocytes engulf the myelin and neuronal debris and present the CNS antigens to the cytotoxic CD8⁺ T cells. The regulatory CD4⁺ T cells (Tregs) suppress the effector CD4⁺ and CD8⁺ T cell activation and also inhibit inflammatory macrophages and microglial response, and the delicate balance between effector and regulatory cell types dictates the course of neuroinflammation. Most often, the inflammatory molecules tune the plasticity of these regulatory cells and promote them into effector lineage

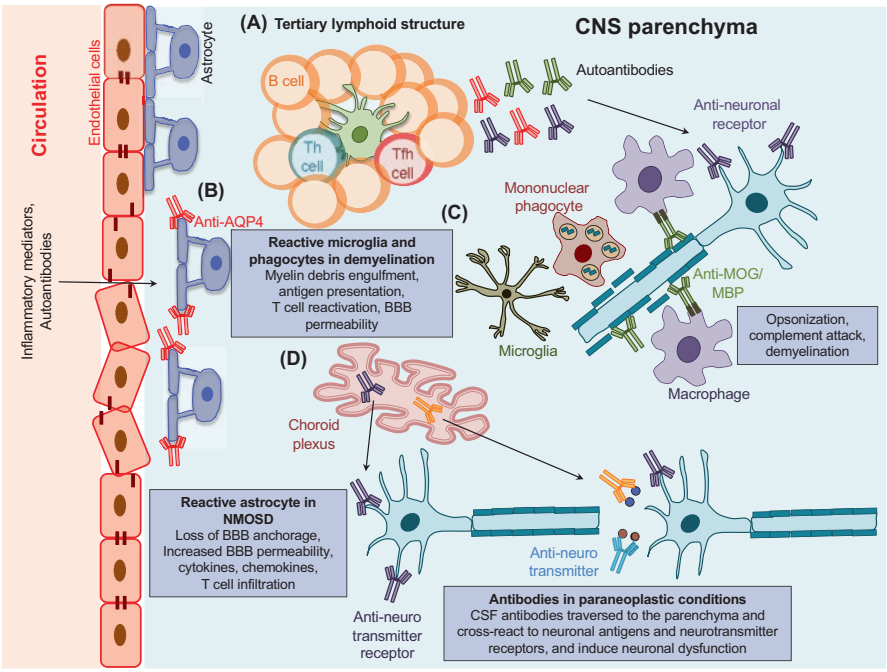

Fig. 3 Consequences of the blood-brain barrier breakdown – the humoral immune response. Disrupted BBB allows the migration of inflammatory mediators, cross-reactive antibodies, autoantibodies, and immune cells in the CNS. (*A*) The CNS-infiltrated B cells form a cellular niche along with T helper (Th) and T follicular helper (Tfh) cells known as tertiary lymphoid structures. Meningeal tertiary lymphoid structures are common in MS, EAE, optic neuritis, and NMOSD. These local lymphoid structures support the autoantibody production that recognizes the myelin, neuronal, and astrocytic antigens. (*B*) The anti-AQP4 antibody recognizes the water channel protein in the astrocytic end-feet that establishes the physical contact with the brain endothelium. Anti-AQP4 disrupts the astrocyte-endothelium interaction, affects the astrocyte polarity and integrity of BBB, and also induces complement-dependent astrocyte damage leading to cellular infiltration and demyelination during optic neuritis, NMOSD, and MS. (*C*) Autoantibodies directed toward myelin antigens (anti-MOG/MBP/MAG) recognize the myelinated neurons and induce opsonization and complement activation-induced myelin sheath damage. Anti-neuronal receptor antibodies and activated M1-like macrophages, reactive microglial cells, and mononuclear phagocytes actively contribute to the inflammatory damage to myelin sheath and demyelination process during several autoimmune inflammatory demyelinating diseases such as MS, ADEM, optic neuritis, transverse myelitis, and NMOSD. (*D*) During paraneoplastic conditions, malignant cells induce the production of antibodies that cross-react to the neuronal antigens. The circulating antibodies may traverse to the CSF-draining spaces via choroid plexus, where they recognize the neurotransmitter receptors, molecules involved in synaptic transmission and vesicular transport. These autoantibodies affect the synaptic transmission and induce neuronal dysfunction, excitotoxicity and hyperexcitability and neuronal depolarization

Roles of Th1 and Th17 cells CD4$^+$ T cells recognize CNS antigens presented by the antigen-presenting cells along with appropriate activation and co-stimulatory signals drive the differentiation of various effector and regulatory CD4$^+$ T cell subsets, and these differentiated cells produce several pro-inflammatory and anti-inflammatory cytokines (Fig. 4). The myelin antigen-reactive CD4$^+$ T cells and IFN-γ-expressing Th1 and IL-17A-expressing Th17 cells are capable of inducing CNS autoimmune demyelination in susceptible animal models [81, 91]. Similarly, Th1 and Th17 cells and their associated pro-inflammatory cytokines are abundantly present in the CSF and at the active CNS lesions of MS patients. Depending on the inflammatory mediators present in the inflamed microenvironment, autoreactive T cells are terminally differentiated cells that can acquire distinct and overlapping immune cell phenotypes [92]. The IFN-γ$^+$IL-17A$^+$ or IFN-γ$^+$IL-17A$^+$GM-CSF$^+$ Th17 cells that co-express lineage-defining transcription factors, T-box transcription factor (*TBX21* or T-bet), and retinoic acid-related orphan receptor gamma t (RORγt) are considered as highly pathogenic in MS [93–95]. Further, exTh17-Th1 cells, which express IFN-γ and granulocyte-macrophage colony-stimulating factor (GM-CSF) and T-bet and derived from the previously IL-17A-expressing Th17 cells, are also shown to have an encephalitogenic function in mouse models of EAE [96, 97]. The IL-17A-expressing γδ T cell counterparts of the Th17 cells also share most of the pathogenic signature and have been reported to play an important role in the pathophysiology of the neuroinflammation [98]. Regardless of their cell phenotypes, autoreactive Th1 and Th17 cells have been shown to induce distinctive CNS pathology during EAE.

Th1-dominated diseases are mainly characterized by the infiltration of the monocytes and other mononuclear cells in the spinal cord and proceeds with typical ascending flaccid paralysis, as seen in MS patients. However, Th17-dominated settings show neutrophil infiltration into the brain and induce atypical EAE symptoms such as ataxia, axial rotation, and involvement of cerebellum [99, 100]. The CCR6 and CXCR3 are important chemokine receptors that drive the migration of the Th17 and Th1 cells in the CNS parenchyma via different vascular routes such as choroid plexus and BBB, respectively [101]. Interestingly, CCR6-dependent migration through the CCL20-expressing choroid plexus is considered as a first wave of the Th17 migration. IL-23-induced signaling changes the preference from CCR6 to CCR2 usage for Th17 migration into the CNS [102]. The importance of Th17 cells in the immunopathogenesis of neuroinflammatory disorders is discussed in more detail in chapter "The Role of Th17 Cells in Immunopathogenesis of Neuroinflammatory Disorders").

Significant progress has been made in identifying and characterizing the role of effector and a regulatory subset of the CD4$^+$ T cells in the CNS inflammation and autoimmunity (Table 1). The effector cytokines IL-17A, GM-CSF, TNF-α, and IFN-γ along with other inflammatory mediators orchestrate the neuroinflammation through activation of monocytes, macrophages, neutrophils, astrocytes, and microg-

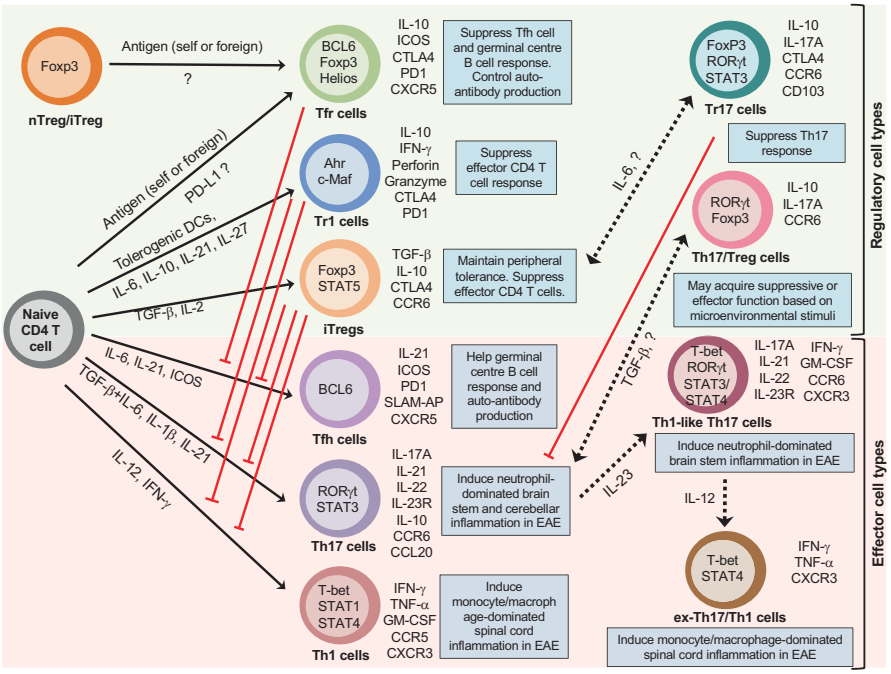

Fig. 4 Differentiation of autoreactive CD4⁺ T helper cells during CNS autoimmunity. Activation of autoreactive naive CD4⁺ T cells occurs in the secondary lymphoid organs during CNS autoimmune conditions. The environmental factors and genetic susceptibility play a key role in dictating the activation of neuronal antigen-specific T cells. The molecular mimicry of pathogenic antigens, bystander immune activation, cross-presentation, and altered CNS homeostasis are thought to provide the initial trigger for autoreactive T cell activation. The differentiated CD4⁺ T cells belong to two main functional categories, effector and regulatory subsets. The main effector cells include Th1, Th17, and Tfh cells. Th1 cells are polarized under the influence of IL-12 or IFN-γ signaling and induce classic EAE symptoms and predominant spinal cord inflammation, whereas TGF-β, IL-6, IL-1β, and IL-21 signaling promotes Th17 differentiation. Th17 cells induce neutrophil chemoattraction and atypical EAE symptoms. Tfh cells need TGF-β, IL-21 and ICOS signaling, and help in the production of autoantibodies. Th17 cells exhibit plasticity and undergo a transition to Th1-like Th17 cells and ex-Th17 Th1 cells under the influence of active IL-23 and IL-12 signaling. These cell types have highest encephalitic activity and are potent inducers of CNS autoimmunity. Contrarily, the regulatory cell types regulate the activation and differentiation process of autoreactive effector T cells and help in controlling the inflammation and tissue damage during CNS autoimmunity. TGF-β and IL-2 signaling promotes the Foxp3⁺-induced Tregs (iTregs) differentiation, which maintains peripheral tolerance, whereas tolerogenic dendritic cell and immunoregulatory cytokines induce T regulatory 1 (Tr1) cells, characterized by Foxp3 and c-Maf expression and production of immunoregulatory cytokines and cytotoxic molecules. The polarizing signals for T follicular regulatory (Tfr) cells are poorly characterized. Tfr possesses the functional characteristics of Tregs and Tfh and home to germinal center, where they actively regulate the Tfh response and autoantibody synthesis. The regulatory cells also exhibit phenotypic and functional plasticity and acquire suppressor or effector functions depending on the microenvironmental cues. Sustained IL-6 signaling induces the conversion of iTregs to Tregs/Th17 transition states or recently identified Tr17 cells. The fine balance between the effector and regulatory functions of primary differentiated CD4⁺ T cells and various transition states and secondary differentiated phenotypes may dictate the course and progression of CNS autoimmune disease

Table 1 The phenotype of various CD4+ T cell subsets and their role in CNS inflammation and autoimmunity

CD4+ T cell subset	Lineage-specific transcription factors	Effector molecules	Homing receptors	Functions	References
Th1	T-bet	IFN-γ, TNF-α,	CXCR3, CCR5	Immunity to intracellular pathogen, antitumor response, and CNS autoimmunity. Induce monocyte/macrophage infiltration in the spinal cord during EAE. Induce M1-like microglia activation in AD and PD mouse models and contribute to neurodegeneration	[100, 228]
Th2	GATA3	IL-4, IL-5, IL-13	CCR3, CCR4, CRTh2	Fight extracellular pathogens and parasitic infections, mostly associated with the recovery of neuroinflammation and autoimmunity. Induce M2-like microglial activation and dampen inflammation in AD and PD. Muscular AChR-specific Th2 cells contribute to myasthenia gravis pathology	[143, 181]
Th17 (less pathogenic)	RORγt	IL-17A, IL-21, IL-22, IL-10, CCL20 GM-CSF	CCR6	Defense against extracellular pathogens and fungal infections, maintain mucosal homeostasis. Do not induce EAE in the adoptive transfer models	[229, 230]
Th17 (highly pathogenic)	RORγt, T-bet	IL-17A, IL-21, IL-22, IFN-γ, GM-CSF	CCR6, CCR2	Associated with autoimmune demyelination during MS, EAE, optic neuritis, and NMOSD. Induce neutrophilic infiltration in the brain during EAE	[100, 102, 141, 142]
Natural Tregs (nTregs)	Foxp3, Helios	TGF-β, IL-10, CTLA-4, PD-1	?	Maintain self-tolerance and regulate the CNS inflammation and autoimmunity (most stable). However, studies show that Treg number and function are impaired in MS and EAE	[115, 231–233]

(continued)

Table 1 (continued)

$CD4^+$ T cell subset	Lineage-specific transcription factors	Effector molecules	Homing receptors	Functions	References
Induced Tregs (iTregs)	Foxp3	TGF-β, IL-10, CTLA-4	CCR6	Maintain self-tolerance and regulate the CNS inflammation and autoimmunity (less stable). However, studies show that Treg number and function are impaired in MS and EAE	[115, 231, 233, 234]
Th9	PU.1	IL-9, IL-10, IL-21	CCR3, CXCR3	Contribute to anti-helminth response, involved in the pathogenesis of asthma. Some reports show the pathogenic role of Th9 in MS and EAE. IL-9 stimulation induces inflammatory changes in astrocytes, microglia, and oligodendrocytes	[91, 235]
T follicular helper (Tfh)	BCL6	IL-21, BTLA, PD-1, ICOS	CXCR5	Help germinal center B cells to produce antibodies, affinity maturation, and class-switching of antibodies in periphery and the tertiary lymphoid structures in the inflamed CNS. Evidence suggest its pathogenic role during Th17-induced EAE	[236, 237]
T follicular regulatory (Tfr)	BCL6, Foxp3 Helios,	IL-10, CD28, ICOS, PD-1, CTLA4	CXCR5	Control the excessive antibody production, germinal center B cell and plasma cell response. Suppress Tfh cell proliferation. The imbalance of Tfh/Tfr function may contribute to the pathology of MS and SLE	[238, 239]
T regulatory 1 (Tr1)	Foxp3, cMaf, Ahr	TGF-β, IL-10, IL-21, perforin, granzyme, CTLA-4, PD-1	?	Dampen the tissue inflammation. Some evidence points to the regulatory role in CNS autoimmune diseases. Most of the MS patients shows defect in Tr1 cell number and function	[240–242]

lia, and contribute to the demyelination and axonal damage. The Th1 response through IFN-γ activates microglia and CNS-infiltrating macrophages and dendritic cells and also induces oligodendrocyte apoptosis. Further details of effector $CD4^+$ T cells in neurological autoimmunity are discussed in detail in chapter "Roles of Effector T Cells in Neurological Autoimmunity".

Roles of Tregs The regulatory $CD4^+$ T cells (Tregs) are marked by expression of transcription factor FoxP3 which plays an important role in maintaining the peripheral tolerance and prevents the activation of autoreactive T cells in the peripheral lymphoid tissues [103–105]. The environmental factors such as coincidental infection and inflammation are known to affect the Foxp3 expression and function of Tregs and influence the neuroinflammation [106–108]. For example, pertussis toxin help in the induction of clinical autoimmune demyelination in mice which are exposed to neuronal antigens by reducing the frequency and suppressive function of Tregs [109, 110] and promote the differentiation of Th17 cells [111]. The Tregs along with autoreactive Th17 cells are also known to enter the inflamed CNS through choroid plexus using chemokine receptor CCR6 [112]. Despite reducing the effector response of the autoreactive T cells during remission of the clinical symptoms [113], Tregs failed to control the proliferation of the autoreactive T cells during relapse leading to chronic inflammation in the CNS [114, 115]. These findings suggest that inflammatory microenvironment dominates over the suppressive Tregs and may transdifferentiate the Tregs into effector $CD4^+$ T cells during neuroinflammation and autoimmunity. The role of Tregs and potential mechanisms subverting Treg function in the context of the CNS inflammatory and autoimmune diseases are discussed in chapter "The Roles of Regulatory T Cells in Central Nervous System Autoimmunity".

Class I MHC locus and $CD8^+$ T cells The genetic polymorphism at class I MHC locus is associated with MS. It has been reported that at the CNS lesions, CSF, and peripheral blood, the frequency of $CD8^+$ T cells is significantly higher than $CD4^+$ T cells in the RR-MS patients [116]. Therefore, $CD8^+$ T cells are also considered as a key player in autoimmune demyelination. A significant number of $CD8^+$ T cells in the CSF of MS patients show effector memory phenotype. The cytotoxic granzyme B-expressing $CD8^+$ T cells are often localized to the demyelinating plaques in the MS and Rasmussen's encephalitis patients [117, 118]. Furthermore, the number of cytotoxic $CD8^+$ T cells correlates with the severity of axonal damage in MS and unihemispheric atrophy during Rasmussen's encephalitis [118].

The $CD8^+$ T cells are class I MHC restricted, and almost all of the CNS-resident cell types upregulate the surface expression of class I MHC molecules in MS and EAE suggesting the scope for activation of neuronal antigen-specific $CD8^+$ T cells. IL-17A-expressing $CD8^+$ T cells, astrocytes, and oligodendrocyte have been reported in the CSF and CNS lesion of the MS patients [119]. The IL-17-expression in the $CD8^+$ T cells are regulated via T-bet and eomesodermin-mediated transcrip-

tional programming and mostly restricted to $CD161^{+}CD8^{+}$ T cell subset [120]. The $IL\text{-}17^{+}CD8^{+}$ T cells are also shown to augment the clinical severity of the EAE by promoting Th17 response [121]. These studies suggest that autoreactive T cells are heterogeneous and employ diverse immunological mechanisms, and together with myeloid cells in the CNS contribute to the initiation and progression of neuronal inflammation and autoimmunity.

Roles of dendritic cells Dendritic cells (DCs) play a critical role in the priming, activation, and differentiation of the autoreactive T cells in the peripheral lymphoid organs as well as T cell reactivation in the inflamed CNS. Under steady state, the subsets of DCs such as $Clec9a^{+}CD8\alpha^{+}$ and $CD11c^{+}MHC\text{-}II^{hi}CD11b^{+}CD103^{+}$ conventional DCs (cDCs) are also present at the vascular barrier of the choroid plexus and meningeal vessels [122, 123]. During neuroinflammation, different subsets of DCs such as plasmacytoid DCs (pDCs), $CD11b^{+}SIRP\alpha^{+}$ cDCs, monocyte-derived DCs (mo-DCs), and $CD11b^{+}CD103^{+}$ DCs infiltrate into the CNS [123].

The DCs perform a protective role during CNS infections, whereas they can also process and present neuronal antigens to the autoreactive T cells and fuel the CNS inflammation and tissue damage. The CNS-infiltrated DCs, particularly cDCs, are potent activators of the myelin-reactive Th1 and Th17 cells in mouse models of myelin antigen or Theiler's virus-induced encephalomyelitis [124, 125]. The $CD8\alpha^{+}$ cDCs promote the activation of cytotoxic $CD8^{+}$ T cells and Th1 cells via IL-12- and IFN-γ-dependent mechanisms. These DCs also exhibit antigen cross-presentation to $CD8^{+}$ T cells and help in epitope spreading [126]. However, a study using DC depletion approach has also shown a protective role of cDCs via promoting the generation of $PD1^{+}$ Tregs in autoimmune inflammation during EAE [127]. The mo-DCs are also capable of presenting myelin antigens to the CNS-infiltrated T cells and selectively promote Th17 differentiation program [128, 129]. The pDCs possess tolerogenic function, control graft-versus-host disease and allergic responses, and promote allograft tolerance. During EAE, pDCs inhibit the effector $CD4^{+}$ T cell differentiation and conversely promote the Treg differentiation in both the lymphoid organs and inflamed CNS, and their depletion causes increased Th1 and Th17 response [130, 131].

The subsets of the DCs known as Tip-DCs which express CD11b, CD103, CD64, TNF-α, and iNOS cross-present the CNS antigens to the $CD8^{+}$ T cells and activate the cytotoxic response, which contributes to the spreading the neuronal epitope during EAE [132]. In contrast, CNS-infiltrated pDCs have regulatory phenotype and inhibit autoreactive T cell response and promote tissue repair [131, 133].

Involvement of B cells in neuroinflammation and autoimmunity The autoantibodies produced by B cells against the neuronal antigens play a significant role in deciding the course and progression of the CNS autoimmune diseases. About 90% of the MS patients, regardless of their clinical course, show the presence of oligoclonal anti-myelin antibodies in the CSF [134]. The autoantibodies are directed against the myelin antigens such as MOG, MBP, and MAG and also against astrocytic contactin-2 and neuronal neurofascin [135, 136]. These autoantibodies

induce various pathogenic mechanisms including antibody-dependent cell toxicity, complement-mediated cell lysis, increased phagocytosis, and opsonization by microglia and mononuclear phagocytes [136]. The B cells function as antigen-presenting cells (APCs) and also serve as a source of the effector cytokines such as IL-6, TNF-α, lymphotoxin-α (LT-α), and GM-CSF in several CNS autoimmunities [136]. The B cell-derived factors such as IL-15, CXCL13, B cell-activating factor (BAFF), and LT-α contribute to the formation and maintenance of the meningeal tertiary lymphoid structures during neuroinflammation [88]. Depletion of B cells using anti-CD20 mAb helps in reducing the severity of the neuronal autoimmunity. In contrast to the pathogenic function of B cells, a subset of B cells, is known to play a regulatory role in the CNS. These regulatory B cells (Bregs) produce anti-inflammatory cytokines such as TGF-β1, IL-10, and IL-35 and also express the inhibitory molecules that effectively help in controlling the neuroinflammation and autoimmunity [137]. Whether autoreactive T cell response or autoantibody response is a primary dominant mechanism of the CNS damage during the inflammation and autoimmunity is not clearly known. Nevertheless, it has been broadly considered that both these mechanisms contribute to the pathophysiology and progression of the clinical symptoms.

Mechanisms of Antibody-Mediated Neuroinflammation and Autoimmunity

The autoantibodies generated against the CNS antigens also play a crucial role in the induction of the several autoimmune demyelinating diseases.

Anti-AQP antibody The classic examples include the anti-MOG, anti-MAG, and anti-MBP antibodies in the MS patients and anti-AQP4 in NMOSD patients. The clinical presentation, lesional pathology, and immunological mechanism are somewhat similar in the MS and NMOSD patients except for the presence of anti-AQP4 IgG1 antibody in about 70–75% patients of optic neuritis and neuromyelitis optica [138]. The AQP4 is a water channel present in the astrocytes especially in the spinal cord gray matter and optic nerves. The anti-AQP4 induces astrocyte destruction via complement activation and antibody-dependent cell-mediated cytotoxicity (ADCC) mechanisms [139]. The anti-AQP4 also induces the internalization and proteolytic cleavage of the AQP4 in the astrocytes [140]. The AQP4 is also shown to form a complex with excitatory amino acid transporter-2 (EAAT2) and maintain glutamate homeostasis, and loss of this interaction in anti-AQP4-positive individuals leads to an alteration in the glutamate uptake and hyperactivation of glutamate receptors [140]. These events may form a basis of oligodendrocyte and axonal pathology in NMOSD. The BBB disruption is associated with the progression of the NMOSD, wherein CNS-infiltrated monocytes, macrophages, eosinophils, and neutrophils contribute to the pathology of the disease. Additionally, anti-AQP4-specific T cells secreting various cytokines such as IL-6, IL-10, and IL-17, and IFN-γ are reported

in NMOSD [141]. The permease, an ATP-binding cassette transporter of *Clostridium perfring*ens, shares about 90% homology with the astrocytic AQP4, emphasizing the molecular mimicry-induced anti-AQP4-reactive Th17 activation during NMOSD [58]. A detailed discussion about the causes of anti-AQP4 production and pathological mechanisms involved in optic neuritis and NMOSD is presented in chapter "Neuromyelitis Optica Spectrum Disorder".

Anti-MOG antibody The anti-MOG antibody-mediated pathology forms a basis of ADEM, a monophasic demyelinating disease of the CNS. The anti-MOG antibodies induce myelin damage and axonal loss by ADCC, complement-mediated cell damage, Fc-receptor-mediated phagocytosis and activation of the immune system. In addition to anti-MOG antibodies, myelin and CNS antigen-specific Th1 and Th17 cells affect the pathophysiology of the ADEM [142], and in recovery phase dominated by IL-4-secreting Th2 cells [143].

Anti-GAD65 antibody The anti-GAD65 antibodies are associated with numerous neurological diseases including limbic encephalitis, stiff-person syndrome (SPS), cerebellar ataxia and epilepsy. The GAD65 is a glutamate decarboxylase that controls GABA biosynthesis. The patients with anti-GAD65 antibody capture GAD65 at the synapses and inhibit the association of GAD65 with the GABAergic vesicles and inhibit the synaptic transmission [144]. The antigen-specific T cells are also involved in anti-GAD65-associated neurological conditions. A shift from Th1 to Th2 dominance in the CSF occurs in anti-GAD65-mediated SPS. In SPS, TNF-α- and IFN-γ-expressing Th1 cells are prevalent in the early stages, whereas IL-4-, IL-5-, and IL-13-secreting Th2 cells takeover in the later stages of the disease [145].

Autoantibodies against neurotransmitter receptors The presence of the CSF and intrathecal autoantibodies against various CNS antigens is a characteristic of several autoimmune diseases. These autoantibodies may be directed toward or cross-react to surface neurotransmitter receptors or intracellular proteins involved in synaptic transmission. Figure 5 illustrates how autoantibodies to neurotransmitter receptors affect the synaptic transmission and induce neural dysfunction. The patients with antibody-mediated neurological diseases show a very good response to plasma exchange, intravenous immunoglobulins, and B cell-directed therapies, suggesting the pathogenic role of autoantibodies. Most of these diseases show the evidence of the intrathecal production of the autoantibodies without disturbing the BBB integrity. For example, the synthesis of anti-NMDA receptor antibodies occurs even with the intact BBB in the CNS in anti-NMDA receptor encephalitis [146]. The complement-mediated neuronal death characterizes the anti-NMDA receptor encephalitis. However, *in vitro* studies have shown that anti-NMDA receptor, anti-AMPA receptor, and anti-$GABA_A$ receptor antibodies decrease the surface expression and synaptic localization of NMDA receptor, AMPA receptor, and $GABA_A$ receptor, respectively, without inducing neuronal death [147–149]. The anti-NMDA receptor disrupts the interaction of NMDA receptor and ephrin receptor 2 (EphB2R) and induces excess levels of the glutamate [150]. The resulting bias in the excitatory

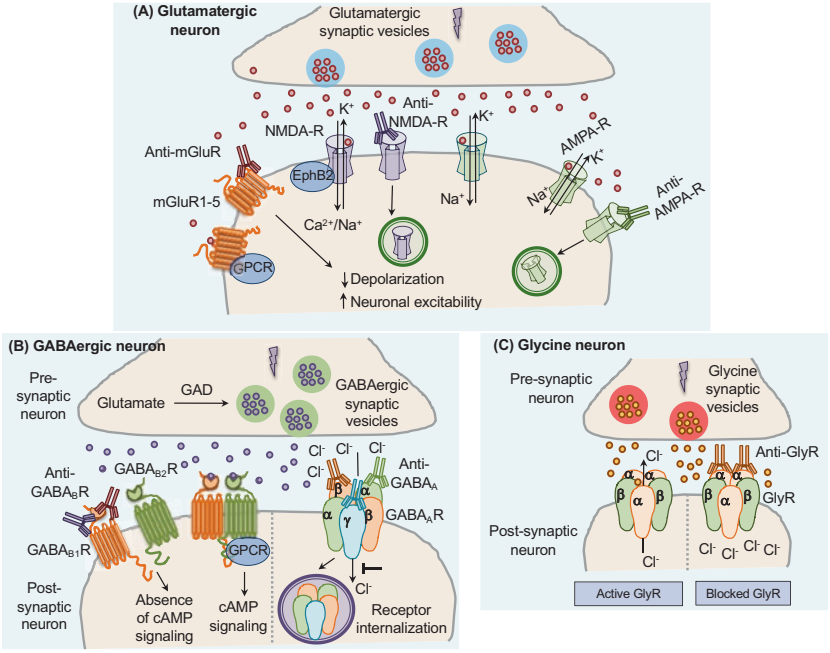

Fig. 5 Pathological mechanisms of autoantibody recognizing neurotransmitter receptors. Autoantibodies cross-reacting or directed toward neurotransmitter receptors are often found in CNS autoimmunity, paraneoplastic neurological complications, and neuropsychiatric diseases. The glutamate receptors, GABA receptors, and glycine receptors are the most common targets among the neurotransmitter receptors. (**a**) Autoantibodies, anti-NMDAR, anti-AMPAR, and anti-kainate receptor recognizing the glutamate receptor ion channels at the synapse region induce the vesicle-driven internalization of target antigens, NMDAR, AMPAR, and kainate receptors, respectively. This leads to the depolarization of postsynaptic glutamatergic neuron, and often causes neuronal excitability. The anti-GluR1 and anti-GluR5 recognize the glutamate receptor, a G-protein coupled receptor (GPCR), and induce Purkinje cell and hippocampal neuron dysfunction. Anti-glutamate receptors induce broad ranges of neurological symptoms such as encephalitis, progressive cerebellar degeneration, neuropathy, cerebellar ataxia, seizures, and psychiatric disorders. (**b**) Anti-GABA antibodies target both $GABA_A$ and $GABA_B$ receptors on GABAergic postsynaptic neurons. Binding of the anti-$GABA_A$ receptor to α, β, and γ subunit of heteropentameric $GABA_A$ receptor chloride channel reduces levels of $GABA_A$ receptors at synapse via vesicle-driven receptor internalization leading to depolarization and hyperexcitability of the postsynaptic neuron. Anti-$GABA_B$ receptor to $GABA_{B1}$ subunit of $GABA_B$ receptor (GPCR) blocks the cAMP-dependent signaling pathway and induces inhibitory synaptic currents in postsynaptic GABAergic neuron during stiff-person syndrome, limbic encephalitis, cerebellar degeneration, progressive encephalomyelitis, epilepsy, and seizures. (**c**) Anti-glycine receptor antibodies are most common in stiff-Person syndrome and progressive encephalomyelitis. The active postsynaptic glycine neuron is a pentameric chloride ion channel, which outflows the chloride ions into the synaptic regions. The binding of anti-glycine receptor blocks this chloride ion channel, increasing the intracellular chloride ion concentration, and induces neuronal excitability and affects the neurotransmission

and inhibitory neuronal transmission is thought to lead to the development of the seizures in the anti-AMPA receptor-positive limbic encephalitis patients [151]. The patients with limbic encephalitis, neuromyotonia, and Morvan's syndrome show the presence of anti-LGI1 antibodies (mainly of IgG4 subclass) in the CSF [25]. The secreted LGI1 has an important role in bridging the presynaptic voltage-gated potassium channel protein, Kv1.1 with the post-synaptic AMPA receptor through interaction with the synaptic receptors ADAM22 and ADAM23 [152]. Anti-LGI1 alters the binding of LGI1 with the ADAM22, and therefore, decreases the post-synaptic levels of AMPA receptors leading to neuronal hyperexcitability [152]. The mechanistic studies show that anti-LGI1 induces complement activation and neuronal death [30]. The anti-glycin receptor antibodies were reported in the progressive encephalomyelitis patients which induce the internalization of the glycin receptors, and its high titers in the patients correlated with the increased GABA and decreased glutamate levels [153]. Together, autoantibody-mediated mechanism drives the development of autoimmune diseases of the nervous system, and compelling evidence suggests the important contribution of autoreactive T cell response is needed to form a full spectrum of the neuropathology. The significance of autoantibodies and its critical role in neuronal autoimmunity are broadly discussed in chapter "Significance of Autoantibodies".

Role of Infections in the Neuroinflammation and Autoimmunity

Divergent infectious strategies Both innate and adaptive immune responses play a crucial role in the protection from the neurotrophic infections. Various clinical observations have revealed that immunodeficiencies and lymphocyte-directed therapies in the MS and psoriasis patients have higher risk of developing neuronal infections such as progressive multifocal leukoencephalopathy (PML) [154]. The neurotrophic pathogens use different strategies like retrograde transfer through infected immune cell transmigration, transendothelial migration across the BBB and axons, and transmigration through choroid plexus into neuronal tissue (i–iv Fig. 1). The viral and bacterial products are known to impair the BBB endothelial junctions and transit through paracellular route, whereas pathogen like *Neisseria meningitidis* adheres to the BBB endothelium and affects the cell polarity and tight junctions [155]. The *Cryptococcus neoformans* and *Toxoplasma gondii* are known to infect the brain endothelial cells before entering into the CNS. However, some neurotrophic arboviruses, JEV, WNV, and Venezuelan equine encephalitis virus, do not induce BBB damage; instead they induce type I IFN signaling in the BBB endothelium and strengthen the barrier. Nonetheless, the related dengue viral nonstructural protein 1 and HIV-1 Tat protein induce the BBB permeability [154]. Some patients with the *Plasmodium falciparum* infection are known to develop cerebral malaria, which is characterized by the sequestration of the parasite-infected RBCs and inflammation in the CNS microvessels. The *P. falciparum*-infected RBCs induce NF-κB-dependent intercellular cell adhesion molecule-1 (ICAM-1), IL-6,

IL-8, CXCL1, and CXCL2 in the human brain endothelial cells and cause BBB disruption during cerebral malaria [156].

Since the majority of the CNS-resident cells such as astrocytes, microglia, neurons, and oligodendrocytes express toll-like receptors (TLRs), RIG-like receptors (RLRs), and mitochondrial anti-viral sensors (MAVS) C-lectin receptors, they can induce and respond to the inflammatory reactions during neuronal infections [154]. Microglia are considered as one of the first cell types that react to the invasion of the pathogen in the CNS by activating several innate inflammatory pathways, including cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) signaling, and promote the further recruitment of the neutrophils, monocytes, and lymphocytes [157].

Regional heterogeneity The regional heterogeneity in the neurons represents a decisive factor in the spatiotemporal extent of the viral replication and spread of infection in the CNS. The MAVS-mediated viral sensing by neurons induces cytotoxic death of the infected cells [158]. The cortical neurons are more permissive for WNV infection as compared to hindbrain. This difference is due to the expression of the innate immune signaling molecules, retinoic acid-inducible gene-1 (RIG-1), MDA5, an interferon-stimulated gene (ISG) 54, and ISG56 molecules on the neurons. Nonetheless, various pathogens have evolved unique mechanisms that subvert the innate immune response in the neuronal tissue. For example, WNV inhibits nonreceptor tyrosine-protein kinase (TYK2), human Zika virus targets signal transducer and activator of transcription 2 (STAT2), and chromatin repressor complex induced during *T. gondii* infection inhibits the STAT1 and IFN-γ signaling in the infected neurons.

Neuroinflammation Recently, the meningeal lymphatic system was identified which helps in regulating the pathophysiology of the neuronal infection and inflammation [159]. The local inflammatory response generated in the nervous system by microglial and other perivascular macrophages induce the vascular inflammation at the BBB, meninges, and choroid plexus that facilitate the infiltration of the T and B lymphocytes in the neuronal tissues. The virus-specific and myelin-specific T cells use somewhat similar strategies to migrate into the inflamed CNS. The microglia, perivascular macrophages as well as CNS infiltrated monocyte-derived macrophages, and dendritic cells reactivate the lymphocytes and orchestrate the antipathogen protective immunity [160]. The $CD4^+$ T cells expressing IFN-γ and TNF-α and $CD8^+$ T cells expressing IFN-γ, TNF-α, perforin, and granzyme are the key players in controlling the parasitic, bacterial, fungal, and protozoan infections of the nervous system [154]. The CCL2-dependent recruitment of the monocytes is linked with the CNS pathology during WNV and HSV infection. The murine models of lymphocytic choriomeningitis virus show increased CNS damage and aggravated

neurological symptoms [161]. Additionally, aberrant humoral response induced during the HSV encephalitis is often associated with the production of the anti-NMDA receptor antibodies that cause post-viral encephalitis syndrome.

Control of excessive neuroinflammation The tissue-resident memory T (Trm) lymphocytes, broadly identified by the expression of the cell surface molecules CD103 and CD69, are also known to play an important role in controlling the neuronal infection and its associated neuroinflammation and tissue repair [162]. Similarly, T cell-derived IL-10 and microglia- or macrophage-derived IL-27 are also known to control the excessive inflammatory response in the neuronal tissues and protect from the fatal pathologies, possibly by restraining the Th17 response [163, 164]. The increased number and function of the $CD4^+$ Tregs during neurotropic viral infections control the effector T cell response and reduce the immunopathology. It also compromises the viral clearance [165]. Together, this suggests that both peripheral and local immune responses help in the protection from the neuronal infections. However, innate and adaptive neuroinflammatory reactions are also a cause of several infection-associated neuropathologies.

Autoimmunity triggered by infections The *C. jejuni* is the most predominant bacterial pathogen associated with GBS, an autoimmune neuropathy affecting both the CNS and PNS characterized either by acute inflammatory demyelinating polyneuropathy or by acute motor axonal neuropathy induced paralysis. About 30–40% GBS patients show *C. jejuni* infection [59]. The immunodominant glycoproteins of *C. jejuni*, lipooligosaccharides (LOS), and lipopolysaccharide (LPS) closely resemble the neuronal gangliosides such as GM1, GD1a, GM1/GD1 complex and induce cross-reactive autoantibodies and effector T cells [59, 166]. Interestingly, *C. jejuni* ganglioside-like LOS structures bind to siglec-7 (sialic acid-binding immunoglobulin-like lectin) present on microglia, oligodendrocytes, and Schwann cells [167]. The *C. jejuni* is a common food-borne pathogen, which after ingestion colonizes the intestinal wall and disturbs the gut immune tolerance and mounts a potent immune response against the closely resembling myelin antigens and gangliosides abundantly present on oligodendrocytes and neurons [168]. The presence of autoantibodies characterizes the GBS patients, complement C3 and membrane attack complex components along with huge perivascular lymphocytic and mononuclear infiltrates, suggesting a role of humoral complement-mediated and T cell-mediated pathology [59, 169]. The γδ T cells which recognize gangliosidic antigens via CD1b presentation manner are also well characterized in GBS patients and animal models [170]. Similarly, respiratory pathogens, *M. pneumoniae* (around 10–12% GBS cases) and *H. influenzae* (around 8–9% GBS cases), are associated with GBS, and the molecular mimicry of bacterial glycolipids with myelin galactocerebroside (Gal-C) is considered as a major trigger of autoimmune response in GBS patients with prior history of *M. pneumoniae* or *H. influenzae* infection [169]. There is evidence that links between *M. pneumoniae* infection and the presence of anti-GQ1b, anti-GM1,

anti-GD1b, and anti-GA1 in Bickerstaff brainstem encephalitis and Mille Fisher / Fisher syndrome patients [59, 169]. These autoantibodies are known to induce demyelination and neuropathic signs. Interestingly, studies with animal models of GBS, experimental allergic neuritis (EAN) showed that autoreactive T cells, cytokine-induced inflammatory pathways, and autoantibodies to neuronal and oligodendrocyte antigens form the basis of demyelinating and axonal neuropathy [171], and some of the disease-modifying strategies of MS work well in EAN models [172, 173]. The primary CMV infections and reactivation of the latent infection are shown to be associated with the GBS with predominant involvement of cranial nerves and sensory impairment [59]. The antibodies to CMV envelope glycoproteins cross-react with the GM2 and GD2 gangliosides on peripheral neurons and induce autoimmune neuropathy [174]. The CMV infection also promotes HLA-G expression on various immune cells of the myeloid lineage and induce immune tolerance state and Th2 cytokine bias, which supports autoantibody production [175]. Furthermore, similar molecular mimicry-induced autoantibody production and the bystander immune activation have been noted in GBS patients with HSV-1, HSV-2, VZV, EBV, and HIV-1 infections [59]. The EBV can directly infect peripheral nerves and induce neuritis symptoms, often characterized by the presence of anti-GQ1b, perivascular lymphocytic infiltration, microgliosis, demyelination, and axonal damage with the involvement of vascular inflammation [176]. In contrast to these mechanisms, hepatitis viruses employ a different strategy to induce neuropathic conditions. The GBS patients also show the presence of HAV, HBV, HCV, and HEV [59]. The HBV surface antigen (HBsAg) immune complexes are shown to be deposited on the peripheral nerves leading to neuropathies, whereas HAV- and HCV-associated immune complexes get deposited on endothelial vasculature and cause vasculitis in perinervous areas [59]. The GBS patients with HEV infections show anti-GM1 and anti-GM2 antibodies [177].

Acute cerebellitis is a rare inflammatory disease characterized by cerebellar ataxia, reduced consciousness, severe headache, and dysmetria, etc. It occurs either due to primary infection or post-infectious or post-vaccination reasons [178]. Various viral and bacterial pathogens such as CMV, measles, mumps, rubella, EBV, HSV, VZV, poliovirus, Coxsackie virus, rotavirus, *B. burgdorferi*, *Bordetella pertussis*, *Coxiella burnetii*, group A streptococcus, *M. pneumoniae*, and *S. typhi* are known to be associated with acute cerebellitis [178]. Some of these infectious agents are recovered from the CSF of the acute cerebellitis patients [179]. Many of these patients are characterized by the presence of serum antibodies reactive to gangliosides of neurons and oligodendrocytes, while VZV-associated cerebellitis show antibodies against the components of the centrosomes [179].

These clinical and pathological findings suggest the molecular mimicry-induced autoantibody production as a major pathological mechanism; however, cerebellar damage and associated mechanisms of the innate immune activation are also thought to contribute to the pathology [178, 179] (Fig. 1).

Neuroimmune Mechanisms in Paraneoplastic Diseases

Association of neoplasm and neuroinflammation and autoimmunity Several clinical evidence have shown the presence of antibodies reactive against the neuronal antigens during malignant conditions leading to neurological manifestations. The association of paraneoplastic autoantibodies with neurological diseases and their possible pathophysiological roles are summarized in Table 2. Most of the neurological antibodies are synthesized in the neuronal tissues during paraneoplastic neuroimmune diseases and can be detected in the CSF. The paraneoplastic neurological disorders show clinical and pathological features similar to CNS autoimmune diseases. The immune activation in the majority of the paraneoplastic neurological disorders occurs due to homology between tumor and CNS antigens, leading to the generation of an immune response against neo-onconeural antigens. Additionally, some of the tumors interfere with the immune tolerance mechanisms and promote the autoimmune responses to neuronal antigen.

Breakdown of immune tolerance in paraneoplastic conditions The immune tolerance is an active state of immune unresponsiveness (lack of immune reaction) to the cells and molecules capable of inducing an immune response. It operates at the level of lymphocyte development and maturation in the primary lymphoid organs, thymus (T cells) and bone marrow (B cells) called the central tolerance and also at the level of lymphocyte activation in the peripheral lymphoid organs called the peripheral tolerance. The type of tumor associated with the particular paraneoplastic neuronal autoimmunity may alter the efficiency of immune tolerance mechanisms. The thymoma, thymic carcinoma, thymic metastasized tumors, and lymphoma are capable of affecting the central tolerance. The evidence suggests that thymoma allows the autoreactive T cells to escape from the negative thymic selection [180]. The acetylcholine receptor (AChR)-specific T and B cells are abundantly present within thymoma [180], and anti-AChR produced by these B cells and AChR-specific Th2 cells induce the myasthenia gravis, an autoimmune disease that affects the voluntary muscles [181]. Interestingly, surgical removal of thymoma leads to the improvement of clinical symptoms of myasthenia gravis. The tumors in such microenvironment can produce numerous neoantigens that are cross-reactive to neuronal ones. The altered thymic selection of lymphocytes reactive to these antigens can produce helper T cells supporting the autoantibody production against the neuronal antigens.

Additionally, subsets of malignant B cells in chronic lymphocytic leukemia serve as APCs and provide co-stimulation (CD80/CD86-CD28) and activation (CD40-CD40L) signals to the autoreactive T cells residing in the secondary lymphoid organs in an anergic state and break the peripheral tolerance. Furthermore, decreased Treg number or activity is also an important mechanism, since increased levels of autoantibody production are often associated with the reduced Treg function. A study has shown that Qa-1-restricted $CD8^+$ Tregs with mutant MHC molecule incapable of binding to the T cell receptor selectively inhibit its function [182]. These mice show delayed tumor growth with enhanced expansion of Tfh cells and

Table 2 The autoantibodies and their pathological role in paraneoplastic neurological diseases

Autoantibody	Antigen	Most common cancers	Neurological complications	Pathological mechanisms	References
Autoantibodies against neurotransmitter receptors and ligand-gated ion channels involved in synaptic transmission					
Anti-NMDA receptor	NMDA receptor	Ovarian cancer, (90%), testicular cancer, lung cancer, SCLC, breast cancer, lymphoma	NMDAR (NMDR) encephalitis, cognitive dysfunction, oral and facial dyskinesia, post-herpes simplex encephalitis	Anti-NMDA receptor targets obligatory GluN1 subunit of heterotetrameric ionotropic glutamate receptor and also disrupts NMDA receptor-EphrinB2 receptor interaction leading to the internalization of glutamate receptor at the synapse. This induces the defect in glutamate uptake and hyperactive glutamatergic pathways.	[147, 150]
Anti-AMPA receptor	AMPA receptor	Breast cancer, lung cancer, thymoma, SCLC	Limbic encephalitis, cerebellar degeneration, neuropathy, ataxia, seizures and psychiatric disorders,	Anti-AMPA receptor mainly targets GluA1 and GluA2 subunit of heterotetrameric AMPA glutamate receptor. Induces internalization of AMPA receptor and decreases receptor availability at synapse leading to imbalance of excitatory and inhibitory synaptic transmission. The anti-NMDA receptor, anti-LGI1, and anti-GABAB receptor are also detected in the patient's CSF	[148, 151]
Anti-metabotropic glutamate receptor 1 and 5	Glutamate receptor 1 and glutamate receptor 5	Hodgkin's lymphoma	Cerebellar degeneration, progressive encephalomyelitis, limbic encephalitis, cerebellar ataxia.	The anti-metabotropic glutamate receptor 1 affects Purkinje cell excitability and also induces its death, while anti-glutamate receptor 5 interferes with synaptic transmission of hippocampal neurons. Both these antibodies cause excess glutamate-induced neuronal hyperexcitability	[64, 66]
Anti-$GABA_A$ receptor and anti-$GABA_B$ receptor	$GABA_A$ receptor and $GABA_B$ receptor	Anti-$GABA_A$: till date, no strong association with cancer found. Anti-$GABA_B$/SCLC (50%)	Stiff-person syndrome, limbic encephalitis, cerebellar degeneration, progressive encephalomyelitis, epilepsy, refractory seizures	These autoantibodies bind to α, β, and γ subunit of heteropentameric $GABA_A$ receptor and $GABA_{B1}$ subunit of $GABA_B$ receptor (GPCR), respectively. Anti-$GABA_A$ receptor reduces levels of $GABA_A$ receptors at synapse. Anti-$GABA_B$ may block $GABA_B$-mediated inhibitory synaptic currents. The patients also show the presence of anti-CRMP5 and anti-ANNA-1 and anti-ANNA-3	[67, 149, 243]

(continued)

Table 2 (continued)

Autoantibody	Antigen	Most common cancers	Neurological complications	Pathological mechanisms	References
Anti-glycine receptor	Glycine receptor	Thymoma, lymphoma	Stiff-person syndrome, progressive encephalomyelitis	These antibodies mainly target glycine receptor α subunit of pentameric glycine channels. Anti-glycine receptor induces internalization of glycine channel in transfected human embryonic kidney cells	[153, 243]
Anti-AChR	Acetylcholine receptor	Thymoma	Myasthenia gravis	About 90% myasthenia gravis patients show the presence of anti-AChR. The antibody induces the complement-mediated cell damage and inflammation at postsynaptic membrane. The AChR cross-linking induces its internalization and lysosomal degradation	[181]
Autoantibodies against neurotransmitter receptors and components of voltage-gated ion channels involved in synaptic transmission					
Anti-LGI1 (voltage-gated potassium channel)	Leucine-rich glioma-inactivated 1	Thymoma	Autoimmune encephalitis, limbic encephalitis, epilepsy, seizures, hippocampal atrophy	Anti-LGI1 binds various epitopes of LGI1. It disrupts the interaction of LGI1 with ADAM22 required to bridge Kv1.1 (presynaptic)-AMPA receptor (postsynaptic) connection and affects synaptic transmission	[152, 243]
Anti-Caspr2 (voltage-gated potassium channel)	Contactin-associated protein 2	Thymoma	Encephalopathy, limbic encephalitis, seizures, cerebellitis	It targets N-terminal discoidin and laminin G1 domain of Caspr2. It interferes with the clustering of Kv1.1/1.2 potassium channels and affects axonal excitability and also inhibits interneuron activity. The patients also show the presence of anti-LGI1 and anti-DPP10	[243]
Anti-P-/Q-type and N-type voltage-gated calcium channels	P-/Q-type voltage-gated calcium channel (Purkinje cells); N-type voltage-gated calcium channel (neuron)	Lung cancer (50%), breast cancer, and ovarian cancer	Lambert-Eaton myasthenic syndrome, cerebellitis, cerebellar ataxia	Astrogliosis and microgliosis in the cortical and cerebellar regions, Perkinje cell (anti-P-/Q-type) and neuronal (anti-N-type) damage, very rare or no involvement of perivascular infiltrated mononuclear cells and lymphocytes	[244, 245]

Autoantibodies against intracellular proteins involved in synaptic transmission					
Anti-ANNA-1, anti-ANNA-2, anti-ANNA-3	ANNA1: Neuronal nuclear RNA-binding proteins (HuB, HuC, and HuD) ANNA2: Neuro-oncological ventral antigen (NOVA)-1 and NOVA-2; RNA-binding and RNA-splicing regulators ANNA3: Unknown	SCLC, breast cancer, lung cancer	Brainstem encephalitis, limbic encephalitis, cerebellar degeneration, myelitis, radiculopathy, sensory neuropathy, Lambert-Eaton syndrome, seizures	The anti-ANNA-1 and anti-ANNA-2 recognize neuronal RNA-binding proteins Hu (HuB, HuC, and HuD) and Ri (NOVA-1 and NOVA-2) antigen, respectively, and induce complement activation and apoptosis of primary neurons. The inflammatory $CD4^+$ and $CD8^+$ T cells and activated microglia also contribute to anti-ANNA-induced inflammation	[63, 246]
Anti-PCA 1, anti-PCA2, and anti-PCA-Tr	PCA1: PCD17/CDR2 (cerebellar degeneration-related protein 2) PCA2: Unknown PCA-Tr: Delta/Notch-like epidermal growth factor-related receptor	Ovarian and breast cancer (PCA-1) SCLC (PCA-2) Hodgkin's lymphoma (PCA-Tr	Limbic encephalitis, brainstem encephalitis, cerebellar degeneration, peripheral neuropathy, cerebellitis	The inflammatory $CD4^+$ and $CD8^+$ T cells and activated microglia also contribute to anti-PCA1-induced inflammation. Autoreactive $CD8^+$ T cell induces neuronal death in neurological conditions associated with anti-PCA1, anti-PCA, and anti-PCA-Tr	[247–249]
Anti-CRMP3 and anti-CRMP5	Collapsin response mediator protein 3 and collapsin response mediator protein 5	SCLC, thymoma,	Brainstem encephalitis, limbic encephalitis, myelitis, peripheral neuropathy, optic neuritis, radiculopathy	The inflammatory $CD4^+$ and $CD8^+$ T cells and activated microglia also contribute to anti-CRMP3/5-induced inflammation. Autoreactive $CD8^+$ T cell induces neuronal death	[250]

(continued)

Table 2 (continued)

Autoantibody	Antigen	Most common cancers	Neurological complications	Pathological mechanisms	References
Anti-Ma1/Ma2	Ma1 and Ma2 proteins	Testicular cancer, ovarian cancer, breast cancer, lung cancer	Brainstem encephalitis, limbic encephalitis, encephalopathy,	Tumor expression of neuronal Ma1/Ma2 antigen induces anti-Ma1/Ma2 autoantibodies and autoreactive T cells. The inflammatory $CD4^+$ and $CD8^+$ T cells and activated microglia also possibly contribute to anti-Ma1-/anti-Ma2-induced inflammation	[251]
Anti-amphiphysin	Amphiphysin 1	SCLC, breast cancer	Limbic encephalitis, brainstem encephalitis, myelitis, sensory and motor neuropathy, stiff-person syndrome	Amphiphysin is intracellular trafficking protein, and antibody binding to it induces internalization of IgG-bound amphiphysin and alters GABAergic and glutamatergic synaptic transmission. Autoreactive $CD8^+$ T cells also contribute to the neuronal death	[252]
Anti-GAD65	Glutamic acid decarboxylase	Thymom, breast, and colon cancer, pancreas cancer	Stiff-person syndrome, limbic encephalitis, brainstem encephalitis, epilepsy, progressive encephalomyelitis	Antibodies recognizing different epitopes of the secreted GAD65 in the synapse are observed. They disrupt the interaction of GAD65 and GABAergic synaptic vesicles leading to reduced inhibitory synaptic transmission. Autoreactive $CD8^+$ T cells also contribute to the neuronal death	[144, 145]

germinal center B cells and increased levels of autoantibodies in the serum [182]. It is quite possible that similar mechanisms operate during the paraneoplastic neurologic disease, where downregulation of Treg function promotes increased autoreactive T cell and B cell response leading to the generation of autoantibodies reactive to neuronal antigens.

Autoantibodies toward neurotransmitter receptors or ion channels Several paraneoplastic neurological diseases are characterized by the presence of the autoantibodies against the ions channels and neurotransmitter receptors such as NMDA receptors, AMPA receptors, $GABA_A$ receptor, α1 and α3 subunits of the AChR, glycine receptor, P/Q-type voltage-gated calcium channels, Caspr2 and dipeptidyl-peptidase-like protein 6 (DPPX) [183]. The mechanisms of neurological manifestations mainly depend on the type of antibodies produced and the nature of the target antigen. The anti-NMDA receptor, anti-AMPA receptor, and anti-$GABA_A$ receptor induce the target receptor internalization and reduce their synaptic localization, which affects the synaptic transmission and neuronal plasticity and excitability [147–149]. Depending on the anatomical areas where target antigens are abundantly present, these antibodies induce characteristic clinical symptoms. The anti-metabotropic glutamate receptor 1 and 5 affect the Purkinje cell in the cerebellar synapses and hippocampal neurons, respectively [64]. The anti-P/Q-type voltage-gated calcium channels target peripheral neurons and also induce neuronal apoptosis [64]. The anti-amphiphysin and anti-$GABA_A$ are known to induce the receptor internalization leading to an imbalance of glutamatergic and GABAergic synaptic transmission, whereas anti-gephyrin causes receptor internalization and neuronal death leading to alteration in the GABAergic and glycinergic synaptic transmission [183].

Autoantibodies toward intracellular antigens Additionally, the intracellular neuronal antigens are also targeted. The anti-ANNA-1, anti-ANNA-2, anti-Ma1/Ma2, anti-collapsin response mediator protein-2 (CRMP2), anti-CRMP5, anti-PCA-Tr, and anti-amphiphysin-associated neurological manifestations are mainly characterized by the CNS infiltration of $CD4^+$ T cells, $CD8^+$ T cells, B cells, macrophage, and the microglial activation. The neuronal death is considered mostly due to cytotoxicity of the antigen-specific $CD8^+$ T cells [183]. However, substantial evidence is needed to pinpoint the role of the antigen-specific T cells in the induction of neuronal damage and associated neurological symptoms in the paraneoplastic conditions.

Neuroimmune Mechanisms in Vascular Inflammatory Diseases

The blood vascular inflammation affects almost all the blood vessels ranging from capillaries, arterioles, venules, arteries, and veins. The vasculitis is characterized by the thick, constricted and narrow blood vessels obstructing the blood flow leading to the tissue damage. The CNS vasculitis is a heterogeneous and rare disease and associated with clinical infections. The primary CNS vasculitis shows a headache,

motor deficits, and cognitive and memory dysfunction. The CNS vasculitis secondary to the infectious and autoimmune inflammation is often involved in pro-inflammatory cytokines and autoantibody-mediated BBB damage. For example, about 20–40% of SLE patients show the presence of the antiphospholipid antibody that induces complement-mediated neuronal damage [184].

BBB inflammation The BBB dysfunction is a hallmark of many neuroinflammatory conditions including MS, and endothelial cells of the BBB actively participate in the inflammatory process [4]. The type I activation of BBB endothelial cells is fast and marked with striking changes in the gene expression patterns, whereas type II activation is slower and induce multiple inflammatory signaling. Various inflammatory mediators have previously been shown to cause endothelial activation and BBB damage. The type I endothelial activation involves Rho activation, increased cytosolic Ca^{2+}, and Ca^{2+}/calmodulin-dependent myosin light-chain kinase (MLCK) phosphorylation leading to adherence and tight junction remodeling [185]. In contrast, type II activation is driven by sustained inflammatory signals that stimulate MAPK-, ERK-, and STAT-dependent signaling pathways. Activation of these pathways upregulates the endothelial adhesion molecules, E- and P-selectins, integrins, and chemokines that help in the transmigration of effector immune cells across the BBB into the CNS parenchyma [185]. The CNS-infiltrating immune cells activate myriad of inflammatory signaling pathways leading to microglial and astrocytic activations which together cause oligodendrocytes and neuronal damage. The redox imbalance in the inflamed endothelial BBB also leads to the activation of inflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and peroxisome proliferator-activated receptor gamma (PPARγ) signaling that in synergy with other inflammatory cytokines induce inflammatory changes in the BBB endothelium [185]. Furthermore, inflammatory molecules also affect the endothelial-glial interaction and induce BBB permeability. The calcium- and zinc-dependent matrix metalloproteinases, MMP2 and MMP9, cleave extracellular basement membrane proteins that anchor BBB endothelial cells to the astrocytic end-feet and also induce microglial IL-1β-dependent and astrocytic VEGF-A-dependent loss of BBB integrity during MS [186]. The levodopa (L-DOPA) administration in a mouse model of PD leads to the increase in VEGF-A expression in the astrocytes and induces BBB permeability dyskinesia [187], and recently, phase 3 trials of CVT-301 (levodopa inhalation powder) were concluded in the PD [188]. However, prostaglandins control the endothelial activation via prostaglandin I2 (PGI2) and prostaglandin F2 alpha (PGFα) signaling and cAMP production and promote barrier integrity in the CNS [189].

Neuroimmune Mechanisms in Neurodegenerative Diseases

The neuroinflammation is a characteristic hallmark of the neurodegenerative diseases and often considered as secondary to the neuronal DAMPs. The microglial cells are the major resident immune cell in the brain and spinal cord, which actively

maintains the neuronal homeostasis and also performs the immunosurveillance and defense functions (Fig. 1).

Vulnerable factor in neuroinflammation Several genome-wide association studies have shown the association of microglial or mononuclear phagocytes molecules with the increased susceptibility to the development of neurodegenerative diseases. For example, TREM2, complement receptor 1, SIGLEC 3 (CD33), and myeloid cell-expressed membrane-spanning 4-domains subfamily A member 6A (MS4A6A) and MS4A4E are known to be associated with AD while CD14, TNF-α, TNF receptor 1, TREM2, and IL-1β and IL-1 receptor antagonist with the PD [190–192]. The TREM2 has also recently been shown to be a risk factor for frontotemporal dementia [193, 194].

Roles of misfolded protein in autoimmunity Majority of the neurodegenerative diseases are characterized by the excessive accumulation of the misfolded protein aggregates or modified proteins, such as tau and amyloid-β in AD, α-synuclein in PD, mutant SOD1 and S100A9-S100A8 amyloidogenic protein complex in ALS, huntingtin (HTT) in HD [190]. The protein aggregates are either formed inside the neurons or deposited in the extracellular microenvironment. The intracellular aggregates directly affect the neuronal survival, whereas extracellular protein aggregates induce various signaling pathways and induce neuronal death and interfere with synaptic function. Microglial cells have capacity to sense the misfolded protein aggregates of tau and amyloid-β, α-synuclein, mutant SOD1, HTT, and S100A9-S100A8 complex released from degenerating neurons possibly through surface receptors, CD14, CD36, CD47, $\alpha_6\beta_1$-integrin and TLR4 and intracellular sensors, nucleotide oligomerization domain (NOD)-like receptors (NLRs), and NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome activation [190, 195]. The microglial inflammatory response is generally characterized by the hyperproliferation, increased phagocytosis, secretion of prostaglandins, and excess production of pro-inflammatory cytokines, TNF-α, IL-1β, IL-18, IL-12p35, IL-12p40, and IL-23p19, inducible nitric oxide synthase (iNOS), nitric oxide and reactive oxygen species, and NADPH oxidase in the AD and PD [190, 196]. The microglial cells in the brain phagocytose and clear the cellular debris via TREM-2-TYRO protein tyrosine kinase-binding protein (TYROBP)-induced signaling.

Specific autoimmunity in AD A mutation in TREM-2 has been shown to be associated with the increased risk of the AD [197]. The NLRP3-deficient or inflammasome component-deficient mouse model of AD shows reduced amyloid pathology [198]. The mitochondrial dysfunction in microglial cells is also shown to play a critical role in the pathophysiology of the AD. The evidence also suggests that microglia-induced inflammation contributes to the formation of neurofibrillary tangles (hyperphosphorylated tau) in the neurons after years from the deposition of amyloid-β aggregates during early stages of the AD.

Specific autoimmunity in PD The role of microglia-induced neuroinflammation is also evident in the PD, where dopaminergic neurons were shown to be protected from the damage in microglia-restricted TLR4-deficient 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mouse models of PD [199].

Specific autoimmunity in ALS The ALS is a fatal neurodegenerative disease affecting the function of motor neurons, and mutation in the SOD1 gene is reported in an approximately 20% ALS patients. The reactive astrocytes, microglial, and CNS-infiltrated mononuclear myeloid cells are present in the CNS of $SOD1^{G93A}$ transgenic mice [200, 201]. The induction of classical NF-kB signaling in these cells contributes to the neuronal pathology in a mutant SOD1 model of ALS. The microglia or astrocytes with mutant SOD1 exhibit activated phenotype, and activate neighboring glial cells, secrete inflammatory molecules, reactive oxygen and nitrogen species, and cause neuronal damage [190]. The aggregates of mutant SOD1 derived from dying cells also activate NLRP3 inflammasome and promote neuroinflammation [202]. SOD1-mutated microglia show defect in the phagocytic clearance of dead and dying neurons. The intervention strategies comprising of giving minocycline, a broad-spectrum antibiotic or pioglitazone, an activator of peroxisome proliferator-activated receptor-γ (PPARγ), show beneficial results in animal models of ALS [203, 204]. These findings suggest the possible role of systemic inflammation.

Specific autoimmunity in HD The activated microglia and astrocytes are present in the early stages HD, an autosomal dominant neurodegenerative disease characterized by the presence of extended CAG trinucleotides in the HTT gene. The activated microglia has been shown to express increased IL-1β and complement C3 and C9 proteins in the affected regions of the brain [205]. The increased level of circulating TNF-α also correlates with the HD progression. The activated microglia expressing mutant HTT induce neuronal death possibly via increased accumulation of ferritin and subsequent induction of inflammatory response characterized by increased IL-1β, TNF-α, IFN-γ, and TGF-β1 in the brain [190]. The activation of microglial cannabinoid receptor 2 leads to neuroprotection, whereas its deficiency in the microglial cells exacerbates disease symptoms in R6/2 mice model of HD [206]. Similarly, astrocyte-restricted mutant HTT expression leads to reduced glutamate uptake and neuronal dysfunction and shows age-dependent neurodegenerative signs [207]. The microglial intracellular TLR7 may sense the single-stranded RNA CAG-repeats generated from the Dicer-mediated cleavage of the CAG-trinucleotide repeats in the mutant HTT mRNA and induce neuroinflammation [208].

Vital roles of innate immune responses in degenerative diseases Although the contribution of the adaptive immune response to the neuroinflammation during the neurodegenerative diseases is not well established, recent evidence suggests that T cell-mediated response plays a crucial role in the neuroinflammatory processes during degenerative events [209]. The reduced $Foxp3^{+}CD4^{+}$ Treg number or activity has shown to reduce the neuroinflammation and improve the clearance of amyloid-β

plaques and control the cognitive symptoms in AD [210]. The effector T cell response helps in the clearance of amyloid plaques while regulatory T cells induce the tissue repair function in the brain of AD patients [211]. During AD, loss of BBB integrity allows the infiltration of antigen-specific peripherally activated T cells in the brain and creates an inflamed milieu, and activated microglia promote amyloid antigen-specific T cell reactivation and neuroinflammation. It has been shown that the modified α-synuclein released by the dying neurons activates surrounding microglia and mononuclear phagocytes and supports activation of effector T cells in the brain [212]. These findings suggest that innate immune response, especially microglia- and astrocyte-induced neuroinflammation, plays a vital role in the development and clinical progression of neurodegenerative diseases.

Neuroimmune Mechanisms in Neuropsychiatric Diseases

Neuroimmune mechanisms underlying ASD The clinical evidence and studies in animal models suggest that maternal infections, allergies, asthma, and autoimmune disease, as well as early childhood infections, increase the risk of the development of ASD, bipolar and obsessive-compulsive disorder, and schizophrenia [213–215]. The exposure of influenza virus infection, bacterial lipopolysaccharide (LPS), or polyinosinic:polycytidylic acid (poly I:C) to the experimental animals during gestation has shown an increased risk of developing ASD and schizophrenia-like neuropsychiatric disorders [216]. The autoantibodies such as anti-NMDA receptors, anti-LGI1, anti-contactin-2, anti-contactin-associated protein 2 (Caspr2), anti-AMPA receptors, and anti-dopamine 2 receptor-induced neuronal apoptosis during CNS autoimmunity are associated with the development of the neuropsychiatric symptoms [217]. It has been shown that a higher percentage of the mothers of the autistic children have serum antibodies that directly react or cross-react with the neuronal antigens [215, 218].

Transplacental transfer of autoantibodies in ASD It is also thought that the mother-to-fetal transfer of such antibodies may occur through crossing the relatively permissive fetal BBB. The presence of coincidental infections and systemic inflammation may direct the prenatal BBB disruption, allowing pathogenic autoantibodies to enter into the CNS. In support of this, several experimental studies with ASD animal models including nonhuman primates have shown that transplacental delivery of serum IgG from mothers of autistic animals during gestational periods leads to the development of motor and sensory deficits and behavioral and social alterations in the offsprings [219, 220]. It has been shown in rodents that maternal immune activation leads to changes in the serotonin and dopaminergic signaling in the offsprings [221]. However, not all maternal infections lead to the development of ASD or schizophrenia in the children, suggesting the involvement of the specific immunological trigger or the multiple immune activation pathways in addition to the genetic and environmental factors. A single injection of IL-2, IL-6, or IL-17A

during gestational periods in mice leads to the development of ASD and schizophrenia in the offsprings, whereas overexpression of IL-10 or neutralization of IL-17A partially controls this [222], suggesting the importance of these cytokines during fetal development.

Peripheral autoimmunity contributes to neuropsychiatric diseases The SLE and RA-associated neuropsychological pathology involves a variety of inflammatory cells and pathways. The RA patients with cognitive dysfunction have also been characterized by increased levels of serum anti-MOG, anti-MBP, and anti-S100β [223]. The human and mouse studies have shown that the subset of anti-DNA antibodies induced during SLE are present in the CSF and cross-react with the NR2 subunit of NMDA glutamate receptors on the neurons and induce their apoptosis leading to cognitive dysfunction [224, 225]. The systemic inflammation and increased levels of CSF pro-inflammatory cytokines such as TNF-α, IL-1β, interferon (IFN)-α, IL-6, and IL-8 contribute to the breach of BBB allowing cross-reactive antibodies to enter in the CNS [78]. The inflammation-induced activation of perivascular macrophages, microglia, and astrocytes further exacerbate the extent of neuronal apoptosis. The astrogliosis and astrocytopathy are considered as a critical factor contributing to the neuroinflammation during schizophrenia, bipolar disorder, obsessive-compulsive disorder, anxiety, depression, and mood disorders [226]. Collectively, this suggests that maternal immune activation, coincidental infections, and systemic and neuroinflammation play a critical role in neuropsychiatric diseases possibly altering the neurotransmission and inducing neuronal apoptosis by antibody-dependent mechanisms, pro-inflammatory cytokine-induced BBB damage, and glial activation.

Future Perspective

Possible targets of immunotherapies In the past decades, substantial progress has been made in the diagnosis, clinical care, and disability management of the patients with the neuroimmune disease. Currently, our limited understanding of the mechanisms of neuroimmune diseases is hampering the development of more precise and safer treatment strategies. The mechanisms may involve predominantly autoreactive T cell response (MS), autoantibody (paraneoplastic neurological diseases), or a combination of both (optic neuritis and NMOSD). However, the clinical course of neuroimmune diseases is quite heterogeneous and may involve different neuroimmunological mechanisms and may need to be targeted at various cell types at different stages of the disease. Since the disease susceptibility is also influenced by the genetic factors, understanding the genetic association with the different clinical form of the diseases is of great importance. The advent in genomics techniques like whole-genome sequencing and exome sequencing would prove beneficial in identifying novel genetic regulators of the progressive phases of the neuroimmune diseases. Such studies would help in formulating strategies to restore the affected

immune pathways to control the difficult-to-treat diseases such as PP-MS and PR-MS.

Neuroinflammation-induced recovery processes Inflammation triggers the pathogen clearance, removes unwanted cells and debris, and repairs the damaged tissue. In the context of the neuroimmune diseases, we have achieved significant progress in understanding the cellular and molecular basis of the inflammatory tissue damage and pathology. However, we have very little understanding of inflammation-triggered disease recovery, remyelination, tissue reparative, and restorative function. Almost all the CNS-resident cells express TLR and DAMP sensors and respond to insult; still, the plasticity of the inflammatory and regulatory function of astrocytes, microglia, oligodendrocytes, neurons, and brain endothelial cells is not completely known. The single-cell omics approaches such as transcriptomics, proteomics, epigenomics, and metabolomics coupled with system-level analysis and functional studies would help in gaining more in-depth insight into the functional and phenotypic heterogeneity of astrocytes and microglia during homeostasis and a specific neuroinflammatory and neurodegenerative condition. Additionally, the $CD4^+$ and $CD8^+$ memory T cells are known for their critical role in immunosurveillance, tissue homeostasis, and repair function. The carefully designed studies using human CSF, tissue specimens, and animal models are needed to look deeper and more closure at the frequency, anatomic localization, and function of memory T cells during demyelination, axonal damage, neurodegeneration, cognitive dysfunction, and reparative stages of neuroimmune diseases. These studies drive a way of controlling the inflammatory cells and promoting the reparative functions.

Although autoantibody produced against onconeural antigen forms a basis of paraneoplastic neuroimmune diseases, our understanding of the association of cancer with the neuroimmune disease is still in its infancy, and requires a vast multicentered clinical data collection across different ethnic groups to derive a conclusive association of a specific graded cancer with the presence of onconeural antigen-specific antibodies in the patients. Furthermore, we have been able to show a pathologic role of very few of the autoantibodies such as anti-NMDA receptor, anti-AMPA receptor, and anti-GAD65, and our understanding of the pathogenesis of other autoantibodies is incomplete. To gain a better insight of their pathologic mechanisms, the ability of purified antibody from patient-derived CSF to transfer the disease in animal models needs to be systematically tested. The primary limitation is that we lack animal models that mimic the pathology of a specific antibody-driven neuroimmune disease. Finally, more fundamental neuroimmunological studies are needed to investigate how a given cancer cell type edits its epitopes that resembles or cross-reacts with the neuronal ones, and how a given autoantibody enter into the neuronal tissue and mediates autoimmune response.

Therapeutic strategies for paraneoplastic neurologic diseases The paraneoplastic neurological diseases can be controlled with the tumor resection. Since paraneoplastic neurologic autoimmunity and neuropathies are triggered mainly by autoantibody-mediated immune activation, the immunotherapy coupled with anti-

seizure or antipsychotic medication represents a valuable approach to control such pathologies. First-line immunotherapy may comprise of corticosteroids (reduction of neuroinflammation and autoimmune activation), plasma exchange or immunoadsorption (removal of circulating autoantibodies), and intravenous immunoglobulin (IVIg; outcompeting the circulating autoantibodies, targeting autoantibody (Fab) and (Fc) regions, etc.) therapy either alone or in combination. Previously, such first-line immunotherapeutic approaches showed good clinical improvements in non-paraneoplastic encephalitis (anti-NMDA receptor, anti-LGI1, anti-Caspr2), GBS, myasthenia gravis, NMOSD, and MS [227]. Patients who respond poorly to first-line choices and those showing relapses can be treated with second-line immunotherapeutic strategies, which include cyclophosphamide, methotrexate, azathioprine, and mycophenolate mofetil alone or coupled with any of the working first-line treatment options. Treatment with B cell-depleting rituximab (anti-CD20) may represent a more direct way of targeting autoantibody production and effector T cell activation and cytokine production; however, it increases the risk of opportunistic infections, such as PML, as previously reported in the case of RR-MS, RA, and SLE [154]. Therefore, considering rituximab or other such lymphocyte-targeting therapies needs careful evaluation and screening for high-risk infections.

The precise understanding of immune-mediated mechanisms would help in devising immunologic strategies to control neuroimmune diseases.

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Roles of Effector T Cells in Neurological Autoimmunity

Yuki Fujita and Toshihide Yamashita

Abstract Neurological autoimmunity is a mistargeted immune response to the central or peripheral nervous system. Multiple sclerosis (MS) is one of many neuroimmune diseases involving autoreactive T cells in the central nervous systems (CNS). In MS, immune cells infiltrate the CNS and attack myelin sheaths, leading to demyelination, axonal damage, and neurological disabilities (Trapp and Nave, Annu Rev Neurosci 31:247–269, 2008; Hauser and Oksenberg, Neuron 52:61–76, 2006; Baxter, Nat Rev Immunol 7:904–912, 2007). The role of CD4+ T helper cells in MS pathology has been widely studied using animal models such as experimental autoimmune encephalomyelitis (EAE). Classically, it is considered that dysregulation of the balance between pro-inflammatory CD4+ T helper 1 (Th1) cells and anti-inflammatory Th2 cells plays an important role in MS development. More recent studies have provided evidence that interleukin (IL)-17-expressing Th17 cells are also essential for disease pathogenesis. Furthermore, CD8+ T cells are predominantly observed in human MS lesion sites. However, their functions in this disease are understudied. In this chapter, we summarize the roles of effector T cells in neuroimmune diseases focusing on findings from studies involving EAE and individuals with MS. Excess inflammatory responses can induce demyelination and progressive neuronal degeneration leading to functional disabilities. We also discuss

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_2

approaches to modulate the immune system and attenuate neuronal degeneration as a therapeutic target for MS.

Keywords Multiple sclerosis · Autoimmune encephalomyelitis · CD4+ T cells · Neuronal degeneration

Introduction

The central nervous system (CNS) has traditionally been considered structurally and functionally unique, in which immune surveillance is limited compared to that in other organs [4]. In neuroimmune diseases, misguided immune responses affect normal nervous systems and cause various symptoms. Multiple sclerosis (MS) is a chronic autoimmune disease of the CNS, in which immune cells infiltrate the CNS parenchyma, leading to demyelination and neurodegeneration [1–3, 5, 6]. Much emphasis has been placed on investigating pathogenetic, diagnostic, and therapeutic aspects of MS, and various animal models have been developed. Experimental autoimmune encephalomyelitis (EAE) is widely accepted as a suitable animal model for MS and is frequently used to investigate the mechanisms of inflammation and neurodegeneration [7]. Many studies using these animal models and humans have demonstrated that CD4+ T helper (Th) cells are critical effector cells for CNS inflammation [8, 9]. Interferon-gamma (IFN-γ)-producing Th1 cells were initially considered a predominant subset of effector CD4+ T cells that induces the pathogenesis of MS. However, more recently, interleukin-23 (IL-23) has been shown to be required for MS pathogenesis [10] and the activation of IL-17-producing T helper 17 (Th17) cells. Specifically, IL-17, IL-17 receptor, or IL-23 receptor deficiency diminishes clinical signs in EAE [11–14]. These observations provided evidence that Th17 cells play important roles in the development of MS pathogenesis. However, another study reported that the inhibition of IL-17A does not prevent EAE development, raising the possibility that other factors or mechanisms might be involved in neurological autoimmunity [15]. Although the role of CD4+ T helper cells in the pathogenesis of MS has been widely studied, CD8+ T cells are the most abundant T cells in CNS lesions of MS and exhibit clonal expansion [16–19]. In recent years, the function and mechanism of CD8+ T cells in MS progression appear to have been elucidated.

It is widely recognized that inflammation causally correlates with demyelination and axonal damage and loss, leading to neurological disability [5, 20]. Furthermore, some studies suggest that neurodegeneration in MS might occur independently from inflammation and could even be the primary cause of MS [1]. Therefore, besides the modulation of immune cells, the inhibition of neurodegeneration and demyelination is possible strategy for MS treatment.

This section summarizes evidence of the pathogenic and regulatory functions of effector T cells in neuroimmune diseases. We mainly focus on MS and its animal model EAE and discuss the dysregulated interactions between the immune and central nervous systems.

Dysregulation of the Th1/Th2 Balance in MS

T lymphocytes play a major role in the pathogenesis of MS. The infiltration of both CD4+ and CD8+ T cells has been observed in MS lesions [21]. The deleterious effects of CNS antigen-activated T cells are evidenced by studies demonstrating that the adoptive transfer of myelin-activated CD4+ T cells can cause EAE [22, 23]. CD4+ T cells recognize peptides that are presented by major histocompatibility complex (MHC) class II molecules on specialized antigen-presenting cells (APCs), and are usually derived from exogenous antigens ingested and processed by these cells [24]. Naïve Th cells differentiate into functional effector subsets based on their cytokine production pattern. Classically, two major subsets have been identified, namely Th1 and Th2 cells [25] (Fig. 1). Th1 cells produce large amounts of interferon (IFN)-γ and interleukin (IL)-2 and are mainly involved in macrophage activation and host defense against intracellular pathogens. Uncommitted naïve T cells can become Th1 cells through IL-12-induced signal transducer and activator of transcription 4 (STAT4) activation. Moreover, Th1 cells express the T-box transcription factor T-bet and secrete large amounts of IFN-γ. In contrast, Th2 cells produce IL-4, IL-5, IL-10, and IL-13, and mainly induce antibody production by B cells. IL-4-mediated STAT6 activation and GATA3 transcription contribute to Th2 differentiation. Th2 cells have key roles in host defense against helminths. Recently, additional Th cell subsets including Th17, T regulatory cells (Treg), Th9, Th22, and T follicular helper cells (Tfh) have been recognized [26–28].

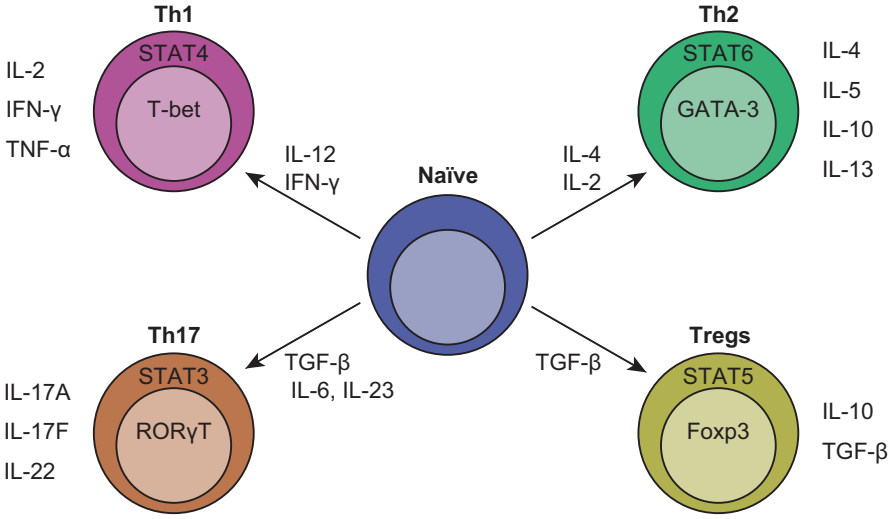

Fig. 1 Differentiation of naïve T cells into CD4+ T cell subsets including Th1, Th2, Th17, or Treg. IL-12 induces Th1 polarization characterized by the expression of T-bet and IFN-γ, whereas IL-4 induces Th2 polarization through STAT6 and GATA-3 signaling. TGF-β and IL-6 promote Th17 commitment, characterized by the expression of IL-17, IL-21, and IL-22, as well as the transcriptional factor RORγt

These studies initially suggested the possible existence of deviations in T cell subsets in 1971. It was also reported that antigen modification via the acetoacetylation of flagellins reduces the antigenic properties of unmodified flagellin in rats [29]. In contrast, this modification also induced increases in delayed-type hypersensitivity responses [30]. These findings suggest an inverse association between antigenic responses and delayed-type hypersensitivity. Although subsequent studies supported the possibility that different T cell populations might exist, there were no available antibodies to detect cell-surface markers and cytokines to determine specific populations at that time [31, 32]. In the mid-1980s, two functionally distinct T cell subtypes were identified. IFN-γ- and IL-2-expressing Th1 cells were distinguished from Th2 cells, which produce T cell growth factor distinct from IL-2 [25]. Further, the addition of supernatants from Th2 cell clones to T cell-depleted mouse spleen cells stimulated with lipopolysaccharide increased IgE production, whereas the Th1 factor IFN-γ suppressed this effect [33].

The balance between Th1 and Th2 cells has long been considered required to regulate immune responses and disrupted in autoimmune diseases [34]. Th1 cells have been implicated in diseases associated with autoimmunity such as type 1 diabetes and multiple sclerosis [8, 35, 36]. Accordingly, the expression of Th1 cytokines was observed in both EAE and MS lesions [37, 38]. Moreover, the shift in Th1 cells toward a Th2 cytokine profile ameliorates EAE symptoms [39, 40], whereas the adoptive transfer of Th1 cells was shown to be sufficient to induce EAE [41–44]. Further, the genetic ablation of T-bet suppressed the development of EAE [45, 46]. In addition, the pathogenic roles of Th1-type immune responses have been reported in an experimental murine arthritis model and in rheumatoid arthritis [47–50]. Treatment with a neutralizing anti-IL-12 antibody prevented the development of collagen-induced arthritis (CIA) not only in wild-type mice but also in IFN-γ receptor-knockout mice, suggesting that IL-12 promotes arthritis independent of IFN-γ production [51]. These observations indicate the deleterious potential of Th1 cells in autoimmunity.

Furthermore, a monoclonal antibody against IL-2 receptors inhibits the activation of T cells in EAE [52]. In addition, the adoptive transfer of Th1 cells contributes to the development of EAE [41]. Therefore, promoting the shift from Th1 toward Th2 cytokines could have a beneficial effect on the clinical symptoms of MS. A synthetic amino acid copolymer, namely, glatiramer acetate (Copaxone ®), which is an approved drug for the treatment of MS, reduces the frequency of relapses and the progression of disability [53]. The mechanism underlying these beneficial effects in animal models and patients seems to be the shift from Th1 to Th2 responses [54–60]. Moreover, glatiramer acetate induces Th2 cell development and increases IL-10 production through the modulation of dendritic cells [61] and patients treated with this drug exhibit a shift from Th1-biased to Th2 cytokine profiles [62–64]. These observations suggest that attenuating the Th1/Th2 cytokine imbalance could alleviate MS symptoms.

Despite this clear evidence showing the encephalitogenic roles of Th1 cells, other studies suggest opposite effects. Specifically, the administration of IFN-γ was found to ameliorate EAE severity, whereas treatment with a neutralizing antibody

against IFN-γ exacerbated EAE clinical disease severity [65–68]. Consistent with these findings, the deletion of IFN-γ or IFN-γ receptor rendered resistant strains susceptible to EAE induction [69–73]. Further, the genetic deletion of other Th1-related molecules such as STAT1 and IL-12 receptor β2 enhances EAE disease severity [45, 74]. Taken together, Th1 responses appear to be not absolutely required for EAE induction.

Th17 Cells in MS/EAE

In 2000, a T cell subset other than Th1 and Th2 was reported [75]. Initially, IL-23 was linked to the differentiation of Th17 cells [76–78]. Since IL-23 receptor (IL-23R) expression is limited, it was found that a combination of IL-6 and TGF-β induces the differentiation of pathogenic Th17 cells from naïve T cells and stimulates the expression of retinoic acid-related orphan receptor-γt (RORγt), which is a crucial transcriptional factor that triggers the expression of IL-23R [11, 79]. More detailed mechanisms underlying the definition and differentiation of Th17 cells, as well as their roles in neuroimmune diseases, are described in the later section by Pourgholaminejad [80].

Accumulating studies have shown that Th17 cells also contribute to the immunopathogenesis of MS. Deletion of Th1-related molecules also causes inflammatory autoimmune diseases. For example, IFN-γ−/− [81], IFN-γR−/− [71], IL-12Rβ2−/− [74], IL-12p35−/− [10, 82], and IL-18−/− mice [83] are susceptible to EAE. These findings suggest that reconsidering the involvement of Th1 cells in the pathogenesis of MS might be warranted. In addition to the important role of Th1 cells in MS, IL-17-expressing Th17 cells are known to be involved in MS immunopathogenesis [13, 84]. Passive transfer studies demonstrated that IL-23-dependent CD4+ T cells are highly pathogenic and play an essential role in the inflammation associated with CNS autoimmunity [77]. A subsequent study revealed that the pathology induced by Th17 cells differs from that of Th1 cells [85]. Th17 cells induce distinct chemokine profiles, and neutrophils are predominantly observed in IL-23-driven lesions, whereas Th1 cell-induced EAE is prominently associated with activated macrophages. These observations suggest that IL-23-mediated immune responses, as well as IFN-γ or IL-12, are critical for the development of EAE. In addition, various models of autoimmune diseases revealed a crucial role for Th17. The deletion of IL-17 suppressed immune induction in a collagen-induced mouse model of arthritis [86] and treatment with an IL-17R antagonist attenuated joint inflammation and bone erosion in a rat model of adjuvant-induced arthritis [87].

However, some studies have reported that IL-17 is not prominent during the development of MS [15, 88]. The IL-17 cytokine family consists of six isoforms, specifically IL-17A to IL17F. IL-17A is primarily produced by Th17 cells and IL-17F is most homologous to IL-17A. However, treating IL-17F-deficient mice with an antagonistic monoclonal antibody against IL-17A resulted in limited

beneficial effects on the development of EAE. These controversial results might be due to differences in protocols and strains of mice.

CD8 T Cells in MS/EAE

In general, T cells can be classified as CD4+ and CD8+. CD4+ T cells recognize peptides that are presented by MHC class II molecules on APCs. In contrast, CD8+ T cells recognize peptides that are presented by MHC class I molecules. Traditionally, CD4+ T cells mainly contribute to autoimmune pathogenesis. However, recent studies have provided increasing evidence for the possible pathological role of CD8+ T cells. Human leukocyte antigen (HLA) class II alleles, which mainly encode MHC class II molecules, have been reported to be associated with an increased risk of autoimmune diseases [89–93], and HLA-DRB1∗15:01 showed the strongest association in European and US populations [94]. This genetic association was first reported for MS pathogenesis, followed by other autoimmune diseases such as type 1 diabetes and rheumatoid arthritis [95]. MHC class I molecules have also been suggested to be linked to MS [96–98], and more recent studies demonstrated positive and negative associations with MHC class I regions. Specifically, HLA-A∗0301 was associated with a twofold increase in MS risk, whereas HLA-A ∗0201 showed a protective effect. The chapter of Multiple Sclerosis by Kira and Isobe more precisely described the genetic association between MHC regions and MS. These genetic associations support the involvement of CD8+ T cells in this disease.

Although the cause of MS is still unclear, the infiltration of immune cells into the CNS has been strongly associated with its pathogenesis [99–101]. Within the T cell population, CD8+ T cells are predominantly abundant in MS lesions [16, 17, 102–104] including human autopsy material from individuals with acute, chronic active, and inactive disease. Further, the micromanipulation and single-cell analysis of MS tissues revealed that CD8+ T cells might be more prevalent than CD4+ cells in various types of MS [17, 19, 105]. CD8+ T cells demonstrated a three to tenfold enrichment compared to CD4+ T cells in acute and chronic MS lesions [16, 17, 100]. CD8+ T cells were also found to be encephalitogenic in EAE [106]. Under pathological conditions, the expression of MHC class I molecules was found to be upregulated in the CNS cells including neurons, astrocytes, and oligodendrocytes, whereas the expression was limited to only vascular and meningeal cells under physiological conditions [107–109]. These observations suggest possible associations of CD8+ T cell infiltration with inflammatory lesions of MS. Recent studies reported that a portion of infiltrating CD8+ T cells in MS lesions show an activated cytotoxic phenotype, whereas the remaining cells have features of tissue-resident memory T cells, which might be focally reactivated in active lesions [103, 110]. Tissue-resident memory cells have been suggested to infiltrate tissues associated with acute inflammation as effector T cells and alter their phenotype to persist within the tissue as long-lived memory T cells. It is considered that tissue-resident memory T cells have important roles in protective immunity in site-specific

inflammatory lesions of the lung and skin after viral infection [111–114]. Acute MS is associated with a high percentage of CD8+ T cells co-expressing CD103, which is a marker of tissue-resident memory T cells. CD8+ cells are also predominant in other inflammatory diseases such as Rasmussen's encephalitis [115]. Future studies will more precisely determine the role and characteristics of tissue-resident memory T cells in MS.

The findings that CD8+ T cells can directly damage axons also support the pathogenic role of CD8+ T cells in autoimmune diseases (Fig. 2). In biopsies derived from MS individuals, acute axonal injury, defined by the accumulation of amyloid precursor protein, correlates best with the number of CD8+ T cells and macrophage/microglia rather than CD4+ T cells [116, 117]. Furthermore, the adoptive transfer of myelin antigen-specific CD8+ T cells causes the development of EAE in mice [107, 118, 119]. Synthetic peptides 35–55 from myelin oligodendrocyte glycoprotein (MOG) activate CD8+ T cells and induce encephalitis in C57BL/6 mice. Another study demonstrated that MOG37-46-specific CD8+ T cells also induce EAE. Further, the adoptive transfer of myelin basic protein 79–87 peptide-specific CD8+ T cells induces EAE symptoms in C3H mice. Since the co-injection of neutralizing antibody with IFN-γ was found to significantly reduce the severity

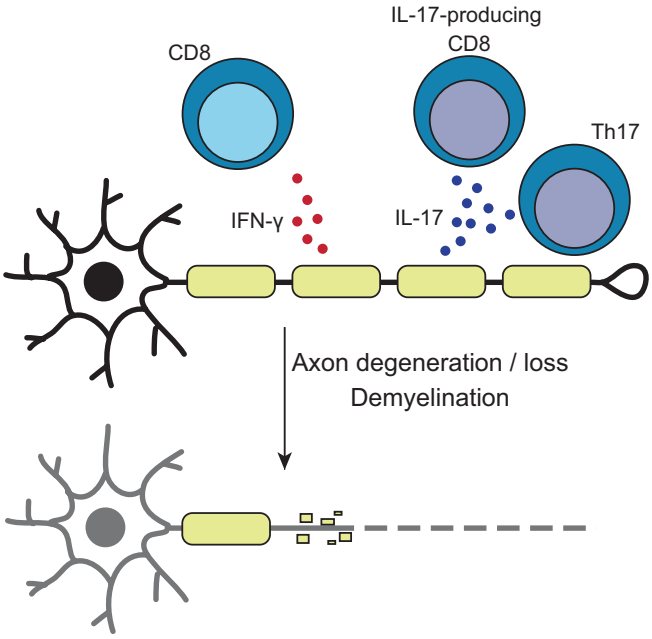

Fig. 2 The function of CD8+ cells in the pathogenesis of multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE). CD8+ T cells induce demyelination and axon degeneration, leading to axonal loss. The pathogenetic significance of antigenic expression remains unclear, and pro-inflammatory cytokines including IFN-γ and IL-17 are mainly involved in the induction of MS/EAE

of disease, IFN-γ might be important for CD8+ T cell-mediated CNS autoimmune disease [107]. Macrophage/microglia are activated in the transgenic mouse model with constitutive IFN-γ expression, suggesting that microglia may promote demyelination through the activation of these cells [120].

In addition to CD4+ T cells, CD8+ cells were found to be equally immunostained for IL-17 in MS tissue. IL-17-producing CD8+ T cells were detected in the lymph nodes and CNS of MOG37-50-induced EAE mice [121]. Moreover, IL-17-production in CD8+ T cells corresponded to decreased expression of granzyme B and IFN-γ, suggesting that these cells have diminished cytotoxic functions compared to classic cytotoxic CD8+ T cells. In addition, an increased number of IL-17-expressing T cells have been observed in acute lesions of MS. Immunohistochemistry revealed that 70 to 80% of T cells in acute lesions or active areas of chronic lesions, but only 17% of those in inactive lesions, showed IL-17 immunoreactivity [122]. These observations suggest that IL-17-producing CD4+ and CD8+ T cells are involved in the induction of EAE. Furthermore, IL-17-producing CD8+ T cells support the infiltration of IL-17-producing CD4+ T cells in the CNS and the induction of EAE via the chemokine receptor CCR6 [123]. These results suggest the indirect contribution of IL-17-producing CD8+ T cells to the initiation of autoimmunity through the pathogenicity of Th17 cells.

In contrast, another group reported that the suppression of CD8+ T cell accumulation has no effect on disease signs and CD4+ T cell motility in MOG35-55-induced EAE. In a further study, EAE was induced in lymphocyte-deficient Rag1−/− mice, which were replenished with only CD4+ T cells, only CD8+ T cells, or a mixture of both cell types, with the MOG35-55 peptide, and disease course was monitored. Mice without functional CD4+ T cells did not develop EAE, whereas mice without functional CD8+ T cells showed no significant difference compared to those with CD4+ CD8+ T cells [124]. Moreover, the in vivo CNS-cross-reactivation of CD8+ T cells was found to have no impact on disease progression in EAE [125]. Thus, the pathogenetic role of CD8+ T cells in the pathogenesis of MS is still under debate. Further studies assessing specific cell populations would be helpful to decipher their role in MS.

Possible Therapeutic Targets for Neurodegeneration and Demyelination in MS and EAE

Multifocal autoimmune-mediated demyelination and axonal loss are considered to have an autoimmune etiology. Therefore, neuroprotection could be a possible therapeutic target for MS. In the CNS, there are various myelin-derived proteins that inhibit axonal regeneration following neuronal damage, resulting in limiting functional recovery. These proteins are expressed mainly in myelin sheaths, which are formed by the oligodendrocyte plasma membrane wrapped around the axon. Three myelin-associated inhibitors—Nogo, myelin-associated glycoprotein (MAG), and

oligodendrocyte-myelin glycoprotein (OMgp)—have been well characterized. These three structurally distinct proteins all bind the same receptor, specifically the Nogo receptor (NgR), and activate RhoA-mediated axon growth inhibition [126–129]. RhoA is one of the Rho family of small GTPases and regulates various cellular functions, including maintenance of neuronal morphology and neurite outgrowth inhibition. Since NgR is a GPI-anchored protein and has no intracellular domain, it is considered unable to transduce signals into neurons and requires a co-receptor(s). The low-affinity neurotrophin receptor p75NTR was found to be a signal transducer of MAG [130], and subsequent studies demonstrated that p75NTR associates with NgR to form a receptor complex for MAG, Nogo, and OMgp [131, 132] (Fig. 3). The CNS transmembrane protein leucine-rich repeat and Ig domain containing 1 (LINGO-1) was also identified as an additional component of the receptor complex of NgR and p75NTR [133]. p75NTR induces the release of RhoA from Rho GDP-dissociation inhibitor (RhoGDI), thus acting as a RhoGDI

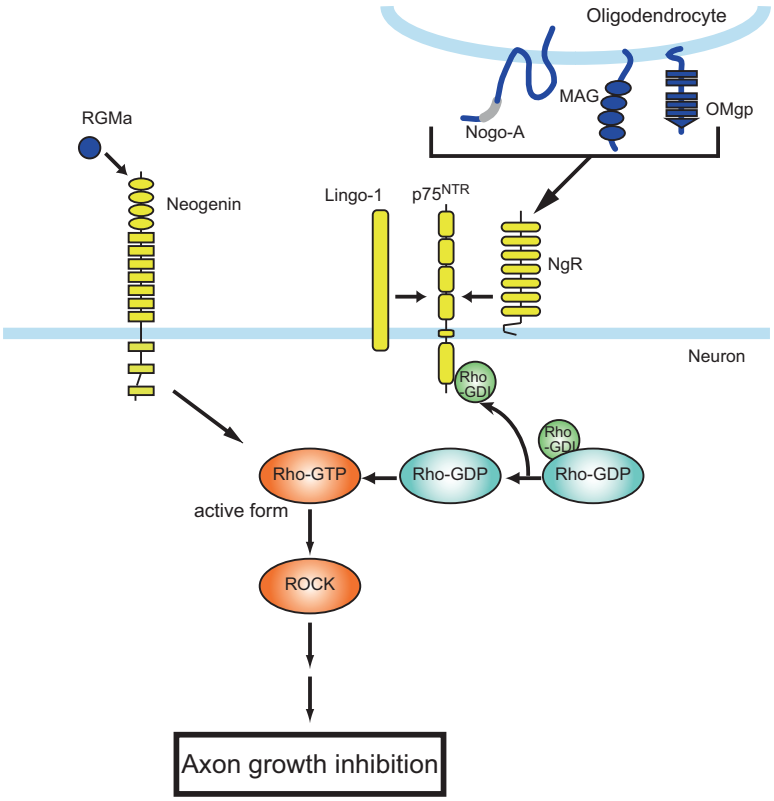

Fig. 3 Representative molecular mechanisms associated with axonal growth inhibition. Myelin-derived inhibitory factors MAG, Nogo, and OMgp bind NgR1, which induces Rho activity via interactions with NgR1 co-receptors, LINGO-1 and p75NTR. RGMa induces Rho activity via its receptor neogenin. Rho activation inhibits axon growth in neurons

dissociator [134]. Then, RhoA is able to change bound GDP for GTP, leading to gain its active conformation. LINGO-1 seems to also regulate the localization of RhoGDI and the activation of RhoA [135]. Further, MAG stimulation was found to mediate the activation of RhoA/ROCK signaling via these receptor complexes. Downstream of the RhoA/ROCK signaling pathway, the inactivation of collapsin response mediator protein-2 (CRMP-2), which interacts with tubulin heterodimers and facilitates microtubule assembly [136], leads to growth cone collapse and axon growth inhibition.

Particularly, LINGO-1 acts as a negative regulator of oligodendrocyte precursor cell differentiation [137]. Further, LINGO-1 antagonists or siRNA-mediated knockdown of this marker promotes oligodendrocyte differentiation, and LINGO-1-knockout mice show early-onset CNS myelination. Furthermore, treatment with an anti-LINGO-1 antibody promotes spinal cord remyelination in MOG-induced EAE and a toxin-induced demyelination model [138, 139]. These findings imply that the inhibition of LINGO-1 could be therapeutically beneficial for the management of MS. Moreover, a humanized anti-LINGO-1 monoclonal antibody (opicinumab, BIIB033, Biogen) has been developed to promote remyelination in individuals with remitting-relapsing MS, and this has been subjected to clinical trials [140]. Although the phase 2b trial of the anti-LINGO-1 antibody failed, fairly strong effects were observed with an intermediate dose, among four doses of the anti-LINGO-1 antibody, with IFNβ-1a (Avonex®) (NCT01864148) [141].

Repulsive guidance molecule (RGM) is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein that is involved in the inhibition of axon growth following CNS injury [142, 143]. The binding of RGMa to its receptor neogenin regulates various functions including axon guidance, neuronal differentiation, and survival during the development of the CNS [144–146]. Although RGMa levels are relatively low in the adult CNS, its expression is increased following CNS damage such as ischemic stroke and spinal cord injury [147, 148]. In an animal model of spinal cord injury, treatment with a neutralizing antibody against RGMa at the lesion site was found to significantly enhance axon regeneration and motor function recovery in both rodents and primates [149, 150]. Because the stimulation of neurons with RGMa induces RhoA and Rho-associated coiled-coil-containing protein kinase (ROCK), resulting in axon growth inhibition, the effect of this antibody might be dependent on the inhibition of this signaling pathway.

In addition, RGMa is involved in the pathophysiology of the autoimmune diseases. The inhibition of RGMa using a neutralizing antibody reduces pro-inflammatory cytokine production, demyelination, and neurodegeneration and relieves neurological deficits in MS and EAE [151–154]. Further, treatment with an RGMa-specific antibody reduces T cell proliferation and pro-inflammatory cytokine production in peripheral blood mononuclear cells from individuals with MS. Treatment with the anti-RGMa antibody also promotes axon regeneration and attenuates clinical manifestations in various EAE models such as MOG-induced and proteolipid protein-induced EAE in mice, Th17-cell-mediated EAE, and a focal model of EAE in rats. A humanized monoclonal antibody against RGMa also ameliorates EAE severity in non-obese diabetic (NOD) mice, a model of secondary

progressive MS. Thus, RGMa is involved in T cell-mediated autoimmune processes and its inhibition exerts both anti-inflammatory and neuroprotective effects in EAE.

Recently, estrogen receptor ligand β (ERβ) ligands have been shown to promote remyelination [155]. Treatment with selective ERβ ligands such as diarylpropionitrile (DPN) or chloroindazole (IndCl) increased myelination in animal models of MS [156–158]. Optimized analogues of IndCl ameliorate disease severity in EAE and improved myelination through the reduced production of the oligodendrocyte toxic molecules IFN-γ and chemokine (C-X-C motif) ligand, CXCL10 [159]. These findings suggest that ERβ ligand β ligands might be attractive candidates for MS therapy.

Conclusion

Accumulating studies have demonstrated the pathobiological roles of effector T cells in neuroimmune diseases. Particularly, rodent EAE models have made important contributions to understanding the molecular mechanisms of inflammation and to preclinical drug development for MS. Potent drugs have been developed for the treatment of relapsing-remitting MS, including glatiramer acetate (Copaxone®), IFN-β (Avonex®, Betaseron®, Extavia®, Rebif®), fingolimod (Gilenya®), natalizumab (Tysabri®), and mitoxantrone (Novantrone®). Recent clinical trials also revealed that a B cell-targeting antibody (humanized anti-CD20 antibody), ocrelizumab (Ocrevus®), shows beneficial effects not only on relapsing forms of MS but also on primary progressive MS [160–162]. These findings led the US Food and Drug Administration to approve ocrelizumab as the first monoclonal antibody for primary progressive MS and secondary progressive MS.

The degeneration of axons might be the major cause of permanent neurological disability in MS. Therefore, manipulating this process could form the basis for MS therapeutics. Indeed, several molecules involved in neurodegeneration have been explored as therapeutic targets for MS. Despite evidence supporting links among inflammation, demyelination, axonal injury/loss, and neurological disability, whether autoimmune processes comprise a direct or indirect response to neurodegeneration needs to be elucidated. However, therapeutic treatment could be improved by a greater knowledge of the molecular mechanisms underlying both immune and neuronal alterations in neuroimmune diseases.

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The Role of Th17 Cells in Immunopathogenesis of Neuroinflammatory Disorders

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Abstract Neuroinflammation, characterized by infiltration of immune cells such as T lymphocyte populations and other immune cells, is a prominent pathological feature of neurodegenerative disorders. However, consequence of neural injury during this inflammation is still unclear. Traditionally, CD4+ T helper (Th) cells have been categorized into various subsets. T helper 17 (Th17) cells are a Th subpopulation that plays an important role in the pathogenesis of neuroinflammatory diseases. The chronic forms of inflammatory milieu induce the Th17 cell polarization from their precursors and then secretion of pro-inflammatory cytokines such as interleukin-17 (IL-17), IL-21, IL-22, IL-23, and IL-6. Both interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) from Th17 cells exacerbate the inflammation. Migrating autoreactive Th17 cells into the nervous system can elicit neuronal apoptosis directly via Fas/FasL interaction. Th17 cells increase migration of other immune cells such as neutrophils into the inflamed CNS through the blood-brain barrier (BBB) and trigger the inflammatory reactions that occasionally lead to irreversible neuronal damages. Therefore, it is not surprising that these cells are implicated in a wide range of neuroinflammatory and autoimmune disorders including multiple sclerosis (MS), Alzheimer disease (AD), Parkinson disease (PD), schizophrenia, and many other neuroimmune disorders. In this chapter, we describe the immunopathogenesis of Th17 cells in neuroinflammations and discuss the neuronal injuries induced by Th17 cells and other Th17-related immune cells.

Keywords Th17 cell · Neuroinflammation · Autoimmune disease · Neurodegenerative disorder

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_3

Introduction

Based on some immunological features such as cytokine profile, transcription factors, phenotypic characteristics, homing receptors and immunological functions, CD4+ T helper (Th) cells are subdivided into the four main subpopulation including Th1, Th2, and Th17 cells and regulatory T (Treg) cells. Other Th subsets such as TFH (T follicular helper), Th9, and Th22 cells have their phenotypic and functional properties [1]. Th1, Th2, and Th17 cells trigger and promote immune response from the different pathways, and these responses eliminate foreign antigens. These subsets alone or with collaboration of other immune cells such as macrophages, neutrophils, dendritic cells, and also B cells can cause inflammatory reactions that lead to hypersensitivity disorders and autoimmunity. Exclusively, Th1 and Th17 cells are involved in the immunopathogenesis of some autoimmune and other chronic inflammatory immune-mediated diseases, whereas Th2 cells play a central role in allergic or atopic diseases. On the other side, one function of Treg cells is to avoid autoimmune responses and to stop the effector reaction against antigens through modulation or regulation of immune cells, when the response itself becomes hazardous for the host [2, 3]. The existence of Th cells is thus critical for proper immune-homeostasis and host defense.

Historically, *Mossman* and *Coffman* identified two subpopulations of effector Th lymphocytes, Th1 and Th2 cells, in 1986. In 2000, the first report on Th17 lymphocytes mentioned the role of these cells in host immune response to the bacterial species of the spirochete class of the genus *Borrelia*, named *B. burgdorferi* [4]. Recently, Th17 cells have been the most studied Th subset distinct from Th1 and Th2 cells with particular phenotypic and functional properties. Despite the fact that Th17 cells were discovered more than 10 years ago, the roles of these cells are not adequately understood [5]. These cells produce pro-inflammatory cytokines such as IL-17 (IL-17A), IL-17F, IL-21, IL-22, IL-23, IL-6, TNF-α, IFN-γ, and also granulocyte-monocyte colony-stimulating factor (GM-CSF). They play an important role in the pathogenesis of neuroinflammatory diseases [6, 7]. It can be stated that IL-17 cytokine family is a group of cytokines which includes six members: IL-17A, B, C, D, E (IL-25), and F [8]. Retinoic acid-related orphan receptor (RORC) (the human ortholog of mouse ROR-γt) is the specific transcription factor of Th17 cells. The chronic forms of inflammatory milieu induce the Th17 cell polarization from their precursors and intensify the inflammatory reactions in the nervous system [9, 10]. It has been demonstrated that generation of pathogenic/inflammatory IL-17-producing Th cells occurs in the presence of pro-inflammatory factors such as IL-1β, IL-6, and IL-23 [10]. These Th17 cells produce simultaneously both IL-17 and IFN-γ and co-express transcription factors T-bet (specific for Th1 cells) and RORC (specific for Th17 cells) [11, 12]. Hybrids of Th1 and Th17 cells are named Th17/1 cells [13]. Th17/1 cells are a new putative subtype of IL-17-secreting Th cells. Interestingly, the pattern of chemokine receptors expression on the surface of Th17/1 cells can isolate them from Th17 cells.

Th17 cells express C-C Chemokine Receptor type-6 (CCR6) and CCR4 whereas Th17/1 cells express CCR6 and CXCR3 [14]. Collectively, the precise origin of Th17/1 cells and their biological effects remain unclear. This research field requires more experiments.

Regarding the issue of Th17 cell differentiation, it has been shown that the transforming growth factor (TGF)-β acts as an anti-inflammatory. Treg-related cytokine is essential for the Th17 cell polarization [15]. Our own studies have also revealed that optimal differentiation of human Th17 cells occurs in the presence of pro-inflammatory cytokines IL-1β, IL-6, and IL-23 independently of TGF-β and TGF-β-related signaling, with TGF-β being a negative regulator of human Th17 cell development [10]. We have shown that in the presence of TGF-β, the production/expression of IL-17, IFN-γ, and IL-22 decreases and the expression of Foxp3 (specific transcription factor for Treg cells) increases (Fig. 1) [10].

Given the pro-inflammatory features of Th17 cells and their active role in neuroinflammation in neuroimmune and neurodegenerative disorders, we focus on the functions of Th17 cells and their mechanisms in Multiple Sclerosis (MS) and other neurodegenerative disorders such as Parkinson Disease (PD), Alzheimer Disease (AD) and Schizophrenia.

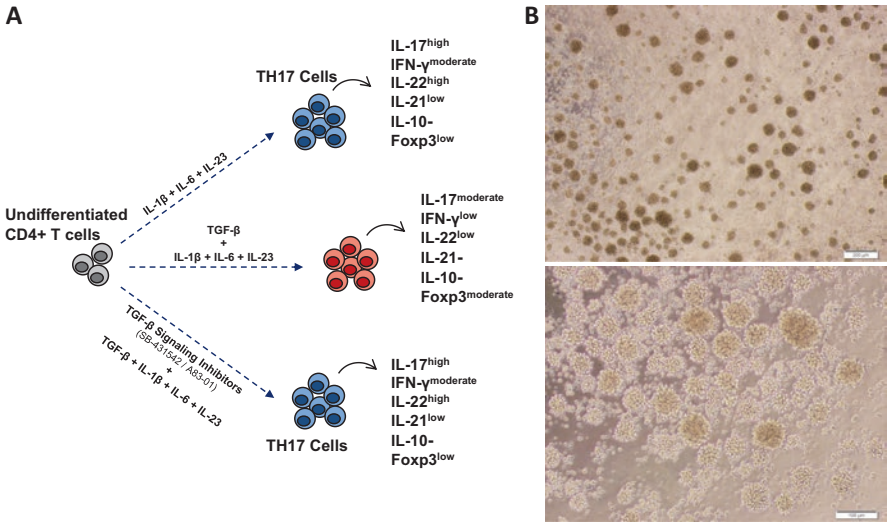

Fig. 1 Human Th17 cell differentiation. (**a**) Scheme of human Th17 cell polarization induced by pro-inflammatory cytokines (IL-1β, IL-6, and IL-23), independently of TGF-β and its related signaling. In the presence of pro-inflammatory cytokines, production of IL-17, IL-22, and IFN-γ is enhanced, while TGF-β decreases the production of IL-17, IL-22, and IFN-γ but upregulates Foxp3 expression. Using TGF-β signaling inhibitors (small molecules; SB-431542 and A83-01) reverse the situation. (**b**) Representative microimages are shown with different magnifications. Colonies of proliferating CD4+ T cells during Th17 cell polarization in the cell culture media are presented. (Courtesy of Dr. Pourgholaminejad, Guilan University of Medical Sciences, Rasht, Iran: [10])

The Role of Th17 Cells in Immunopathogenesis of Multiple Sclerosis

Roles of Th1 and Th17 Cells in Pathogenesis of Multiple Sclerosis

MS is a multifactorial disease, and as well MS patients suffer from a variety of clinical indications including sensory loss, visual problems, muscle weakness, and difficulties in speech [16]. Clinically, MS is a heterogeneous disease, and most patients (>80%) experience initial relapsing-remitting (RRMS) period followed by the secondary progressive MS (SPMS) characterized by neurological disabilities [17]. MS initiates with the acute neuroinflammatory lesions characterized by disruption of BBB, and through these spaces, leukocytes especially T cells enter into the CNS. We can consider that MS is a T cell-mediated demyelinating disease of the human CNS with irreversible loss of myelin sheaths leading to chronic inflammation. In other words, MS is an autoimmune neurodegenerative disorder in which the underlying immunological mechanisms cause demyelination and progressive degeneration of gray and white matter [18]. The factors behind the initiation of inflammatory reactions remain unclear yet. Both innate and adaptive immune systems are implicated in the etiology of MS, and abnormality in the immune response is among the leading causes of MS, owing to the fact that the innate immunity along with adaptive immune cells particularly T lymphocytes could induce neurodegeneration by producing pro-inflammatory cytokines, enzymes, oxidative products, and reactive oxygen species (ROS) [19, 20].

Many findings point toward a central role of abnormal adaptive immune cells that are associated with immunopathogenesis of MS. Although the pathogenesis of MS remains elusive, recent studies have demonstrated that different Th subsets and Th-specific polarizing factors are also implicated in MS pathogenesis. Some systemic infections cause the upregulation of adhesion molecule expression on the surface of endothelium of the brain and spinal cord. This leads to the entering of leukocytes into the normally immunological privileged CNS. These inflammatory cells trigger other cascades of inflammatory events, resulting in the formation of CNS lesions and plaques [16]. CD4+ Th1 and Th17 cells are two inflammatory Th subsets that are considered as contributors in pathogenesis of MS through different mechanisms [21, 22]. Both Th1 and Th17 cells migrate to the CNS through endothelial cells. IL-17-induced Th cells attach to the brain endothelium better than IFN-γ-secreting T cells. This is due to the CD49d, CD6 and melanoma cell adhesion molecule (MCAM/CD146) expression on Th17 cell surface more than Th1 cells. Moreover, Th17 cells have a higher proliferative ability and are less susceptible to suppression than Th1 cells [23]. In another study, it has been shown that the expression of CCR6 is high in Th17 subsets that enhance infiltration of myelin-specific Th17 cells into the CNS. The CCR6 ligand (CCL20) is expressed

on choroid plexus epithelial cells in mice and humans. The CCR6-CCL20 interaction is an important factor in the development of experimental autoimmune encephalomyelitis (EAE), and animal model of MS [24]. The pattern of CNS infiltration of Th17 cells is mediated by the integrin LFA-1 (αLβ2), whereas Th1 cells use the integrin VLA-4 (α4β1) for their CNS migration. In the absence of VLA-4, Th17 cells could settle in the CNS [25]. Th17 cells through production of their hallmark pro-inflammatory cytokines disrupt BBB tight junctions and by CCR6-CCL20 interaction can infiltrate into the CNS (Fig. 2). Th17 cells show a high pathogenic potential in MS [6].

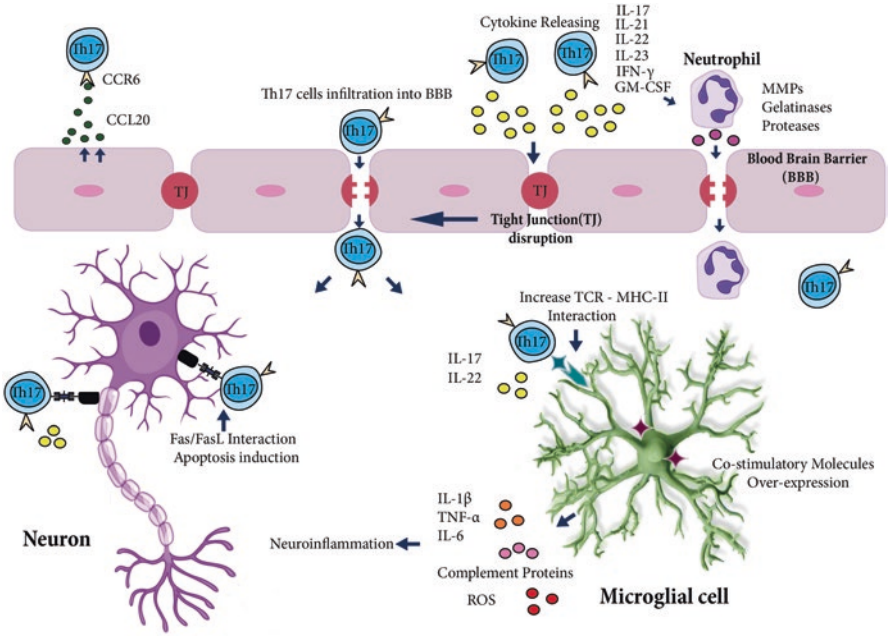

Fig. 2 Th17 cell functions in immunopathogenesis of neuroimmune disorders. Th17 cells by producing inflammatory cytokines such as IL-17, IL-21, IL-22, IL-23, IFN-γ, TNF-α, and GM-CSF can disrupt BBB tight junctions and through CCR6-CCL20 interaction migrate into the CNS. Th17 cell-derived cytokines enhance neutrophil infiltration, neutrophil enzymes, and their secretions in addition to BBB disruption. This causes neural damage and neuroinflammation. Autoreactive Th17 cells activate microglia to secrete other pro-inflammatory cytokines (IL-1β, TNF-α, and IL-6), complement proteins, and reactive oxygen species (ROS). These inflammatory factors can switch on apoptotic pathways in neurons. Also, Th17 cell-associated inflammation stimulates the expression of co-stimulatory and MHC molecules on microglia surfaces, leading to enhancement of TCR-MHC interaction between Th17-microglia cells. Moreover, Th17-neuronal interaction through Fas-FasL molecules induces neuronal apoptosis that finally lead to neural degeneration. (Courtesy of Dr. A. Pourgholaminejad, Guilan University of Medical Sciences, Rasht, Iran [7])

Actions of Divergent Pro-Inflammatory Cytokines

Myelin-specific T cell activation and production of pro-inflammatory cytokines are believed to have a crucial role in the development of MS pathogenesis. It was reported that the quantity of IL-17-producing T cells was elevated during clinical exacerbation in peripheral blood and cerebrospinal fluid (CSF) of MS patients [26]. Elevated levels of Th1- and Th17-derived cytokines including IL-2, IL-6, TNF-α, IFN-γ, IL-17, IL-22, and also IL-23 are found in progressive MS patients [27–29]. Recent data suggest that Th17 cells, Th17/1 cells, and also Th1 cells are involved in MS pathogenesis as well [30–32]. For the process of Th cell differentiation, the presence of the transcription factor interferon regulatory factor 4 (IRF4) is essential [33]. According to Yang et al., inflammatory Th1 and Th17 cells are suppressed by the inhibition of IRF4. This leads to an increase in Treg cell infiltration and a decrease in Th1 and Th17 cell infiltration that eventually ameliorates MS symptoms in mice [34]. It is currently suggested that Th17 cells have a major role in the immunopathogenesis of MS because of the pro-inflammatory cytokine profiles including IL-17, IL-6, IL-21, IL-22, IL-23, and TNF-α that could be associated with neuroinflammatory reaction, a primary feature of MS pathophysiology [35]. The frequency of autoreactive Th17 cells in the inflamed CNS is high in EAE [36]. The level of Th17 cells in the CSF of RRMS patients remains high when the patients are in the relapsing phase as compared to the remission phase [23]. In addition to Th17 cells, it is also believed that IL-17-secreting gamma-delta T (γδ T) cells play a crucial pathogenic role in CNS inflammation in MS patients [37]. IL-17-secreting γδ T cells are a primary early source of IL-17 and IL-21 production that results in a considerable amount of IL-17 production by Th17 cells in autoimmune diseases [37]. Some studies have reported that brain autoimmunity specifically MS is associated with specific microbiota modifications and increased proliferation of Th17 cells in the intestine. Increased frequency of mucosal IL-17+, IL-22+, and Th17 cells correlates with high disease activity, and since the gut is an important environment for development of mucosal Th17 cells, myelin-specific autoreactive Th17 cells are driven in small intestinal mucosa and then migrate into the CNS [38].

Th17 Cell Plasticity

Since the autoreactive Th17 cells are the critical pathological cells in the pathophysiology of MS and EAE, CNS-resident natural killer (NK) cell enrichment ameliorates MS by interacting with microglia and suppressing myelin-reactive Th17 cells, which may substantiate Th17 cell key role in MS immunopathogenesis [39]. Some reports have demonstrated that IFN-γ-deficient mice, anti-IFN-γ-treated mice, and IFN-γ receptor-deficient mice develop EAE which is a classical Th1-associated disorder [40, 41]. Evidence suggests that IL-17- and IL-22-secreting Th cells are implicated in the early stages of MS [42]. The debate over the stability of Th17 cells was heated when it was reported that T cells express IL-17 and IFN-γ simultaneously

under the inflammatory situations [43, 44]. It has also been reported that Th17/1 cells were present in the CNS of EAE mice and CNS of MS patients [27, 45], and circulating lymphocytes harvested from MS patients were found to have an increased propensity to polarize into IL-17/IFN-γ double secretors [27]. Kebir et al. have demonstrated that lymphocytes isolated from the blood of MS patients show considerable potential to expand into IFN-γ-secreting Th17 cells. Accordingly, IFN-γ + Th17 cells pass through the human BBB during the active phase of MS and accumulate in the CNS in mice [27]. This causes Th17 cells plasticity, impacts on phenotype instability and autoimmunity [30, 46]. IL-17-secreting Th cells that convert into IFN-γ producers (that so-called ex-Th17 cells) have been incriminated in the immunopathogenesis of EAE models [47]. About 15–20% of T cells in active MS injuries express GM-CSF, and the majority co-express IL-17 and IFN-γ [47]. Langrish et al. have demonstrated that a more severe EAE occurs when Th17 cells are transferred as compared to Th1 cells [32]. Although autoreactive Th1 and Th17 cells induce similar disease, the pathology of the disease induced by these cells appears significantly different [48]. In fact, Th1- and Th17-mediated forms of EAE reveal different patterns of feedback to the same immunomodulatory agent [48].

Multiple Actions of IL-17

Since the hallmark cytokines of Th17 cells have pro-inflammatory effects, the consequences of the irreversible CNS damages are due to the high level of these cytokines in the CNS environment. IL-17 as a hallmark cytokine of Th17 cells affects a wide range of cells such as endothelial cells, epithelial cells, fibroblasts, and myeloid cells. IL-17 stimulates and triggers production of other inflammatory factors such as endogenous pyrogens (IL-1, IL-6, and TNF-α) and some chemoattractants including IL-8, CXCL1, CXCL6, and MIP-2 [49]. IL-4 (Th2-related factor) and IFN-γ (Th1-related factor) negatively regulates IL-17 production from effector Th cells, and, also, neutralizing anti-IL-17 antibody suppressed chemokine expressions and leukocyte infiltrations in the CNS during EAE [50]. Furthermore, the development of EAE is inhibited in IL-17$^{-/-}$ mice. These mice show delayed onset of disease, declined severity scores, improved histological changes and early recovery from disease [51]. IL-17 affects the function of myeloid cells such as neutrophils and microglia. The principal function of IL-17 is the breakdown of BBB. IL-17 selectively recruits neutrophils into the inflamed CNS via the release of neutrophil-specific chemokines [52]. Neutrophil-related enzymes such as matrix metalloproteinases (MMPs), gelatinases, and other proteases can powerfully disrupt BBB (Fig. 2). The production of ROS, which is stimulated by IL-17, enhances endothelial cell adhesion molecule expression that leads to the infiltration of other inflammatory cells such as monocytes and macrophages [53]. Macrophage infiltration and delayed-type hypersensitivity (DTH), predominated in Th1-mediated neuroinflammation, occur, whereas in the Th17-mediated damages, neutrophils are predominant [48].

Th17-Related Inflammation and the Neuronal Apoptosis

Brain-resident microglia/macrophage and CNS-infiltrated T cells play important roles in neuropathology of MS. Activated microglia and T cells can be found in close proximity in CNS lesions [54]. Resident microglia/macrophages are thought to play major roles in demyelinating lesion formation through re-stimulation of T cells within the CNS. They act as antigen-presenting cells to restimulate T lymphocytes in the CNS [55]. The recruitment of monocytes/macrophages is mediated by CCL2-CCR2 signaling. Hypertrophic astrocytes in active MS lesions produce CCL2, while CCR2 is expressed on monocytes/macrophages [56]. Thus, macrophages play major roles in antigen presentation. Microglia/macrophages express major histocompatibility complex (MHC class-I and MHC class-II) molecules along with several co-stimulatory molecules (such as B7.1 and B7.2) [57]. B7 molecules can interact with CD28 on T cell surfaces. B7-CD28 interaction causes T cell activation, proliferation, differentiation and cytokine production. Other co-stimulatory molecules such as OX40 ligand and CD40 ligand express on the microglia/macrophages that can re-activate auto-inflammatory infiltrated T cells. This microglia/macrophages-T cell interaction through MHC and co-stimulatory molecules leads to a cascade of inflammatory events resulting in neuronal damage [54].

Treatment of microglial cells with IL-17 increase production of nitric oxide (NO), IL-6, MIP-2, and neurotrophic factors. Amazingly, Kawanokuchi et al. have shown that IL-17 is produced by microglia in response to IL-23 or IL-1β. Theses authors showed that microglia produce IL-1β and IL-23 itself. These cytokines may act in an autocrine manner to induce IL-17 secretion by microglial cells [58]. Infiltrated Th17 cells cause overexpression of MHC and co-stimulatory molecules on microglia surfaces and, through the TCR-MHC interaction, activate microglial cells producing inflammatory cytokines (IL-1β, IL-6, and TNF-α), complement proteins, and ROS. This leads to enhancement of other immune cell migrations and CNS inflammation of MS patients (Fig. 2). These neuroinflammations ultimately lead to neuronal apoptosis and neural degeneration. Neuronal apoptosis is mediated by Fas/Fas-ligand interaction. The expression of Fas molecule is upregulated when the neurons are exposed to inflammatory situations such as inflammatory cytokines. It has been revealed that IFN-γ can upregulate the expression of Fas on the neuronal surfaces [59], and also the formation of immunological synapse between T cells and neurons through MHC-peptide and TCR complex promotes the polarized release of IFN-γ, which would in turn enhance neuronal Fas expression and susceptibility to apoptosis [60]. In this regard, Th17 cells predominantly express Fas-L, and the neuronal apoptosis takes place through Fas/Fas-L interaction between neuron and Th17 cells (Fig. 2) [61]. IL-17 also interferes with re-myelination process, reducing survival and inducing oligodendrocyte apoptosis, the myelin-forming cells [62, 63]. Paintlia et al. have shown that synergistic activity of IL-17 and TNF-α increases oxidative stress-mediated oligodendrocyte apoptosis [63]. Another pathway of neuronal apoptosis that is mediated by Th17 cells is through the oxidative stress. Oxidative stress is the state of imbalance between the level of antioxidant defense

mechanism and secretion of the ROS such as hydrogen peroxide, superoxide free radicals, and nitric oxide [64]. ROS generation is induced by Th17- and Th1-related cytokines and this leads to cytokine-induced oligodendrocyte/neuron apoptosis [63]. ROS also react with cellular macromolecules through oxidation and causes the neurons/oligodendrocyte undergo apoptosis. Inflammation-associated oxidative burst in activated microglia/macrophages has a critical function in demyelination and ROS-mediated CNS injury. Neuroinflammation can trigger oxidative stress by two mechanisms: secretion of ROS by activated microglia and astrocytes and arachidonic acid signaling through the cyclooxygenase pathway [65]. ROS contribute to many mechanisms underlying the pathogenesis of MS. Upon interaction of immune cells such as monocytes with brain endothelium, ROS are produced which leads to BBB tight junction alterations, loss of BBB integrity and subsequent infiltration of leukocytes into the CNS. Furthermore, migratory leukocytes secrete ROS in high levels which induce neuronal and axonal injury [66, 67]. In contrast to the active functions of IL-17 to progression of MS and EAE, some reports have pointed out the negative or minor function of IL-17. IL-17-deficient mice are susceptible to the induction of EAE, and upregulation of IL-17 in murine T cells has no major effect on the development and severity of the disease [68].

Multiple Actions of IL-22, IL-23, and GM-CSF

IL-22 and IL-23 as well as Th17-derived factors play crucial functions in neuroimmune diseases such as MS. The production of IL-22 is induced by IL-23, increases during the peak of EAE, and decreases after neurological recovery [69]. IL-22 is also produced by Th22 cells, another Th cell subset involved in neuroinflammatory diseases [70, 71]. IL-17 together with IL-22 disrupt BBB tight junctions, because BBB expresses IL-17R and IL-22R. Autoreactive Th17 cells migrate toward the CNS through this cavities (Fig. 2) [72]. IL-23 is a member of IL-21 cytokine family, as an inducer of Th17 cell expansion. It helps the Th17 cell survival and maintenance of IL-17 production. Moreover, IL-23 has a role in late-stage differentiation and survival of Th17 cells. Like IL-12, IL-23 is produced by dendritic cells (DC) and other phagocytes. Monocyte-derived DCs secrete and express elevated amounts of IL-23 in MS patients [73]. IL-23R is expressed on the surface of Th17 cells. IL-23 acts as an autocrine factor. Moreover, development of Th17/1 cells is triggered by IL-23 and other inflammatory factors (Fig. 1) [74]. In the absence of IL-23, Th17 cell polarization is inhibited at the early activation stage. This leads to less proliferation and migration of Th17 cells toward the CNS from lymph nodes [75]. An important finding about the role of IL-23 in the pathogenesis of MS is that the anti-IL-23-specific antibody blocks the IL-23 function, leading to the inhibition of acute EAE. Furthermore, anti-IL-23 treatment reduces the serum level of IL-17, as well as CNS expression of IFN-γ, IL-17, IL-6, and TNF-α mRNA [76]. Blockade of IL-23 by monoclonal antibodies can be considered as an effective strategy of MS therapies. GM-CSF is another MS-related Th17-derived cytokine that has recently

gained more attention in the pathogenesis of MS. A recent study has indicated that encephalitogenicity of Th17 cells is dependent on IL-1- and IL-23-induced secretion of the GM-CSF [77]. IL-23 and the transcription factor RORγt drive the expression of GM-CSF in Th17 cells. Autoreactive Th17 cells with lacking GM-CSF fail to initiate neuroinflammation. It has been shown that GM-CSF-producing Th cells with a deficiency of IL-17- and IFN-γ can induce EAE [78]. So, GM-CSF production by Th17 cells is critical for their capacity to induce MS/EAE. Moreover, GM-CSF helps the recruitment of other immune cells such as activated monocytes, macrophages, and granulocytes into the CNS [79].

Open Problems and Therapeutic Applications

Inflammation in the brain parenchyma is primarily induced by Th17 cells rather than Th1 which triggers substantial increase of IL-17. Spinal cord parenchyma inflammation may be caused by a wide range of Th17/Th1 ratio [80]. The number of Th1 cells remains constant in the peripheral blood of active MS patients, but the Th17 cells expand and increase [81]. Research findings pertaining to the importance of Th17 cells and its associated cytokines in the pathogenesis of MS have been confusing and contradictory. There are several studies pointing toward the crucial role of Th17 cells and their hallmark cytokines, IL-17, IL-22, and IL-23, in the pathogenesis of MS. However, other studies have demonstrated a minor role of IL-17 and IL-22 in the development of MS [68, 82]. Several factors such as differences in protocols, strains of mice, and immunization strategies could underlie these controversies. The concept of Th17 cells' role in CNS inflammation, specifically MS, is still a matter of debate.

Nowadays, different approaches directed against Th17 cells and their related cytokines, including IL-17, IL-23, and GM-CSF, have been developed. Some therapies are currently being tested in clinical trials. It has been proposed that IFN-β, one of the first-line MS-modifying agents, inhibits Th17 cell development. IFN-β has been used over the past 20 years as a primary therapy in RRMS, and the effect of IFN-β is multifactorial. IFN-β shifts the dendritic cells to produce anti-inflammatory factors such as IL-27 rather than the pro-inflammatory cytokines IL-1 and IL-23 [83]. Also, IFN-β inhibits IL-17 production and induces IL-10 secretion. IFN-β directly suppresses human Th17 differentiation by inhibition of RORC expression in CD4+ CD45RA+ T cells [84]. FTY720 (Fingolimod) is a sphingosine 1-phosphate (S1P) receptor modulator that has shown efficacy in phase II and III clinical trials in MS patients. FTY720 suppresses lymphocyte egress from lymph nodes and spleen into the peripheral blood circulation. FTY720 reduces IL-17-producing T cells in the blood circulation [85]. The fully humanized neutralizing anti-IL-17A antibody called AIN457 (Secukinumab) (clinicaltrial.gov) is already accepted for the first-line treatment of psoriasis. It showed a reduction by 63% of new MRI lesions compared with placebo-treated MS patients [86]. Ixekizumab is another anti-IL-17 monoclonal antibody with higher potential for the treatment of

MS patients (clinicaltrial.gov). Ixekizumab is also currently tested in psoriasis [87]. Human monoclonal antibody against GM-CSF called MOR103 (clinicaltrial.gov) was tested in randomized phase 1 trial in MS patients. However, it did not show the expected efficacy [88]. The neutralizing antibody of p40 subunit of IL-12 and IL-23 (ustekinumab) (clinicaltrial.gov) has not shown the efficacy in reducing neuroinflammation of MS patients in phase II clinical trial [89, 90]. Specific neutralizing antibodies of p19 subunit of IL-23, such as tildrakizumab, guselkumab, AMG-139, BI-655066, and LY-3074828, have been developed and are currently tested in other autoimmune diseases [86, 91]. Preclinical studies have been also conducted for MS treatment in animal models. Digoxin, a small molecule which binds RORγt, and its derivatives and also a component ursolic acid interfere with the transcription phenomenon leading to the suppression of murine Th17 cell differentiation and also reduction of EAE severity [92, 93]. These representative clinical and preclinical reports could lead to the design and development of new strategies aimed at modulating the immune response in MS.

The Role of Th17 Cells in Immunopathogenesis of Alzheimer Disease

Link Between Immune System and Neurodegeneration

Alzheimer Disease (AD) is a chronic neurodegenerative disorder and the most common form of dementia [94]. It was estimated that approximately 47 million people worldwide were afflicted with AD in 2015 [95]. The pathogenesis of AD is considered to be multifactorial. Accumulation of amyloid plaques in the brain is one of the neuropathological hallmarks [96]. These plaques that are extracellular precipitations of the β-amyloid peptide (Aβ) are composed primarily of amyloid β peptide-40 (Aβ-40) and amyloid β peptide-42 (Aβ-42) derived from amyloid precursor protein (APP) by a proteolytic cleavage [97, 98]. In addition, Aβ oligomer species were found to be fundamental for neuropathogenesis of the AD [99]. Oxidative stress is elevated in the AD and contributes to the pathogenesis and disease progression leading to the inflammatory process and neuronal death [100]. Microglias, the immune cells of the brain are activated by the inflammatory process and upregulated. Microglia produce pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α [101]. Biometal dyshomeostasis and elevated concentrations of some redox-reactive metal ions such as iron and copper ions are also associated with AD. his dysregulated metal homeostasis may contribute to increasing the production of ROS and oxidative stress levels in the brain of AD patients [102]. According to our study, high concentrations of these metals cause morphological changes in β-amyloid aggregates which directly associated with AD neuropathogenesis [102].

The link between the immune system and neurodegeneration in AD is a topic that has recently attracted a lot of attention [103]. Most of the studies are about the role of innate immunity in the AD rather than adaptive immunity, and the precise role of

adaptive immune cells in the neuropathogenesis of AD has not been completely clarified [104]. Even though we will discuss more the role of adaptive immunity especially Th17 cells in the immunopathogenesis of the AD, to the best of our knowledge, there is no sufficient evidence available to suggest autoimmune nature of the AD. It is suggested that Aβ deposits mediate inflammatory mechanisms by activating the complement pathway [105]. In order to develop therapeutic strategies, signaling pathways of the pro-inflammatory cytokines associated with AD should be clarified. Some studies investigated the neurodegenerative and neuroprotective roles of pro-inflammatory cytokines [101]. Inflammatory molecules produced by activated microglia and astrocytes, complement proteins, and ROS produce extracellular signals to neuronal cells, and consequently several intracellular signaling pathways evoked by these extracellular signals to neuronal cells trigger neurodegeneration [106]. High ROS levels within cells can cause nuclear factor kappa B (NF-kB) to induce extra-production of cytokines associated with neuroinflammation [107]. According to several studies, the immune system plays an essential role in responding to AD by increasing level of cytokines, chemokines, and microgliosis in the AD brains [103, 108]. On the other hand, microglial cells phagocyte Aβ-plaques and initiate inflammatory cytokine release. So, they have a protective function because of clearing Aβ aggregates by phagocytosis [107].

Th17-Mediated Mechanisms in Alzheimer Disease

Although the total numbers of CD4+ and CD8+ T cells were not unchanged in AD patients, the amount of circulating immune cells particularly lymphocytes that secrete IL-17, IL-6, and IFN-γ is increased [109]. The role of Th17 cells in the AD has been studied but not entirely clarified completely. In a study by Zhang et al., a rat model of AD was established by Aβ-42 injection into the brain. The study has provided evidence of BBB disruption and Th17 cell infiltration into the brain parenchyma of AD rat model. These events may cause neuroinflammation by releasing inflammatory cytokines such as IL-17 and IL-22. Increased level of these cytokines in the CSF and serum were found [110]. Co-culturing Aβ-specific Th1 or Th17 cells with glial cells resulted in increased MHC-II expression and Aβ-induced cytokine generation. In addition, the expression of co-stimulatory molecules on the microglia was increased when it was co-cultured with Th1 or Th17 cells, because glial cells are antigen presenting cells (APC) for both Aβ-specific Th1 and Th17 cells [111]. The cytokines, specifically IL-21 released by Th17 cells, also bind to their receptors on neurons. IL-21R expression is upregulated in neurons. Cytokines ultimately switch on the apoptotic pathway and lead to neurodegeneration [112]. Kebir et al. have illustrated that Th17 cells migrate through the epithelial cells of BBB by producing IL-17 and IL-22. These cytokines bind to their receptors expressed in endothelial cells of BBB. Cytokines help Th17 cells to disrupt BBB tight junctions and promote their infiltration into the brain in inflammatory diseases [113]. Serum concentration of IL-17 and IL-23 is also elevated in Chinese AD patients [114]. Elevated

levels of IL-1β in the brains of AD mice may induce upregulation of IL-17 [115]. According to a meta-analysis on investigating peripheral blood cytokine level, several pro-inflammatory cytokines such as TNF-α and IL-6 are also increased in AD patients [116]. High level of TNF-α is observed in the CSF of AD patients [117, 118]. Zhang et al. have demonstrated that IL-6 and TNF-α and IL-1β are decreased. The inhibited inflammatory responses might attenuate the neurodegeneration and ultimately improve learning and memory in AD rat models [119]. Not only Th17 cytokines including IL-17 and IL-23 are increased in AD brains, but also the level of transcription factor RORγt is elevated in the brain of AD rats. This is an indication of Th17 cells polarization [120]. In contrast, Treg-related cytokines, TGF-β and IL-35, are decreased in the brain of AD rats [120]. There is an imbalance in Th17/Treg cytokines in the brain of AD rats. Another study has indicated that the concentration of IL-6, IL-21, and IL-23 and also RORγt is remarkably increased in the AD, which is associated with Th17 cell activity [121]. Fas/FasL direct interaction between neurons and the Th17 cell can switch on the apoptotic pathway [110, 122]. Moreover, Fas and FasL expression are significantly upregulated in the brain of AD rats [110]. Marciani et al. have claimed that promising AD vaccines should inhibit Aβ-induced Th1 and Th17 immunities without abrogating them and induce Th2 immunity in response to Aβ. So, it could limit or even prevent neuroinflammation and neurodegeneration [123].

The Role of Th17 Cells in Immunopathogenesis of Parkinson Disease

Immune-Mediated Mechanisms in Parkinson Disease

Parkinson disease (PD) is a long-term progressing neurodegenerative and motor system disorder which is known as a second common inflammatory neurodegenerative disorder after AD [124]. PD presents clinically with tremor, rigidity, impairment of balance, and postural abnormalities [125]. It affects approximately 2–3% of the population of elderly people [126]. The symptoms result from abnormally low dopamine levels in the striatum and consequently profound loss of dopaminergic neurons in the substantia nigra (SN) of the brain [127]. The histopathological hallmark of PD is the existence of intracellular alpha-synuclein (α-syn) in the form of Lewy bodies in susceptible neurons [128]. α-syn and posttranslationally modified α-syn are neuropathologically linked to PD because they are the major components of Lewy bodies [129, 130]. Their presence increase the production of pro-inflammatory cytokines and chemokines, leading to subsequent activation of microglial cells and ultimately infiltration of T lymphocytes into substantia nigra and neuronal death [131]. Upregulated levels of pro-inflammatory cytokines and immune cells are found in CSF and brains of PD patients [132]. Components of both the innate and adaptive immune systems are involved in PD [133]. Microglia

and CD4+ and CD8+ T cells have been found in the brain of postmortem human PD specimens [134]. Although several inflammatory immune cells and pro-inflammatory cytokines are implicated in the neuropathogenesis of PD, the autoimmune nature of the PD has not been demonstrated yet. PD is classified as an inflammatory neurodegenerative disorder. Several subsets of T cells may be involved in the PD-associated neuroinflammation [135]. Each T cell subpopulation may have distinct impact, in particular, in terms of cytotoxicity on the neuronal cells in the brain of PD patients [135]. Various immunotherapeutic approaches should be developed in attempt to treat PD. Data on the neuroprotective and neurotoxic effects of T cells would be really helpful in for this approach [135]. According to the microarray studies conducted by Hu et al., Th17 cells, among all Th cell subsets, have a leading role in PD [136]. Experimental models have offered insights into the understanding the role of Th17 cells in neurodegeneration in the PD [137].

Th17 Cell Role in Parkinson Disease

PD is characterized by some autoimmune features against α-syn. Th17 cells might have an underestimated role in the etiology and immunopathogenesis of the PD [136]. Based on recent findings regarding upregulation of some of the effector molecules in PD, it has been suggested that Th17 cell immunological pathway is switched on in PD [136]. The number of Th17 cells may be different in normal conditions and in PD. However, Peng et al. posited that Th17 cells can exist in some specific regions of the brain even in the healthy brain [138]. Still, increased proportion of Th1 and Th17 cells have been reported in the PD patients [139]. Engagement of the Fas receptor by its Fas ligand would trigger neuronal apoptosis by a direct contact between Th17 cells and neurons, which results eventually in cell death [140]. Niwa et al. have shown that Th17 cells are not predominant in PD, without increased quantity in PD [141]. Pro-inflammatory cytokines released by Th17 cells bind specifically to their receptors on neuronal surfaces and transduce their apoptotic signals [112].

IL-17-producing T cells could trigger production of inflammatory cytokines such as IL-1β, IL-6, and TNF-α, ultimately make up and promote both inflammatory reactions and neuronal apoptosis [135]. High levels of inflammatory cytokines, IL-1, IL-6, and TNF-α, have been also detected in serum, CSF, and brain of PD patients [136]. IL-6 and TNF- α produced by Th17 cells might also promote cytokine/chemokine secretion in an autocrine manner [136]. According to several studies, the pro-inflammatory cytokines such as IL -1, IL-6, and TNF-α, are key cytokines that shape the development of Th17 cells [142, 143]. There is an association between overexpression of IL-1 and microglial activation which induces upregulation of α-syn in PD [144]. Also, the presence of Th1 and Th17 lymphocytes upregulates the secretion of free radicals (NO, superoxide, and hydrogen peroxide) from microglia, as well as inflammatory cytokines (IL-1, TNFα). This results in neuronal damage [135]. IL-1, TNFα, and IL-6 produced by Th17 cells

can induce neural cells to go through Wallerian-like degeneration which may occur in many neurodegenerative diseases [136]. The aggregates of α-syn increases the production of molecular chaperones by neurons such as heat shock protein (HSP)-60 and HSP70 which ultimately induce cytokines such as IL-6 and TNF-α by triggering Toll-like receptors (TLR)-2, 4, 5, and 7 [136, 145]. Approximately, all HSPs are overexpressed in the brain of PD patients which can be recognized by upregulated TLR5 and TLR7 which induce Th17 cell immunity [136]. Not only both IL-17 and TGF-β receptors in Th17 cells are upregulated in PD, but also several Th17 cell-driven transcription factors such as CCAAT/enhancer-binding protein (CEBP)-β, CEBP-γ, and RORα are overexpressed in leukocytes of PD patients which are associated with Th17 cell immunopathogenesis [136]. The expression of RORγt which is the master transcription factor of IL-17 expression and Th17 cells has also been enhanced by pro-inflammatory cytokines IL-1 and IL-23 [146] promote inflammation. The presence of IL-32 suppresses NF-kB and STAT3 expression. Downregulation of IL-32 may induce expression of Th17-related transcription factors NF-kB and STAT3 in PD [136]. Furthermore, upregulation of other effector molecules associated with Th17-related inflammations such as iNOS and cyclooxygenase has been reported in the brain of PD patients, which could switch on inflammatory pathways and ultimately lead to irreversible neural damage in PD [147].

The Role of Th17 Cells in Immunopathogenesis of Schizophrenia Disease

Immunopathology of Schizophrenia Disease

Schizophrenia is a multifactorial mental disorder characterized by neurodevelopment impairment and neurodegeneration after illness onset [148, 149]. Although the etiopathology of schizophrenia is largely unknown, evidence powerfully indicates a major role of inflammation and immunity in the pathogenicity of schizophrenia. Schizophrenia could be considered to be a chronic inflammatory disease of the brain [150]. Recent studies demonstrate an immune system dysfunction such as alterations in the expression of immune-related cytokines in the brain and CSF of schizophrenia patients [151–155]. However, the etiopathology of the disease and the role of immune system in the pathogenicity of schizophrenia are still unknown [150, 156]. There is no clear evidence to indicate the primary autoimmune nature of the schizophrenia. According to a recent meta-analysis performed by Kesteren et al., the overall increase in gene expression and protein transcription level of pro-inflammatory cytokines is observed in patients. However, the levels of transcription and translation of anti-inflammatory cytokines are similar in schizophrenia and controls [157]. Immune system abnormality and dysfunction in nervous system might have a central role in schizophrenia development [158]. Neuroinflammation

in CNS is evident in schizophrenia patients and [155, 159]. Overexpression of some inflammatory genes and schizophrenia-associated upregulated immune markers indicates an association between schizophrenia and neuroinflammation [160, 161]. According to a genome-wide expression analysis by Gardiner et al., there is a significant dysregulation of immunological pathways in schizophrenia [162] and specific cytokines in the peripheral blood of schizophrenia patients have been considered as trait markers which could provide insight into the exact role of immune system in the disease [163]. Impaired BBB and infiltration of T cells and B cells, and microglial activation may be associated with the disease pathophysiology [163]. Several studies have explored the roles of both innate and adaptive immunities [150, 164–167]. Alteration and disruption in dopaminergic signaling and population of peripheral immune cell have been observed in some schizophrenia patients [168]. Dopamine abnormalities have been reported in specific regions of the brain in schizophrenia patients. Dopamine is a neurotransmitter that has an important role in the risk and progression of this disorder. Dopamine is involved in the modulation of T cells trafficking and proliferation [150].

Immunopathology of Schizophrenia from Th17 Cell Viewpoint

The role of Th17 cells in the pathogenesis of schizophrenia has also been investigated [169–171]. Drexhage et al. have emphasized the role of activated T cell network in schizophrenia [171]. Th17-associated pro-inflammatory cytokines such as IL-17 and IL-22 would disrupt the BBB and help Th17 cell infiltration, which may lead to neuroinflammation and neurodegeneration in schizophrenia [53, 170]. CCL20 chemokine is constitutively expressed by epithelial cells of choroid plexus, and its receptor CCR6 on Th17 cells facilitates Th17 cell infiltration into intact or inflamed CNS. This interaction appears to be actively involved in the role of immunity in schizophrenia [172]. IL-23 may also induce BBB disruption which helps the Th17 cell to enter the brain parenchyma [173]. Nevertheless, several reports have pointed out contradictory results about the role of Th17 cells in schizophrenia. Some studies have shown increased quantities of Th17 cells in schizophrenia, but others have underlined decreased levels of these cells. According to one study, the number of Th17 cells and the level of IFN-γ and IL-6 are higher in schizophrenia patients in comparison with healthy controls [169].

Th17 cells can cause microglial activation and production of IL-1β, TNF-α, and IL-6 locally [170]. By contrast, a report by Borovcanin et al. has demonstrated that the level of IL-17 and the ratio of IL-17/TGF-β and IFN-γ are decreased in schizophrenia patients. Elevated level of Th17-suppressing cytokines including IL-4 and IL-27 has been observed [174].The concentration of IL-17 might decrease in patients with chronic antipsychotic medication rather than healthy controls [175]. Elevated levels of IL-6 and TNF-α have been described [176–178]. Overall, little is known about the precise role of Th17 cells and related pro-inflammatory cytokines in the immunopathogenesis of schizophrenia. Further investigations are clearly required.

Conclusion

Although emerging studies have demonstrated the roles of Th17 cells and their related cytokines in the pathogenesis of MS and other neuroinflammations such as AD, PD, and schizophrenia, our knowledge is still poor in this area. One major reason is that the pathophysiology of these neuroinflammations is multifactorial. Th17 cells are not the unique factor determining disease severity. Some therapeutic agents that target Th17 cells have not reduced neuroinflammation. On the other side, many reports have indicated the critical functions of Th17 cells in disease promotion. The specific targeted-therapy of Th17 cells by chemicals and monoclonal antibodies may regulate the neuroinflammation. Nowadays, the concept of Th17/Treg interplay and Th17/1 cells and the existence of anti-inflammatory Th17 cells (Treg-like Th17 cells) in different environments extend our knowledge on the concept that Th17 cells are more complex than other Th cell subpopulations. Some studies report that the Th17 cells are more unstable than Th1 cells and the conversion of Th17 cells to the Tregs are more frequent. Hence, Th17/Treg interplay is currently an amazing research area. Th cells plasticity and their conversion from inflammatory to anti-inflammatory subsets occur via cytokines and other stimuli such as accessory immune cells and inflammation niche.

During the recent years, the issue of cell therapy in autoimmune and neuroimmunological diseases is highlighted. Pro-inflammatory cytokines (IL-1β, IL-6, and IL-23; the cytokines necessary for Th17 cell differentiation) upregulate the immunomodulatory functions of mesenchymal stem cells (MSCs) [179]. MSCs are a population of adult stem cells with immunoregulatory properties and immune-suppression functions that could be used in cell-based therapy of degenerative and neuroinflammatory disorders. MSCs through production of anti-inflammatory cytokines such as TGF-β and cell-cell contact manner inhibit human Th1 and Th17 cell differentiation (unpublished data). MSCs induce Treg cells during differentiation process of Th1 or Th17 cells. MSCs as immunomodulatory stem cells can be used for cell therapy of autoimmune disorders such as MS and other neuroimmune diseases.

In summary, the role of Th17 cells as pathogenic inflammatory lymphocytes has been demonstrated in the pathogenesis of neuroinflammation, especially MS and other neurological immune-mediated disorders. There are many Th17-mediated mechanisms in the pathophysiology of these diseases such as promotion of inflammation through production of pro-inflammatory cytokines, enhancement of other immune cells migration into the CNS and causing neurodegeneration through induction of microglia activation and neuronal apoptosis. However, in spite of these findings, there are numerous unknown aspects of the Th17 cells properties and their role in immune-mediated inflammations, exclusively neuroimmune diseases. Further research is needed.

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Significance of Autoantibodies

Christiane S. Hampe

Abstract The field of autoimmune neurological disorders is rapidly expanding, and novel autoantibodies and their neuronal antigens continue to be discovered. Autoimmunity targeting brain proteins is enigmatic, because traditionally, the central nervous system (CNS) is viewed as immune-privileged. However, the discovery of the lymphatic and glymphatic circulation in the CNS demonstrates the interaction between the CNS and the immune response. Furthermore, the barriers protecting the brain from direct exposure to the immune response can be compromised by inflammations, infection, or injury. A compromised blood–brain barrier, or blood–cerebrospinal fluid barrier, will allow egress of neuronal antigens to regional and peripheral lymphoid organs and may lead to the initiation of an autoimmune response. Peripheral autoantibodies or intrathecally produced autoantibodies can reenter the CNS. Besides being useful diagnostic markers, these autoantibodies may be involved in the pathogenesis of the disease by mechanisms such as complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), and modulation of receptor function. The effect of a neural autoantibody depends not only on the nature of its antigen but also on the antibody's Ig isotype or IgG subclass. We will discuss different causes of neurological autoimmunity and pathogenic mechanisms involved in neurological autoimmune diseases. Finally, we will discuss naturally occurring IgM autoantibodies and IgG4 autoantibodies with protective and reparative functions and appropriate treatment options.

Keywords Autoantibodies · Blood–brain barrier · Blood–cerebrospinal fluid barrier · Neurological disorders

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_4

Abbreviations

AChR	Acetylcholine receptor
ADCC	Antibody-dependent cell-mediated cytotoxicity
AEBP1	Adipocyte enhancer-binding protein-1
AMPAR	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic glutamate receptor
AQP4	Aquaporin-4
ASD	Autism spectrum disorder
BBB	Blood–brain barrier
CA	Cerebellar ataxia caspr: contactin-associated protein 1
CDC	Complement-dependent cell death
CDR2L	Cerebellar degeneration-related protein 2-like
CIDP	Chronic inflammatory demyelinating polyneuropathy
CNS	Central nervous system
CRMP	Collapsing response mediator protein
CSF	Cerebrospinal fluid
GABA	Gamma-aminobutyric acid
GAD65	65kda isoform of glutamate decarboxylase
GBS	Guillain–Barré syndrome
GFAP	Glial fibrillary acidic protein
hnRNP-A1	Heterogeneous nuclear ribonuclear protein-A1
HSV-1	Herpes simplex virus-1
HSVE	HSV encephalitis
LDH	Lactate dehydrogenase
LEMS	Lambert–Eaton myasthenic syndrome
LRP4	Lipoprotein receptor-related protein 4
MAC	Membrane attack complex
MAG	Myelin-associated glycoprotein
MAR	Maternal autoantibody-related autism
MBP	Myelin basic protein
MG	Myasthenia gravis
MOG	Myelin oligodendrocyte glycoprotein
MS	Multiple sclerosis
MuSK	Muscle-specific kinase
MYEOV2	Myeloma overexpressed gene 2
NMDAR	N-Methyl-D-aspartate glutamate receptor
NMDARE	NMDAR encephalitis
NMJ	Neuromuscular junction
NMO	Neuromyelitis optica
PCD	Paraneoplastic degeneration
PEM	Paraneoplastic encephalomyelitis
PNS	Paraneoplastic neurologic disorder
PSMD4	Proteasome non-ATPase regulatory subunit 4
RE	Rasmussen's encephalitis

SCI	Spinal cord injury
SCLC	Small-cell lung cancer
SGPG	Sulfoglucuronosyl paragloboside
SLE	Systemic lupus erythematosus
SPS	Stiff person syndrome
STIP1	Stress-induced phosphoprotein 1
SUMPPs	Small myelin protein-derived peptides
TBI	Traumatic brain injury
TG	Transglutaminase
VGCC	Voltage-gated calcium channel
VGKC	Voltage-gated potassium channel

Introduction

The central nervous system (CNS) has long been viewed as immune-privileged, referring to an absence of an interaction between the immune response and the CNS [1, 2]. This view has however been challenged [3, 4], and today the consensus is that while there is considerably less interaction between the CNS and the immune response as compared to other organs, the immune privilege is not absolute. Particularly, there are areas of the brain that are less immune-privileged, including the ventricles and the meninges. In these regions, B cells can produce antibodies, from where the latter may diffuse to the parenchyma [4]. Inflammatory conditions lead to a dramatic increase of leukocyte numbers in the cerebrospinal fluid (CSF) [5], and B cells can make up 30% of the cell population residing within the CSF [6–8]. Notably, the migration of leukocytes into the CSF occurs across the choroid plexus, while migration from the blood into the parenchyma involves the blood–brain barrier (BBB). Because the BBB is more stringent than the blood–CSF barrier [9], most of the intrathecal antibody production stems from B cells residing in the CSF.

In addition to intrathecal antibody production, antibodies may also gain access to the brain by crossing the BBB. In humans, the BBB is maturing during fetal development [10], potentially allowing access antibodies and other components of the immune system to the fetal brain during early fetal development. Such a scenario has been suggested in a subset of autism spectrum disorders (see below). Even after the BBB is fully established, specific circumstances can allow the passage of large molecules, including antibodies [11]. These conditions include stress, trauma, infection, and inflammation [12–15] and more severe damage caused by traumatic brain injury or stroke [16, 17]. Even under regular conditions, the BBB is permeable to systemic circulating antibodies to some extent [18].

A compromised BBB allows increased access of immune cells and antibodies to the CNS and at the same time an increased egress of autoantigens from the CNS to secondary lymphoid tissue via blood and/or primitive lymphatics [19, 20]. Exposure of antigens to B cells residing in the CSF can initiate an autoimmune response within the brain, leading to intrathecal antibody production, while exposure of antigens to

the peripheral immune system may trigger the peripheral immune system to react to CNS autoantigens, leading to the production of CNS-specific autoantibodies. These autoantibodies may gain access to brain tissue as discussed above. Intrathecal autoantibody production is indicated by the presence of oligoclonal bands in the CSF, although these are not always present. Typically, these autoantibodies recognize different antigens and/or antigen epitopes than autoantibodies present in the periphery. CNS autoantibodies may have a pathogenic effect and lead to neurological diseases or may simply serve as biomarkers for the associated disorder.

Theme A: Causes of Antibody Development

In the following section, we will discuss different triggers of development of autoantibodies directed against neuronal proteins and highlight each mechanism with a clinical example.

Paraneoplastic Neurologic Disorders (PNS)

In rare cases, cancer can trigger *paraneoplastic neurologic disorders* (PNS). In these disorders, the neurological symptoms are not caused by the tumor itself but rather by the immune system's response to the cancer. The neuronal target antigens are expressed by the tumor cell, and exposure of these proteins may initiate an autoimmune response. Thus, the associated autoantibody is often specific to the associated cancer. PNS typically develop in individuals with lung, ovarian, lymphatic, or breast cancer. As the neurological symptoms often occur prior to tumor detection, the diagnosis may aid in the identification of the underlying cancer. Examples for PNS are listed in Table 1.

Clinical Example

Anti-Yo Antibody-Associated Paraneoplastic Cerebellar Degenerations

Anti-Yo antibodies are present in patients with paraneoplastic cerebellar degenerations (PCD) associated with ovarian or breast carcinoma [21]. These antibodies recognize cytoplasmic proteins that are expressed in Purkinje cells in the cerebellum [22]. Importantly, at least one of these proteins (cerebellar degeneration-related protein 2-like, CDR2L) is found overexpressed in the majority on anti-Yo-positive ovarian carcinomas associated with PCD [23]. In these tumors, genetic alterations of the *CDR2L* gene and massive infiltration by immune cells can be observed [23–26]. Further studies suggest that the overexpression of CDR2L triggers an autoimmune response, possibly through the expression of neo-epitopes [27]. This

Table 1 Examples for PNS, associated autoantibodies, and associated cancer

Antigen	Neurological disorder	Cancer	References
Hu	Encephalitis, PCD, subacute sensory neuropathy	SCLC	[200]
CV2/CRMP5	Chorea, optic neuritis, PEM, peripheral neuropathy	SCLC, thymoma	[201]
Ma	Limbic, brainstem, and hypothalamic encephalitis	Testicular tumors	[202]
Yo	Cerebellar degeneration	Carcinoma of the ovary, breast, or uterus	[21]
Ri	Opsoclonus–myoclonus, PCD	Breast carcinoma, lung carcinoma	[203]
Amphiphysin	Stiff person syndrome, PEM	Breast carcinoma, SCLC	[204]
Glycine receptor	PCD	Lung carcinoma	[205]
VGCC	Lambert–Eaton myasthenic syndrome +/− cerebellar degeneration	SCLC	[206, 207]
Muscle AChR	Myasthenia gravis	Thymoma, SCLC	[208, 209]
Neuronal AChR	Autonomic neuropathy	Thymoma, SCLC	[210]
NMDAR	Anti-NMDAR encephalitis	Ovarian teratoma	[211]
AMPAR	Limbic encephalitis, atypical psychosis	SCLC, thymoma, breast cancer	[212]
GABA(B) receptor	Limbic encephalitis	SCLC	[213]
Anti-retinal bipolar cell	Retinopathy	Melanoma	[214]
LGI1	Limbic encephalitis	Thymoma, SCLC	[215]
Caspr2	Encephalitis, Morvan syndrome, and acquired neuromyotonia (Isaacs syndrome)	Thymoma	[215]
Anna-3	Cerebellar ataxia, limbic encephalopathy	Lung carcinoma, upper airway carcinoma	[216]

Abbreviations: AChR, acetylcholine receptor; AMPAR, α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic glutamate receptor; NMDAR, N-methyl-D-aspartate glutamate receptor; PCD, paraneoplastic degeneration; PEM, paraneoplastic encephalomyelitis; SCLC, small-cell lung cancer; VGCC, voltage-gated calcium channel

autoimmune response is characterized by the presence of anti-Yo antibodies in both the periphery and the CSF. Anti-Yo antibodies in the cerebellum can be taken up by Purkinje cells [28–30], where they induce cell death [28, 29]. These studies suggest that anti-Yo antibodies play a direct role in the pathogenesis of cerebellar injury. The exact mechanism involved in the cell death is not yet fully understood. Studies by Schubert et al. suggest a perturbation of neuronal calcium homeostasis as a mechanism for anti-Yo cytotoxicity [27], while Hida et al. suggest that Purkinje cell death might be caused by an interference with protein synthesis [31], and yet other studies indicate that the antibodies may interfere with CDR2's interaction with c-Myc and subsequent disruption of c-Myc cytoplasmic pathways leading to accelerated neuronal apoptosis [32].

Infection

While in PNS the originating antigen is a neuronal autoantigen expressed by the tumor, in neuronal autoimmune disorders triggered by infections, the initial trigger of the immune response can also be a foreign antigen. Examples for infections that trigger neuronal autoimmune diseases include *Campylobacter jejuni*, *Streptococcus*, *Mycoplasma pneumonia*, and herpes simplex virus (Table 2). Infections may trigger autoimmunity through several mechanisms including molecular mimicry, bystander activation, presentation of cryptic antigens, or epitope spreading [33]. In *molecular mimicry*, the pathogen has properties similar to a self-antigen, and antibodies originally formed against the pathogen may be cross-reactive to the self. In *bystander activation*, the inflammatory environment evoked by the infection can activate autoimmune cells. Infection may also lead to the processing and presentation of "*cryptic antigens*," leading to the development of autoimmunity toward these epitopes that are usually hidden from the immune response [34]. Lastly, in *epitope spreading,* the infection damages host cells, leading to the release of self-antigen, which may trigger an autoimmune response. Examples for infections triggering neuronal autoimmunity are listed in Table 2.

Table 2 Examples of neurological disorders associated with infectious agents

Infectious agent	Autoantigen	Neurological disease	References
Campylobacter jejuni, *Haemophilus influenzae*	Myelin, ganglioside GM1, tubulin, GFAP	Guillain–Barré syndrome	[217–219]
Human T-lymphotropic virus type 1 (HTLV-1)	hnRNP-A1	HTLV-1-associated myelopathy/tropical spastic paraparesis	[220]
Plasmodium falciparum	VGKC	Post-malaria neurological syndrome	[221]
HSV	NMDAR	Acute encephalitis (NMDAR)	[36]
Streptococcus pyogenes	Lysoganglioside, dopamine D2 receptor, tubulin	Sydenham's chorea	[222, 223]
Multiple viruses	multiple	MS	[224, 225]
Borrelia burgdorferi	MBP	Myelitis and peripheral neuropathy	[226, 227]
AS03-adjuvanted pH1N1 influenza vaccination, *Streptococcus pyogenes*, seasonal influenza A infections	Ganglioside GM3	Narcolepsy	[228–230]
Treponema pallidum subsp. *pallidum*	Phospholipid	Neurosyphilis	[231, 232]

Abbreviations: GFAP, glial fibrillary acidic protein; hnRNP-A1, heterogeneous nuclear ribonuclear protein-A1; MBP, myelin basic protein; NMDAR, N-Methyl-D-aspartate glutamate receptor; VGKC, voltage-gated potassium channel

Clinical Example

Herpes Simplex Virus-1 (HSV-1) Encephalitis and NMDAR Encephalitis

HSV-1 infections of the brain may cause HSV encephalitis (HSVE) either during the primary infection or after a reactivation of a latent virus [35]. In rare cases, relapsing neurologic symptoms occur in the absence of a reactivation of the virus. In these cases, an autoimmune-mediated etiology is suggested, supported by the presence of autoantibodies to the N-methyl-D-aspartate glutamate receptor (NMDAR) [36]. Upon binding of glutamate to the glutamate binding site, NMDAR ion channels open and allow the passage of cations across the membrane, causing a depolarization of the neuron. NMDARs are involved in processes of synaptic plasticity, critical in learning and memory. NMDAR antibody-positive encephalitis (NMDARE) is the most common antibody-associated acute autoimmune encephalitis (210). NMDAR autoantibodies are present both in the periphery and in the CSF of affected individuals (212–214) and recognize an extracellular, conformation-dependent epitope region at the GluN1 subunit of the NMDAR. Binding of the autoantibody does not interfere with glutamate binding, but cross-links NMDAR, thereby initiating the internalization of the receptor. Reduced NMDAR density on the neuronal surface results in neuronal dysfunction [37] (see also section "Modulation of Receptor Function"). This process is reversible after removal of autoantibodies and may explain the good recovery of patients after immunotherapy [38]. Pathogenicity of the NMDAR autoantibodies has been established in passive transfer experiments, where CSF of patients with NMDAR-E was intraventricularly infused into the mice brain, causing a decrease in NMDAR density (233).

The mechanisms by which HSV-1 infection induces NMDAR antibodies are unclear. In cases where the initial HSV-1 infection affected the CNS, the development of NMDAR immunoreactivity may result from the release of neuronal antigen during the HSV-1-mediated brain injury that exposes NMDAR to the immune system [36]. However, a recent study of young patients with NMDARE without a clinical history of HSVE showed increased frequencies of circulating HSV-1 antibodies, suggesting a previous non-encephalitic HSV-1 infection. Based on these findings, non-encephalitic HSV-1 infections may trigger NMDAR antibody formation via molecular mimicry in the periphery [39].

Injuries to the CNS

Brain traumas including spinal cord injury (SCI) and traumatic brain injury (TBI) cause cell death and a compromise to the BBB integrity. In the first hours following the trauma, the immune response is focused on the recognition and removal of injured neurons. This initial immune response is characterized by the release of cytokines and development of neuroinflammation. This inflammatory milieu weakens the BBB, allowing the leakage of neuronal proteins into the blood and increased

leakage of brain interstitial fluid into the CSF [40, 41]. Thereby, brain antigens may enter cervical lymph nodes and other peripheral immune organs [20, 42–44] and potentially triggering an autoimmune response [45, 46]. Autoantibodies are a characteristic component of this autoimmune response [47] and can serve as biomarkers when they are correlated to injury severity [48, 49]. Examples of autoantibodies associated with injuries to the CNS are listed in Table 3.

Clinical Example

Long-Term Neurological and Systemic Complications in SCI Patients

After the initial SCI, the injured site is infiltrated by peripheral immune cells, and although the ensuing neuroinflammation is part of the natural healing process, it can also damage the neural tissue [50] and trigger a long-lasting neuroinflammation with a significant humoral involvement [51, 52]. The presence of B cells and autoantibodies in SCI-associated neuroinflammation is facilitated by the specific inflammatory milieu seen in SCI [53, 54].

B-cell-deficient mice show enhanced functional recovery after SCI, emphasizing the importance of B cells in complications following SCI [52]. Moreover, passive transfer of antibodies purified from SCI mice to the spinal cord of wild-type mice induces neurotoxicity similar to that observed in mice with SCI [52]. The targets of autoantibodies in SCI include a large number of CNS proteins (Table 3). The antibodies accumulate in the injured spinal cord and co-localize with astrocytes and neurons during the subacute phase of injury [51]. Mechanistically, B cell and their autoantibodies appear to be involved in mediating axonal and myelin pathology, involving complement activation and recruitment of FcR-bearing immune cells [52]. Other studies in humans show that autoantibodies against GM1 gangliosides may prevent functional recovery by inhibiting GM1 function.

Interestingly, the immune response induced by SCI is dependent on the level of the injury. While injuries at the mid-thoracic level induce the above pathogenic B-cell-mediated immune response [51], injuries at higher levels induce a profound

Table 3 CNS injuries and associated autoantigens

CNS injury	Autoantigen	References
TBI	S100B, phospholipids, AMPAR and NMDAR, GFAP	[48, 49, 233–238]
SCI	Galactocerebroside, MAG, AMPAR and NMDAR, tubulin, MBP, GFAP, GM1 ganglioside, S100B, PSMD4, AEBP1, and MYEOV2	[239–247]
Stroke	Neurofilaments, NMDAR, MBP, and S100β	[248, 249]

Abbreviations: AEBP1, adipocyte enhancer-binding protein; AMPAR, α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic glutamate receptor; GFAP, glial fibrillary acidic protein; MAG, myelin-associated glycoprotein; MBP, myelin basic protein; MYEOV2, myeloma overexpressed gene; NMDAR, N-methyl-D-aspartate glutamate receptor; PSMD4, proteasome non-ATPase regulatory subunit 4

immune suppression and diminished B-cell activation [55]. This may be caused by a disruption of sympathetic innervations of the lymphoid tissue [56, 57]. This disruption is referred to as SCI-induced immune deficiency syndrome (SCI-IDS) [58, 59], which may contribute to the increased susceptibility of SCI patients to infections.

Finally, autoantibodies directed against brain antigen can also be found in the CSF and sera of patients following a stroke [60, 61]. Examples of neuronal proteins targeted in stroke patients are listed in Table 3. The clinical relevance of these autoantibodies is uncertain, but specific autoantibodies such as NMDAR may have pathogenic effects as described below.

Transfer of Maternal Antibodies to the Fetal Brain

The passage of maternal IgG antibodies across the placenta to the fetus usually provides passive protection for the newborn child [62]. Until recently, it was believed that the fetal BBB is not fully mature, allowing access of maternal IgG to the fetal brain. However, this concept has since been disputed, and the BBB appears to be fully functional already at week 12 of gestation [63]. Animal studies in developing mice fetuses demonstrated that maternal antibodies are present in the fetal brain very early during development but that this transfer is progressively limited in later stages of gestation [64]. Thus, maternal antibodies (including autoantibodies) appear to have access to the fetal brain during the early stages of fetal development.

Well-established examples of pathogenic autoantibodies present in pregnant women that may impact fetal development and cause CNS abnormalities are acetylcholine receptor autoantibodies in myasthenia gravis [65] and NMDAR autoantibodies in mothers with systemic lupus erythematosus (SLE) [66]. Unless the autoantibody persists or causes developmental defects, the neurological symptoms in the newborn are transient. These different scenarios and the involved autoantigens are listed in Table 4.

Recently, a role of maternal autoantibodies in the fetal brain development has been discussed in the development of autism spectrum disorder (ASD).

Table 4 Transfer of maternal pathogenic autoantibodies

Neurological disorder	Autoantigens	References
Neonatal MG	AChR, MuSK, LRP4	[96]
Neonatal lupus erythematosus	Ro/SSA, La/SSB	[250–252]
ASD	Lactate dehydrogenase A and B, cypin, stress-induced phosphoprotein 1, collapsin response mediator proteins 1 and 2, and Y-box-binding protein	[72]

Abbreviations: AChR, acetylcholine receptor; LRP4, lipoprotein receptor-related protein 4; MuSK, muscle-specific kinase

Clinical Example: ASD

The observation of an association of autism and maternal autoantibodies was made already in 1990 [67]. This association between autism and autoimmunity is further supported by a higher frequency of autoimmune diseases of mothers with children with ASD [68].

Autoantibodies directed against brain protein can be detected in children with ASD and their mothers [69–71]. So far, seven primary antigens associated with maternal autoantibody-related (MAR) autism have been identified as lactate dehydrogenase A and B (LDH), cypin, stress-induced phosphoprotein 1 (STIP1), collapsin response mediator proteins 1 and 2 (CRMP1, CRMP2), and Y-box-binding protein [72]. These proteins are expressed at significant levels in the human fetal brain and have established roles in neurodevelopment [73, 74]. In all, a total of 23% of mothers of children with ASD had autoantibodies directed to two or more of the target proteins [72]. In animal studies, passive transfer of these autoantibodies induced altered ASD-associated behavior, thus indicating that maternal autoantibodies may be of pathogenic significance related to the occurrence of ASD [75, 76]. Notably, different combinations of these autoantibodies are found in different phenotypes of ASD [77–80]. The mechanisms by which these autoantibodies are involved in the pathogenesis of ASD are currently under investigation.

Primary Autoimmune Disorders

In other cases of neurological autoimmune disorders, the trigger of the autoimmune response is unknown; these are primary, or idiopathic, autoimmune disorders. While many of these diseases can also be triggered by the above-discussed pathways, in the absence of a clinical history of infection, cancer, or CNS injury, an idiopathic etiology is assumed. Different neurological autoimmune disorders with unknown trigger are listed in Table 5.

Table 5 Neurological autoimmune disorder without known triggers

Autoimmune disease	Autoantigen	References
MS	Synapsin 1, MOG, MAG, neurofascin and contactin-2, potassium channel (KIR4.1)	[253–260, 261, 262]
Neuropsychiatric SLE	Alpha-internexin, phospholipid, ribosomal P protein, NMDAR, ganglioside M1, GABA receptor	[263–266]
Amyotrophic lateral sclerosis	Desmin, LRP4, VGCC l-type, Fas receptor, GM1 ganglioside, SGPG	[267–272]
Stiff person syndrome	GAD65, amphiphysin, GABA receptor, gephyrin	[83, 273–275]

Abbreviations: LPP4, LDL receptor-related protein 4; MAG, myelin-associated glycoprotein; MOG, myelin oligodendrocyte glycoprotein; NMDAR, N-Methyl-D-aspartate glutamate receptor; SGPG, sulfoglucuronosyl paragloboside; VGCC, voltage-gated calcium channel

Clinical Example

GAD65Ab in Stiff Person Syndrome

Stiff person syndrome (SPS) is an autoimmune CNS disease characterized by progressive muscle stiffness, trigger-induced spasms, spinal deformity, and autoantibodies directed against neuronal antigens, including the smaller isoform of glutamate decarboxylase-65 (GAD65) [81–83]. GAD65 is one of two enzymes that convert glutamate to gamma-aminobutyric acid (GABA). GAD65 is also found in non-neuronal tissues such as the beta cells of pancreatic islets, testes, and oviducts. The physiologic role of GAD65 in these non-neuronal tissues is unclear.

GAD65Ab are found both in the periphery and the CSF of SPS patients [84], and an active intrathecal immune response is suggested by the presence of oligoclonal bands and epitope-specific GAD65Ab in the CSF [85]. The pathophysiology of SPS includes decreased concentrations of GABA in the brain and CSF [86]. Initially, a pathogenic role for GAD65Ab was disputed based on the intracellular location of GAD65, but uptake of GAD65Ab by Purkinje cells present in the cerebellum was demonstrated both in vivo and in vitro [87–89]. GAD65Ab may interfere with one or both roles of GAD65 in GABAergic neurotransmission, namely, the synthesis of GABA from glutamate and the axonal transport of GABAergic synaptic vesicles to the synaptic cleft [90, 91]. GAD65Ab in SPS interfere with both GAD65 enzyme activity [84] and the association of GAD65 with the cytosolic side of synaptic vesicles, necessary for the axonal transport [88], resulting in a decrease in vesicular GABA contents with low release probability [88]. This interference with GABAergic neurotransmission is consistent with the motor hyperexcitability, changes in behavior, and cognitive operations observed in animals intrathecally injected with GAD65Ab [88, 92, 93].

Theme B: Antibody Effector Mechanisms

In the following, we will discuss the different mechanisms by which antibodies exert their physiological effects in neurological disorders with relevant clinical examples (Fig. 1).

Complement-Dependent Cytotoxicity (CDC)

The deposition of immune complexes consisting of autoantibodies and autoantigens can trigger inflammatory responses through activation of complement [15]. There are three distinct complement pathways: the classical pathway, the lectin pathway, and the alternative pathway. Each of these pathways leads to the generation of anaphylatoxins (C3a and C5a), opsonins (C3b and C3d), and the terminal membrane

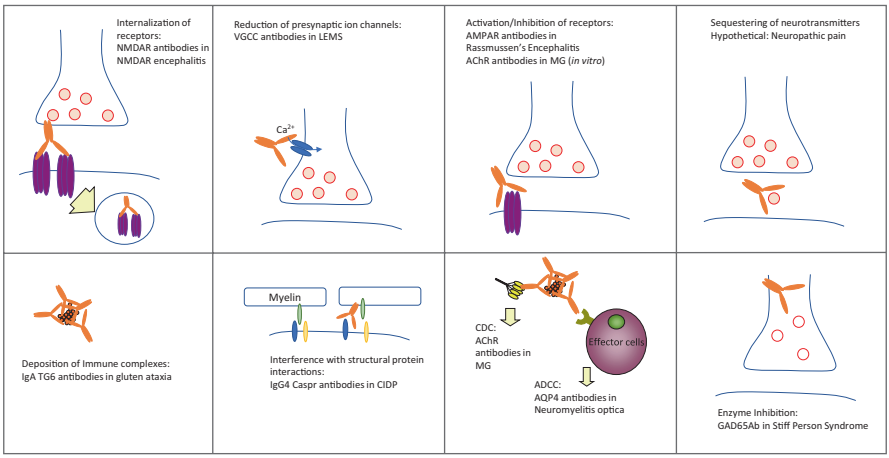

Fig. 1 Autoantibody-mediated effector mechanisms in neurological disorders. Antibodies mediate neurological dysfunction via different pathways
They can:

Cross-link adjacent receptors, facilitating their endocytosis and degradation, and inactive ion channels, thereby reducing the amplitude of the incoming signal

Activate or inactivate neurotransmitter receptors,

Sequester neurotransmitters in the synaptic cleft and thereby reduce the transmitted signal

Form immune complexes that are deposited

Interfere with the interaction of proteins in protein complexes

Bind and initiate the alternative complement pathway leading to cell lysis

Activate effector cells via binding of Fc receptors, inactive enzymes involved in neurotransmission

attack complex (MAC, C5b-9). Anaphylatoxins are proinflammatory molecules that attract and activate leukocytes, opsonins bind to antigen and thereby label it for phagocytosis, and the MAC inserts into cell membranes to form a pore, resulting in cell lysis. The classical pathway is activated by immune complexes consisting of IgM or IgG in complex with antigen. Binding of the Fc portion of the antibody by the complement molecule C1q triggers the complement cascade leading to the above effector molecules (Fig. 1). Of the five human Ig isotypes, only IgM and IgG can activate complement.

Clinical Example: Myasthenia Gravis

Myasthenia gravis (MG) is characterized by impaired synaptic transmission at the neuromuscular junction (NMJ), the site of synaptic transmission between motor neurons and muscle fibers [94–96]. Under healthy conditions, an action potential that reaches the nerve terminal triggers the release of the neurotransmitter acetylcholine (ACh) from the nerve terminal into the synaptic cleft. ACh binds to its receptor (AChR) on the postsynaptic membrane, causing ion channels to open, eventually leading to muscle contraction.

The majority of patients with MG present autoantibodies directed against the AChR [97], which can inhibit the above-described signal transduction. The AChR is composed of four distinct membrane-spanning proteins, α, β, δ, and ε, which form the five subunits (two α-subunits and one copy of each of the three other distinct subunits). ACh binding sites are present on the α-subunits. AChR autoantibodies can block the binding of ACh to AChRs, accelerate the internalization of AChRs, and activate complement [94, 96]. In the classical complement pathway, AChR/AChR antibody immune complexes are bound by C1q. The subsequent complement activation damages the postsynaptic membrane at the NMJ, reducing the overall membrane surface area and the number of AChRs [98, 99]. As a consequence, the patient develops the characteristic muscle weakness associated with MG [94].

Immune Complexes and Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

Antibody-dependent cell-mediated cytotoxicity (ADCC) is the killing of an antibody-coated target cell by a cytotoxic effector cell through a nonphagocytic process, mediated by the release of the content of cytotoxic granules or by the expression of cell death-inducing molecules. In ADCC, the target antigen present on the cell surface is being bound by the antibody, which thus coats the cells and marks it as a target for the immune response. The Fc portion of the antibody is recognized by the Fc receptor present on effector cells. In the periphery, these effector cells include natural killer (NK) cells, macrophages, eosinophils, and neutrophils, while in the CNS, microglia take over most of these functions. Binding of the effector cells to the target cells induces receptor cross-linking on the effector cells, which triggers a signal transduction cascade, resulting in the release of cytotoxic granule content.

Clinical Example: Neuromyelitis Optica

Neuromyelitis optica (NMO), or NMOSD, is an inflammatory demyelinating autoimmune disease that affects mainly the spinal cord (myelitis) and the optic nerve (optica) [100–103]. Patients present with autoantibodies directed against aquaporin-4 (AQP4) [104, 105]. AQP4 is the main water channel in the brain and facilitates water movement across membranes. The protein is found at high concentrations expressed as a transmembrane protein in the end-feet expansions of astrocytes at the blood–brain barrier and in ependymal cells at brain–cerebrospinal interfaces [106]. AQP4 expression is particularly high in the optic nerve and spinal cord, the major tissues affected in NMO. Patients show damage of astrocytes, inflammation, macrophage infiltration, and deposition of activated complement. Notably, AQP4-Ab titers are directly associated with disease activity and decline after immunosuppressive therapy [101, 107, 108]. Binding of AQP4 by its autoantibody does not inhibit AQP4 water permeability [109–111] but activates both complement and ADCC [112–114], recruiting Fc receptor expressing neutrophils, eosinophils, and macrophages to

NMO lesions [115, 116]. Animal studies supported the ADCC involvement and AQP4-IgG without ADCC effector function produced reduced NMO pathology in vivo, and Fc receptor knockout mice showed reduced pathology [116, 117].

Modulation of Receptor Function

Agonist Effect

Clinical Example: Certain Forms of Epilepsy, Specifically Rasmussen's Encephalitis

Roughly 30% of patients with autoimmune epilepsy present autoantibodies [118–123], most commonly, patients with Rasmussen's encephalitis (RE), a rare and severe childhood epilepsy with unknown etiology [124]. One group of autoantibodies reacts against peptide B—aa 372–395 of the GluR3 subunit of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptors [120]. The AMPAR is an ionotropic glutamate receptor and constitutes a tetrameric ligand-gated cation channels that induce depolarization of the postsynaptic membrane [125]. Dysfunctional AMPAR signaling has been implicated in epileptic seizures. Although the region differs from the glutamate binding site, binding of the GluR3 by the autoantibody leads to activation and opening of the receptor's ion channel [126–129] and results in the induction of excitotoxic neuronal death by allowing excessive Ca^{2+} influx through the receptor-operated cation channels [130, 131]. Mice immunized with Glu3R produce specific Glu3R antibodies, and the animals showed higher propensity to seizures and displayed higher anxiety [132].

Antagonist Effect

Clinical Example: MG

Another mechanism by which autoantibodies in MG affect the pathogenesis is through blockage of AChR. Serum IgG from MG patients has been shown to block the ACh binding sites of AChR in cultured mammalian muscle cells [133], inducing acute and severe muscle weakness in the absence of inflammation or necrosis [134, 135]. Whether this mechanism plays a role in human MG is unclear.

Internalization of Receptors

Antibodies can cross-link receptors via their two Fab fragments when one Fab fragment binds to one receptor and the other Fab fragment binds to an adjacent receptor. These antibody-linked receptors are rapidly endocytosed, internalized, and

degraded. Through this mechanism, the number of receptors present on the cell surface is significantly decreased [136–138], and consequently, the neuron's ability to respond adequately to increased neurotransmitter signals is reduced.

Clinical Example: NMDAR Encephalitis

Patients with NMDAR encephalitis present with autoantibodies directed against the *N*-methyl-d-aspartate receptor (NMDAR) [139–141]. NMDARs are ionotrophic glutamate receptors presenting as heterotetramers consisting of two GluN1 subunits and two GluN2 subunits. NMDAR autoantibodies target the extracellular N-terminal domain of the GluNR1 subunits [142] and cause a significant reduction in NMDAR surface expression without neuronal loss in neuronal cultures [143, 144] and in vivo in animals after intrathecal administration [143–145]. This loss of receptor density on the surface is mediated by receptor internalization, and consequently, the neuron shows a reduced ability to respond to glutamate signaling, leading to the characteristic memory and behavioral alterations [143–145].

Ion Channel Function Modulation

Clinical Example: Lambert–Eaton Myasthenic Syndrome

LEMS is a paraneoplastic disorder, with a strong association to small-cell lung cancer tumor [146]. The disease is characterized by skeletal muscle weakness, malfunctions of the autonomic system, and reduced tendon reflexes [147]. Autoantibodies present in LEMS patients target multiple subunits of the voltage-gated P/Q-type Ca^{2+} channels [148] and facilitate the internalization and destruction of the Ca^{2+} channels. The reduction in the number of P/Q-type voltage-gated Ca^{2+} channels at the presynaptic terminal of the NMJ [149, 150] causes a decrease in the amount of action potential-evoked ACh release and results in less effective initiation of muscle contraction, and patients with LEMS experience debilitating muscle weakness. Passive transfer of LEMS patient serum or IgG via intraperitoneal injections induces a significant reduction of presynaptic calcium channels in mice, supporting the above scenario [151].

Sequestering of Neurotransmitters

Hypothetically, autoantibodies directed against neurotransmitters could affect neurotransmission by reducing the level of the respective neurotransmitter in the synapsis. Only few examples for such a pathogenic mechanism have been reported so far, and none of them included human reports.

Example: Neuropathic Pain

Vetrile et al. describe the development of autoantibodies directed against neurotransmitters in rats in response to neuropathic pain [152]. These autoantibodies are inversely correlated with severity of neuropathic pain syndrome and may have protective activity [153].

Enzyme Inhibition

Only few neurological autoimmune disorders are associated with autoantibodies directed toward enzymes. In Stiff person syndrome, autoantibodies against GAD65 may be causal to the decreased GABA levels observed in these patients. The involved mechanisms are discussed in section "GAD65Ab in Stiff Person Syndrome."

Theme C: Determinants of Pathogenic Effects of Autoantibodies

When evaluating the pathogenic effect of an autoantibody, we need to recognize that the effectiveness of any given autoantibody depends on the nature and location of the antigen, the isotype and IgG subclass of the autoantibody, its titer and affinity, and the presence of effector cells. Titers of pathogenic autoantibodies do not necessarily correlate with severity of disease, even if a pathogenic role of the autoantibodies has been established [154–156]. This lack of correlation may be due to variabilities in epitope specificities, affinity, and/or immunoglobulin isotypes.

Epitope Specificity of Autoantibodies

AChR antibodies in MG can target different epitopes of the receptor [157]. Depending on the epitope specificity, these autoantibodies can facilitate receptor internalization via cross-linking [158], block ACh binding sites [135], or initiate complement-mediated cytotoxicity [159]. The relative frequency of the different antibody specificities will determine the dominant pathogenic mechanism and severity of symptoms. Similar observations have been made for epitope specificities of GAD65Ab associated with SPS, type 1 diabetes, and GAD65Ab-associated cerebellar ataxia [88, 92].

Ig Isotypes and IgG Subclasses: Effector Functions

Another parameter determining the effect of autoantibodies are the different Ig isotypes and IgG subclasses. Human immunoglobulins comprise of five isotypes (IgG, IgE, IgM, IgD, and IgA) with clearly defined functions. Four major IgG subclasses are identified (IgG1–4), which differ in their relative frequency in human serum, half-life, and effector function. Thus, the isotype and IgG subclass of an autoantibody are critical determinants of the antibody's effect. Much of the antibody's effect is dictated by the Fc receptors present of different effector cells. An in-depth discussion of IgG subclasses and their receptors is given elsewhere [160]. In the following, we will discuss examples of isotype and/or IgG subclass-dependent pathogenic mechanisms associated with specific autoantibodies. Most autoantibodies are of the IgG isotype, although neurological autoimmune disorder with autoantibodies of the IgM, IgA, and IgE isotypes has been described. Among the IgG isotype, the IgG1 subclass is the most frequent autoantibody subclass. Here, we highlight examples of autoantibodies of less frequent IgG subclasses and Ig isotypes. To date, no studies support a pathogenic role of autoantibodies of the IgG2 subclass or the IgD isotype in autoimmune neurological disorders. Different Ig isotypes and IgG subclasses and their effector function as relevant to neurological autoimmune disorders are listed in Table 6.

Clinical Examples

Autoantibodies of the IgG3 Subclass in Chronic Inflammatory Demyelinating Polyneuropathy

Chronic inflammatory demyelinating polyneuropathy (CIDP) is characterized by an acute Guillain-Barre-like phase, followed by a chronic phase with progressive symptoms. Patients show autoantibodies to components of the Ranvier protein complex that links the myelin sheath and the axon [161]. Of specific interest are autoantibodies directed against the Contactin-associated protein 1 (Caspr) because here, different IgG subclasses are associated with the different phases of the disease. IgG3 Caspr autoantibodies were found in patients who were tested during the acute

Table 6 Effector functions of Ig isotypes and IgG subclasses relevant to autoimmune neurological disorders

	IgM	IgE	IgA	IgG1	IgG2	IgG3	IgG4
Activation of traditional complement	+++			+++	+	+++	
Binding of activating FcR	++	+++	++	+++	+	+++	
Binding of inhibitory FcR							+++
Internalization of receptors				+++			
Block of protein–protein interaction							+++
Enzyme activity inhibition				+++			

GBS-like phase of CIDP, while IgG4 Caspr autoantibodies were present during the chronic phase of disease [162]. This finding supports the idea of a switch from IgG3 to IgG4 at the chronic phase of disease and may in part explain the IgG3-mediated complement activation during the acute phase [163], whereas autoantibodies of the IgG4 subclass block protein interactions in the paranode structure during the later disease stages [164].

Autoantibodies of the IgG4 Subclass in MG

In contrast to IgG subclasses 1–3, IgG4 cannot facilitate binding to protein C1q of the classic complement cascade [165], and shows a reduced binding to activating Fcγ receptors. However, it shows strong binding to the inhibitory FcγRIIb [166]. Without the ability to activate the classic complement pathway or activate immune cells, the only pathogenic mechanisms associated with IgG4 are Fc-independent, e.g., blockage of protein–protein interaction or activation/inactivation of enzymes or receptors [167]. An example for IgG4 autoantibodies in neurological disorders are muscle-specific kinase (MuSK) autoantibodies in MG. These IgG4 autoantibodies effectively block the interaction between MuSK and its ligand [168, 169].

IgA Autoantibodies in Gluten Ataxia

IgA antibodies against tissue transglutaminases are prevalent in patients with celiac disease (CD). Reactivity to gluten can also manifest itself as cerebellar disorders (gluten ataxia), even in the absence of intestinal symptoms. In these patients, the major target of IgA autoantibodies is transglutaminase 6 (TG6) [170], a transglutaminase expressed predominantly by neuronal cells [171]. Cerebellar IgA deposits that contained TG6 have been identified in postmortem tissue from patients with gluten ataxia, and a pathogenic role of these deposits has been suggested [170].

IgE Autoantibodies in MS

IgE antibodies bind to IgE-specific Fcε receptor (FcεRI) expressed on mast cells and basophils, and cross-linking of the FcεRI by IgE–antigen complexes initiates degranulation of these cells. Autoantibodies of the IgE isotype are rare but have been described in patients with MS [172]. These autoantibodies are directed against small myelin protein-derived peptides (SUMPPs) [172]. Mechanistically, IgE activate mast cells, and it is feasible that mast cell degranulation in the CNS might occur as a consequence of intravascular myelin-reactive IgE penetrating the BBB. However, the pathogenic relevance of this finding needs to be further investigated.

IgM Autoantibodies in Peripheral Neuropathy

Peripheral neuropathy is a frequent complication of IgM monoclonal gammopathy [173] and often presents with IgM autoantibodies directed against a number of neural antigens [174], including myelin-associated glycoprotein (MAG), sulfatide, chondroitin sulfate C, cytoskeletal proteins, and several gangliosides [175–177]. Most of these patients experience a chronic, progressive, symmetric, and predominantly distal neuropathy, consistent with dysfunction or loss of large myelinated fibers. Pathological studies on nerve biopsies show segmental demyelination with deposits of IgM and complement [178], and ultrastructural studies show a unique splitting of the outer myelin lamellae [179, 180]. Animal studies support complement-mediated demyelination of nerves [181], suggesting a pathogenic role of these autoantibodies in the disease.

Theme D: Protective and Reparative Autoantibodies

Until now, we focused on autoantibodies with potential pathogenic roles in autoimmune neurological disorders. However, autoantibodies can serve important regulatory functions and improve tissue repair in the CNS. Protective autoantibodies are mainly of the IgM isotype or the IgG4 subclass.

Natural IgM Autoantibodies

One class of beneficial autoantibodies is represented by natural IgM autoantibodies. As characteristic for IgM, they show few, if any, somatic mutations and are polyreactive with low affinity [182]. Importantly, IgM cross the BBB and localize to normal and injured CNS tissues [183]. Once in the CNS tissue, the antibodies bind to surface antigens [184] and activate cell signals that promote remyelination [185, 186]. Specific targets of the IgM were identified in mouse models of MS [187] and include surface glycolipid antigens [188, 189]. One of the proposed reparative mechanisms involves the induction of a transient Ca^{2+} influx in oligodendrocytes [190, 191], which in turn activates mitogen-activated protein (MAP) kinases and eventually downregulates caspase-3 and caspase-9 activation [192].

Another pathway of IgM autoantibodies may be the facilitation of the opsonization and clearance of injured and dying cells by macrophages [187]. This mechanism may also be employed by natural IgG autoantibodies. Within hours of CNS injury, IgG can be observed at the region surrounding a brain injury. These antibodies bind to neurons associated with the initial injury, which have been injured and are in advanced stages of cell death [193]. Oponization marks these cells for rapid clearance [194]. It has been suggested that these autoantibodies bind to neuronal proteins that have been modified or presented in an unusual position as part of cell death [195, 196].

Protective Autoantibodies of the IgG4 Subclass

As discussed above, the IgG4 subclass does not activate complement and binds preferably to the inhibitory FcγRIIb [166]. These characteristics are consistent with the observation that IgG4 is associated with anti-inflammatory immune responses. IgG4 may to protect against antibodies of other IgG subclasses by competition for antigen without exerting an effector function, thus blocking the epitope to prevent the harmful effect of other antibody classes or subclasses. Animal studies support this assumption as IgG4 subclass autoantibodies against the AChR protect against the pathogenic effects of IgG1 of the same idiotype in rhesus monkeys [197].

Neutralization of Pathogenic Autoantibodies by Anti-idiotypic Antibodies

Another example of protective autoantibodies are anti-idiotype antibodies. Anti-idiotypic antibodies recognize the idiotype of antibodies and may effectively neutralize autoantibodies.

Theme E: Treatment Options

Treatment of neurological autoimmune diseases with intrathecal autoantibody production and CNS inflammation is hindered because of the BBB, while systemic autoantibody-mediated diseases may show better treatment responses. Other determining factors are the involved pathogenic mechanisms and duration of disease. For some neurological autoimmune disorders, removal of the autoantibody is sufficient to improve symptoms, while in other diseases, extensive neuronal damage has occurred and the disease progress may only be halted, without the ability to restore lost function.

The first-line treatment of many of these autoimmune disorders is often a combination of corticosteroids with either plasma exchange or IVIG or both.

Corticosteroids are often employed, based on their efficient anti-inflammatory activity. However, some neurological disorders show further deterioration [198]. This may be due to corticosteroid-mediated upregulation of the Na^+/K^+ pump activity and the associated axonal hyperpolarization.

IVIg preparations combine IgG pooled from thousands of donors. The involved mechanisms include both Fc-mediated and Fc-independent effector functions. Fc-independent mechanisms include the anti-idiotypic antibody-mediated neutralization of pathogenic autoantibodies (see above). Fc-mediated mechanisms include FcγR blocking, induction of anti-inflammatory cytokine production, and blockade of complement proteins. IVIg has been successfully used in the treatment of Guillain–Barré syndrome, CIDP, multifocal motor neuropathy (MMN), and SPS.

In therapeutic *plasmapheresis*, the patient's blood components are separated, and plasma, including autoantibodies, is removed. Patients with MG, CIDP, GBS, SPS, and NMDAR-E often show good responses. However, symptoms may reappear together with increased autoantibody titers. Immunosuppression due to removal of plasma from the body can occur and patients are prone to systemic infections.

Another potent immunosuppressant is *cyclophosphamide*, mediated by its apoptotic effect on proliferating lymphocytes, but the often serious side effects need to be considered, particularly in long-term treatment.

A more targeted and potentially curative approach is achieved by *B-cell depletio*n. B cells can be specifically targeted and depleted by a number of drugs. Rituximab is a monoclonal antibody targeting the B-cell marker CD20. CD20 is expressed on most B-cell stages and is lost during final maturation to plasma cells. Rituximab depletes B cells from the circulation; and importantly, rituximab is detectable also in the CSF after i.v. administration, thus depleting B cells both in the periphery and in the CNS. Rituximab treatment has been used successfully in MS patients with relapsing–remitting multiple sclerosis disease, NMO, MG (particularly in those with MuSK autoantibodies), and SPS. Plasma cells can also be targeted by a specific inhibition of proteasome function based on the strong need for protein production in these cells. Bortezomib inhibits proteasome function and is currently tested for treatment of NMDAR-E. Prevention of maturation of B cells into mature, antibody-secreting cells is in part facilitated by specific cytokines. IL-6 aids in the development of B cells into mature, antibody-secreting cells, and an anti-IL-6 monoclonal antibody has been used with promising results in the treatment of NMO and autoimmune encephalitis.

Experimental Approaches

Novel approaches that are currently being tested in clinical trials are listed in Table 7.

Disease Duration

The importance of an early diagnosis of disease in the successful treatment of neurological diseases cannot be emphasized enough and is exemplified by GAD65Ab-associated cerebellar ataxia (CA) [199]. In GAD65Ab-associated CA, GAD65Ab impair GABA release by interfering with the packaging of this neurotransmitter into the synaptic terminal vesicles as well as shuttling of the vesicles to the synaptic cleft. The decrease in GABA neurotransmission induces an upregulation of glutamate release from neighboring synapses and subsequent neuronal cell death caused by continuous glutamate excess (excitotoxicity). To avoid the progression of cerebellar pathology, the correct diagnosis needs to be established as soon as possible.

Table 7 Novel therapeutic approaches for the treatment of neurological autoimmune disorders

Mechanism of action	Drug name	Disorder
Monoclonal antibody directed against complement C5 Inhibition of complement	Eculizumab	MG Guillain–Barré syndrome NMO
Fc fragment, blockage of FcRn	Efgartigimod	MG
Monoclonal antibody directed against the 26S proteasome Inhibition of protein metabolism	Bortezomib	NMO, NMDAR-E
Inhibitor or inosine monophosphate dehydrogenase, inhibition of T- and B-cell proliferation	Mycophenolate mofetil	CIDP
Binding of BAFF, inhibition of B-cell activation	Belimumab	MG
Monoclonal antibody directed against selective adhesion molecule. Reduction of transmission of immune cells into CNS	Natalizumab	MS
Monoclonal antibody directed against CD52. Depletion of T and B lymphocytes	Alemtuzumab	CIPD, MS
Monoclonal anti-IL6 antibody. Prevention of maturation to plasma cells	Satralizumab	NMO

Immunotherapies have been shown to be beneficial when initiated during the early phases of disease due to the remarkable self-recovery capacities of the cerebellum. However, once a critical number of neurons have been destroyed, the condition can only be halted without restoration of lost function.

Conclusions

Neurological autoimmune diseases with autoantibody association are diverse regarding their antigenic targets, clinical phenotypes, pathogenic mechanisms, and initiating triggers. The design of an appropriate treatment plan is complicated by different triggers and different autoantibody mediated pathogeneses involved in the same disease. Moreover, a given autoantibody can be exhibited in a variety of clinical appearances, while a specific neurological symptom can be associated with different autoantibodies. At the same time, an early diagnosis is crucial for a favorable outcome. Current treatment options consist of traditional immune suppression and antibody and B-cell depletion. Further research is critical to develop novel treatment strategies that allow specific targeting of pathogenic pathways with minimal side effects.

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Immune Tolerance in Autoimmune Central Nervous System Disorders

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Abstract Multiple sclerosis (MS) afflicts genetically predisposed individuals and is associated with T lymphocyte-mediated damage to the myelin sheath of neurons in the central nervous system, resulting in severely impaired signal transmission. The mechanisms of the induction and manifestation of MS are not entirely understood. The control of autoimmune disorders is accomplished by both central tolerance in which autoreactive T lymphocytes are eliminated in the thymus and by tolerance mechanisms that operate in the periphery. Among the many mechanisms described, T regulatory (Treg) cells derived from the thymus (tTregs) and induced (iTregs) in the periphery as well as T regulatory type 1 cells (Tr1) are involved in many disease models. However, the precise details of the generation and perpetuation of these various Treg subsets and their relevance to the regulation of autoimmune diseases remain elusive. In this review, we critically analyze the current knowledge of the tolerance mechanisms involved in the regulation of MS and its animal model, experimental autoimmune encephalomyelitis.

Keywords Anergy · Autoimmune diseases · Blood-brain barrier · Central nervous system · Cerebrospinal fluid · Foxp3 · GM-CSF · Human leukocyte antigen · Interferon-γ · Interleukin 17 · Myelin basic protein · Multiple sclerosis · Myelin oligodendrocyte glycoprotein · Neuromyelitis optica · Proteolipid protein · Th1 · Th17 · T regulatory cells · Trichostatin A · Tumor necrosis factor-α, Tolerance

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_5

Introduction

The tenet of the immune system is the protection of the host against both invading pathogens and autoimmune diseases that arise in genetically susceptible individuals. For the former, it is essential to mount robust immune responses, both T-cell-mediated immunity and antibody production, against a myriad of pathogens. After the elimination of the source of "foreign antigenic determinants," the adaptive immune responses must contrive to restore the normal clonal T- and B-cell repertoire. Activation-induced cell death or apoptosis is credited with the restoration of the clonal size of primarily antigen-activated T lymphocytes at the end of productive immune responses [1]. The failure to do so can result in overt immune responses which can cause more harm than benefit to the host. An example of overt immune responses causing damage to endogenous tissues is the production of the multipotent, noxious cytokine, tumor necrosis factor-α (TNF-α) in inflammatory conditions including sepsis [2]. In addition to the need to control these adaptive immune responses, autoimmune responses need to be kept in check to minimize or eliminate adverse reactions against the host tissues. CD4+ T lymphocytes systematically control these seemingly opposite versions of immune responses. The various regulatory mechanisms involved in controlling autoimmune diseases have been the subject of intense investigation over many decades [3–8].

Among the 80 known autoimmune diseases, only a few are specifically directed against the central nervous system (CNS). Although evidence for the involvement of autoimmunity in many CNS disorders is weak or nonexistent, indications of autoimmunity exist in some cases. Neuromyelitis optica (NMO) is an autoimmune, demyelinating disorder of the CNS with typical clinical manifestations of optic neuritis and acute transverse myelitis attacks [9]. Although NMO was believed to be a variant of MS, it is now considered as an independent disorder. NMO is characterized by the presence of antibodies against the water channel proteins aquaporin-4 and aquaporin-1 and myelin oligodendrocyte glycoprotein (MOG). The characteristic phenotype is disruption of astrocyte function and demyelination of the spinal cord, optic nerves, and particular brain regions. Lack of self-tolerance to water channel proteins is likely to be the cause of NMO [10]. However, the underlying pathogenic mechanisms have not been fully elucidated. Alzheimer's disease is characterized by dementia, immunoglobulin in the brain parenchyma, and deposition of complement components in neurons [11]. The autoimmune encephalitis is a group of disorders characterized by autoantibodies directed to synaptic surface antigens (NMDA- and AMPA-type glutamate receptors, GABA (B) receptor, and LGI1) resulting in severe neurological symptoms [12]. Antibodies against glutamic acid decarboxylase, the rate-limiting enzyme for GABA synthesis, are associated with the autoimmune neurological syndromes, namely, stiff person syndrome, cerebellar ataxia, epilepsy, limbic encephalitis, and abnormal eye movements [13]. Whereas these characteristics are indicative of the underlying autoimmunity, detailed studies are required to fully understand the autoimmune nature of these diseases and importantly regulatory mechanisms useful for controlling these diseases. Importantly,

there is a paucity of experimental models to dissect the immunological mechanisms involved in these various neurological disorders.

Multiple sclerosis (MS) is a well-characterized neuronal disorder with an underlying autoimmune basis [14–17]. Whether MS is a non-immunological disorder and primarily a neurodegenerative disease with unknown etiology is heavily debated over the years [18, 19]. Accounts of various T-cell tolerance mechanisms involved in immune responses to nominal antigens and self-determinants including neuronal antigens have been described previously [reviewed in Ref. 3–8]. In this chapter, we focus on MS and its animal model, experimental autoimmune encephalomyelitis (EAE), to highlight the underlying common regulatory mechanisms and point out the disparity between these systems. Finally, we discuss the limitations of the data obtained in various EAE models to their translation into clinical practice for treatment of differing forms of MS.

Effector T Cells and Pathology of Multiple Sclerosis

MS is a chronic disease lasting over many decades and has highly variable presentations [14–17]. After the initial diagnosis, clinically isolated syndrome, a majority (85%) of patients display the common relapsing-remitting form of MS. After 10–15 years of diagnosis, 50% of untreated patients develop secondary progressive MS, whereas in 15% of patients, the disease progresses without remission, referred to as primary, progressive MS. Most current treatments are directed to relapsing-remitting MS, and none of them is effective on primary or secondary progressive MS [14, 16, 17, 20, 21]. These drugs are directed to cull the autoimmune component [14–17], whereas modalities to treat the neurodegenerative component of MS [18, 19] are scarce [20, 21]. The CNS is an immunologically privileged site and lacks a potent innate immune response in healthy individuals [22]. Immune cell infiltration across the blood-brain barrier (BBB) promotes inflammation, demyelination, gliosis, and neuroaxonal degeneration, resulting in disruption of neuronal signaling [14–17]. To better understand the impact of T-cell tolerance on MS, knowledge of the critical underlying pathological features is vital. MS is thought to be mediated by $CD4^+$ T-cells, although $CD8^+$ T cells and antibody-producing B cells are known to contribute to disease pathogenesis [14–17]. CNS-intrinsic events such as activation of microglia and astrocytes as well as chemokines have also been implicated in MS [23–25].

Autoreactive T lymphocytes are thought to mount aberrant immune responses against CNS autoantigens. Susceptibility to develop MS has been linked to the class II human leukocyte antigen HLA-DRB1∗15:01 allele, which is expressed on antigen-presenting cells, implying a role for distinct antigen presentation to T lymphocytes necessary for disease induction [26]. Although the etiology of MS is unknown, poorly understood stochastic events and environmental factors influence the disease penetrance. Infiltration of immune cells from the periphery is prominent in relapsing-remitting MS, and T cells appear early in lesion formation. Inflammation

of the brain and spinal cord is evident in all MS patients, which declines with age and duration of the disease [14–17]. An open question is whether MS is triggered in the periphery such as in draining lymph nodes or originates in the CNS. Peripheral T lymphocytes specific to neuronal antigens are thought to be activated by "molecular mimicry" [26], and subsequent reactivation by the CNS resident antigen-presenting cells leads to a cascade of events resulting in neuronal damage [14–17, 27].

Although the specificity of autoreactive T cells in MS remains obscure, recognition of autoantigens such as MOG and myelin basic protein (MBP) by $CD4^+$ T lymphocytes from both healthy individuals and MS patients has been demonstrated [28–30]. The relative frequency of these cells in healthy individuals and MS cases remains contentious. Thus, it is not clear whether the observed self-reactivity of T lymphocytes in MS patients reflects the underlying pathological events or a stochastic event resulting from molecular mimicry and breach of self-tolerance. Although earlier studies implicated interferon-γ (IFN-γ)-producing Th1 cells as the sole pathogenic T cells, recent investigations also support a role for Th17 cells expressing IL-17 as they are found in the peripheral blood, CNS, and cerebrospinal fluid (CSF) of MS patients. Furthermore, Th cells with a mixed phenotype (double-producers) expressing both IFN-γ and interleukin 17A (IL-17A) have a higher capacity to infiltrate the CNS as inferred from studies of postmortem MS brain tissues [14–17, 31–33]. These results indicate a pivotal role of double producers in MS pathogenesis.

Interestingly, $CD8^+$ T cells found in the active lesions of MS patients produce IL-17, similar to mucosal-associated invariant T cells [34]. In addition to the consistent participation of $CD4^+$ T cells, B cells have been proposed to be strong candidates for autoimmune effector cells in MS [35]. Although significant differences in B cells were found in the CSF, they were neither predictive of disease and disease progression (EDSS, expanded disability status scale) nor conversion to clinically definite MS following diagnosis of the clinically isolated syndrome [36].

MS pathology is characterized by confluent demyelinated areas known as plaques or lesions in the white and gray matter of the brain and spinal cord, indicating a loss of myelin sheaths and oligodendrocytes [14–17]. Damage of axons and neurons correlates with disease severity. Astrocytes form multiple sclerotic glial scars in white matter lesions. Demyelination of the gray matter of the cortex, nuclei, and spinal cord is also associated with MS. Inflammation is more pronounced in acute than in chronic phase. Invading immune cells and macrophages indicates BBB leakage. Macrophages, $CD8^+$ cells, $CD4^+$ cells, B cells, and plasma cells are represented in descending proportions. In the early stages, little damage outside of the plaques, called normal-appearing white matter, is present in both the brain and spinal cord despite general brain atrophy. During disease progression, diffuse T-cell and B-cell infiltrates, microglia and astrocyte activation, and diffuse myelin reduction and axonal damage are evident. Although the numbers of T cells do not change, the frequencies of B cells and plasma cells increase, whereas microglia and macrophages remain in a chronic state of activation throughout the disease. In secondary,

progressive MS, tertiary lymphoid structures are evident in the meninges contributing to cortical demyelination and tissue damage at later stages [14–17].

Effector Mechanisms in EAE

The earliest description of EAE was the occurrence of acute disseminated encephalomyelitis in monkeys repeatedly given intramuscular injections of normal rabbit brain emulsions and extracts [37]. This observation explained that the induction of encephalomyelitis observed earlier in humans vaccinated with rabies virus grown on rabbit spinal cord was due to the immune response triggered by the spinal cord contaminant of the vaccine [38]. Since then, monophasic EAE has been induced in guinea pigs, rats, mice, and primates by immunization with spinal cord homogenates or peptides derived from MOG, MBP, and PLP [see Ref. 14 for citations; [39–52]. Complete Freund's adjuvant along with pertussis toxin is required for EAE induction in guinea pigs, rats, and mice. However, incomplete adjuvant without *Mycobacterium tuberculosis* was sufficient to induce EAE in some strains of rats and marmosets.

Interestingly, EAE could be induced in the susceptible rat strain Dark Agouti without pertussis toxin. Several explanations have been put forward including breaching of BBB, breaking of self-tolerance, and enhancing immunogenicity of the inoculum to explain the dependence of pertussis toxin for EAE induction. Although questions were raised as to the validity of results obtained in EAE models for translation into the treatment of MS patients [53], arguments were also made in support of the fact that when used wisely EAE will provide beneficial information for clinical application [41].

Most rodent EAE models are characterized by ascending flaccid paralysis reflecting preferential targeting of inflammation to the spinal cord, referred to as classic EAE, which manifests in different forms. Immunization with the immunodominant $MOG_{35–55}$ peptide induced a mild, monophasic form of EAE in the C57BL/6 strain (H-2^b haplotype). In this model, the clinical symptoms peak around 9–12 days after immunization, followed by spontaneous resolution by 30 days [42, 43]. However, the pathology of monophasic EAE in C57BL/6 mice does not parallel that of an MS form [44, 45]. On the contrary, similar immunization induced a long-lasting (>75 days, Ref. 46–51), severe disease in NOD (H-2^{g7}) mice characterized by paralysis of fore and hind limbs with [49–51] or without discernible remissions [46–48], respectively, representing secondary and primary progressive MS. Regardless, in NOD mice EAE occurs with a high frequency that shares unique features with MS including lifelong disease, prominent demyelination, axonal loss, and astrogliosis [46–51]. On the other hand, immunization with $PLP_{139–151}$ peptide induced relapsing-remitting EAE in SJL/J (H-2^s) mice characterized by the appearance of clinical signs 6–20 days after priming and relapses first appearing at 30–45 days [52].

In the common classic EAE induced by various peptide antigens in mice, inflammation is preferentially targeted to the spinal cord. In a small number of

antigen-specific models, brain rather than the spinal cord is selectively targeted, referred to as atypical EAE [54–56]. It was initially observed in C3H/HeJ mice immunized with $PLP_{190–209}$ peptide [54] and confirmed subsequently in IFN-γ knockout Balb/c mice immunized with MBP peptides and in C3HeB/FeJ mice immunized with MOG [54–56]. Atypical EAE is presented as a movement disorder, with proprioception defects, ataxia, spasticity, and axial rotation of the head and trunk, and characterized by predominant cerebellar or brainstem involvement. These various animal models are useful in gaining insights into the underlying immunological mechanisms of variant forms of MS. However, the display of complex and variable clinical features and spontaneous remission in certain models render the interpretation of results difficult. Since each variant of EAE recapitulates some but not all features of MS, it is useful to ascertain the efficacy of treatment procedures in a preclinical model that closely mimics the select form of MS in question.

Other models of demyelinating diseases include viral infections and administration of toxic substances [see Ref. 40 for citations]. Chronic demyelinating encephalomyelitis is induced by intracranial introduction of Theiler's virus (BeAn strain or Daniel's strain) or nasal infection with mouse hepatitis (Corona) virus. Inflammatory infiltrates consist of T cells and activated macrophages/microglia in the CNS. Although viral models may reflect critical features of MS-like inflammatory inflammation, it is complicated by the involvement of virus-induced immune-mediated mechanisms. Importantly, evidence for the role of viruses in MS pathogenesis has not yet been obtained. Demyelination induced by toxic models using cuprizone, a copper chelator, is useful for understanding mechanisms of demyelination and remyelination but does not fully reflect aspects of MS pathology and pathogenesis [40].

Although the role of $CD4^+$ T cells in EAE is well established, controversy exists as to the identity of T helper subsets involved. Whereas IFN-γ-producing Th1 cells have been shown to be crucial early during EAE, IL-17A-expressing Th17 cells participate at a later stage [57–58]. In contrast, Th17 cells and double producers, those expressing both IFN-γ and IL-17A, migrate to the CNS before the arrival of Th1 cells [59] or ex-Th17 cells that lost the expression of IL-17A and gained IFN-γ during clinical disease manifestation [60]. Other studies indicated that Th1 [57] or Th17 cells [61] alone could mediate EAE upon adoptive transfer into naïve mice. However, contamination of various degrees of IFN-γ-producing cells in the Th17 cell preparations raised questions about the independent role of Th17 cells in EAE manifestation. In the primary, progressive EAE, Th1 cells were found in the spinal cord, whereas Th1 and Th17 cells but not Th1/Th17 cells infiltrated the spinal cord later during the disease [47]. However, Th1/Th17 cells were prominent in peripheral lymphoid tissues. The plasticity of Th17 cells further complicates the role of distinct T helper subsets in EAE manifestation [62]. Despite enormous effort to understand the role of various lymphokines, cytokines, and accessory cell-associated determinants, their roles in EAE pathogenesis remain obscure. Although Th17 cells have been dubbed as "encephalitogenic" T cells, neither IL-17A nor IL-17F contributes to EAE [63]. The only cytokine that has been attributed a role in EAE is

GM-CSF [64]. In the primary progressive EAE model, amelioration of the disease by treatment with the most potent histone deacetylase inhibitor, trichostatin-A (TSA), was associated with repression of GM-CSF-producing $CD4^+$ T cells in the secondary lymphoid organs and the CNS [47–48]. A closer analysis indicates that GM-CSF is dispensable for EAE induction but is essential for chronic tissue damage and neutrophil accumulation in the brain [48, 65, 66]. Blockade of the GM-CSF receptor α ameliorated relapses in mice [67]. Lesions from secondary progressive but not primary progressive MS patients contained GM-CSF receptor α^+ myeloid cells, indicating a possible target for disease intervention [67]. Collectively, these data suggest an essential role for GM-CSF in the effector arm of the inflammatory disease. The identity of the cytokines crucial for the induction of the autoimmune CNS disease remains to be delineated.

The pathological mechanisms varied with the EAE models [14, 39–41, 44–47, 49, 51]. The acute monophasic EAE was characterized by multifocal, confluent areas of mononuclear inflammatory infiltration and demyelination in the peripheral white matter of the spinal cord. In the brain, in addition to meningitis, perivascular inflammatory cuffing in the cerebellum and hindbrain white matter was prominent. In the relapsing-remitting EAE induced by $PLP_{139–151}$ immunization, lesions of the optic nerve, brainstem, spinal cord, cerebellum, and cerebral cortex along with perivascular and meningeal lymphocyte and neutrophil filtration were evident. Besides, white matter damage and gliosis and demethylated axons were observed. In the primary, progressive EAE induced by immunization of NOD mice, inflammatory cuffs around dilated blood vessels in the white matter with penetration into the gray matter were observed in the spinal cord during the acute phase of the disease [47]. The chronic phase was accompanied by severe damage of the periphery with numerous vacuoles in the dorsal and dorsolateral funiculus. Inflammatory cells were abundant in both the white and gray matter and in the subarachnoid space of ventral funiculus. Luxol fast blue staining indicated severe demyelination of neurons in the spinal cord. Bielschowsky's silver impregnation method unraveled lack of neurofilaments in both the white and gray matter, indicative of severe axonal loss during this form of EAE [47]. Although controversy exists as to whether macrophages play a pathogenic or protective role in classic EAE, increased accumulation of neutrophils during the acute phase of EAE was evident in the spinal cord of mice with primary, progressive EAE [48], a feature attributed to atypical EAE [54–56]. Thus, it appears that the pathological mechanisms involved in monophasic, relapsing-remitting, primary, and secondary progressive EAE as well as atypical EAE are not remarkably distinct and seem to overlap.

Peripheral Immune Tolerance Mechanisms

Autoreactive T and B cells are deleted in the thymus, referred to as central tolerance, which accounts for the lack of overtly self-reactive lymphoid cells in apparently healthy individuals [68]. However, the deletional mechanism is not absolute,

and a fraction of self-reactive T lymphocytes escape thymic selection and exit to the periphery. This may explain the presence of T cells specific to the MBP in the peripheral blood of asymptomatic relatives of MS patients [69, 70]. Similarly, MOG-specific $CD4^+$ T cells were detected in the peripheral blood of healthy individuals albeit at a lower level compared to that of MS patients following in vitro expansion with a MOG peptide [71]. Although these self-reactive T cells can potentially trigger autoimmune diseases when appropriately activated by antigen presentation, they do not elicit autoimmunity in healthy people due to restraints imposed by peripheral tolerance mechanisms. Although reactivity to multiple neuronal determinants such as MBP, MOG, and PLP has been demonstrated in MS, the identity of the causative autoantigen involved in the induction of this disease remains obscure.

Similarly, the initiating autoantigen in most other autoimmune diseases including type 1 diabetes also remains unknown [72]. The lack of this critical information has negatively impacted on developing successful antigen-specific tolerance strategies for the manipulation of MS [73, 74]. However, the utility of other tolerance strategies to control the chronic neurodegenerative disease MS remains incompletely understood. Substantial data have been generated in EAE, a model of MS, supporting a role for immunoregulatory T cells in this autoimmune neurodegenerative disease. Although analogous $CD4^+$ T-cell-mediated immunoregulation may operate in MS, the available data are limited and are often contentious. Herein, we discuss these data critically and evaluate their impact on MS.

One mechanism that was implicated in the prevention of autoimmune diseases is T-cell anergy, physical existence of T cells without displaying functional competence [75]. Exposure of cloned mouse Th1 cells expressing interleukin-2 (IL-2) and IFN-γ to chemically modified antigen-presenting cells pulsed with antigenic peptide induced a state of unresponsiveness, termed anergy. These anergic Th1 cells were unable to produce IL-2 when challenged subsequently with unmodified antigen-presenting cells pulsed with the specific peptide antigen in vitro. Interestingly, this form of tolerance is transient since activation with IL-2 reversed anergy and restored the ability of anergic Th1 cells to respond in an antigen-specific manner [76] subsequently. Demonstration of peripheral blood T lymphocytes reactive to self-antigens such as MBP and MOG in asymptomatic relatives of MS patients exemplifies the existence of potentially autoreactive T cells in the absence of overt autoimmunity, akin to anergic T cells [69–71]. Notably, antigen presentation by cloned murine thymic macrophages induced anergy in Th1 cells without requiring chemical modification, indicating the possibility that specific native antigen-presenting cells are capable of inducing anergy despite the optimal expression of co-stimulatory determinants necessary for T-cell activation [77]. However, the transient and reversible nature of T-cell anergy imposes severe restrictions in applying antigen-specific tolerance strategy to silence the autoreactive T cells in vivo. Another primary mechanism of peripheral tolerance intensely studied during the 1970s and 1980s involved the participation of subsets of antigen-specific T suppressor (Ts) cells governed by idiotype-anti-idiotype interactions and influenced by the unresolved genetic restriction element, I-J [3, 4]. The ensuing result was suppression of antigen-specific immune responses as well as linked suppression of

unrelated immune responses [78]. However, the lack of robust biochemical and molecular evidence discouraged further studies of antigen-driven Ts cells.

Tolerance by T Regulatory Cells

During the decline of interest in antigen-specific Ts cells, the concept of regulation of autoimmune responses by Foxp3-expressing $CD4^+$ T lymphocytes derived from the thymus, termed the thymic T regulatory (tTreg) cells, emerged [79–83]. Also, a subset of Treg cells called the induced Treg (iTreg) generated during the activation of conventional $CD4^+$ T cells with antigen, IL-2 and transforming growth factor-β (TGF-β) [84], and type 1 Treg (Tr1) cells [85] have been described. The relationship between these Treg subsets and antigen-specific Ts cells described earlier as well as their relative contribution to the regulation of immune responses to nominal antigens and autoimmunity remains incompletely understood.

The notion that immunoregulation is accomplished by T lymphocytes distinct from conventional effector $CD4^+$ T cells was fueled by the critical observation that neonatal thymectomy led to the emergence of many autoimmune diseases in mice due to the depletion of $CD4^+CD25^+$ tTreg cells [79, 80]. Notably, autoimmune neuronal diseases were not among those unleashed by the removal of tTreg cells. The tTreg cells are enriched for T-cell receptors and exhibit high affinity for self-peptides. The description of Foxp3 as the critical transcription factor for the development, function, and stability of Treg cells revitalized the study of tTreg cells in mice [81, 82] as well as humans [83]. Whereas tTreg cells control most autoimmune diseases [79, 80], iTreg cells generated from conventional $CD4^+$ T cell with low affinity for self-antigens is thought to play a prominent role in general immune regulation [84]. Although these three Treg subsets are treated as distinct entities, confirmatory phenotypic features that can distinguish between them remain unknown [5–8, 79–86]. Although the Treg cells also exert non-specific immune suppression [87] similar to antigen-specific Ts cells [3, 78], the molecular nature of immunosuppression remains unclear in both cases.

Initially, the human counterparts of mouse Treg cells were identified in the thymus and peripheral blood of healthy individuals as $CD4^+CD25^{high}$ cells which function similar to mouse Treg cells [88, 89]. The frequency, phenotype, and function of Treg cells have been extensively studied in MS patients. In some studies, the frequency of $CD4^+CD25^{hi}$ Treg cells in the peripheral blood of MS patients was similar to that of healthy individuals irrespective of the disease activity [90–93]. Notably, removal of IL-7 $receptor^+$ ($CD127^+$) cells from the analysis unraveled that both the number and function of Treg cells in MS patients did not differ from that of healthy individuals [93]. Paradoxically, the number of Treg cells was higher in the cerebrospinal fluid than in the peripheral blood of MS patients [94]. Chronic MS patients had a higher frequency of memory $CD4^+CD25^+CD127^{lo}CD45RO^+$ Treg cells in the peripheral blood [95]. Surprisingly, $CD4^+CD25^+FOXP3^+$ Treg cells were also significantly increased in MS patients when compared to healthy individuals [96].

On the contrary, in relapsing-remitting MS, the numbers of $CD4^+CD25^+FOXP3^+$ Treg cells were reduced in the peripheral blood [97]. Interestingly, both $CD4^+CD25^+FOXP3^+$ cells and *FOXP3* expression were lower during relapses than remission [98]. The numbers of $CD31^+$ recent thymic emigrants of the $CD4^+CD25^+CD45RA^+CD45RO^-FOXP3^+$ Treg phenotype within the peripheral blood decline with age and are significantly reduced in MS patients [99]. Interestingly, the Treg cells expressing CD39, an ectoenzyme that hydrolyzes ATP, were diminished in MS patients [100]. On the contrary, in another study the frequency of $CD4^+CD25^+CD127^{lo}FOXP3^+CD39^+$ Treg cells in MS patients was comparable to healthy controls [101]. To date, little consensus exists as to the phenotype and frequency of the Treg population in various clinical forms of MS, a disease that lasts for several decades. Longitudinal analysis of Treg cells using uniform phenotypic markers during different stages of the disease will help to delineate whether the Treg cell populations correlate with the clinical presentations.

The Complex Role of FOXP3 in Immunoregulation

The description of Foxp3 as a master regulator of tTreg cells led to its adoption as a surrogate marker of mouse [81, 82] and human Treg cells [7, 8, 83, 96–98, 100, 101]. The IPEX (immune regulation, polyendocrinopathy, enteropathy, and X-linked inheritance) syndrome is characterized by diarrhea, diabetes mellitus, hemolytic anemia, eczema, autodestruction of endocrine glands, and thyroiditis with absolutely no evidence of MS [102]. The IPEX syndrome is fatal and without aggressive immunosuppression or bone marrow transplantation, and male patients rarely survive beyond the second decade of life [103]. Although insulin-dependent diabetes was diagnosed in IPEX patients [102], association between variation of the *FOXP3* gene and the common type 1 diabetes was not subsequently found [104]. IPEX is associated with mutations of the human *FOXP3*, the ortholog of the gene mutated in scurfy mice that develop autoimmune disorders [105, 106].

On the other hand, MS is diagnosed as clinically isolated syndrome typically during the second or third decade of life and requires several decades for full manifestation [14]. Therefore, it is highly unlikely that IPEX patients can develop the full spectrum of MS symptoms within two decades of life. Another unexpected complication is that *FOXP3* mutation affects not only Treg cells but also the effector $CD4^+$ T cells. This was indicated by the ability of $CD4^+CD25^{hi}FOXP3^+$ cells from IPEX patients to suppress the proliferation of normal but not autologous responder T cells [107]. Thus, IPEX syndrome is associated with the development of resistance in responder T cells to suppressor signals and not impaired suppressor function of Treg cells [107]. Although there is no evidence linking IPEX syndrome and MS, sharing of similar defective functions of Treg cells is likely serendipitous (vide infra).

Studies in experimental models unraveled that the role of Treg cells in immunoregulation is complex and complicated. No spontaneous CNS inflammation was observed in *Foxp3* mutant mice or after targeted depletion of $Foxp3^+$ Treg cells in

wild-type mice [108], indicating that the loss of Treg function alone is insufficient to cause EAE. Paradoxically, the same group observed that targeted and acute depletion of Foxp3+ Treg cells resulted in limited autoimmune inflammation by controlling the T effector cell proliferation and mobility within the CNS [109], indicating a role for Treg cells in EAE regulation. This finding is in contrast to another study which showed that the Treg cells accumulated in the CNS at the peak of EAE but were unable to suppress the proliferation of CNS-derived T effector cells in vitro [110]. In several studies, anti-CD25 antibody administration was used to demonstrate the participation of Treg cells in EAE [43, 111].

Interestingly, anti-CD25 antibody administration repressed the secondary but not primary remission [112]. However, CD25 is not a bona fide marker of Treg cells since these antibodies persist in the circulation for an extended period and also could affect the activated T effectors cells expressing CD25 [109]. Moreover, neither the administration of the anti-CD25 antibody nor adoptive transfer of Treg cells obtained during the resolution of monophasic EAE was determined to be antigen-specific [43]. Thus, further analysis is required for a full evaluation of the role of Treg cells in EAE.

Many other complications impede the understanding of the role of Treg cells in EAE. The expression of Foxp3 is not sufficient for the full expression of the suppressor phenotype and requires several "partner proteins" including the transcription factors Gata-3, NFAT, and Runx1, which influence Treg cell functions [113]. The critical importance of partner proteins in immunoregulation was demonstrated in Foxp3 reporter NOD mice in which the disruption of the interaction between Foxp3 and cofactors such as the histone acetyltransferase Tip60, histone deacetylase 7 (HDAC7), and Eos accelerated the development of autoimmune diabetes [114]. Another difficult aspect of Treg cells is their "plasticity." In the peripheral blood of MS patients, increased frequency of $CD4^+CD25^{hi}CD127^{low/-}$ $FOXP3^+$ IFN-γ^+ secreting Th1-like Treg cells with lower suppressive ability was observed, indicating the instability of human Treg cells [115]. The conversion of $Foxp3^+$ Treg cells into IL-17-producing Th17 cells has been implicated in the pathogenesis of collagen-induced arthritis in mice and rheumatoid arthritis [116]. Use of a dual lineage tracing model indicated that the conversion of effector Treg cells into central Treg cells was accompanied by increased Foxp3 stability in vivo [117]. Also, acquisition of Th2-like Treg cells expressing Gata-3, activation of STA6, and secretion of IL-4 have been reported [118]. Although Treg cells appear to populate specific tissues including muscles, the skin, lungs, and the gastrointestinal tract, their involvement in tissue homeostasis remains speculative [119]. An additional complication of Treg cells is the so-called ex-Foxp3 cells, a small proportion of tTreg cells that lost Foxp3 expression during EAE induction and express IFN-γ and the ability to mediate EAE [120]. Although the levels of FOXP3 mRNA and protein are decreased in MS patients [121], it is unclear whether this reflects the transition to "ex-FOXP3 cells" during the disease pathogenesis. Thus, despite extensive investigations on the phenotypic and functional characteristics of $CD4^+FOXP3^+$ Treg cells, several key issues await further elucidation for a better understanding of how these cells contribute to immune homeostasis in humans.

Defective Function of Treg Cells in MS Patients and its Clinical Relevance

Regardless of the complex nature of the phenotype of the Treg subsets, some investigations indicated that these cells are functionally impaired in MS patients [90, 92, 96–98, 100, 101]. Although both $CD39^+$ and $CD39^-$ Treg subsets suppressed the proliferation of responder T cells and IFN-γ production, interestingly only the $CD39^+$ Treg subset suppressed IL-17 production, which is also defective in MS patients [100, 101]. Since IL-17-producing T cells are enriched in active MS lesions and considered crucial for MS pathogenesis [14, 122], these data suggest that compromised Treg cell function may exaggerate IL-17-mediated disease symptoms. This observation could provide possible mechanistic insights into the control of MS. Interestingly, impaired suppressor function of $CD4^+CD25^{hi}$ Treg cells was also noted without numerical reduction of these cells in the peripheral blood of some but not all MS patients [90, 92]. As mentioned above, impaired suppressor function correlated with diminished expression of FOXP3 protein and mRNA in MS patients [121]. Diminished suppression of MBP-induced proliferation of peripheral T cells observed in 45% of untreated MS patients was paradoxically associated with increased frequency of $CD4^+CD25^{intermediate}$ Treg cells [96].

Interestingly, the ability of $CD4^+CD25^+$ Treg cells was depressed in relapsing-remitting but not secondary progressive MS patients, despite comparable number and phenotype of these cells [123]. However, the status of Treg cells in primary progressive MS in which neuronal deficits accrue without remission [14, 20] is currently not known. Thus, these studies suggest that despite the variability in number and phenotype, diminished suppressor function of Treg cells may have a significant impact on MS pathogenesis. However, the clinical value of depressed suppressor function of various Treg cells has been challenged [8, 124] and should be treated with caution. The uncertainty of the functional aspect of human Treg cells is attributed to technical issues associated with in vitro assay systems used for the functional assessment [8, 124]. These include the type of stimulation of responders (anti-CD3 antibody + antigen presenting cells along with anti-CD28 antibody vs. immobilized anti-CD3 antibody), blocking IL-2 production to maximize suppression in some cases, different cellular targets of suppression (responder T cells vs. antigen presenting cells such as dendritic cells), requirement for enormous numbers of Tregs (1:1 ratio of Treg cells and responders), and the lack of suitable animal models to determine the suppressive activity of human Treg cells in vivo. Importantly, using autologous responder and Treg cells, it will be hard to distinguish between the acquisition of resistance in responder cells to Treg cell-mediated suppression and compromised suppressor function of Treg cells. These technical issues render the results of in vitro suppression assays somewhat uninterpretable [8, 124].

Disease-Modifying Therapies Failed to Impact Treg Cells

Since it is difficult to determine the impact of phenotype, defective number, and function of human Treg cells on disease progression during long duration such as in MS, a correlation was sought between these parameters and the health status of MS patients at specific time points. A majority of disease-modifying treatments available today are directed to relapsing-remitting MS, and there is a paucity of drugs to treat secondary and primary progressive MS [14, 20]. Treatment of MS patients with IFN-beta-1a reduces relapses without altering the circulating numbers of $CD4^+CD25^{hi}$ Treg cells [95]. Another study demonstrated that treatment of relapsing-remitting MS patients with IFN-beta-1a increased the proportion of $CD4^+CD25^+GITR^+$ Treg cells above the baseline [125]. Glatiramer acetate treatment of relapsing-remitting MS patients reconstituted naïve Treg cells and increased total Treg cell numbers [126]. Combined treatment with IFN-beta-1a and glatiramer acetate reduced the numbers of naïve ($CD4^+CD25^+CD127^{low}CD45RA^+$) Treg cells without affecting the memory type Treg cells ($CD4^+CD25^+CD127^{low}CD45RO^+$) in chronic MS patients [96]. Glatiramer acetate treatment of MS patients improved the Treg cell function by expanding $CD4^+CD25^+FOXP3^+$ T cells [96].

Interestingly, IFN-1a-beta treatment redistributed tTreg subset to central memory-like Treg population expressing CCR27 and the increased Tr1-like subset that expressed IL-10 and CD46 mRNA [127]. Natalizumab, a monoclonal humanized antibody targeting the α-4 chain of the very late antigen 4 (VLA-4) integrin, reduces relapses independent of alterations in Treg cell frequency or function [128]. Although these disease-modifying therapies (IFN-1a-beta, glatiramer acetate, and natalizumab) reduce relapses in MS patients, they do not provide robust protection against MS or reverse axonal degeneration [20]. It will be exciting and appropriate to determine the alteration of the number and function of Treg cells during various stages of the disease and after treatment with more effective disease-modifying drugs when they become available.

Pharmacological Modulation of Treg Cells

Posttranslational modifications of histones by acetylation, phosphorylation, and ubiquitylation are powerful epigenetic modulations that have a substantial influence on gene expression [129]. Epigenetic markers including acetylation and methylation of histones and cytosine-guanosine (CpG) dinucleotide methylation have been reported at the *Foxp3* locus [130]. Naive $CD4^+CD25^-$ T cells, activated $CD4^+$ T cells, and TGF-β-induced adaptive Treg cells, but not in natural Tregs, CpG dinucleotides are methylated at the *Foxp3* locus. It has been proposed that Treg cells can be manipulated via epigenetic modification of the transcription factor Foxp3 in mice. In support of this notion, it was shown that treatment of mice with TSA enhanced Treg cell-mediated suppression of homeostatic proliferation and decreased

inflammatory bowel disease [131]. In conjunction with low-dose rapamycin, TSA induced permanent Treg cell-dependent cardiac and islet allograft survival and donor-specific allograft tolerance [131].

In contrast to these data, attrition of the naturally occurring type 1 diabetes and primary, progressive EAE in autoimmune-prone NOD mice by TSA treatment was not associated with the modulation of $CD4^+CD25^+Foxp3^+$ Treg cells or function [132, 47]. Global gene expression analysis indicated up- and downregulation of many genes in uninduced splenocytes from TSA-treated mice including novel pro-inflammatory genes specifically expressed in macrophages [133] but not Foxp3 transcription (unpublished data). On the contrary, TSA treatment induced histone hyperacetylation and reduced inflammation, demyelination, and axonal damage in the spinal cord [47]. Interestingly, drug treatment diminished the generation of $CD4^+$ memory T cells and induced antigen-specific tolerance (Fig. 1) as indicated by abrogation of T-cell proliferation when draining lymph node cells and splenocytes were stimulated with $MOG_{35–55}$ in vitro albeit normal proliferation to a T-cell mitogen. However, activation with IL-2 restored the ability of tolerized T cells to respond to antigen stimulation, indicating a reversal of anergy. Tolerance was characterized by the reduced antigen-induced production of IL-17A, IFN-γ, and GM-CSF but not IL-4.

The consecutive appearance of double producers (IL-17A + IFN-γ) and Th1 cells occurred in peripheral lymphoid tissues and was susceptible to repression by TSA treatment. In the CNS, only Th1 cells appeared during the acute phase, while Th1 cells, Th17 cells, and GM-CSF-expressing cells were found in the chronic phase. Importantly, TSA treatment diminished the frequencies of these cells in the CNS

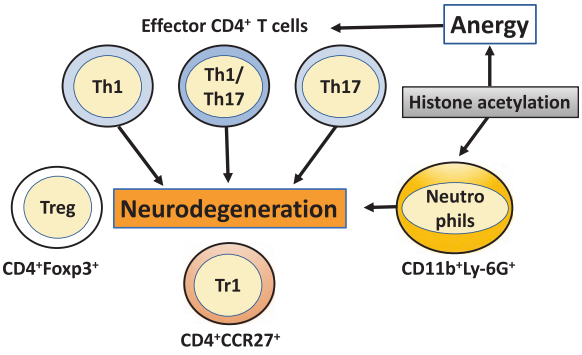

Fig. 1 Overview of mechanisms involved in the regulation of autoimmune neurodegeneration. In the experimental model of MS, drug-mediated histone hyperacetylation induces anergy in effector IFN-γ-producing Th1 cells, IL-17A-expressing Th17 cells, and cells with mixed phenotype (Th1/Th17). In addition, the neutrophil expansion is also subject to regulation by the epigenetic modifier. Together, they contribute to the amelioration of neurodegeneration in the mouse model. It remains to be determined whether similar manipulation of IL-17A- and IFN-γ-producing Th1/Th17 cells found in MS patients by pharmacological intervention could benefit patients with MS. Defective numbers and function of Treg cells have been reported in MS patients, suggesting a role for these cells in disease pathogenesis. Restoration of functional Treg populations in MS patients may potentially provide therapeutic advantages. A role for Tr1 cells in MS disease pathogenesis is also possible

[47]. In the primary, progressive EAE model, TSA treatment also reduced the abundance of mature $CD11b^{+}Ly\text{-}6G^{dim}$-activated neutrophils in the secondary lymphoid tissues and their influx into the spinal cord [48]. Thus, in addition to myelin-specific T-cell tolerance induction, selective repression of mature neutrophils and PD-$L1^{+}$ cells is critically involved in the epigenetic regulation of primary, progressive EAE. These data indicate that epigenetic regulation by histone acetylation ameliorates autoimmune diseases such as type 1 diabetes and EAE by modulating gene expression without involving the Treg population or *Foxp3* transcription.

Interestingly, treatment with various small molecule inhibitors of histone deacetylases increased the suppressive function of both freshly isolated and in vitro-expanded human Treg cells [134]. This functional change was accompanied by increased expression of the negative regulator of immune response, CTLA-4, indicating that epigenetic drugs can be promising pharmacologic agents that can improve the immunosuppressive potential of T lymphocytes. Since TSA, the most potent inhibitor of histone deacetylases is effective in reversing naturally occurring type 1 diabetes and immunization-induced EAE without causing undesirable side effects [132–134, 48], this strategy may be of potential use to treat patients with autoimmunity.

Administration of the lipid-lowering drug atorvastatin alleviated EAE without increasing IL-4-producing Th2 cells or Treg population, implicated in protection against neurodegeneration [135]. This observation is consistent with the ability of statins, inhibitors of cholesterol biosynthesis, to affect IL-17-producing Th17 cells [136]. Since Th17 cells are pivotal for MS pathogenesis [14], inhibition of IL-17 production by statins could be useful for the treatment of MS.

Antigen-Specific Tolerance-Inducing Strategies Failed to Block MS Progression

Since MS is considered as an autoimmune disease, induction of antigen-specific tolerance is the best approach to annihilate this debilitating disease via inactivation of autoreactive T cells without causing undesirable side effects. As MS is genetically predisposed, self-reactive T lymphocytes recognizing neuronal antigens in the context of HLA-DRB1 hypothetically escape thymic deletion (central tolerance), which can be subsequently reactivated by the peripheral antigen-presenting cells causing dire consequences. As discussed above, the induction of anergy could restrain these autoreactive T cells from causing neuronal destruction. Toward this goal, several clinical trials were conducted in MS patients by administering peptides derived from MBP, MOG, and PLP via various routes [Ref. 73, 74 and citations therein]. Also, complexes of HLA class II molecule HLA-DR2 and $MOG_{35–55}$, and myelin peptides ($MBP_{85–99}$, $MOG_{35–55}$, and $PLP_{139–151}$), a plasmid containing MBP protein, referred to as DNA vaccine, attenuated autologous T cells specific to MBP, MOG, and PLP were candidates for tolerance induction in MS patients. Furthermore, peripheral blood mononuclear cells coupled with seven myelin peptides ($MOG_{1–20}$, $MOG_{35–55}$, $MBP_{13–32}$, $MBP_{83–99}$, $MBP_{111–129}$, $MBP_{146–170}$, and $PLP_{139–154}$) were also

tested for their efficacy to produce a favorable outcome in MS patients. Not surprisingly, oral administration of MBP failed to protect MS [73, 74], as observed previously in type 1 diabetes [137]. Altered ligand peptide analogs of immunogenic peptides that have been modified to interact with the T-cell receptor while retaining the ability to bind the HLA motifs proved fatal in clinical trials [138]. Although most of these approaches did not have safety issues, no breakthrough as to clinical benefits has been achieved. Recent efforts to establish an antigen-specific tolerance in autoimmune patients include administration of tolerogenic dendritic cells [139] and tolerogenic immune-modifying nanoparticles [140]. So far, successful "tolerance-inducing" strategies have not emerged as standard-of-care clinical use. The identification of the disease-instigating antigen(s) will pave the way for the successful design of antigen-specific tolerance for the treatment of the debilitating CNS disease.

The Future Perspectives

It is now well established that peripheral tolerance is of paramount importance in the homeostatic control of the T-cell repertoire and for curtailing autoimmunity. The concerted effort for over half a century has unraveled unexpectedly diverse types of Treg cells in the mouse. By analogy, Treg cells with multiple phenotypes have been reported in humans as well. Attempts to understand the mode of immunosuppression mediated by these cells yielded clues to several mechanisms that need to be carefully discerned. Limited studies conducted in EAE models do not support in vivo manipulation of Treg cells, mostly the thymic-derived, "naturally occurring" Treg cells, by epigenetic or pharmacological strategies (Fig. 1). Although disease-modifying therapies provide some benefits to MS patients, it is unclear whether disease protection accompanies changes in the Treg cell number, phenotype, or function. Adoption of uniform parameters for evaluation of phenotypic markers may help to improve our understanding of the status of the Treg subsets during the variable and prolonged duration of chronic neurodegeneration. It is important to emphasize the need for refining in vitro conditions required for assessing the "suppressive" function of human Treg cells. The fact that the Treg cells are scarce in the brain lesions but abundant in the cerebrospinal fluid of MS patients [141] suggests that anatomical constraints are responsible for this uneven distribution of Treg cells. It is essential to know whether pharmacological intervention or administration of biologicals to improve MS symptoms may influence the Treg cell trafficking to the brain. Finally, since the Treg cells producing IL-10, type 1 Treg (Tr1), were also reported to be impaired in MS patients [127, 142], it will be informative whether disease-modifying treatments can also impact this cellular compartment.

Acknowledgments Arathi Jayaraman is acknowledged for comments on the manuscript.

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The Roles of Regulatory T Cells in Central Nervous System Autoimmunity

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Abstract Regulatory T (Treg) cells are a population of T cells that can functionally supress an immune response and are fundamental in maintaining T cell tolerance to self-antigens and immune homeostasis in the healthy individual. They exert strong suppressive functions through a variety of mechanisms, including modulation of antigen-presenting cell maturation or function, metabolic disruption, the production and secretion of anti-inflammatory cytokines and direct cytotoxicity. Treg cells are generally thought to have a beneficial role in most immune-mediated contexts, and a loss of suppressive capability and altered numbers in a variety of neurological conditions can occur. This review examines the role of Treg cells in the context of central nervous system (CNS) autoimmunity, and how they contribute to both relatively common and more rare diseases involving demyelination or degeneration of the CNS, including multiple sclerosis, neuromyelitis optica, acute disseminated encephalomyelitis, anti-NMDAR encephalitis, and narcolepsy with cataplexy. Although the role of Treg cells in some of these conditions is still very much in the preliminary stages, it is a feasible notion that with more research, harnessing the innate suppressive abilities of these potent immune cells will contribute to the development of novel therapeutics in autoimmune disorders of the CNS.

Keywords Regulatory T cells · Autoimmunity · Central nervous system · Anti-inflammatory · Suppressive

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_6

Abbreviations

ADEM	Acute disseminated encephalomyelitis
APCs	Antigen-presenting cells
AQP4	Aquaporin 4
$A_{2A}R$	Adenosine receptor 2A
BBB	Blood–brain barrier
cAMP	Cyclic adenosine monophosphate
CIS	Clinically isolated syndrome
CNS	Central nervous system
CSF	Cerebrospinal fluid
CTLA4	Cytotoxic T lymphocyte antigen 4
DC	Dendritic cell
DEREG	DEpletion of REGulatory T cells
EAE	Experimental autoimmune encephalomyelitis
Ebi3	Epstein–Barr virus-induced gene 3
FoxP3	Forkhead box protein 3
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HLA	Human leukocyte antigen
IBD	Inflammatory bowel disease
IDO	Indoleamine 2,3-dioxygenase
IFN	Interferon
IgG	Immunoglobulin G
IL	Interleukin
iTreg	Inducible regulatory T cell
LAG3	Lymphocyte-activation gene 3
LH	Lateral hypothalamus
MBP	Myelin basic protein
MG	Myasthenia gravis
MHV	Mouse hepatitis virus
MOG	Myelin oligodendrocyte glycoprotein
MS	Multiple sclerosis
NMDAR	N-methyl-D-aspartate receptor
NMO	Neuromyelitis optica
NMOSD	Neuromyelitis optica spectrum disorders
nTreg	Natural regulatory T cell
NT1	Narcolepsy type 1
PBMCs	Peripheral blood mononuclear cells
PLP	Proteolipoprotein
PPMS	Primary progressive multiple sclerosis
RRMS	Relapsing–remitting multiple sclerosis
SPMS	Secondary progressive multiple sclerosis
TCRs	T cell receptors
TGF	Transforming growth factor

Th	T helper cell
TNF	Tumour necrosis factor
Treg	Regulatory T cell

Introduction

Regulatory T (Treg) cells are a small subset of lymphocytes with potent suppressive capacities that regulate most types of immune responses, including allergy, autoimmunity, inflammation, and reactions to microbes and tumours [1]. Treg cells maintain immunological self-tolerance and immune homeostasis via suppression of activation, proliferation, and effector functions on a myriad of immune cells, including T cells, B cells, antigen-presenting cells (APCs), and natural killer cells [2]. They constitute ~10% of CD4+ T cells in lymphoid organs, and ~2% of peripheral blood CD4+ T cells [3]. Emerging evidence indicates that Treg cells also reside in non-lymphoid tissues, and assist in resolving tissue inflammation and tissue healing [4]. Importantly, they play an essential role in the inhibition of autoimmunity, acting as an innate braking mechanism to ensure immune responses occur in synchrony with complementary beneficial inflammatory responses.

Treg cells are most commonly defined by their expression of the interleukin (IL)-2 receptor α chain (CD25) and the transcription factor, forkhead box protein 3 (FoxP3). Despite also being present in activated non-suppressive CD4+ T cells [5] and on subsets of macrophages [6], FoxP3 is considered the most specific Treg cell marker, and is fundamental for Treg cell function and development [7, 8]. Further, high-level FoxP3 expression is capable of eliciting a suppressive phenotype to normal non-Treg cells [1]. It is well established that FoxP3+ Treg cells may be further classified into two main subsets, thymus-derived natural Treg (nTreg) and inducible Treg (iTreg) cells [9]. FoxP3+ nTreg cells are derived as a functionally mature population from the thymus [10]. Following maturation, these cells migrate to the periphery, and are involved in regulating and preventing autoimmunity, with deleterious alterations initiating a myriad of autoimmune conditions in rodents, many of which share similarities to corresponding human diseases [11]. Conversely, iTreg cells differentiate from conventional CD4+ T cells in peripheral lymphoid tissue, can be generated in vitro and re-establish immunological tolerance, and are heavily involved in regulating immune responses to foreign antigens [12].

Autoimmune pathogenesis often involves autoreactive effector T helper (Th) cells, such as interferon (IFN)-γ-producing Th1 cells and IL-17-producing Th17 cells. Treg cells play an important role in controlling the expansion and activation of autoreactive effector T cells, as well as APCs. Treg cells exert immunosuppressive functions through a variety of mechanisms, including (i) modulation of dendritic cell (DC) maturation or function, (ii) metabolic disruption, (iii) production and secretion of anti-inflammatory cytokines, and (iv) direct cytotoxicity [13]. Figure 1 summarises the mechanisms of Treg cell-mediated immunosuppression.

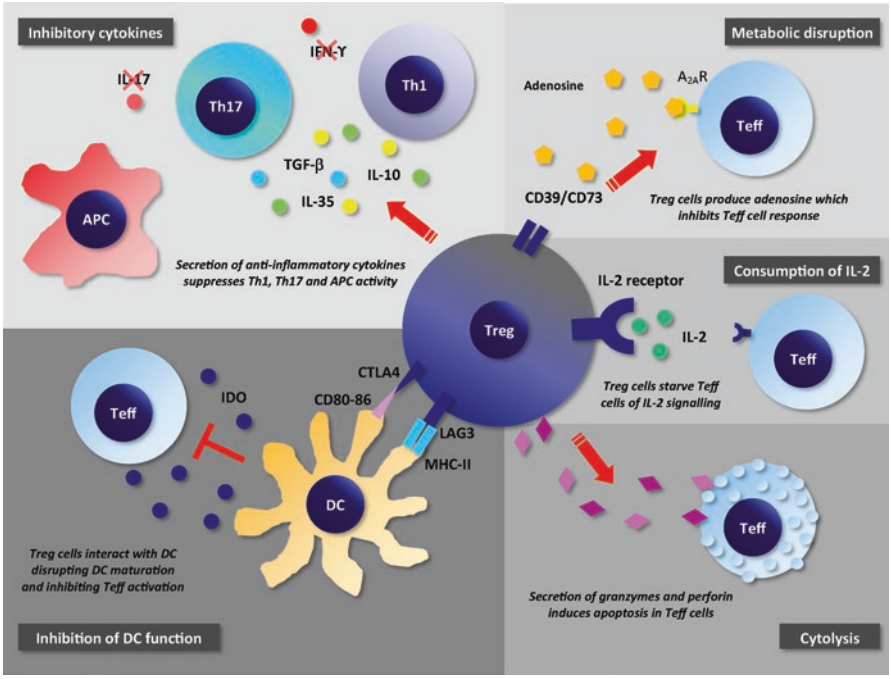

Fig. 1 Mechanisms underlying Treg-mediated immunosuppression. Treg cells control immune responses by suppressing the functions of effector T cells (Teff) and antigen-presenting cells (APCs) through diverse mechanisms, including (i) modulation of dendritic cell (DC) function and prevention of DC maturation by the interaction of CTLA4 and LAG3 expressed by Treg cells and the CD80/86 costimulatory molecules and MHC class II expressed by DC, respectively, leading to IDO production and prevention of Teff cell activation; (ii) metabolic disruption, Treg cells can disrupt metabolic functions by the expression of the ectoenzymes CD39/73 allowing adenosine generation and binding of adenosine to the adenosine receptor 2A ($A_{2A}R$) expressed on Teff cells, or by IL-2 deprivation; (iii) production and secretion of the anti-inflammatory cytokines IL-10, IL-35, and TGF-β that inhibit Th1 and Th17 immune responses and the production of IFN-γ and IL-17, respectively; and (iv) direct cytotoxicity, Treg cells can also induce direct killing of effector cells via the release of granzyme A, granzyme B, and perforin, which induce apoptosis in the target cells

Modulation of dendritic cell (DC) maturation or function Disruption of DC maturation or function has been proposed as a possible suppressive pathway through which Treg cells perform. In vivo studies utilising intravital microscopy have proposed that Treg cells interact directly with DCs, which are necessary for activation of effector T cells [13]. These studies showed Treg cell interaction with DCs in a cytotoxic T lymphocyte antigen 4 (CTLA4)-dependent manner, a co-stimulatory molecule constitutively expressed by Treg cells [14, 15]. Specifically, it has been shown that CTLA4-deficient Treg cells or the use of CTLA4-specific blocking antibodies in the absence of functional CTLA4 reduces Treg cell-mediated suppression of effector T cells via DCs [16, 17]. Research has also shown that Treg cells may condition DCs to express the potent regulatory molecule indoleamine

2,3-dioxygenase (IDO) [13]. IDO suppresses effector T cell responses by inducing catabolism of tryptophan, which produces pro-apoptotic metabolites via a mechanism reliant on interactions between CTLA4 and CD80 and/or CD86 [18, 19].

Studies suggest lymphocyte-activation gene 3 (LAG3, also known as CD223) inhibits maturation of DCs. LAG3 is a CD4 homologue which binds to MHC class II molecules with high affinity, intrinsically limits Treg cell proliferation, and is essential for maximising Treg cell-mediated suppression [13, 20, 21]. Induction of an immunoreceptor tyrosine-based activation motif-mediated inhibitory signalling pathway following LAG3 binding to MHC class II molecules has been shown to suppress DC maturation and their ability to elicit stimulation of the immune system [22]. As well, neuropilin-1 facilitates prolonged interactions with immature DCs and Treg cells [23]. Treg cells differentially express neuropilin-1 and so this may be advantageous over naïve T cells for the modulation of DCs [13] and a suppression of immune responses.

Metabolic disruption Metabolic disruption of effector T cells has also been proposed as a mechanism through which Treg cells exert immunosuppression, including the induction of intra- and extracellular adenosine nucleosides [13]. Studies have shown that expression of the ectoenzymes CD39 and CD73 produces pericellular adenosine, and through the activation of the adenosine receptor 2A ($A_{2A}R$), can ameliorate effector T cell function [24–26]. Further, Zarek and colleagues demonstrated that binding of adenosine to $A_{2A}R$ both inhibits effector T cell responses and enhances the generation of iTreg cells through inhibition of IL-6 expression and promoting transforming growth factor (TGF)-β secretion [27]. IL-6 inhibits Treg cell differentiation, and as such, IL-6 inhibition has interesting implications regarding the maintenance of Treg cells. It has also been shown that Treg cells transfer the inhibitory secondary messenger cyclic adenosine monophosphate (cAMP) into effector T cells via membrane gap junctions [28], supporting metabolic disruption as a means through which Treg cells can reduce immune responses.

Cytokine deprivation-mediated apoptosis may also be a means through which Treg cells disrupt normal cellular metabolism, and induce immunosuppression. Disagreement within the field exists as to whether high expression of CD25 allows Treg cells to ‘consume’ local IL-2, in turn starving dividing effector T cells by depleting the IL-2 necessary for their survival [13, 29, 30]. This mechanism has been revisited in recent years following evidence that Treg cells do indeed induce cytokine deprivation-mediated apoptosis, specifically referring to IL-2. Pandiyan et al. showed that the pro-apoptotic protein Bim is essential for Treg cell-induced effector T cell death and that CD4+ T cells incubated with Treg cells had lower activation levels of the pro-survival kinase Akt, complemented with less phosphorylation of the pro-apoptotic protein Bad, while also confirming Treg cells do indeed ‘starve’ effector T cells by utilising IL-2 without producing it [31]. These findings lend credibility to the notion that cytokine deprivation-mediated apoptosis is a prominent inhibitory mechanism of Treg cells. However, a study using human Treg cells has concluded that IL-2 deprivation alone is not necessary for Treg cells to

suppress functioning of effector T cells [32], and so more research is clearly warranted to elucidate the precise mechanism through which Treg cells disrupt metabolism.

Production and secretion of anti-inflammatory cytokines Treg cells produce the anti-inflammatory cytokines IL-10, IL-35, and TGF-β, and their production and secretion are common and well-supported proposed mechanisms of Treg cell-mediated suppression. In animal models of allergy and asthma, research suggests that both nTreg and iTreg cells modulate disease through a mechanism that is partly dependent on IL-10 and TGF-β [33, 34]. Kearley and colleagues showed that, following allergen challenge, CD4+ effector T cells are stimulated to produce large quantities of IL-10 in the lung following adoptive transfer of Treg cells, and that this is sufficient to control disease and could be reversed upon administration of an IL-10 receptor-specific antibody [35]. It is important to note, however, that allergic inflammation and airway hyper-reactivity were still suppressed following the transfer of IL-10-deficient Treg cells and elevated IL-10 levels remained suggesting suppression of Th2-driven allergen responses is contingent on IL-10, yet production of IL-10 by Treg cells alone is not solely responsible for the observed suppression [13]. In contrast, Treg cell-specific ablation of IL-10 expression was shown to increase allergic inflammation and hyper-reactivity in the lung [36], highlighting the need for more research in this area. Numerous studies have shown beneficial and protective roles for IL-10-producing Treg cells in a variety of disease contexts [37–40], and while precise mechanisms may remain unclear, the advantageous role of IL-10 is undisputed.

Similarly, studies have shown that Treg cell-produced TGF-β may be directly involved in the suppression of effector T cells, and potentially general Treg cell maintenance [13]. For instance, in a mouse model of inflammatory bowel disease (IBD), TGF-β-resistant effector T cells could not be suppressed by Treg cells [41]. TGF-β produced by Treg cells is also thought to be involved in limiting anti-tumour immunity in follicular lymphoma [42] and head and neck squamous-cell carcinoma [43]. While it is important to note that the exact importance of TGF-β for correct functioning of nTreg cells as opposed to iTreg cells remains to be confirmed [13], the therapeutic contributions of this anti-inflammatory cytokine are well documented in a variety of conditions. Kursar and colleagues showed that Treg cell-produced TGF-β is important in mediating the host immune response to *M. tuberculosis* [44], with similar beneficial roles observed in prevention of colitis in an IBD model [45] and the suppression of allergic responses [34]. Interestingly, TGF-β associated with tumour exosome membranes is thought to improve the suppressive capability of Treg cells and lead T cells away from typical effector functions in favour of a more regulatory phenotype [46], while ovalbumin-induced airway inflammation can be ameliorated by heme oxygenase-1 via membrane-tethered TGF-β and IL-10 [47].

IL-35 is the most recently discovered cytokine produced and secreted by Treg cells, and is required for their maximal suppressive function [48]. IL-35 is a member of the IL-12 heterodimeric cytokine family and is formed through the pairing of

Epstein–Barr virus-induced gene 3 (*Ebi3*) and p35 [13]. The importance of IL-35 for maximal suppressive capacity of Treg cells was initially highlighted by Collison et al. [48]. Here it was shown that both *Ebi3*$^{-/-}$ and p35$^{-/-}$ Treg cells were significantly restricted with regulatory functions in vitro, with a failure to resolve IBD and control homeostatic proliferation of effector T cells in vivo. Collison and colleagues also showed that IL-35 is sufficient to induce and maintain Treg cell activity as ectopic expression of IL-35 lends regulatory properties to naïve T cells, and recombinant IL-35 can suppress T cell proliferation in vitro [48]. Since its discovery, IL-35 as a potent modulator of immunity has been investigated in numerous disease contexts. IL-35-producing B cells are now known to be essential regulators of host immune responses in both autoimmune and infectious diseases, with mice lacking these cells losing their ability to recover from experimental autoimmune encephalomyelitis (EAE), a T cell-mediated demyelinating disorder closely resembling human multiple sclerosis (MS) [49]. Further, Treg cell stimulation with IL-35 has been shown to confer protection against collagen II-induced arthritis via the production of IL-10 [50]. The extensive literature investigating IL-35, as well as IL-10 and TGF-β, in healthy and pathogenic environments has shown that these inhibitory cytokines are key mediators of Treg cell function; however, more research is warranted to completely clarify the underlying mechanisms.

Direct cytotoxicity Direct cytotoxicity is yet another suggested mechanism through which Treg cells procure immune regulation and is thought to involve cytolysis through granzyme A- and B-dependent, and perforin-dependent killing mechanisms. In human Treg cells, an amalgamation of CD3 and CD46 stimulation has been shown to induce granzyme A, initiating apoptosis in activated target cells such as natural killer cells, APCs, and effector T cells [51], supporting cytolysis as a possible Treg cell-mediated suppressive pathway. Granzyme B is also thought to be involved in Treg cell-mediated suppression. Several research laboratories have shown that, upon activation, Treg cells can kill APCs or responder/effector T cells in vitro in a granzyme B-dependent manner [52, 53] and have identified increased levels of granzyme B in murine Treg cells [54]. Gondek et al. first demonstrated that granzyme B-deficient Treg cells are inhibited in their suppressive activity in vitro and that this effect appeared to be a perforin-independent result involving Treg cell-induced apoptosis of effector T cells [52], but studies have also shown a partially perforin-dependent mechanism through which Treg cells can induce apoptosis via granzyme B [53]. As well, in vivo studies reinforce cytolysis as a mechanism for Treg cell-mediated suppression as they show granzyme B is essential in preserving Treg cell-dependent skin graft tolerance [55].

Although the CNS is thought to be an immune-privileged site with minimal immune responses, emerging evidence in recent years has forced a review of this notion. The CNS is now known to undergo constant immune surveillance [56, 57], which is tightly regulated. However, in certain cases where peripheral tolerance is lost, autoimmune responses involving autoreactive T cells or auto-antibodies against CNS antigens ensue, leading to demyelination or neurodegeneration within the CNS. A critical player in these autoimmune disorders is the Treg cell, with evidence

indicating a defect in either the number or function of Treg cells isolated from the peripheral blood of patients [58] and some degree of Treg cell instability and plasticity [59]. While not a complete list, here we discuss the contribution of Treg cells in both common and rare autoimmune disorders of the CNS, including multiple sclerosis (MS), neuromyelitis optica (NMO), acute disseminated encephalomyelitis (ADEM), N-methyl-D-aspartate receptor (NMDAR) antibody encephalitis, and narcolepsy with cataplexy.

Involvement of Treg Cells in Multiple Sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease of the CNS and is most often characterised by widespread areas of demyelination, gliosis, neurodegeneration, and neuroinflammation within the brain and spinal cord. MS predominantly affects individuals in their early adult life [60] and is one of the most common CNS inflammatory disorders with an estimated prevalence of 2.5 million people affected globally [61]. The precise pathogenesis of MS remains largely unknown; however, evidence suggests that a compromise in the integrity of the blood–brain barrier (BBB) precedes an influx of myelin-specific autoreactive T cells into the CNS [2]. The entrance of these autoreactive cells is believed to prompt a chronic inflammatory response which promotes the formation of disease plaques, characterised by focal areas of demyelination, glial reactivity, immune cell infiltration, and axonal damage. The aetiology of MS also remains unknown, although metabolic abnormalities, environmental factors, including vitamin D deficiency and obesity, and a genetic predisposition coinciding with a non-genetic trigger (e.g. virus) are implicated [62].

There are several forms of MS, which may be categorised based upon clinical manifestation. These include clinically isolated syndrome (CIS), relapsing–remitting MS (RRMS), primary progressive MS (PPMS), and secondary progressive MS (SPMS). RRMS remains the most prevalent form of the disease with approximately 85% of patients presenting with distinct episodes of neurological deficit [63]. This is due to focal white matter injury involving autoimmune targeting of myelin sheath components, infiltration of numerous immune cells, and activation of glial cells in the CNS [2]. Motor deficits, coordination problems, sensory disturbances, optic neuritis, and eye-movement aberrations are among the most common complications experienced by MS patients [64]. Periods of disease exacerbation are followed by periods of remission, before a subsequent relapse occurs. The physiology of clinical remission in MS is unknown, but resolution of inflammation, remyelination, and recovery of an immune balance are thought to be involved [65, 66]. In contrast, progressive MS is characterised by steadily worsening neurological function with diffuse tissue injury, including degeneration of chronically demyelinated axons, damage to or dysfunction of astrocytes, and microglial activation.

EAE is the most widely used animal model in MS research, sharing both clinical and pathological characteristics of the disease, including demyelination, neuronal

damage, and neuroinflammation [2]. EAE is induced through active immunisation with self-antigenic epitopes of myelin, such as proteolipoprotein (PLP), myelin basic protein (MBP), or myelin oligodendrocyte glycoprotein (MOG), and has contributed valuably to understanding various aspects of MS. EAE has been extensively implemented to investigate the contribution of Treg cells in the progression of the disease, and how their innate anti-inflammatory characteristics may be used in the development of novel therapeutics for MS [67]. Preclinical studies of MS utilising animal models have shown Treg cell number is elevated in the CNS during stages of clinical recovery [68–70], with the surrounding inflammatory milieu dictating their role in disease. For instance, it has been shown that antigen-specific FoxP3+ Treg cells accumulating centrally can inhibit IFN-γ production through CNS-derived effector cells [71] yet are incapable of modulating effector T cells that produce tumour necrosis factor (TNF) and IL-6 [68].

The importance of Treg cell function in EAE has also been highlighted using transgenic animals which allow the selective targeting and depletion of Treg cells. Using the transgenic DEpletion of REGulatory T cells (DEREG) mice, Koutrolos et al. selectively depleted animals of Treg cells 4 days post MOG_{35-55}-induced EAE disease onset and reported both intensified clinical symptoms and elevated T cell proliferation and pro-inflammatory cytokine production within the CNS of these EAE-affected animals [72]. Similarly, in a PLP-induced EAE model, intravenous adoptive transfer of CD4+ CD25+ cells previously isolated from naïve SJL mice ameliorated disease severity via IL-10. McGeachy and colleagues also showed that intravenous transfer of CD4+ CD25+ cells derived from the CNS of mice in the recovery stage of EAE into recipient animals protects the recipients from disease development [69]. Interestingly, in the latter study, the researchers also showed that transfer of the same number of naïve CD4+ CD25+ cells derived from lymph nodes elicits no effect in recipient animals, demonstrating the increased potency of Treg cells derived from the CNS, which in turn confirms the importance of centrally located Treg cells in the natural resolution of EAE [67].

There exists much evidence which confirms a therapeutic role of Treg cells in EAE, however, much debate continues about the beneficial role of Treg cells in human MS patients. Frequency of Treg cells in the blood of untreated MS patients has been reported to be both unchanged [73–75] and decreased [76, 77] in comparison to healthy controls. As well, it has been reported that untreated patients with RRMS have a reduced number of Treg cells and lower FoxP3 expression in the peripheral blood when compared to both healthy controls and untreated SPMS patients [58]. Periods of remission in RRMS are thought to coincide with increased levels of FoxP3+ Treg cells in the blood of patients not receiving treatment in the 3 months prior to their most recent relapse [67, 78]. Some researchers also contend that only a specific subset of Treg cells are decreased in the blood of RRMS patients. Fletcher et al. showed that both CD39+/− Treg cells (i.e. FoxP3+ CD39+/−) are able to suppress IFN-γ production and T responder cell proliferation in vitro, but IL-17 production was only suppressed through the CD39+ population; interestingly, the CD39- population of Treg cells actually produced IL-17 [79]. Fletcher and colleagues also showed that the ability of residual CD39+ Treg cells from treatment-naïve

MS patients to suppress IL-17 production from responder T cells is diminished when compared with healthy individuals. Despite this evidence, a recent meta-analysis concluded that the frequency of Treg cells is not a risk factor for the development of MS [80].

Treg cells isolated from patients with MS consistently display phenotypic alterations compared with healthy controls, and these may be involved in the development and maintenance of the disease. Studies have shown that regulation of effector T cell and antigen-specific T cell proliferation in untreated MS patients through Treg cell-dependent mechanisms is dysfunctional [73, 74], when compared with Treg cells isolated from healthy individuals. Similarly, elevated numbers of IFN-γ-producing, Th1-like FoxP3+ Treg cells have been detected in patients with untreated RRMS [81], supporting the notion of a dysfunction within the Treg cell population in human MS patients. Treg cell suppression is known to decrease with age [67], yet Treg cells in a paediatric variant of MS displayed impaired regulatory properties in patients not receiving treatment with corticosteroids, compared to age-matched healthy controls, in the weeks preceding collection of Treg cells [82]. Taken together, a defect in numbers and/or function of Treg cells in MS patients seems plausible.

It is also worth noting that a recent study by Dombrowski and colleagues has revealed a new regenerative function of Treg cells within the CNS which is distinct from immunomodulation [83]. In this study, Treg cells were shown to promote oligodendrocyte differentiation and (re)myelination in a lysolecithin-mediated animal model of demyelination. By utilising this model along with other models with minimal peripheral immune influence, the authors showed that CCN3, a growth regulatory protein with bioactivity in extracellular, nuclear, and cytoplasmic compartments, is produced by Treg cells and is implicated in the regeneration of various tissue types [84–86]. It was determined that CCN3 within the CNS accelerates both oligodendrocyte differentiation and (re)myelination, thereby expanding the classically accepted function of Treg cells in nervous system pathologies and may contribute to therapies targeting tissue regeneration in a variety of disorders.

Involvement of Treg Cells in Neuromyelitis Optica

Neuromyelitis optica (NMO), also known as Devic's disease, is also a demyelinating disease of the CNS with a strong inflammatory component. NMO is characterised by severe attacks of myelitis and optic neuritis which differ from those typically seen in MS by commonly sparing the brain in the early stages [87]. These lesions are often large and necrotic, and typically affect the optic nerves and spinal cord [88]. Episodes of myelitis and optic neuritis among NMO patients are generally sequential rather than simultaneous, and the period between these episodes may be years or decades. Typical features of NMO include loss of vision with ocular pain, and myelitis with symmetric paraplegia, bladder dysfunction, sensory loss below

the level of lesions, paroxysmal tonic spasms, and Lhermitte's phenomenon (an electric shock-like sensation that occurs as a result of flexion of the neck). Like MS, the majority of NMO patients are female, with researchers believing women are up to nine times more likely than men to develop the disease [89]. The median age of onset for NMO is thought to be around 40 years of age; however, the disease may also affect children and the elderly.

Also similar to MS, most patients presenting with NMO exhibit with relapsing episodes of optic neuritis and myelitis as opposed to a monophasic, progressive course, with relapse occurring within 3 years in 90% of patients. Once a maximum clinical deficit has been reached, remission generally occurs in the weeks or months following; however, recovery is typically incomplete [87]. Some researchers speculate that within 5 years of disease onset, more than 50% of presenting patients with relapsing NMO are blind in one or both eyes and/or require ambulatory help. NMO spectrum disorders (NMOSD) is a term that has been used to describe patients who do not meet the diagnostic criteria of NMO [90], and complex immune interactions are involved in all conditions. However, this review will specifically focus on the involvement of Treg cells in NMO.

NMO is a complex disorder and while the precise cause of the disorder is unknown, it is generally accepted that an interaction between genetic (e.g. human leukocyte antigen, HLA) and environmental factors is responsible [91]. A serum immunoglobulin G (IgG) auto-antibody, NMO-IgG, has previously been shown to be highly specific to NMO [92], and Lennon et al. have shown that NMO-IgG binds selectively to the aquaporin-4 (AQP4) water channel, the predominant water channel found on astrocytes within the CNS [93]. AQP4 is an essential protein in astrocytic plasma membranes and is present in high concentrations in foot process domains facing microvessels, where it interacts with dystrophin-associated proteins. However, there does exist a subset of NMO patients who exhibit seronegativity for anti-AQP4, suggesting the myelitis and optic neuritis seen in NMO may be caused by alternative mechanisms [91]. These alternative mechanisms are thought to include paraneoplastic disorders, infectious diseases, and connective tissue dysfunctions [94]. There is also a well-established association between NMO patients and multiple systemic autoimmune conditions, including myasthenia gravis (MG), systemic lupus erythematosus, and Sjögren syndrome, suggesting individuals living with NMO may have a genetic predisposition to abnormal, pathogenic autoimmunity [91].

Histopathological analyses of active NMO lesions show perivascular deposition of antibodies, with immune complexes also deposited along myelin sheaths [95] and typically degenerated penetrating spinal vessels associated with a neutrophil and macrophage-predominant inflammatory infiltrate. Various subsets of T cells are also thought to play a role in the development and maintenance of lesions in NMO. Researchers have demonstrated an elevated number of activated AQP4-specific and PLP-specific T cells in clinical relapses in AQP4-IgG positive NMO patients [91], and a direct correlation between NMO disease activity and severity and the level of T cell activation has also been established [96].

Treg dysfunction through an inability to suppress self-reactive T cells is thought to be a key component in the development of many autoimmune disorders, and studies exist to support this notion in the context of NMO. Uzawa et al. quantified levels of various cytokines and chemokines in the cerebrospinal fluid (CSF) of patients with clinically diagnosed NMO [97]. IL-10, a Treg-associated cytokine, was found to be elevated in the CSF of NMO cases as compared to patients with non-inflammatory neurological disorders. The concurrence of NMO with MG, a peripheral autoimmune disease, has also been reported and T cell subsets are believed to be involved in this simultaneous development [98]. In this study, a decreasing frequency of Treg cells among peripheral CD4+ T cells was reported in patients with concurrent MG and NMO, and alterations in Treg cell number and regulatory function have been supported numerous times in the context of MG [99–101]. As well, examining AQP4-specific T cells reveals a significant decrease in frequency of Treg cells in NMO patients in response to recombinant human AQP4, but not to p61-80 (an immunodominant AQP4 T cell determinant) [102]. Recently, a distinctive Treg gene signature in the peripheral blood, as well as significantly decreased FoxP3 mRNA expression in peripheral blood mononuclear cells (PBMCs) of NMO patients versus healthy controls was demonstrated [103]. Thus, it seems plausible that Treg cells, more specifically reduced cell numbers or a loss of suppressive function, may play a role in the pathogenesis of NMO.

As is the case with many neurological disorders, animal models in the context of NMO have allowed for greater understanding of the underlying pathological mechanisms of the condition. EAE is an animal model most widely used in MS research, but with slight modifications this model is also a useful tool in NMO settings. Bradl and colleagues induced EAE in rats through typical methods, but when first clinical symptoms presented, the immune system was supplemented with NMO-IgG containing AQP4-specific antibodies, which then entered the CNS [104–106]. These antibodies adhered to the surface of astrocytes in a pattern typically seen in the human condition and initiated the formation of astrocyte-destructive lesions. This NMO/EAE animal model demonstrates the involvement of T cells in the opening of the BBB, showing that T cells are required for the entry of these antibodies into the CNS [105, 107]. NMO/EAE studies further suggest Th17 cells may encourage lesions to localise to the optic nerve and spinal cord in NMO patients [108], and Th17 cells and IL-17 are increased in the peripheral blood of patients [109], and as such the suppressive capabilities of Treg cells upon this class of effector T cell may have potential as a therapeutic option within this context. Bar-Or et al. posit that adoptive transfer of AQP4-restricted Treg cells into NMO patients could modulate pathogenic immune cells and elicit a beneficial response [110], with animal models of colitis [111] and haemophilia [112] providing ‘proof of concept’. The potential of Treg cell immunotherapy in NMO and NMOSD patients is unknown, but the known involvement of T cells in disease pathogenesis and preliminary data in other immune-based conditions suggests a promising avenue for future research.

Involvement of Treg Cells in Acute Disseminated Encephalomyelitis

Acute disseminated encephalomyelitis (ADEM) is usually an acute, monophasic, multifocal immune-mediated disorder of the CNS and most commonly affects the paediatric age group, with a mean age of 5–8 years [113, 114], but has also been reported in adults [115]. Symptoms typically include headache, nausea, vomiting, and fever, with a broad spectrum of neurological abnormalities also involved, affecting males and females almost equally [116]. These can include ataxia, depressed consciousness, meningeal aberrations, visual deficits, cerebellar disturbances, spinal cord abnormalities, and seizures [114, 117–119]. Grey matter involvement has also been recorded [115]. Due to a lack of clear diagnostic criteria, epidemiological evidence for ADEM must be interpreted with caution, particularly in adult populations. It is generally accepted that disorders such as transverse myelitis [120], disorders involving recurrences [121], or conditions affecting both the peripheral and central nervous systems have been incorrectly diagnosed as ADEM. As previously mentioned, this disorder most commonly affects paediatric patients, usually following a viral infection [115]. Lacking a biomarker and diagnostic criteria suitable for adult patients, diagnosis is generally based on a combination of clinical symptoms, imaging, and CSF testing, as well as elimination of other inflammatory and infectious neurological conditions.

Typical ADEM pathology involves perivenular sleeves of demyelination paired with pro-inflammatory infiltrates of T and B cells, myelin-laden macrophages, granulocytes and plasma cells [122]. ADEM generally develops following an infection (usually of the upper respiratory tract) or, in rare instances, following a vaccination [123]. The infectious aetiology of ADEM is supported by seasonal fluctuations in disease frequency, with peaks in winter and spring [114, 124]. The precise pathogenesis of ADEM is unknown but is thought to predominantly involve T cell-mediated cross-activation and an immune response against myelin proteins (including MOG, MBP, and PLP), via molecular mimicry [125]. It has also been suggested that ADEM arises due to a non-specific self-sensitisation of reactive T cells against myelin proteins within the CNS, secondary to infections [123, 126, 127]. An autoimmune basis of the condition is supported by the presence of anti-MOG antibodies in the CSF of patients, and a reduction of these antibodies during disease resolution [128]. Despite this, it is not thought that anti-MOG antibodies are indicative of ADEM persistence or that there is a relationship between anti-MOG levels at disease onset and disease severity [123]. Inflammatory cells have also been confirmed within the CNS of ADEM patients, suggesting a disruption to the integrity of the BBB in this condition [127, 128].

Despite the uncertainties surrounding ADEM pathogenesis, there is no doubt that the immune system plays an intricate role. Martino and colleagues have elucidated on cytokine secretion and T cell activation in the various phases of ADEM [129]. During the hyperacute stage of ADEM, an elevation of various adhesion molecules typically expressed on endothelial cell membranes and leukocytes was found

and believed to contribute to the breaching of the BBB seen in early ADEM. Serum concentrations of two metalloproteinases, enzymes produced by T cells, were also elevated during this stage. Interestingly, Th1 lymphocytes and their associated cytokines dominate the acute stage of ADEM [129]. IFN-γ, TNF-α, IL-1, IL-6, and IL-8 were found to be increased in the CSF and serum of ADEM patients during the acute stage, with a shift to a Th2/anti-inflammatory cell-predominant environment during clinical resolution. This stage of remission found an elevation of the anti-inflammatory cytokines IL-4, IL-10, and TGF-β, with a concurrent downregulation of the previously implicated adhesion molecules [129].

Treg cells in the specific context of ADEM have not been extensively explored, but Treg cell research in other autoimmune conditions may be able to illuminate a role for these cells in ADEM, particularly as demyelination is known to share pathogenic mechanisms regardless of a viral induction or autoimmune causes [130]. The suppressive effects of Treg cells are well documented, so it may be reasonable to expect these cells would be able to dampen host immune responses, and potentially alleviate disease. A transfer of bulk populations of Treg cells into mouse hepatitis virus (MHV)-affected C57BL/6 or $RAG^{-/-}$ mice improved survival rates, decreased the amount of demyelination seen in affected animals, and reduced the number of CNS-infiltrating inflammatory cells [131, 132]. It has also been seen that Treg cell depletion prior to viral infection increases demyelination at later time points [133].

Investigations into paediatric MS may also allow for extrapolation in the context of ADEM, as the conditions also share some pathological features. A study assessing responses of T cell subsets in both adult and paediatric MS patients to MOG and MBP revealed preferential and comparable responses to particular antigenic epitopes across both groups [134]. It has also been shown that children with MS have fewer Treg cells than their healthy counterparts, and that the suppressive function of these Treg cells is also impaired [82].

Involvement of Treg Cells in Anti-NMDAR Encephalitis

The N-methyl-D-aspartate receptor (NMDAR) is a mediator of glutamatergic neurotransmission, and is central to many processes thought to involve synaptic plasticity [135]. Anti-NMDAR encephalitis is a relatively newly described condition in which antibodies attack NMDA receptors at central neuronal synapses. It commonly affects young women and an ovarian teratoma is sometimes associated with the syndrome [136]. The condition may also develop in the weeks following a viral infection [137]. Further, men and children can develop the disorder, albeit less commonly. Affected patients typically develop a characteristic set of neurologic deficits, as well as prominent psychiatric manifestations. Symptoms include changes in mood, personality and behaviour, which can resemble acute psychosis, and clinically progress to include depressed level of consciousness, seizures, autonomic instability, dyskinesias, and hypoventilation [138–141]. An immune-mediated pathogenesis was suggested after it was noted that patients generally recovered

following immunotherapy and removal of the teratoma [142] and that all patients have antibodies within the CSF and serum that react with the cell surface of neurons [113].

NMDARs are heteromers of NR1 subunits that bind glycine, and NR2 subunits that bind glutamate [143]. Anti-NMDAR encephalitis has since been characterised as a disorder in which these NR1–NR2 heteromers are the target for antibodies [142], predominantly the extracellular region of the NR1 subunit. Dalmau and colleagues assessed the effect of antibodies from patients on neuronal cultures, in particular the effect of these antibodies on NR1 clusters [143] in postsynaptic dendrites. They showed that neurons treated with CSF from NMDAR encephalitis patients for 3 or 7 days had a reduction in the number of clusters per length of postsynaptic dendrite compared to neurons treated with CSF from healthy controls. Further, neurons treated for 3 days with patient CSF and then 4 days with control CSF had comparable numbers of NR1 clusters with neurons only treated with control CSF, demonstrating a selective yet reversible decrease of NR1 clusters in postsynaptic dendrites as a result of patient antibodies. Recovery from anti-NMDAR encephalitis is slow and subject to relapses, and CSF antibody titres require more time than those of serum to return to baseline during neurological improvement [141].

The inflammatory reaction of the CNS in the context of anti-NMDAR encephalitis has not been extensively researched with only a handful of immunopathology available from autopsy cases revealing gliosis, microglial cell proliferation and IgG deposits, with scarce mononuclear cells and some plasma cells and perivascular B lymphocytes [142, 144]. A similar case study reported perivascular inflammatory B cell accumulation, as well as macrophage and T cell infiltration into the brain parenchyma, which is indicative of an encephalitic process [145]. Treatment focuses on immunotherapy, which is already known to benefit patients with the condition [143]. NMDAR antagonists are a promising drug class in many neuronal disorders, and some researchers argue that through their modulation of T cell receptors (TCRs) and the T cell responses, these drugs may be a viable option for immunosuppression/modulation in anti-NMDAR encephalitis [146]. Repeated administration of a low dose of NMDAR antagonist to differentiating Th cells has been shown to decrease IL-2 and IFN-γ production in Th1 cells, while concurrently increasing the production of IL-10 and IL-13, both known immunosuppressive cytokines involved in recovery in various neurological conditions [147, 148].

NMDAR antagonists are also able to target potassium channels, and this has been proposed as a potential mechanism through which Treg cells may be modulated in immune conditions to produce a desirable phenotype. It has been shown that inhibition of T cell function and migration can be induced by blocking the $K_V1.3$ and $K_{Ca}3.1$ potassium channels [146]. $K_V1.3$ is the main potassium channel on effector memory T cells, with $K_{Ca}3.1$ dominating both naïve and early memory T cells. Treg cells are known to express similar numbers of both $K_V1.3$ and $K_{Ca}3.1$ as naïve T cells [149, 150], as such NMDAR antagonists may potentially modulate Treg cell function as well. The role of Treg cells in anti-NMDAR encephalitis is largely unknown, yet given their fundamental roles and beneficial capacity in a myriad of other CNS autoimmune conditions, it seems feasible to expect an

involvement of these cells in the current disorder. However, extensive research in this area in the context of anti-NMDAR encephalitis remains to be performed.

Involvement of Treg Cells in Narcolepsy with Cataplexy

Narcolepsy with cataplexy (referred to as narcolepsy type 1, NT1 onwards) is a neurological condition characterised by excessive daytime fatigue, hypnagogic hallucinations, sleep paralysis, cataplexy (muscle paralysis triggered by strong emotion), and disrupted nocturnal sleep patterns. The condition affects approximately 0.02% of the population worldwide [151] and typically initiates in adolescence or early adulthood [152]. Hypocretins, also known as orexins, remain one of the most important scientific breakthroughs in the search for the cause of narcolepsy [153–155]. These molecules are synthesised exclusively in the lateral hypothalamus (LH) and derive from prepro-hypocretin, a single protein precursor [151]. NT1 is caused by defective neurotransmission via hypocretins, which results in irreversible selective loss of hypocretinergic neurons within the LH [156]. The precise aetiology of narcolepsy remains unknown, but the condition has a strong genetic association with the *HLA-DQB1∗06:02* allele, which has been reported in more than 98% of clinically diagnosed narcoleptic patients [157, 158]. HLA class I alleles and TCR-α polymorphisms have also been identified as narcolepsy-associated genes with relevance to immune responses [159–161], contributing to the shifting notion that narcolepsy is an autoimmune condition involving autoaggressive lymphocytes. Also supporting the autoimmune basis of NT1, autoantibodies have been identified in some patients with narcolepsy, although the pathogenic relevance of these remains to be determined [156, 162–165]. Further, the 2009 vaccination against the H1N1 influenza virus with the European preparation Pandemrix was directly associated with eliciting narcolepsy in people specifically carrying the *HLA-DQB1∗06:02* allele [166].

A T cell-mediated pathogenesis of narcolepsy with cataplexy in which hypocretin neurons are targeted in genetically susceptible individuals has been proposed by various researchers [167, 168]. Specific HLA-TCR interactions have been identified, which support this hypothesis [169]. A recent study by Latorre and colleagues revealed the existence of autoreactive CD4+ memory, and in some patients CD8+, T cells that target self-antigens on neurons that produce hypocretin [170]. This highlights the potentially pathogenic role of both CD8+ and CD4+ T cells in the context of NT1. It has been shown that CD8+ T cells are capable of directly killing hypocretin-producing neurons in the LH, leading to the development of sleep attacks, neuronal destruction, and cataplexy [171]. As is the case in other autoimmune disorders of the CNS, CD4+ T cells are thought to be involved in the disruption of the integrity of the BBB in NT1, initiating an influx of pathogenic antibodies and effector inflammatory cells, through the production of high levels of IFN-γ and GM-CSF (granulocyte-macrophage colony-stimulating factor) [172].

Treg cells have also been implicated in the development and maintenance of NT1. An elevated frequency and absolute count of CD4+ Treg cells in the peripheral blood of NT1 patients has previously been identified, and these cells were shown to be more activated, attributed to elevated levels of activated and memory effector CD4+ T cells [173]. It is suggested that a weak yet global inflammation in NT1 patients contributes to activation of all T cell subsets, including Treg cells, but that a defect in the ability of these Treg cells to maintain peripheral tolerance may be present. The prevailing defects of Treg cell populations in autoimmune conditions, including type 1 diabetes, lend credibility to this concept [174, 175]. Other studies have reported no significant changes regarding overall frequency of Treg cells in narcolepsy; however, it should be noted that the definition of Treg cells differs among the literature, which may account in some way for this [156].

Lecendreux et al. postulate the increase in Treg cell numbers they observed are an attempt for patients' bodies to dampen inflammation and restore tolerance, but there may be a variety of mechanisms through which this is impeded [173]. It is possible impaired suppression may be caused by Treg-intrinsic deficits or through effector T cell subsets developing resistance to Treg cell-mediated regulation. This regulation resistance is particularly true within the Th17 effector T cell subset. As well, microenvironment alterations, such as elevated levels of pro-inflammatory cytokines and altered functioning of APCs, can increase development of resistance to regulation via Treg cells [173]. Polymorphisms in TCR-α and β loci have been attributed to NT1 development, and in these patients lower polyclonality levels among altered Treg TCRs could also be a potential factor in the loss of tolerance seen in NT1 [173].

Conclusion

Treg cells are essential for maintaining self-tolerance and homeostasis [67], and their anti-inflammatory properties appear to strongly influence the pathogenic milieu in a variety of disorders involving autoimmunity within the CNS, including MS, NMO/NMOSD, ADEM, anti-NMDAR encephalitis, and NT1. Table 1 summarises the role of Treg cells in these conditions. Many studies demonstrate the beneficial roles of Treg cells in disease pathogenesis implicating defects in Treg cell number and/or function, and recent studies highlight newer concepts of Treg cell instability and plasticity contingent upon the surrounding environment, and potential roles of tissue-specific Treg cells in the CNS [59]. Thus, although Treg cells play a critical role in preventing autoimmunity, there are situations in which altered Treg cell activity suppresses protective immune responses in the CNS [67]. Overall, the potency of Treg cells makes them attractive targets for many CNS immune-mediated conditions; however, many challenges regarding clinical translation of Treg cell-based therapeutics remain. These include technical issues, such as antigen specificity, specific *ex vivo* expansion and isolation of the Treg population, and optimal timing and dosing of adoptive cell therapy. Moreover, developing antigen-specific

Table 1 Reported contributions of regulatory T cells in autoimmune conditions of the CNS

Human disease	Animal model	Involvement of Treg cells	References
MS	EAE	Numerous reports of: - Altered Treg cell numbers (e.g. elevated numbers in the CNS during stages of clinical recovery) and protective role of Treg cells in EAE. - Decreased or unchanged frequency in the peripheral blood of MS patients. - RRMS patients show decreased FoxP3 expression in peripheral blood. - Altered Treg cell functions (e.g. IFN-γ-producing, Th1-like FoxP3+ Treg cells) in MS.	[58, 68–70, 73–77, 81]
NMO	NMO/EAE: EAE supplemented with NMO-IgG containing AQP4-specific antibodies	Reports suggestive of Treg cell dysfunction: - IL-10 elevated in CSF of NMO patients. - Decreased FoxP3 mRNA expression in PBMCs of NMO patients. - Decreased frequency of peripheral Treg cells in patients with concurrent NMO and MG.	[97, 98, 103]
ADEM	–	Role of Treg cells is largely extrapolated from other diseases: - Anti-inflammatory cell-predominant environment associated with clinical resolution in ADEM patients. - Bulk transfer of Treg cells in MHV models decreased demyelination and decreased CNS-infiltrating inflammatory cell numbers.	[129, 131, 132]
Anti-NMDAR encephalitis	–	Role of Treg cells is unknown, but treatment with NMDAR antagonists may modulate Treg cell cytokine secretion and Treg cell function through potassium channels.	[147, 148]
Narcolepsy with cataplexy	–	Reports suggestive of Treg cell dysfunction, and altered numbers: - Elevated or unchanged frequency of Treg cells in the peripheral blood of NT1 patients. - Defect in Treg cells prevents regulation of global inflammation in NT1 patients.	[173]

Treg cell therapy is likely to provide a more effective and safer approach than the use of polyclonal Treg cells (with broad undefined specificity), which can potentially suppress protective immunity against tumours and infectious diseases. A more comprehensive understanding of the mechanisms Treg cells play in autoimmune conditions of the CNS undoubtedly will lead to improved Treg-cell therapies.

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Disruption of the Blood-Brain Barrier During Neuroinflammatory and Neuroinfectious Diseases

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Abstract As the organ of highest metabolic demand, utilizing over 25% of total body glucose utilization via an enormous vasculature with one capillary every 73 μm, the brain evolves a barrier at the capillary and postcapillary venules to prevent toxicity during serum fluctuations in metabolites and hormones, to limit brain swelling during inflammation, and to prevent pathogen invasion. Understanding of neuroprotective barriers has since evolved to incorporate the neurovascular unit (NVU), the blood-cerebrospinal fluid (CSF) barrier, and the presence of CNS lymphatics that allow leukocyte egress. Identification of the cellular and molecular participants in BBB function at the NVU has allowed detailed analyses of mechanisms that contribute to BBB dysfunction in various disease states, which include both autoimmune and infectious etiologies. This chapter will introduce some of the cellular and molecular components that promote barrier function but may be manipulated by inflammatory mediators or pathogens during neuroinflammation or neuroinfectious diseases.

Keywords Blood-brain barrier · Neuroinfectious diseases · Tight junctions · Innate immunity · Central nervous system

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_7

Abbreviations

AJ	Adherens junction
ANG-1	Angiopoietin-1
APC	Antigen-presenting cell
AQP4	Aquaporin 4
BBB	Blood-brain barrier
bFGF	Basic fibroblast growth factor
BMEC	Brain microvascular endothelial cell
Cav-1	Caveolin-1
CBF	Cerebral blood flow
CHIKV	Chikungunya virus
CNS	Central nervous system
CSF	Cerebrospinal fluid
CSPG	Chondroitin sulfate proteoglycan
CTL	Cytotoxic T cell
DP1	Prostaglandin D2 receptor 1
dsRNA	Double-stranded ribonucleic acid
EC	Endothelial cell
ECM	Experimental cerebral malaria
ECM	Extracellular matrix
ERK	Extracellular signal-regulated protein kinase
ET	Edema toxin
gd-MRI	Gadolinium MRI
GDNF	Glial cell line-derived neurotrophic factor
HiV	Hendra virus
HIV-1	Human immunodeficiency virus type 1
HSV	Herpes simplex virus
ICAM-1	Intercellular adhesion molecule 1
IFN	Interferon
IFNAR	Type I IFN receptor
IL	Interleukin
iRBC	Infected RBC
JEV	Japanese encephalitis virus
LCMV	Lymphocytic choriomeningitis virus
MAPK	Mitogen-activated protein kinase
MAV-1	Mouse adenovirus type-1
MAVS	Mitochondrial antiviral-signaling protein
MDA5	Melanoma differentiation factor 5
MerTK	Tyrosine-protein kinase Mer
Mfsd2a	Major facilitator superfamily domain-containing protein 2a
MHV	Mouse hepatitis virus
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging

MS	Multiple sclerosis
Msp	Meningococcal serine protease
NADPH	Nicotinamide adenine dinucleotide phosphate
NiV	Nipah virus
NLR	Nucleotide oligomerization domain-like receptor
NMOSD	Neuromyelitis optica spectrum disorder
NVU	Neurovascular unit
OPN	Osteopontin
PAFR	Platelet-activating factor receptor
PDGF-BB	Platelet-derived growth factor BB
PDGFRβ	Platelet-derived growth factor receptor β
PECAM-1	Platelet-associated cell adhesion molecule 1
PG	Proteoglycan
PGD2	Prostaglandin D2
PI3K	Phosphatidylinositol 3 kinase
pIgR	Polymeric immunoglobulin receptor
PKB	Protein kinase B
PLC	Phospholipase C
PPMS	Primary progressive multiple sclerosis
PRR	Pattern recognition receptor
RABV	Rabies virus
Rac-1	Ras-related C3 botulinum toxin substrate
RBC	Red blood cell
RhoA	Ras homolog gene family, member A
RLR	Retinoic acid-inducible gene 1 like receptor
ROS	Reactive oxygen species
RRMS	Recovery and remission multiple sclerosis
S1P	Sphingosine-1-phosphate
SAS	Subarachnoid space
sCD40L	Soluble CD40L
SHH	Sonic hedgehog
SPMS	Secondary-progressive multiple sclerosis
ssRNA	Single-stranded ribonucleic acid
TBEV	Tick-borne encephalitic virus
TEER	Transendothelial electrical resistance
TIMP	Endogenous tissue inhibitor of MMP
TJ	Tight junction
TLR	Toll-like receptor
TMEV	Theiler's murine encephalitis virus
TNFα	Tumor necrosis factor alpha
VCAM-1	Vascular cell adhesion molecule 1
VEEV	Venezuelan equine encephalitis virus
VEGF	Vascular endothelial growth factor
VSV	Vesicular stomatitis virus
WNV	West Nile virus

Introduction to BBB Structure and Function

Over 100 years ago, a publication by Lina Stern, Professor and Head of the Department of Physiological Chemistry at the University of Geneva, coined the term "blood-brain barrier (BBB)" to describe the finding that systemically administered dyes are excluded from the developing mammalian brain [1]. Since then, physicians and scientists have appreciated the unique diffusion barrier between the blood and the brain and its stringent regulation of central nervous system (CNS) entry of molecules, immune cells, and pathogens [2–4]. The BBB occurs at the level of postcapillary venules and capillaries and is comprised of a variety of physical specializations including inter-endothelial tight and adherens junctions (TJ and AJ), endothelial cells with polarized expression of protein receptor influx and efflux transporters, and transcytosis systems limited to albumen and histones [5]. Vasculature at the BBB is enveloped by pericytes and astrocyte end feet, which leads to the development of dual basement membranes with a complicated extracellular matrix (ECM) separating blood contents from perivascular spaces within the CNS parenchyma. The CNS ECM is comprised of hyaluronic acid and proteoglycans (PG), mainly chondroitin sulfate proteoglycans (CSPG) [6]. Heparan sulfate proteoglycans (HSPG), especially the negatively charged heparan sulfate (HS), bind and sequester pro-inflammatory molecules, including the endothelial cell-derived chemokine CXCL12 ([7] and see below), which regulates the recruitment and effector functions of leukocytes that infiltrate the CNS during neuroinflammatory diseases [8]. BBB TJ are heterodimeric proteins comprised of occludin and members of the claudin family of proteins, including claudin-3 or -5, that link to the cytoskeleton via the scaffolding and regulatory proteins ZO-1, -2, -3, and cingulin (reviewed in [9]). Similarly, AJ are comprised of E-cadherin proteins that link to actin filaments via α-, β-, and γ-catenin. The length of actin fibers, which are regulated by the activation of RhoGTPases, controls the integrity of both AJ and TJ complexes [10]. Activation of Rac1 promotes stabilization of TJ and AJ, while RhoA promotes destabilization. Junctional integrity is critical for two separate capacities of the BBB, termed "gate" and "fence" function [11]. Gate function refers to the importance of inter-endothelial junctional complexes in limiting the movement of molecules and cells from the blood to the brain parenchyma. RhoA activation may therefore reduce gate function and allow BBB penetration without loss of junctional proteins. Fence function refers to the role of TJ and AJ in the maintenance of BBB polarity, preventing the rotation and diffusion of proteins and other biomolecules within the cell membrane between abluminal and luminal surfaces. Thus, BBB permeability can also be increased without complete destruction of junctional proteins through alterations in the locations of proteins involved in transport or transcytosis.

The transcellular transport of macromolecules across endothelial barriers occurs in peripheral organs via a variety of pathways including macropinocytosis and clathrin- and caveolae-mediated endocytosis. The BBB, however, exhibits low levels of macropinocytosis and lack of clathrin expression. Caveolae-mediated

transcytosis is strictly regulated at the BBB by the major facilitator superfamily domain-containing protein 2a (Mfsd2a), which is exclusively expressed on brain endothelial cells and induced by pericytes [12]. Consistently, *Mfsd2a*$^{-/-}$ mice exhibit increased BBB permeability, caused by enhanced caveolae-mediated transcytosis [12]. Caveolae are flask-shaped plasma membrane invagination enriched in cholesterol and sphingolipids. They contain the major structural protein caveolin-1 (Cav-1), which undergoes extensive oligomerization prior to interacting with cavin-1 to form caveolae. Genetic ablation of either Cav-1 or cavin-1 results in a complete loss of caveolae in related tissues, suggesting their essential role in caveolae formation [13, 14]. Previous studies identified a close association between caveolae and stress fibers, a feature absent in clathrin-coated vesicles [15]. These interactions are critical for both stabilizing and entry of caveolae at the plasma membrane and are also regulated by the small RhoGTPases, including Ras homolog gene family, member A (RhoA) and Ras-related C3 botulinum toxin substrate (Rac)-1 [15]. Caveolae internalization is further regulated by kinases and phosphatases. In general, BBB endothelial cells exhibit low level of formation of caveolae due to the effects of Mfsd2a. However, levels of this protein are decreased during intracranial hemorrhage, suggesting that serum inflammatory mediators might increase BBB permeability via their effects on caveolae-mediated transcytosis.

The polarized expression of proteins at the CNS vascular barriers is also important for normal immune surveillance of the CNS. There is a growing body of evidence that lymphocytes, including effector memory CD4 and CD8 T cells, normally reside within the cerebrospinal fluid (CSF) compartment [16–22]. The CSF compartment includes both the subarachnoid space (SAS) and the ventricular system, the latter of which contains the choroid plexus, a plexus of microvessels with modified ependymal cells that form a barrier between its fenestrated capillaries and the CSF compartment (reviewed in [23]), which connects with lymphatics that provide mechanisms for leukocyte egress out of the CNS [24, 25]. The choroid plexus is the main producer of CSF, which circulates via a combination of directed bulk flow, and both pulsatile and continuous bidirectional movement at the BBB and at the borders between CSF and CNS interstitial spaces (reviewed in [26]). The SAS occurs between meningeal arachnoid and pia maters and contains fenestrated capillaries where immune cells may exit the blood and migrate along abluminal surfaces into perivascular spaces within the brain parenchyma at sites with BBB specializations. The localization of lymphocytes along CNS vasculature is accomplished via polarized expression of chemokines, including CXCL12 [27], which promotes interactions between T and perivascular antigen-presenting cells (APCs) in the setting of neuroinfectious diseases. Infiltrating T cells express CXCR4, a G protein-coupled signaling receptor of CXCL12 that is downregulated after T cell receptor activation, which allows T cell egress out of perivascular compartments [28, 29]. The abluminal localization of CXCL12 stands in stark contrast to its expression pattern at high endothelial venules within lymph nodes, where luminal CXCL12 promotes the homeostatic circulation of lymphocytes between the blood and lymphoid compartments [30], whereas BBB CXCL12 instead limits T cell entry into the CNS parenchyma [27, 28]. The level of CNS expression of CXCL12 vascular barriers is

accomplished at both transcriptional and protein expression levels, the latter of which occurs via the CXCL12 scavenging receptor CXCR7 [31]. As the CXCR7 promoter contains eight NF-kB binding sites, multiple cytokines may alter the level of its expression at the BBB during neuroinflammation, including interleukin-1, -8, -17, and interferon-γ. Alterations in the patterns of localizing cues at the BBB could promote excessive leukocyte entry, which may lead to further alterations in the BBB functions.

Cellular Constituents of the NVU Regulate BBB Formation and Function

The NVU is comprised of brain microvascular endothelial cells (BMECs), abluminal pericytes, and astrocyte terminal processes, known as end feet, the latter of which receive neuronal signals that modulate BBB influx and efflux transporters in response to parenchymal demands or damage [5]. Pericapillary pericytes extend their processes along pre- and postcapillary vessels, receiving signals from BMECs, astrocytes, and neurons that induce them to form, maintain, and regulate BBB function [32]. Studies in pericyte-deficient and transgenic mice with aberrant signaling between endothelial-derived platelet-derived growth factor BB (PDGF-BB) and platelet-derived growth factor receptor β (PDGFRβ) in pericytes have thus identified critical roles for these cells from embryonic development to adulthood [33]. *Pdgfb* and *Pdgfrβ* homozygous knockout mice completely lack pericytes, which causes embryonic lethality via cerebral blood vessel rupture and microhemorrhages. While $Pdgfr\beta^{+/-}$ mice and mice with modified PDGF-BB bioavailability are viable, they exhibit reductions in pericyte coverage along vasculature, leading to poor maintenance of BBB function and increased permeability [33]. These mice also exhibit dysregulated cerebral blood flow (CBF) leading to eventual loss of neurons in the cortex and hippocampus. These data indicate the importance of maintaining adequate pericyte numbers for proper BBB function.

Both pericytes and astrocytes are important in the preservation of BMEC TJs through the regulation of junctional proteins occludin, claudin, and ZO-1. Astrocyte end feet also contact the abluminal surfaces of BMECs and enwrap neuronal synapses, enabling simultaneous modulation neuronal activity and blood flow in response to elevations in intracellular Ca^{2+} levels [34, 35]. Astrocyte end feet are also highly polarized and express specialized molecules such as Kir4.1 K^+ channels and aquaporin 4 (AQP4), which each regulate BBB ionic concentrations, and protein transporters such as glucose transporter-1 and P-glycoprotein, the latter of which promotes the efflux of toxic substances away from brain parenchyma [36, 37]. Astrocytes may exchange signals through gap junctions forming a functional syncytium that coordinates BBB responses and communicates with neurons [38, 39]. Astrocytes critically develop and maintain BBB characteristics through the release of vascular endothelial growth factor

(VEGF), glial cell line-derived neurotrophic factor (GDNF), basic fibroblast growth factor (bFGF), and angiopoietin (ANG)-1 [39, 40], which form TJ, promote enzymatic systems, and polarize expression of transporters [41].

The full integration of NVU responses that regulate and maintain BBB function relies on multiple signaling pathways and proteins that regulate TJ integrity, including calcium, protein kinase A, protein kinase C, G proteins, calmodulin, cAMP, and phospholipase C [42, 43]. Heterotrimeric G proteins and protein kinase C signaling pathways, in particular, act via altering intra- and/or extracellular levels of calcium, which promotes TJ integrity [44]. Phosphorylation additionally regulates transmembrane and accessory proteins of TJs. Both serine and threonine phosphorylation of occludin, which regulates its subcellular localization, are highly correlated with the reassembly of TJs following alterations in BBB integrity [45] The PAR3-PAR6-APKC pathway and the evolutionarily conserved signaling complex related to the *Drosophila* Stardust-Disc lost-Crumbs complex (equivalent to the mammalian Pals1-PATJ-Crumbs complex) [43] have also been implicated in regulation or modulation of TJ assembly. As PAR-complex APKC and PAR3 may be downregulated upon activation of NF-kB and, in turn, act to inhibit NF-kB-mediated signaling, these pathways may provide additional mechanisms for the alteration of BBB function during neuroinflammation.

In summary, the BBB has evolved numerous cellular, subcellular, and molecular mechanisms to stringently regulate the CNS access of solutes, molecules, cells, and pathogens. BBB function, however, may become dysfunctional or derailed via intrinsic and/or extrinsic effects in the setting of neuroinflammatory diseases, including those caused by autoimmune, infectious, or neurodegenerative processes.

Mechanisms of BBB Disruption During Pathological Conditions

During CNS disease, the NVU may undergo cytoarchitectural modulations that promote BBB permeability without significant alterations in structural integrity. TJs and their associated proteins are dynamically regulated and able to undergo alterations in transcription, translation, and posttranslational modifications, subcellular localization, and protein-protein interactions in normal and diseased states. Thus, acute and subtle changes in BBB permeability with accompanying mild elevations in CSF protein levels may occur without severe CNS symptoms. Prolonged alterations in NVU structure and function, however, can lead to complete TJ disruption leading to brain edema and neural cell damage and irreversible in brain injury. Here, we will discuss the role of primary or secondary BBB dysfunction in the etiology, progression, and repair of neuroinflammatory diseases.

BBB Disruption During CNS Autoimmunity

Failure of BBB function is a critical event during the development and progression of autoimmune diseases of the CNS, including neuromyelitis optica spectrum disorders (NMOSD) and multiple sclerosis (MS). NMOSD are rare, relapsing immune-mediated CNS disorders characterized by inflammation and demyelination of the optic nerves and spinal cord with evidence of BBB dysfunction in up to 70% of cases and two-thirds of patients exhibiting elevations in serum anti-AQP4 autoimmune IgG antibodies (classified as NMO patients) (reviewed in [46]). While patient serum levels of anti-AQP4 IgG are not a predictive biomarker for overall disease course [47], they are positively correlated with the extent of spinal lesions; BBB permeability, as assessed via albumin index; levels of CSF myelin basic protein concentration; and serum C3 [48]. Anti-AQP4 IgG contribute to pathogenesis via effects at astrocyte end feet within the NVU, which express AQP4, and bind to the abluminal surfaces of microvessels in NMO patients, in conjunction with lesions containing complement proteins, infiltrating neutrophils and eosinophils, and loss of AQP4 [49]. Human data are consistent with a significant role for anti-AQP4 IgG in the pathogenesis of NMO, which is further supported by the clinical efficacy of plasma exchange and B cell depletion [50]. In animal studies, targeted deletion of AQP4 or administration of anti-AQP4 IgG-positive sera plus complement from NMO patients leads to loss of BBB integrity and impaired water homeostasis within astrocyte end feet [51–53]. Similarly, using an in vitro human BBB model administration of human anti-AQP4 IgG and complement increased the migration of granulocytes across BMECs and led to astrocyte injury and decreased transendothelial electrical resistance (TEER) [54]. While the mechanism of anti-AQP4 IgG entry at the BBB, including access to astrocyte end feet, is unclear, endothelium-specific antibodies, VEGF, and matrix metalloproteinase (MMP)-9 are all elevated in NMO [52, 55]. Activation of BMECs via endothelium-specific antibodies may lead to concomitant upregulation of intercellular adhesion molecule (ICAM)-1 [52], promoting capture of leukocytes, and secretion of TNF and VEGF. The release of MMP-9 from infiltrating neutrophils could play a role in the degradation of the BBB ECM [55] by allowing anti-AQP4 IgG access to astrocyte AQP4. Further development of animal models of NMO could help identify therapeutic targets to prevent these effects.

The role of BBB dysfunction in the induction and progression of MS is a subject of controversy [56], mostly due to the lack of models that faithfully reproduce the diseases observed in patients. MS is a heterogeneous group of demyelinating syndromes in which patients may present with a relapsing-remitting form, characterized by periods of disease exacerbation followed by recovery and remission (RRMS). RRMS may be followed by the onset of continued progression of disease (i.e., secondary-progressive (SP)MS) or a primary progressive form in which patients continue to develop neurologic deficits without remission (PPMS) [57]. The characteristic CNS lesion observed in MS patients is a focal area of

inflammatory-mediated demyelination surrounding postcapillary venules within white matter [58]. In severe cases of MS, patients may also exhibit demyelinating lesions within cortical gray matter, often adjacent to meninges. Defects in BBB function are observed in all lesions, with gadolinium extravasation observed using magnetic resonance imaging (MRI). However, while frank TJ disruption is not observed in MS lesions, as assessed in early studies using electron microscopy [59], the exact mechanisms of BBB impairment are unclear, as is the timing of these events as primary or secondary to the effects of immune cells.

Evidence for primary causes of BBB dysfunction include altered BMEC expression of molecules involved in the stabilization of TJs, including sphingosine 1-phosphate receptor 2 (S1P2) and claudin proteins [60, 61]. S1P2 is one of the five subtypes of G protein-coupled receptors (S1P1-5) that are targeted by S1P, a signaling, blood-borne sphingolipid that regulates angiogenesis, vascular stability, and permeability and may also be important in the pathogenesis of neurodegenerative diseases (reviewed in [62]). S1P also regulates the trafficking of T and B cells within lymphoid tissues and directly suppresses TLR-mediated immune responses from T cells [63]. At the BBB, S1P1 and S1P3 activation promote Rac1-mediated tightening of inter-endothelial junctions, while S1P2 leads to their disassembly via RhoA [64]. In murine models of RRMS, disassembly of BBB TJs and AJs is associated with loss of polarized expression of CXCL12 with increased capture and CNS entry of CXCR4-expressing T cells [61]. Patients with MS exhibit loss of BBB polarity within white matter lesions [65], and women with RRMS exhibit significantly higher levels of S1P2 at the NVU within hindbrain regions compared with male MS patients [61].

Loss of polarized expression of CXCL12 may also be the result of BMEC expression of the CXCL12 scavenger receptor CXCR7. Studies in animal models of MS suggest that interleukin(IL)-17-secreting CD4 and γδ T cells may drive CNS autoimmunity, especially with regard to access to CNS parenchyma from perivascular spaces [66]. γδ T cells, which do not require antigen processing and major histocompatibility complex (MHC) presentation of peptide epitopes and instead may recognize lipid antigens, are also sources of IL-1 within the inflamed CNS [67, 68]. CXCR7 reporter mice exhibit expression of the receptor along postcapillary venules, which is increased during induction of CNS autoimmune disease, leading to loss of abluminal expression of CXCL12 and increased CNS access of myelin-specific T cells [31]. In vivo targeting of CXCR7 in animal studies using small molecule inhibitors maintains polarized expression of CXCL12 and limits the egress of immune cells out of perivascular spaces during induction of EAE. In vitro studies examining the regulation of CXCR7 expression on BMECs demonstrated that IL-17 and IL-1 increase the expression and activity of the receptor, respectively, consistent with in vivo studies demonstrating roles for these cytokines in driving neuropathology and the clinical effectiveness of therapies that target IL-17 or IL-1 in patients with autoimmune diseases [69–71]. Novel therapeutics targeting CXCR4 and/or CXCR7 are under development [72] and may prove beneficial for the treatment of MS.

Although BBB disruption is clearly evident on gadolinium (gd)-MRI of MS patients, the notion that this is due to direct alterations in TJ protein expression has been controversial. Early reports examining the levels of expression of claudin-5, a major component of CNS TJs, did not reveal differences in CNS specimens from patients with and without MS [73]. More recently, claudin-11, which co-localizes with claudin-5 in CNS capillaries, was found to be significantly decreased in CNS tissue of MS patients and of mice with EAE [60]. Multiple studies, however, show leakage of serum proteins including fibrinogen, albumen, and IgG, into CNS parenchyma within MS lesions [74, 75], which is consistent with the overall loss of BBB function. Whether this extravasation is the result of loss of gate and/or fence function, the latter of which might include alterations in BMEC intracellular endocytic pathways, remains to be determined.

BBB Disruption During Neuroinfectious Diseases

The meningeal barriers, which cover the surface of the brain and spinal cord and are comprised of the dura, arachnoid, and pia maters, effectively limit the ability of a majority of bacterial, fungal, and viral pathogens to gain access to the CNS parenchyma. Thus, only neurotropic viruses, molds, and certain parasites are able to cross the BBB and infect CNS parenchyma. Certain bacteria that gain access to the subarachnoid space within the meninges may also enter perivascular spaces of postcapillary venule, leading to BBB disruption and parenchymal infection. However, this extent of infection occurs late in the course of bacterial meningitis and is generally associated with severe and fatal outcomes. Here, we will delineate mechanisms of BBB disruption during neuroinfectious diseases, focusing on pathogens that infect immunocompetent hosts (Table 1).

Induction of BBB Disruption and Parenchymal Invasion by Bacteria

A variety of Gram-positive and Gram-negative bacteria display a predilection for CNS invasion, predominantly spread hematogenously within the subarachnoid space into the CSF. Most bacterial infections lead to robust inflammatory responses leading to extensive neutrophilic infiltrates throughout the meninges and, if untreated, result in vasogenic edema, disruption of the BBB, coma, and death [76]. Although bacterial infections within the CNS generally cause meningitis and are limited to the CSF compartment, host inflammatory responses and, in some cases, bacterial products may lead to BBB disruption with bacterial invasion of the CNS parenchyma. With few exceptions, most bacteria are unable to invade neural cells, leading instead to their encapsulation by glial elements and abscess formation [77]. Here, we will discuss the specific mechanisms by which bacteria interact with subarachnoid vasculature and the molecular events that may lead to parenchymal invasion.

Table 1 Mechanisms of BBB disruption by various pathogens, including bacteria, viruses, and parasites, depicted in pink, blue, and gray, respectively

Pathogens	Mechanisms of BBB disruption	References
Group B Streptococcus (GBS)	GBS activates ERK1/2/MAPK signaling pathway in BMECs, leading to the induction of host transcriptional repressor Snail1, which in turn suppresses the expression of TJ proteins	[238]
Listeria monocytogenes	Bacterial proteins InlA and InlB interact with host cellular receptors E-cadherin and MET, respectively, on choroid plexus epithelium and brain endothelium, respectively	[86]
Bacillus anthracis	Reduce the expression of ZO-1 induced by bacterial edema toxin (ET) Bacterial toxins also reduce the expression of VE-cadherin by inhibiting Rab11/Sec15-dependent endocytic recycling pathway	[239, 240]
Haemophilus influenzae	Porin, OmpP2 causes endothelial damage via binding to the common carboxy-terminal domain of LR, and pili interact with platelet-activating factor receptor (PAFR), both expressed by BMECs	[95, 96]
Neisseria meningitidis	Degradation of TJ proteins and ECM via the induction of MMP8 Delocalization of TJ proteins in BMECs induced by bacterial pili	[241–243]
WNV (*Flavivirus*)	Degradation of TJ and AJ proteins mediated by virus-induced elevation of MMP-1,-3, and-9	[104, 244]
JEV (*Flavivirus*)	Disruption of TJ complexes by virus-induced inflammatory cytokines (e.g., IP-10 and TNFα) in the CNS	[109]
TBEV (*Flavivirus*)	Virus-induced cytokine/chemokine overproduction in the brain	[172]
HIV-1 (*Retrovirus*)	Inducing lesion in brain ECs and activation of MMPs by gp120 Release of s-CD40L by Tat-induced platelet activation	[135, 136, 245]
HeV and NiV (*Henipavirus*)	Syncytium formation in brain ECs. Induction of inflammatory cytokines in the brain and peripheral tissues	[129–131]
VEEV (*Alphavirus*)	Increased expression of MMP9 Monocytes infiltration and release of inflammatory cytokines	[102, 105]
RABV (*Lyssavirus*)	Downregulation of TJs mediated by IFN-γ from infiltrating CD4 T cells	[182]
MHV3 (*Coronavirus*)	Reduced expression of TJ and AJ proteins due to impaired production of IFN-β by infected BMECs	[111]
LCMV (*Arenavirus*)	CTL-mediated recruitment of neutrophils and monocytes into the CNS leads to vascular damage	[181]
Influenza A virus (*Orthomyxovirus*)	Disruption of tight junction protein ZO-1, likely by virus-induced inflammatory cytokines	[246, 247]
TMEV (*Cardiovirus*)	Degradation of TJs by perforin secreted from CD8 T cells	[184]
HSV-1 (*Simplexvirus*)	Virus-induced upregulation of MMP9	[248]

(continued)

Table 1 (continued)

Pathogens	Mechanisms of BBB disruption	References
P. falciparum (HCM)	Parasite-induced structural change in the membrane of iRBC makes them adherent to vascular endothelium, resulting in apoptosis and disruption of TJ proteins in BMECs	[193]
P. berghei ANKA (ECM)	Platelets deposition and leukocyte arrest on brain vascular endothelium Degradation of TJs and extracellular matrix by CD8 T cells	[204–206]
Toxoplasma gondii	Upregulation of CAMs and inflammatory cytokines in the CNS. Sustain and intense interaction/adherence of leukocytes with brain endothelium Production of nitric oxide and MMPs in the CNS	[35–38]
Trypanosoma brucei	Enhanced production of pro-inflammatory cytokines by activated microglial and astrocytes. T cell-mediated activation of MMPs	[219]
Acanthamoeba	Degradation of TJ proteins. Induction of cell cycle arrest and apoptosis in BMECs through activation of PI3K	[228]

Streptococcus pneumoniae (aka Pneumococcus)

S. pneumoniae are Gram-positive, facultative anaerobic bacteria that reside in the respiratory tract. There are over 90 serotypes of *S. pneumoniae* that differ in virulence and susceptibility to antimicrobials. Pneumococcal infections generally originate in the nasal cavities but, in young children and the elderly, may become invasive, with hematogenous spread to multiple organs including the CNS. Within the subarachnoid space, *S. pneumoniae* may adhere to fenestrated endothelium via a number of interactions between bacterial and host proteins. Thus, the major adhesion protein of *S. pneumoniae* pilus-1, RrgA, binds both polymeric immunoglobulin receptor (pIgR) and platelet-associated cell adhesion molecule (PECAM)-1 on endothelial cells, while the bacterial choline-binding protein (PspC) binds only pIgR [78]. These interactions trigger Toll-like receptor-mediated expression of inflammatory mediators by meningeal endothelial cells including interleukins (IL)-1, -6, -10, tumor necrosis factors (TNF), and cytokine-induced neutrophil chemoattractant (CINC)-1 [79, 80]. The recruitment of neutrophils and lymphocytes heralds the onset of meningitis symptoms, including fever, photophobia, and meningismus [81]. Untreated, inflammatory infiltrates may gain access to the CNS parenchyma via migration along venules from the meningeal compartment. Neutrophils and macrophages secrete barrier destabilizing cytokines, IL-1, and TNF, which activate RhoA within BBB endothelial cells, which disrupts TJs [15]. In severe infections, *S. pneumoniae* may also gain access to the brain parenchyma, as animal studies demonstrate that pneumococcal pneumolysin may damage endothelial cell membranes or TJs [82].

Listeria monocytogenes

Listeria monocytogenes is a facultative intracellular bacterium that is tenfold more effective at invading the CNS other than neuroinvasive Gram-positive bacteria [83]. *L. monocytogenes* spreads hematogenously from the gastrointestinal tract after the consumption of contaminated food, gaining access to the CNS parenchyma through a variety of routes including invasion of meningeal endothelium, transportation across the BBB within infected monocyte, or retrograde migration along cranial nerve axons [84, 85]. Bacterial proteins, including internalins (InlA and InlB), interact with host cellular receptors E-cadherin and mesenchymal-epithelial transition (MET), respectively, and are expressed by choroid plexus epithelium and brain endothelium, respectively [86]. Listeriolysin O (LLO), a pore forming toxin, activates NF-kB within brain endothelial cells in vitro, leading to increased expression of P- and E-selectin, ICAM-1 and VCAM-1, as well as IL-6, -8, and CCL2, which may promote the adhesion and recruitment of neutrophils and monocytes [87]. Invasion and infection of brain endothelial cells with the ensuing activation of glial cells and recruitment of leukocytes in patients with severe CNS *L. monocytogenes* infections may lead to abscess formation or cerebritis [88].

Bacillus anthracis

Bacillus anthracis, a spore-forming Gram-positive bacterium, causes the disease anthrax, which has three clinical forms: cutaneous, inhalational, and gastrointestinal [89]. Untreated, anthrax disseminates hematogenously to the CNS, causing fatal hemorrhagic meningitis. Anthrax toxins, such as InhA and BsIA, induce destruction of brain endothelial cell TJs, leading to increased BBB permeability and hemorrhage [90, 91]. BsIA has also been demonstrated to act as an adherence factor for all endothelial cells and to be required for CNS infection [92]. Finally, the anthrax toxin pXO1 downregulates innate immune responses, allowing dissemination of the pathogen throughout the CNS [93].

Haemophilus influenzae

Haemophilus influenzae is a Gram-negative bacterium that was a leading cause of childhood meningitis until its near eradication through the introduction of a the highly effective conjugate HiB vaccine [94]. In vitro studies have implicated *H. influenzae* porin, OmpP2, in endothelial damage via binding to the common carboxy-terminal domain of LR, and *H. influenzae* pili have also been shown to interact with platelet-activating factor receptor (PAFR), which are both expressed by BMECs [95, 96]. In vivo studies have shown that targeting leukocyte CD11/CD18 integrins in conjunction with systemic treatment with corticosteroids reduces life-threatening CNS inflammation and prevents TJ disruption [97], the latter of which is now standard of care in the treatment of patients with *H. influenzae* meningitis [98].

Neisseria meningitidis

Neisseria meningitidis, a Gram-negative bacterium that may colonize the oropharynx and genital tract, causes fulminant meningococcemia and meningococcal meningitis, which often occur together [99]. *N. meningitidis* adheres to host endothelial cells via pili surface proteins Opa and Opc followed by bacterial adhesin PilQ interaction with the common carboxy-terminal domain of LR [96]. Additional determinants of host cell binding include complex protein ACP and the autotransporter meningococcal serine protease (Msp) A [100, 101].

In summary, while bacterial invasion of the CNS is primarily limited to the meningeal compartment, numerous species exhibit pili surface proteins that are able to interact with BMECs via binding to pIgR and/or LR, which lead to endothelial cell activation, with upregulation of PAFr, CD31, and/or intercellular adhesion molecules [95]. PAFr activation leads to dilation of vessels, aggregation of platelets, and increased BBB permeability, which are all terminal events during bacterial meningitis.

Effects of Viruses on BBB Structure and Function

Many neurotropic viruses with barrier disrupting properties (e.g., Japanese encephalitis virus (JEV), West Nile virus (WNV), Venezuelan equine encephalitis virus (VEEV)) enter the CNS in the absence of BBB opening, suggesting that barrier disruption results from the local virus replication in the CNS [102–106]. Viruses can compromise the integrity of BBB by either infecting or inducing cellular damage to the NVU or by eliciting innate and adaptive immune responses leading to neuroinflammation. Thus, a combination of host and virus-related factors contributes to BBB opening during neurotropic viral infection.

Virus Factors that Impact BBB

Infection of mice with mouse adenovirus type-1 (MAV-1) induces BBB disruption in the absence of inflammation, suggesting that the barrier loss is primarily caused by viral infection rather than inflammatory responses [107]. MAV-1 infects brain vascular endothelium in vivo [108] and dampens expression of TJ proteins in vitro [107]. Indeed, reduced expression of TJ and AJ proteins is a characteristic feature of BBB disruption by neurotropic viruses such as JEV, WNV, and human immunodeficiency virus type 1 (HIV-1) in vivo [104, 109, 110]. Viruses accomplish this either by downregulating transcription levels of TJ mRNA or promoting protein degradation [104, 111].

Disruption of TJ complexes is often associated with enhanced generation of reactive oxygen species (ROS). Viral infection in target cells can induce mitochondrial damage or NADPH oxidase activation, resulting in robust ROS generation [112, 113]. While low levels of ROS are required for normal cell function, unchecked

level of these reactive intermediates can exert detrimental effects. Indeed, ROS can target virtually all biological molecules, including lipid, protein, and nucleic acid, resulting in the release of various cytokines and proteases that damage vasculature. Cellular component of NVU can be a source and target of ROS. While brain endothelial cells are highly susceptible to oxidative stress, astrocytes are less prone to such damages. However, exposure to viral proteins (e.g., HIV-1 Nef) augments astrocyte sensitivity to redox insults [114]. Activation of metalloproteinases (MMPs) is one of the mechanisms by which ROS dysregulate TJ complexes [115–117]. Elevated levels of MMPs have been reported in brain tissue of mice infected with neurotropic viruses such as WNV, JEV, and VEEV [104, 105, 118] and in cerebrospinal fluid (CSF) of human patients infected with WNV [118]. Infected microglia and astrocytes robustly elevate the expression of MMP-2 and -9 in vitro and in vivo [119, 120]. MMPs are known to disrupt the BBB integrity by cleaving TJ proteins, AJ proteins, and the extracellular matrix (ECM) [115]. Activity of these MMPs is controlled by regulating gene expression, activation, and inhibition mediated by endogenous tissue inhibitors of MMPs (TIMPs) [121]. Nonetheless, viral infection (e.g., HIV-1) can perturb the fine balance between MMPs and TIMPs, resulting in enhanced MMP levels and BBB leakage [122]. Consistently, pharmacological blockage or genetic ablation of MMPs is reported to protect BBB integrity upon viral infection in murine models [118, 123].

Additionally, ROS trigger the small GTPase RhoA, PI3 kinase, and protein kinase B (PKB/Akt) signaling pathways. This results in the reorganization of the actin cytoskeleton, altered localization of TJ proteins, and consequently increased BBB permeability [124, 125]. Furthermore, ROS can cause barrier dysfunction by activating inflammasome via signaling pathways involving mitogen-activated protein kinases (MAPK) and extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) [126].

Viruses can also infect brain endothelial cells and induce syncytium resulting in vascular damage and hemorrhage [127, 128]. For instance, Nipah and Hendra viruses (NiV and HeV, respectively) invade the CNS by infecting brain endothelial cells. Virus infection induces syncytium in brain endothelium resulting in extensive vascular damage associated with influx of inflammatory cells [129–131]. Additionally, neurotropic viruses induce apoptosis in brain endothelial cells causing BBB dysfunction in vitro [132]. Secretory viral proteins also trigger barrier permeability. For instance, HIV-1 Tat protein is actively released from the infected cells and crosses the cellular membrane [133]. Intravenous injection of mice with HIV-1 Tat reduces the expression of TJ proteins in brain vasculature, partly by upregulating cyclooxygenase-2 expression [134]. Additionally, HIV-1 Tat enhances serum levels of soluble CD40L (sCD40L) by activating platelets [135, 136], a phenomenon also observed in HIV-infected patients [137, 138]. sCD40L alters barrier permeability by increasing the expression of cell adhesion molecules on brain endothelial cells in a JNK-dependent manner [139]. This culminates in enhanced leukocyte adhesion to brain endothelium leading to BBB dysfunction [135].

In summary, evidence indicates that neurotropic viruses can directly induce BBB permeability by disrupting TJs and AJs between brain endothelial cells. This is

mainly achieved by inducing ROS generation in the CNS, which in turn activates several tyrosine kinases, MMPs, and small GTPase RhoA. The cumulative effect of these activities leads to the loss of BBB function.

Innate Immune Responses to Viruses that Impact BBB Function

Microbes possess pathogen-associated molecular patterns (PAMPs) that are recognized by pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), retinoic acid-inducible gene 1 (RIG-I)-like receptors (RLRs), nucleotide oligomerization domain-like receptors (NLRs), and cytosolic DNA sensors. These PRRs are expressed by various cell types in the CNS (reviewed in [140]). Viruses contain single- or double-stranded RNA molecules (dsRNS and ssRNA, respectively), present either in the viral genome or generated during virus replication. Recognition of viral PAMPs by TLR3 (dsRNA) or TLR-7 (ssRNA) triggers signaling pathway related to NF-κB, resulting in the production of pro-inflammatory cytokines and type I interferons (IFNs). Similarly, RLRs, which include RIG-I and melanoma differentiation factor (MDA)-5, are activated by dsRNA and ssRNA sequences containing 5′-triphosphate [141]. RLR activation stimulates mitochondrial antiviral-signaling protein (MAVS), which in turn elicits the expression of inflammatory cytokines via induction of the NF-κB signaling pathway. While PRR-induced expression of type I IFNs restricts virus replication in the CNS [142, 143], enhanced production of pro-inflammatory cytokines and chemokines can lead to neuroinflammation. Studies have shown that TLR3 signaling contributes to both enhancement and protection of CNS inflammation during WNV infection in murine models [144, 145]. Similarly, TLR3 signaling has been associated with neuropathogenesis of rabies virus (RABV) in mice [146] while limiting infection of herpes simplex virus (HSV)-2 in the CNS through the activation of IFNAR signaling in astrocytes [142].

The NLR family is involved in the maturation of pro-inflammatory cytokines, produced by other PRRs (e.g., TLRs and RLRs) in response to viral infection. Viral sensing by NLRs triggers the assembly and activation of inflammasome complex, resulting in the maturation and release of interleukin-1β (IL-1β) and IL-18 from infected cells (reviewed in [140]). Seemingly, IL-1β acts in synergy with type I IFNs to suppress virus replication in cortical neurons, thus providing protection against lethal WNV infection in mice [147]. In contrast, enhanced production of IL-1β is linked to neuropathology associated with JEV infection in murine models [148]. IL-1β also abrogates the protective effect of astrocytes on BBB integrity by suppressing astrocytic expression of sonic hedgehog (SHH), a protein that upregulates the expression of TJs in BMECs [149]. Additionally, IL-1β and IL-18 activate microglia and astrocytes to generate more inflammatory molecules potentiating inflammation. Activation of microglia is often reported during encephalitic viral infection [104, 150–153], which is regulated by IFNAR signaling in astrocytes and neurons [154]. While microglia play a crucial role in viral clearance in the CNS [154–156], uncontrolled gliosis can disrupt BBB integrity through induction of pro-inflammatory

cytokines and matrix metalloproteases [153]. Similarly, astrocytes mount a strong innate immune response upon recognition of viral PAMPs via RLR and TLR signaling pathways. In fact, astrocytes are the main producers of type I IFNs during infection with several distinct neurotropic viruses, including La Crosse virus, rabies virus (RABV), vesicular stomatitis virus (VSV), and Theiler's murine encephalitis virus (TMEV) [157, 158]. Deletion of *IFNAR* signaling specifically in astrocytes results in severe encephalomyelitis and mortality during otherwise nonlethal mouse hepatitis virus (MHV) [159]. Nonetheless, activated astrocytes can release excess amount of IP-10 during encephalitic viral infection [109]. IP-10 enhances the expression of tumor necrosis factor alpha (TNFα) in a JNK-dependent manner, leading to barrier disruption [109]. Consistently, injection of mice with neutralizing antibodies against IP-10 [109] or TNF-α ameliorated the decrease in TJ proteins and improved BBB integrity during JEV infection [160].

Alternatively, viral-induced inflammasome activation triggers pyroptosis, a highly inflammatory form of programmed cell death [161]. Although pyroptosis plays a crucial role in controlling virus spread [162], it can cause neuronal necrosis and gliosis [163], features associated with BBB disruption. In the CNS, inflammasome activity is regulated by mechanisms involving osteopontin (OPN) and prostaglandin D2 (PGD2), upon viral infection [164, 165]. OPN inhibits the caspase-1-dependent inflammasome activation by reducing the expression of inflammasome components in the brain [165]. However, PGD2 can exert both pro- and anti-inflammatory effects depending on the receptor involved. Engagement of D-prostanoid receptor 1(DP1) on microglia by PGD2 upregulates PYDC3 (an inflammasome inhibitor), which protects against IL-1β-mediated neuroinflammation [164]. Deficiency in DP1 also correlates with reduced expression of IFN-I and -III, augmenting viral titer in the brain. Interestingly, upon viral infections, IFNAR signaling in BMECs reduces expression of IL-1β [15], likely via inhibition of inflammasome activity [166]. Additionally, type I IFNs act in synergy with MerTK (a member of TAM receptor tyrosine kinases) to activate Rac-1, which in turn improves TJ integrity [167].

Taken altogether, these studies suggest that innate immunity plays a central role in restricting viral replication in the CNS. This has the potential to be protective or detrimental, depending on the virus and magnitude of host immune response. The protective effect is mainly attributed to IFNAR signaling in CNS residential cells that not only limits local virus replication but also restricts additional viral entry or leukocyte infiltration by retaining BBB integrity.

Adaptive Immune Responses to Viruses that Impact BBB Function

Leukocyte migration across the BBB requires expression of ICAM-1 and VCAM-1 on brain endothelial cells (ECs). As mentioned above, under normal conditions, these molecules are expressed minimally on brain ECs to restrict immune cell interaction and extravasation into the CNS. However, elevated expression of ICAM-1 and VCAM-1 has been frequently observed in infection with several neurotropic

viruses [168–172]. Altered expression of these adhesion molecules mainly results from the activation of NF-κB by ROS or ERK signaling pathway. For instance, HIV-1 Tat protein induces NADPH oxidase in astrocytes, which results in the upregulation of CAMs expression via NF-κB signaling [173, 174]. Similarly, JEV infection augments ICAM-1 expression on brain endothelial cells through activation of ERK signaling pathway [171]. Interestingly, expression of CAMs on brain endothelium is downregulated by IFNAR signaling in astrocytes, which promotes BBB integrity during encephalitic viral infection [170]. Additionally, IFNAR signaling in astrocytes influences the composition of inflammatory cells recruited to the CNS upon viral infection [159].

Although immune cell infiltration is crucial for viral clearance in the CNS [147, 175, 176], it can cause BBB disruption and neuronal damage by potentiating neuroinflammation [104]. Studies have shown that immune cell infiltration precedes BBB disruption in mice infected with VEEV and tick-borne encephalitic virus (TBEV) [102, 172]. This is associated with increased expression of RANTES, CCL2, IP-10, ICAM-1, TNF-α, IL-6, and IL-1β in brain tissues [172]. Similarly, enhanced levels of CCL2 and RANTES have been reported in serum samples of TBE-infected human patients [177]. Activated monocytes produce CCL2 in response to viral infections, which promotes barrier permeability via alteration in the actin cytoskeleton and localization of TJ proteins [178, 179]. Additionally, infiltrating neutrophils and monocytes produce high levels of MMP8, which promotes myelomonocytic cell extravasation and vascular leakage upon infection with lymphocytic choriomeningitis virus (LCMV) [180]. Consistently, depletion of both monocytes and neutrophils in LCMV-infected mice promotes BBB integrity and prolonged survival [181]. Notably, individual depletion of either cell type does not protect against vascular permeability.

As with monocytes, infiltrating lymphocytes can also induce BBB disruption by secreting inflammatory cytokines. Infected neurons produce CXCL10, which is a chemoattractant for CD4 and CD8 T cells [176]. Upon infection with rabies virus, CD4 T cells infiltrate into the CNS and differentiate into Th1 and Th17 that produce IFN-γ and IL-17, respectively [182, 183]. While IFN-γ reduces the expression of TJ proteins (i.e., occludin, claudin-5, and ZO-1), elevated levels of IL-17 disrupt TJ complexes in infected mice [182]. Consistently, blockage of IFN-γ ameliorated BBB integrity in vivo, presumably by restoring expression of TJ proteins in brain endothelial cells [182]. Administration of IFN-γ-neutralizing antibody also alleviated BBB disruption in JEV-infected mice [103]. CD8 T cells are also involved in vascular leakage during viral infection. They promote BBB disruption during infection with TMEV (Theiler's murine encephalomyelitis virus), by releasing perforin that disrupts TJ proteins [184]. Additionally, it has been suggested that interaction of CD8 T cells with neurons upregulates VEGF, which in turn promotes barrier dysfunction by dysregulating TJ complexes [184, 185]. Another mechanism by which cytotoxic T cells (CTLs) can cause vascular leakage involves degradation of the basal membrane via secretion of granzyme B. This allows CTLs to extravagate across brain vasculatures [186]. Upon extravasation, CD8 T cells release several

chemoattractants (e.g., CCL2, CCL3, and CCL4), which recruit monocytes and neutrophils to the CNS, thus indirectly leading to the loss of BBB function during viral infection [181].

Notably, physical interaction of infiltrating leukocytes with ICAM-1 on brain endothelium per se can promote vascular permeability by triggering generation of ROS in a NADPH oxidase and Rac-1-dependent manner [187–189]. Enhanced generation of ROS activates downstream tyrosine kinases (e.g., c-Src and PYK2), resulting in phosphorylation of VE-cadherin. This phosphorylation dissociates interaction of VE-cadherin with the actin cytoskeleton resulting in the disruption of adherent junctions. Likewise, TNFα and VEGF that are generated during viral infection [109, 120] trigger Rac-1-mediated ROS generation. ROS in turn promotes phosphorylation and internalization of VE-cadherin, leading to barrier dysfunction [188, 190, 191].

Collectively, the above studies suggest that upon viral infection, CNS residential cells release inflammatory cytokines/chemokines, which activate brain endothelium allowing immune cell infiltration. Infiltrating leukocytes provide microglia with costimulatory signals to eliminate infected cells. Additionally, cytotoxic T cells can directly kill infected cells contributing to viral clearance. Nonetheless, an excessive immune cell infiltration imposes severe structural damages to the cells of NVU, leading to barrier dysfunction.

Effects of Parasites on BBB Structure and Function

As with other pathogens, neurotropic parasites have evolved several strategies to disrupt the BBB promoting their entry into the brain. These include infection and lysis of brain ECs (e.g., *Toxoplasma gondii*), secretion of proteases and toxins (e.g., *trypanosoma* and a*canthamoeba)*, and induction of inflammatory cytokines or matrix metalloproteinases [192].

Cerebral Malaria and BBB Function

Plasmodium falciparum, the causative agent of human cerebral malaria (HCM), is associated with disruption of BBB and severe vasculopathy. Infection of red blood cells (RBCs) by *P. falciparum* induces structural changes in their membrane that make them adhesive to other cell types. This results in the formation of mircoaggregates that can obstruct blood flow, leading to hypoxia, hypertension, and alteration of metabolites in the CNS [192]. Additionally, infected RBC (iRBC) can directly interact with brain vascular endothelium and promote BBB dysfunction. Indeed, adhesion and sequestration of iRBCs in brain vasculature are linked to the loss of BBB function in humans [193, 194]. In vitro studies also have shown that adherence of iRBCs to brain endothelium triggers barrier permeability via induction of apoptosis and disruption of TJ proteins (e.g., ZO-1) [195–197]. In mouse models

of experimental cerebral malaria (ECM), BBB dysfunction correlates with platelet deposition and leukocyte arrest on the endothelium of postcapillary venules [198, 199]. Activated platelets augment BBB permeability by potentiating vascular damage induced by iRBC and impairing vascular repair. Similarly, leukocyte arrest, along with increased production of vasoconstrictive factors, impairs venous efflux from the CNS. This results in enhanced intracranial hypertension, vascular leakage, and hemorrhages [200, 201]. Among leukocytes, antigen-specific CD8 T cells and ICAM-1$^+$ macrophages are particularly involved in the development of ECM [198]. *Plasmodium* species upregulate the expression of ICAM-1, VCAM-1, P-, and E-selectins both in human and mice, thus promoting immune cell extravasation into the CNS [202, 203]. Antigen-specific CD8 T cells trigger BBB permeability by secreting granzyme B and perforin that target TJ proteins and induce apoptosis in brain endothelial cells [204–206]. Nonetheless, endothelial cell death is not correlated with barrier dysfunction or development of ECM in other studies [198, 199]. Instead, BBB disruption is related to increased paracellular or transcellular transport, mediated by the interaction of leukocytes with postcapillary venules [199]. Furthermore, antigen-specific CD8 T cells activate brain endothelial cells by releasing IFN-ɣ [207], which upregulates the expression of adhesion and antigen-presenting molecules selectively on cerebrovascular ECs but not peripheral ECs [207, 208]. Consistently, deficiency in IFN-γ or depletion of CD8 T cells results in complete protection against ECM by preventing barrier permeability and vascular hemorrhage [207, 209]. Likewise, co-infection with chikungunya virus (CHIKV) protects mice from ECM mortality by preventing pathogenic CD8 T cells from migration into the CNS [210].

Toxoplasmosis

Toxoplasmosis is one of the most common parasitic diseases that is caused by *Toxoplasma gondii*. In healthy individuals, infection is either mild or asymptomatic; however, it can cause life-threatening CNS complications in developing fetus or immunocompromised patient [211]. Upon infection, *T. gondii* can enter CNS via Trojan horse as well as direct infection of brain endothelial cells [192, 212]. During acute phase, the parasite induces an exacerbated inflammatory response, which then subsides during the chronic phase. Inflammatory response upregulates expression of VCAM-1 on brain endothelium to promote migration of CD4 T cells into the CNS, which is required for controlling parasite replication [213]. Nonetheless, sustained and intense leukocyte-endothelium interaction in postcapillary venules leads to the formation of plugging, which can interfere with blood flow and cause cerebral hypoperfusion [214, 215]. Additionally, *T. gondii* can infect, lyse, and induce structural and functional defects in brain endothelial cells [192, 215]. Furthermore, elevated levels of MMPs, inflammatory cytokines, and nitric oxide are reported during parasite infection, features associated with BBB disruption [216, 217].

Trypanosomiasis

Human African trypanosomiasis (HAT), also known as "sleeping sickness," is caused by *Trypanosoma brucei*. HAT is divided into two clinical stages: during the first stage, parasite replicates in the blood and lymphatic system. This is followed by a second stage when the parasite enters and establishes infection in the CNS, which can cause meningoencephalitis [218]. *T. brucei* initially enters the CNS through choroid plexus and circumventricular organs, likely by secreting a protease that degrades the basal lamina [219, 220]. During the early phase of CNS infection, production of IL-6 and IL-10 protects against neuroinflammation [221]. However, later in disease, activated microglia and astrocytes produce high levels of inflammatory cytokines (e.g., IL-1β, CXCL-8, CCL-2, and TNF-α), which can lead to severe neuropathology [222]. These inflammatory cytokines upregulate the expression of cells adhesion molecules (i.e., ICAM-1, VCAM-1, and E-selectin) on brain endothelial cells, which promotes leukocyte migration into the CNS. Infiltrating lymphocytes are particularly involved in the entry of parasite across BBB. They secret IFN-γ, which in turn activates MMP9, a protease that degrades astrocytic basement membrane allowing paracellular entry of parasite into the CNS [219]. Consistently, enhanced expression of MMPs is reported during *Trypanosoma* infection [223, 224]. Additionally, IFN-γ augments the expression of CXCL-10 by astrocytes, which recruits more lymphocytes into the CNS [225]. Furthermore, *T. brucei* releases cysteine proteases that trigger protease-activated receptors (PARs) on BMECs, thereby promoting BBB dysfunction through enhancement of intracellular calcium level [226]. Interestingly, production of nitric oxide by perivascular macrophages restricts the entry of both parasites and activated T cells into the CNS by preserving BBB integrity [224].

Amoebic Encephalitis and BBB Effects

Acanthamoeba castellanii is a fatal infection of immunocompromised individuals and is associated with BBB dysfunction and has been shown to cause granulomatous encephalitis in immunocompromised patients. It invades the CNS through hematogenous pathway following disruption of the BBB [227]. *A. castellanii* interacts with BMECs through a mannose-binding protein that is expressed on the surface of its trophozoites. These interactions trigger degradation of TJ proteins (e.g., occludin and ZO-1) in a Rho kinase-dependent manner [228]. Additionally, parasite interaction can induce cell cycle arrest and apoptosis through activation of phosphatidylinositol 3 kinases (PI3K) and inhibition of proteins that are involved in cell cycle progression [229, 230]. Similarly, cell cycle arrest and apoptosis of brain endothelial cells have been reported for *Balamuthia mandrillaris*, another parasite that is known to cause fatal amoebic meningoencephalitis [231]. Notably, host immune response plays a major role in the disruption of BBB during infection with *A. castellanii* and *Naegleria fowleri* [227, 232]. Since these amoebae are relatively

large in size, they elicit an amplified immune response that not only compromises the BBB integrity but also causes neuronal damage [232].

Neurotropic parasites (e.g., *Trypanosoma*, *Acanthamoeba, and Balamuthia* species) are also known to produce and release a variety of proteases (e.g., cysteine and serine proteases and metalloproteinases) that target TJ proteins and the basal membrane of the BBB, leading to barrier dysfunction [233–236]. Proteases interact with protease-activated receptors on BMEC and stimulate calcium release from intracellular stores by activating phospholipase C (PLC) [226]. Increased calcium levels result in calmodulin activation of myosin light chain, which in turn augments intracellular contraction, leading to disruption of TJs between brain ECs [237].

Future Perspectives

Since the initial demonstration and appreciation of the specialized nature of the CNS microvasculature, researchers have learned that it is less an impermeable barrier and more a dynamic interface that senses and responds to the periphery. These responses are generally neuroprotective, such as the IFNAR-mediated increase in TJ integrity during viral invasion or the stringent regulation of T cell access that can promote efficient clearance of pathogens, such as *T. gondii*, without excessive immunopathology. Pathogens have evolved various mechanisms to exploit cellular and molecular processes that control the CNS access, such as the reduction in expression of ZO-1 induced by the *B. anthracis* edema toxin (ET). Host responses, in turn, regulate immune cell infiltration into the CNS via antigen-specific events that allow leukocyte localization, interactions, and egress from perivascular spaces such that inflammation is efficiently directed at pathogen elimination. The interaction of immune cells with BMECs destabilizes junctional molecules via cytokine-mediated signaling events that alter the structural properties of these cells. Thus, the most severe outcomes in the context of neuroinfectious diseases that enter the CNS via the BBB arise from host inflammatory responses rather than due to direct effects of pathogens themselves. This is particularly evident in the context of autoimmune diseases of the CNS where leukocytes gain inappropriate access to the CNS and cause extensive damage without acute infection.

While we continue to improve our understanding of these processes, the challenge will be to better identify mechanisms that promote efficiency in immune-mediated pathogen clearance while enhancing the CNS' own neuroprotective mechanisms. The use of animal models of neuroinfectious diseases that focus on various aspects of these processes in conjunction with the development of methods to isolate cellular participants, such as RiboTag or single cell RNA sequencing, in conjunction with cell-specific gene deletion strategies will permit cell-type-specific evaluation of mRNA expression and protein functions during the course of in vivo pathogen invasion, infection, and clearance. The advent of techniques in which human-induced pluripotent stem cells (hiPSCs) can be differentiated into all members of the NVU which are then incorporated into three-dimensional, fluid-based

models of the human BBB also holds promise for identifying molecular players in this process and validating results in human systems. Future studies are likely to uncover novel neuroimmune pathways that may be safely targeted to prevent or treat infections of the CNS while also providing strategies for manipulating BBB function for the purposes of drug delivery or immunotherapies for noninfectious neurologic diseases.

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Structural and Functional Characteristics of the Human Blood-Nerve Barrier with Translational Implications to Peripheral Nerve Autoimmune Disorders

Eroboghene E. Ubogu

Abstract Peripheral nerves and nerve roots comprise of three structural compartments: the outer epineurium consisting of longitudinal arrays of collagen fibers responsible for structural integrity and the inner perineurium consisting of multiple concentric layers of specialized epithelioid myofibroblasts that surround the innermost endoneurium which consists of myelinated and unmyelinated axons embedded in a looser mesh of collagen fibers. Axons are responsible for signal transduction to and from the central nervous system required for normal physiological processes and are targeted by the immune system in autoimmune disorders. A highly regulated endoneurial microenvironment is required for normal axonal function. This is achieved by tight junction-forming endoneurial microvessels that control ion, solute, water, nutrient, macromolecule and leukocyte influx and efflux between the bloodstream and endoneurium, and the innermost layers of the perineurium that control interstitial fluid component flux between the epineurium and endoneurium. Endoneurial microvascular endothelium is considered the blood-nerve barrier (BNB) due to direct communication with circulating blood. The mammalian BNB is considered the second most restrictive vascular system after the blood-brain barrier (BBB). Guided by human in vitro studies using primary and immortalized endoneurial endothelial cells that form the BNB, in situ studies in normal and pathologic human peripheral nerves, and representative animal models of peripheral nerve autoimmune disorders, knowledge is emerging on human BNB molecular and functional characteristics, including its array of cytokines/cytokine receptors, selectins, and cellular adhesion and junctional complex molecules that may be employed during normal immune surveillance and altered in autoimmune diseases, providing potential targets of efficacious immunotherapy.

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_8

Keywords BNB · Endoneurium · Immune system · Leukocyte trafficking · Peripheral nerve

Abbreviations

BBB	Blood-brain barrier
BNB	Blood-nerve barrier
CIDP	Chronic inflammatory demyelinating polyradiculoneuropathy
DSP	Distal sensory polyneuropathy
EAN	Experimental autoimmune neuritis
FITC	Fluorescein isothiocyanate
GBS	Guillain-Barré syndrome
GDNF	Glial-derived neurotrophic factor
HIFs	Hypoxia-inducing factors
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
ICAM-1	Intercellular adhesion molecule-1
IFN-γ	Interferon-γ
IL-1β	Interleukin-1β
IL-2	Interleukin-2
MAPK	Mitogen-activated protein kinase
RET	"rearranged upon transformation"
RNA	Ribonucleic acid
SAPP	Spontaneous autoimmune peripheral polyneuropathy
TEER	Transendothelial electrical resistance
TGF-β	Transforming growth factor-β
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial cell growth factor
ZO	Zonula occludens

Anatomy of Human Peripheral Nerves

Human peripheral nerves serve to facilitate afferent and efferent communication between the central nervous system (brain and spinal cord) and the periphery (internal and external organs, such as the gastrointestinal tract and skin, respectively, secretory organs, and muscle) required for normal physiological processes needed to healthy bodily function. Human peripheral nerves comprise of three compartments: the outer epineurium which consists of longitudinal arrays of collagen fibers that are important for maintaining the structural integrity of the peripheral nerve, the inner perineurium which consists of concentric layers of specialized cells, and the innermost endoneurium which consists of a looser mesh of collagen fibers. A nerve

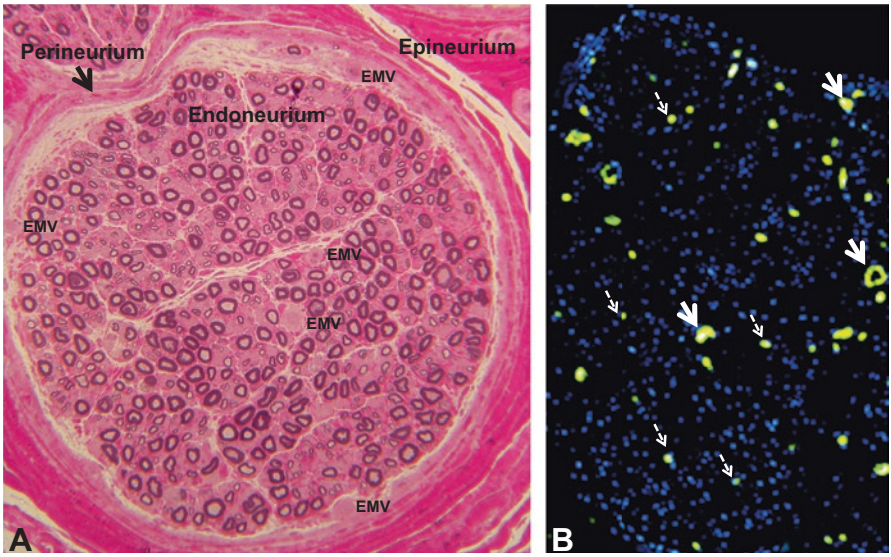

Fig. 1 Digital light photomicrograph of a normal adult sural nerve (plastic embedded semi-thin axial section stained with Toluidine Blue and counterstained with basic fuchsin) showing the three compartments in peripheral nerves and endoneurial microvessels (EMV) that form the BNB (**a**) and an indirect fluorescent digital photomicrograph of a normal adult sural nerve (cryostat thick section stained with fluoresceinated *Ulex europaeus* agglutinin-1) showing epineurial macrovessels (solid arrow) and endoneurial microvessels (broken arrow) (**b**)

fascicle consists of the endoneurium and its surrounding perineurium, initially described in 1876 (Fig. 1a) [1–4].

The epineurium consists of arteries, arterioles, venules, and veins that are considered collectively as epineurial macrovessels. The macrovessels are derived from and communicate with the extrinsic vascular supply to individual peripheral nerves known as the vasa nervorum. Lymphatic vessels are also present within the epineurium. The perineurium consists of specialized epithelioid myofibroblasts that form concentric layers, consisting of single cells, around the endoneurium (1–15 layers dependent on nerve diameter), forming fascicles, as well as smaller diameter macrovessels that communicate with the epineurium and endoneurium. The endoneurium consists of axons that are responsible for electrical impulse signal transduction to and from the central nervous system. These axons are myelinated or unmyelinated, are dependent on axonal size and function, and are aligned in the longitudinal axis of the peripheral nerve [1–5].

Schwann cells are the glial cells in peripheral nerves responsible for myelinating segments of large and small diameter axons needed to facilitate rapid salutatory action potential conduction, or surround bundles of small diameter unmyelinated axons (known as a Remak bundle), providing physiological support to these axons [6]. Motor neurons (axonal cell bodies) are located in the brain (for cranial nerves) and spinal cord (for somatic nerves), while sensory neurons are located in collections

of cell bodies called ganglia (e.g., dorsal root ganglia for somatic nerves). The endoneurium also consists of capillary-like microvessels that lack smooth muscle walls (Fig. 1b), as well as rare resident leukocytes (macrophages and mast cells) and fibroblasts [1–5].

The sciatic nerve is the largest nerve in mammals, compromising of 50–80 fascicles in adult humans in the mid-thigh region (and as many as 140 fascicles in the gluteal region) [2, 7, 8] and 1–4 fascicles in adult mice and rats [9–11]. The commonly studied human sural nerve typically consists of 8–10 fascicles in adults [12]. It is important to recognize the rodent sciatic nerve consists of a thin epineurial layer with loose connective tissue in contrast with the more extensive and fibrous human epineurium. This significant structural difference between human and rodent peripheral nerves is important when extrapolating in vivo or in situ experimental observations made in rodents to human peripheral nerves, particularly with reference to nerve injury and local drug delivery (e.g., anesthetics and analgesics).

Identification and Definition of Blood-Nerve Barrier

The importance of maintaining a highly regulated ionic microenvironment to facilitate axonal impulse conduction in peripheral nerves is intuitive and led to the proposal of a blood-nerve barrier (BNB) akin to the blood-brain barrier (BBB). In vivo permeability studies performed in different animal species following intravenous Evans blue albumin and fluoresceinated albumin or dextran administration demonstrated restricted macromolecules within endoneurial microvessel lumens without extravasation into the endoneurium despite diffuse entry into the epineurium (which was in contrast with the diffuse lack of brain parenchymal entry), implying that restrictive interfaces exist in peripheral nerves and nerve roots [13–17].

Subsequent ultrastructural assessment of human peripheral nerves demonstrated that the impermeable endoneurial microvessels consist of endothelial cells that form tight intercellular junctions and share their basement membrane with surrounding pericytes, lack fenestrations, and possess very few 50–100 nm pinocytic vesicles. This was in contrast with permeable epineurial macrovessels that contain a layer of endothelial cells that possess fenestrations and are surrounded by a smooth muscle wall. Furthermore, the innermost concentric perineurial cell layers (i.e., closest to the endoneurium) are connect by intercellular tight junctions, lack fenestrations, and possess pinocytic vesicles (with higher density in the outermost layers). Thus, the internal microenvironment of the endoneurium is deemed to be regulated by tight junction-forming endoneurial endothelial cells and the cell layers of the innermost perineurium [2, 3, 5].

Endoneurial endothelial cells are in direct contact with circulating blood, including hematogenous leukocytes, while perineurial cells are in contact with interstitial fluid from the epineurium and endoneurium. As a consequence, endoneurial endothelial cells form the BNB, while perineurial cells form critical interfaces between the endoneurial and epineurial interstitial fluid compartments which are also important

for maintaining peripheral nerve homeostasis. Since cross-talk between the systemic immune system and peripheral nerves largely depends on hematogenously derived circulating leukocytes, it is important to understand the structural, molecular, and functional characteristics of the human BNB in health in order to elucidate biologically relevant alterations that may occur in disease states such as peripheral nerve autoimmune disorders.

Characteristics of the Human BNB in Health

Basic knowledge of the structural, molecular, and functional characteristics of the human BNB in health and disease is emerging, guided by data from the human BBB and studies performed on peripheral nerve biopsies in situ and primary and immortalized human endoneurial endothelial cells in vitro; however, our knowledge is far from complete. Structurally, human endoneurial endothelial cells that form the BNB possess electron-dense intercellular tight junctions in situ and in vitro (Fig. 2) [3, 7]. In vitro, these tight junctions consist of occludin, members of the claudin family such as claudin-5, as well as cytoplasmic adaptors such as members of the zonula occludens (ZO) family, e.g., ZO-1 and ZO-2 (also known as tight junction proteins 1 and 2, respectively), based on immunocytochemistry of confluent cultures [7, 18–20], while claudin-5 and ZO-1 had been previously demonstrated in situ [21–23]. Data has emerged over the past 15 years on the importance of the

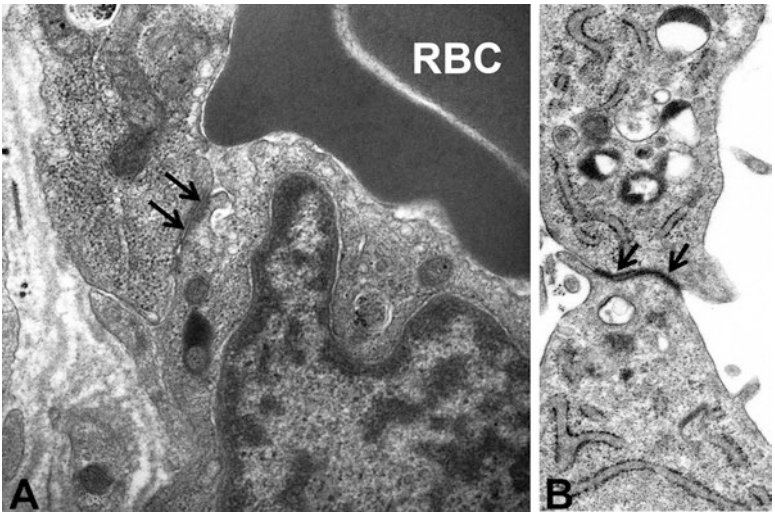

Fig. 2 Digital electron ultramicrographs from an adult sural nerve (**a**) and cultured semipermeable transwell inserts (**b**) showing human endoneurial endothelial cells with electron-dense intercellular tight junctions (black arrows). A red blood cell (RBC) is present in the lumen of the endoneurial microvessel

intercellular junctional complex, consisting of tight, adherens, and gap junctions and their associated adaptor proteins and interacting cytoskeletal components in normal specialized endothelial and epithelial cell function [22, 24–29].

Recent work elucidating the normal adult human BNB transcriptome based on conserved transcripts expressed by early- and late-passage primary human endoneurial endothelial cells and laser-capture microdissected endoneurial microvessels from four histologically normal adult sural nerve biopsies demonstrated expression of 133 intercellular junctional complex molecules (22 tight junction or junction-associated, 45 adherens junction or junction-associated, and 52 cell junction-associated or adaptor molecules), with in situ protein expression of α1 catenin, cadherin-5, cadherin-6, claudin-4, claudin-5, crumbs cell polarity complex component lin-7 homolog A, gap junction protein A1, multiple PDZ domain crumbs cell polarity complex component, protocadherin-1, vezatin, ZO-1, and zyxin demonstrated on endoneurial microvessels by indirect fluorescent immunohistochemistry [22]. This complexity may exist to provide significant molecular redundancy needed to maintain a structurally normal BNB due to its essential homeostatic role in normal peripheral nerve function.

Restrictive intercellular tight junction formation is a critical observation that differentiates endoneurial microvascular endothelial cells from epineurial macrovascular endothelial cells in human peripheral nerves. Endoneurial endothelial cells express receptors for specific mitogens such as glial-derived neurotrophic factor (GDNF, GFRα1), vascular endothelial growth factor (VEGF, VEGFR2), basic fibroblast growth factor (bFGF, FGFR1), transforming growth factor-β (TGF-β, TGFRI/II), and glucocorticoids (GR) [18, 19, 30–32], implying that autocrine or paracrine mitogen secretion by endothelial cells, Schwann cells, pericytes, mast cells, or endoneurial fibroblasts could regulate BNB composition and function in health. Schwann cells, the glial cells of the peripheral nervous system present in the endoneurium, have been shown to secrete GDNF in vitro and in vivo [33, 34], and GDNF has been demonstrated to influence restrictive human BNB characteristics in vitro at low nanomolar concentrations in a dose-dependent manner via RET-tyrosine kinase-mitogen-activated protein kinase (MAPK) signaling and enhance murine BNB restrictive characteristics in vivo following non-transecting nerve injury using a tamoxifen-inducible conditional knockout model [30, 35]. This suggests that GDNF is an essential paracrine regulator of BNB formation that may also have an important role during BNB formation during development and maintenance in health, with some redundancy demonstrated in vitro by other less efficacious mitogens, such as basic fibroblast growth factor.

In addition to the junctional complex, specialized influx and efflux transporters that regulate ionic, water, molecular, nutrient, drug, and xenobiotic entry into or removal from the peripheral nerve endoneurium exist at the human BNB, controlling the endoneurial microenvironment. In vitro, these include alkaline phosphatase, glucose transporter-1 (also known as SLC2A1), monocarboxylate transporter-1 (also known as SLC16A1), creatine transporter (also known as SLC6A8), large amino acid transporter-1 (also known as SLC7A5), γ-glutamyl transpeptidase, and p-glycoprotein (also known as ABCB1) expressed by primary and immortalized

human endoneurial endothelial cells (messenger RNA or protein) [7, 32], with glucose transporter-1 previously demonstrated on human endoneurial microvessels in situ [36].

The human BNB transcriptome demonstrated 509 transporter transcripts, including 196 members of the solute carrier transport family, 76 cation channel, 33 members of the ATP-binding cassette family, 14 zinc transporter, 13 anion channel, 4 solute carrier organic transporter, and 3 aquaporin molecules. ABCA8, ABCB1, AQP1, SLC1A1, SLC2A1, SLC3A2, SLC5A6, SLC16A1, and SLC19A2 were demonstrated on BNB-forming endoneurial endothelial cells in normal human sural nerve biopsies by indirect immunohistochemistry in situ [22]. The extensive repertoire of transcripts that comprise the healthy human BNB cellular components (i.e., cell junction, cell part, extracellular matrix, extracellular region, macromolecular complex, membrane, organelle, and synapse) and their protein classes has been recently published, recognizing that not all transcripts undergo translation to functional protein. Although there are major similarities, structural differences and molecular heterogeneity in the composition of the BNB probably exist between different species [5, 37], limiting the degree of extrapolation feasible between data derived from animal models in vitro and in vivo and the human BNB. Figure 3 depicts a schematic figure summarizing essential structural and molecular components of the human BNB.

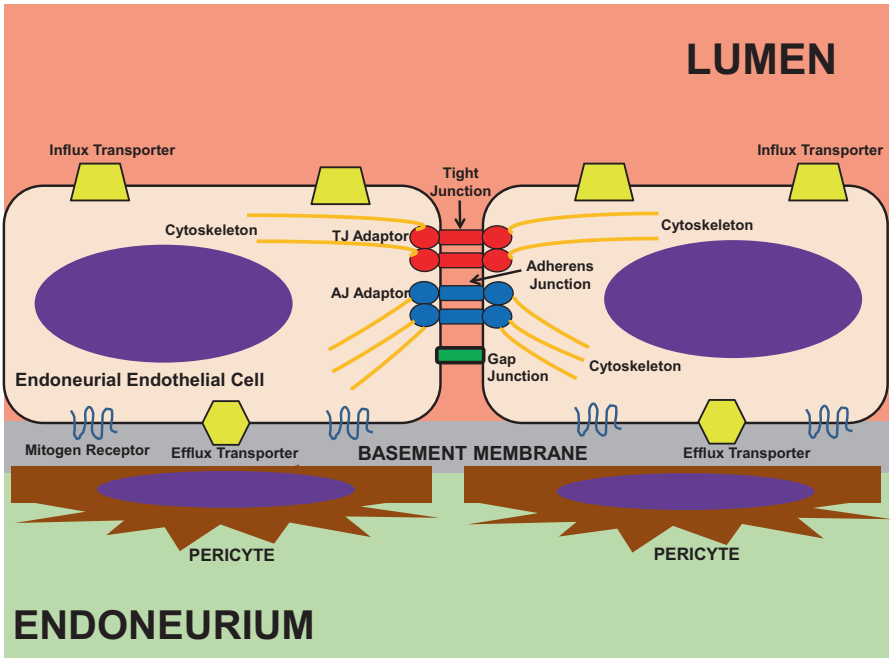

Fig. 3 Schematic figure showing the structural and essential molecular components of the human BNB

Human BNB Physiology

The human BNB, similar to other specialized tight junction-forming microvascular systems such as the BBB, blood-retina barrier, and blood-testis barrier, is expected to possess high transendothelial electrical resistance (TEER), low permeability to solutes and macromolecules, and low transendothelial water flux (hydraulic conductivity). In support of this, comparative animal studies have determined that the BNB is the second most restrictive microvascular tissue barrier in mammals, after the BBB. Unlike the human BBB, supported by the glia limitans (which consists of astrocyte and microglial foot processes), there is no physical support of the BNB by Schwann cells. It has not been conclusively established whether endoneurial microvascular pericytes (that lack intercellular junctions and share a basement membrane with endoneurial endothelial cells) provide trophic support to the human BNB.

The human BNB TEER in vivo is unknown; however, it is expected to be >1500 $\Omega.cm^2$, based on BBB data [38–41]. Similarly, its permeability coefficients and hydraulic conductivity in vivo are also unknown, although some work has been published in other mammalian and nonmammalian species evaluating solute permeability and interstitial fluid flux in peripheral nerves following intravenous electrolyte and tracer injections, followed by timed nerve procurement [17, 42–44]. Human BNB TEER has been measured to be as high as ~180 $\Omega.cm^2$ in confluent cultures by a voltohmmeter applying a direct current across transwell inserts and as high as ~900 Ω when recorded in specialized culture wells with gold electrodes using a fixed alternating current at 4000 Hz via electrical cell impedance sensing [7, 20, 32, 35].

Solute permeability to sodium fluorescein (molecular mass 376 Da) and 70 KDa fluoresceinated dextran (dextran-70-FITC) across primary and immortalized human endoneurial endothelial cells is typically <5% of input at 15 minutes using static transwell systems in vitro, with higher values (~3–15-fold) seen with sodium fluorescein when directly compared to dextran-70-FITC using the same batch of endothelial cells in concurrent experiments [7, 20, 32]. Human BNB transendothelial water flux under the influence of hydrostatic pressure, otherwise known as hydraulic conductivity, has been measured in vitro (~2.0×10^{-7} cm/s/cm H_2O) using a customized transwell diffusion chamber-bubble track system [45]. Consistent with prior observations, the human BNB was the second most restrictive human or mammalian microvascular endothelial cell type after the BBB in terms of water flux [17, 43–45].

Hematogenous leukocyte trafficking across microvascular endothelium in vivo (based on intravital microscopy) or in vitro under flow is a sequential coordinated process that involves leukocyte attraction from circulating blood to the endothelial cell luminal surface (mediated by specific chemokines bound to glycosaminoglycans on the endothelium and chemokine receptors expressed by leukocytes), rolling (mediated by selectins expressed on the endothelium and their glycoproteins or

carbohydrate moiety counterligands expressed on leukocytes), leukocyte arrest and haptotaxis on the endothelial cell surface (mediated by chemokines and chemokine receptors), integrin activation and firm adhesion (via leukocyte integrin binding to endothelial cell adhesion molecules) that induces a conformation change in leukocyte shape from round to flat with formation of pseudopodia, and leukocyte transmigration via the paracellular (i.e., through intercellular junctions) or transcellular (i.e., through endothelial cells) routes followed by basement membrane disruption at the abluminal surface (via secretion of specific matrix metalloproteases) required for complete passage into tissues [46–52]. There is in vitro data using a flow-dependent leukocyte-BNB trafficking model providing evidence that this sequential process (also known as the multistep paradigm of leukocyte trafficking) occurs in peripheral nerves [53–55].

The presence of rare endoneurial macrophages, mast cells, and T lymphocytes in normal human peripheral nerve endoneurium implies some physiological cross talk between the systemic immune compartment and peripheral nerves at the BNB. The human BNB transcriptome supports the expression of human leukocyte antigen (or major histocompatibility complex) class I and II molecules in normal healthy endoneurial microvessels in situ [22], suggesting that the human BNB may directly participate in innate and adaptive immune responses in peripheral nerves (Tables 1 and 2). Furthermore, specific chemokine transcripts were also expressed by the normal healthy adult BNB based on this transcriptome. These include CCL2, CCL14, CCL28, CXCL3, CXCL12, CXCL16, and CX3CL1 [22].

These chemokines could facilitate the interaction of hematogenous monocytes (CCL2, CCL14, CX3CL1), T lymphocytes (CCL2, CX3CL1), natural killer T cells (CXCL16), and neutrophils (CXCL3) with endoneurial microvascular cells during normal immunosurveillance or part of an early immune response to injury, while CXCL12 and CCL28 may be important in endothelial cell migration and vascular repair. A more complex array of chemokines including CXCL9, CXCL10, and CXCL11 that facilitate CXCR3+ CD4+ T-helper 1 lymphocyte migration were expressed by the basal human BNB in vitro [22, 55], implying some degree of endothelial cell activation in vitro or dysregulated chemokine expression in situ.

Endoneurial microvascular endothelial cells also express selectins (e.g., P-selectin, E-selectin) and cell adhesion molecules (e.g., intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), fibronectin Type III connecting segment) under basal conditions that were upregulated or underwent alternative splicing following stimulus with physiological concentrations of pro-inflammatory cytokines tissue necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) in vitro (Fig. 4) [55]. The constitutive expression of these cell adhesion molecules known to facilitate leukocyte adhesion and transmigration supports the notion the endoneurial microvessels participate in cross talk between subsets of circulating leukocytes that are components of systemic immune compartment and peripheral nerves.

Table 1 List of known molecules involved in the innate immune response expressed by the human BNB transcriptome in health that may be relevant in physiological cross-talk between the systemic immune compartment and peripheral nerve endoneurium and in peripheral nerve autoimmune disorders

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	**Panther protein class**
HUMAN|HGNC = 9066|UniProtKB = P16885	ENSG00000197943	1-Phosphatidylinositol 4,5-bisphosphate phosphodiesterase gamma-2; PLCG2; ortholog	1-PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE PHOSPHODIESTERASE GAMMA-2 (PTHR10336:SF25)	Calcium-binding protein(PC00060); guanyl-nucleotide exchange factor(PC00113); phospholipase(PC00186); signaling molecule(PC00207)
HUMAN|HGNC = 9553|UniProtKB = P62333	ENSG00000100519	26S protease regulatory subunit 10B; PSMC6; ortholog	26S PROTEASE REGULATORY SUBUNIT 10B (PTHR23073:SF31)	Hydrolase(PC00121)
HUMAN|HGNC = 9547|UniProtKB = P62191	ENSG00000100764	26S protease regulatory subunit 4; PSMC1; ortholog	26S PROTEASE REGULATORY SUBUNIT 4 (PTHR23073:SF24)	Hydrolase(PC00121)
HUMAN|HGNC = 9549|UniProtKB = P17980	ENSG00000165916	26S protease regulatory subunit 6A; PSMC3; ortholog	26S PROTEASE REGULATORY SUBUNIT 6A (PTHR23073:SF7)	Hydrolase(PC00121)
HUMAN|HGNC = 9551|UniProtKB = P43686	ENSG00000013275	26S protease regulatory subunit 6B; PSMC4; ortholog	26S PROTEASE REGULATORY SUBUNIT 6B (PTHR23073:SF8)	Hydrolase(PC00121)
HUMAN|HGNC = 9548|UniProtKB = P35998	ENSG00000161057	26S protease regulatory subunit 7; PSMC2; ortholog	26S PROTEASE REGULATORY SUBUNIT 7 (PTHR23073:SF13)	Hydrolase(PC00121)
HUMAN|HGNC = 9552|UniProtKB = P62195	ENSG00000087191	26S protease regulatory subunit 8; PSMC5; ortholog	26S PROTEASE REGULATORY SUBUNIT 8 (PTHR23073:SF12)	Hydrolase(PC00121)

HUMAN|HGNC = 9554|UniProtKB = Q99460	ENSG00000173692	26S proteasome non-ATPase regulatory subunit 1; PSMD1; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 1 (PTHR10943:SF2)	Enzyme modulator(PC00095)
HUMAN|HGNC = 9555|UniProtKB = O75832	ENSG00000101843	26S proteasome non-ATPase regulatory subunit 10; PSMD10; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 10 (PTHR24126:SF24)	
HUMAN|HGNC = 9556|UniProtKB = O00231	ENSG00000108671	26S proteasome non-ATPase regulatory subunit 11; PSMD11; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 11 (PTHR10678:SF2)	
HUMAN|HGNC = 9557|UniProtKB = O00232	ENSG00000197170	26S proteasome non-ATPase regulatory subunit 12; PSMD12; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 12 (PTHR10855:SF1)	Enzyme modulator(PC00095)
HUMAN|HGNC = 9558|UniProtKB = Q9UNM6	ENSG00000185627	26S proteasome non-ATPase regulatory subunit 13; PSMD13; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 13 (PTHR10539:SF0)	Enzyme modulator(PC00095)
HUMAN|HGNC = 16889|UniProtKB = O00487	ENSG00000115233	26S proteasome non-ATPase regulatory subunit 14; PSMD14; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 14 (PTHR10410:SF5)	Metalloprotease(PC00153); transcription factor(PC00218)
HUMAN|HGNC = 9559|UniProtKB = Q13200	ENSG00000175166	26S proteasome non-ATPase regulatory subunit 2; PSMD2; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 2 (PTHR10943:SF1)	Enzyme modulator(PC00095)

(continued)

Table 1 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	**Panther protein class**
HUMAN|HGNC = 9560|UniProtKB = O43242	ENSG00000108344	26S proteasome non-ATPase regulatory subunit 3; PSMD3; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 3 (PTHR10758:SF2)	Enzyme modulator(PC00095)
HUMAN|HGNC = 9561|UniProtKB = P55036	ENSG00000159352	26S proteasome non-ATPase regulatory subunit 4; PSMD4; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 4 (PTHR10223:SF0)	Enzyme modulator(PC00095)
HUMAN|HGNC = 9563|UniProtKB = Q16401	ENSG00000095261	26S proteasome non-ATPase regulatory subunit 5; PSMD5; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 5 (PTHR13554:SF10)	
HUMAN|HGNC = 9564|UniProtKB = Q15008	ENSG00000163636	26S proteasome non-ATPase regulatory subunit 6; PSMD6; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 6 (PTHR14145:SF1)	
HUMAN|HGNC = 9565|UniProtKB = P51665	ENSG00000103035	26S proteasome non-ATPase regulatory subunit 7; PSMD7; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 7 (PTHR10540:SF7)	Metalloprotease(PC00153)
HUMAN|HGNC = 9566|UniProtKB = P48556	ENSG00000099341	26S proteasome non-ATPase regulatory subunit 8; PSMD8; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 8 (PTHR12387:SF0)	Enzyme modulator(PC00095)
HUMAN|HGNC = 9567|UniProtKB = O00233	ENSG00000110801	26S proteasome non-ATPase regulatory subunit 9; PSMD9; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 9 (PTHR12651:SF1)	Enzyme modulator(PC00095)

HUMAN|HGNC = 8816|UniProtKB = O15530	ENSG00000140992	3-Phosphoinositide-dependent protein kinase 1; PDPK1; ortholog	3-PHOSPHOINOSITIDE-DEPENDENT PROTEIN KINASE 1-RELATED (PTHR24356:SF163)	Annexin(PC00050); calmodulin(PC00061); non-receptor serine/threonine protein kinase(PC00167); transfer/carrier protein(PC00219)
HUMAN|HGNC = 5261|UniProtKB = P10809	ENSG00000144381	60 kDa heat shock protein, mitochondrial; HSPD1; ortholog	60 KDA HEAT SHOCK PROTEIN, MITOCHONDRIAL (PTHR45633:SF3)	
HUMAN|HGNC = 23575|UniProtKB = O75179	ENSG00000132466	Ankyrin repeat domain-containing protein 17; ANKRD17; ortholog	ANKYRIN REPEAT DOMAIN-CONTAINING PROTEIN 17 (PTHR23206:SF1)	
HUMAN|HGNC = 16608|UniProtKB = Q9ULZ3	ENSG00000103490	Apoptosis-associated speck-like protein containing a CARD; PYCARD; ortholog	APOPTOSIS-ASSOCIATED SPECK-LIKE PROTEIN CONTAINING A CARD (PTHR10454:SF203)	Cysteine protease(PC00081); protease inhibitor(PC00191)
HUMAN|HGNC = 590|UniProtKB = Q13490	ENSG00000110330	Baculoviral IAP repeat-containing protein 2; BIRC2; ortholog	BACULOVIRAL IAP REPEAT-CONTAINING PROTEIN 2 (PTHR10044:SF79)	Protease inhibitor(PC00191)
HUMAN|HGNC = 591|UniProtKB = Q13489	ENSG00000023445	Baculoviral IAP repeat-containing protein 3; BIRC3; ortholog	BACULOVIRAL IAP REPEAT-CONTAINING PROTEIN 3 (PTHR10044:SF99)	Protease inhibitor(PC00191)
HUMAN|HGNC = 712|UniProtKB = P32121	ENSG00000141480	Beta-arrestin-2; ARRB2; ortholog	BETA-ARRESTIN-2 (PTHR11792:SF20)	Enzyme modulator(PC00095)

(continued)

Table 1 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	**Panther protein class**
HUMAN|HGNC = 29938|UniProtKB = Q8WUQ7	ENSG00000105298	Cactin; CACTIN; ortholog	CACTIN (PTHR21737:SF6)	
HUMAN|HGNC = 9380|UniProtKB = P17612	ENSG00000072062	cAMP-dependent protein kinase catalytic subunit alpha; PRKACA; ortholog	CAMP-DEPENDENT PROTEIN KINASE CATALYTIC SUBUNIT ALPHA (PTHR24353:SF82)	
HUMAN|HGNC = 9381|UniProtKB = P22694	ENSG00000142875	cAMP-dependent protein kinase catalytic subunit beta; PRKACB; ortholog	CAMP-DEPENDENT PROTEIN KINASE CATALYTIC SUBUNIT BETA (PTHR24353:SF116)	
HUMAN|HGNC = 16393|UniProtKB = Q9BXL7	ENSG00000198286	Caspase recruitment domain-containing protein 11; CARD11; ortholog	CASPASE RECRUITMENT DOMAIN-CONTAINING PROTEIN 11 (PTHR14559:SF4)	
HUMAN|HGNC = 16391|UniProtKB = Q9H257	ENSG00000187796	Caspase recruitment domain-containing protein 9; CARD9; ortholog	CASPASE RECRUITMENT DOMAIN-CONTAINING PROTEIN 9 (PTHR14559:SF3)	
HUMAN|HGNC = 1509|UniProtKB = Q14790	ENSG00000064012	Caspase-8; CASP8; ortholog	CASPASE-8 (PTHR10454:SF162)	Cysteine protease(PC00081); protease inhibitor(PC00191)
HUMAN|HGNC = 2527|UniProtKB = P07858	ENSG00000164733	Cathepsin B; CTSB; ortholog	CATHEPSIN B (PTHR12411:SF16)	Cysteine protease(PC00081); protease inhibitor(PC00191)
HUMAN|HGNC = 2536|UniProtKB = P43235	ENSG00000143387	Cathepsin K; CTSK; ortholog	CATHEPSIN K (PTHR12411:SF55)	Cysteine protease(PC00081); protease inhibitor(PC00191)

HUMAN|HGNC = 2537|UniProtKB = P07711	ENSG00000135047	Cathepsin L1; CTSL; ortholog	CATHEPSIN L1 (PTHR12411:SF411)	Cysteine protease(PC00081); protease inhibitor(PC00191)
HUMAN|HGNC = 2545|UniProtKB = P25774	ENSG00000163131	Cathepsin S; CTSS; ortholog	CATHEPSIN S (PTHR12411:SF525)	Cysteine protease(PC00081); protease inhibitor(PC00191)
HUMAN|HGNC = 1527|UniProtKB = Q03135	ENSG00000105974	Caveolin-1; CAV1; ortholog	CAVEOLIN-1 (PTHR10844:SF18)	G-protein modulator(PC00022); membrane traffic protein(PC00150); structural protein(PC00211); transmembrane receptor regulatory/adaptor protein(PC00226)
HUMAN|HGNC = 16016|UniProtKB = Q5KU26	ENSG00000158270	Collectin-12; COLEC12; ortholog	COLLECTIN-12 (PTHR24023:SF910)	
HUMAN|HGNC = 1243|UniProtKB = Q07021	ENSG00000108561	Complement component 1 Q subcomponent-binding protein, mitochondrial; C1QBP; ortholog	COMPLEMENT COMPONENT 1 Q SUBCOMPONENT-BINDING PROTEIN, MITOCHONDRIAL (PTHR10826:SF1)	
HUMAN|HGNC = 2348|UniProtKB = Q92793	ENSG00000005339	CREB-binding protein; CREBBP; ortholog	CREB-BINDING PROTEIN (PTHR13808:SF1)	Acetyltransferase(PC00038); chromatin/chromatin-binding protein(PC00077); transcription cofactor(PC00217)

(continued)

Table 1 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	**Panther protein class**
HUMAN|HGNC = 13257|UniProtKB = Q9UMR7	ENSG00000111729	C-type lectin domain family 4 member A; CLEC4A; ortholog	C-TYPE LECTIN DOMAIN FAMILY 4 MEMBER A (PTHR22802:SF357)	Cell adhesion molecule(PC00069); immunoglobulin receptor superfamily(PC00124)
HUMAN|HGNC = 2551|UniProtKB = Q13616	ENSG00000055130	Cullin-1; CUL1; ortholog	CULLIN-1 (PTHR11932:SF81)	Ubiquitin-protein ligase(PC00234)
HUMAN|HGNC = 21367|UniProtKB = Q8N884	ENSG00000164430	Cyclic GMP-AMP synthase; MB21D1; ortholog	CYCLIC GMP-AMP SYNTHASE (PTHR10656:SF35)	
HUMAN|HGNC = 2577|UniProtKB = P13498	ENSG00000051523	Cytochrome b-245 light chain; CYBA; ortholog	CYTOCHROME B-245 LIGHT CHAIN (PTHR15168:SF0)	
HUMAN|HGNC = 17294|UniProtKB = Q5VWQ8	ENSG00000136848	Disabled homolog 2-interacting protein; DAB2IP; ortholog	DISABLED HOMOLOG 2-INTERACTING PROTEIN (PTHR10194:SF26)	G-protein modulator(PC00022)
HUMAN|HGNC = 215|UniProtKB = P78325	ENSG00000151651	Disintegrin and metalloproteinase domain-containing protein 8; ADAM8; ortholog	DISINTEGRIN AND METALLOPROTEINASE DOMAIN-CONTAINING PROTEIN 8 (PTHR11905:SF20)	Metalloprotease(PC00153)
HUMAN|HGNC = 6846|UniProtKB = P52564	ENSG00000108984	Dual specificity mitogen-activated protein kinase kinase 6; MAP2K6; ortholog	DUAL SPECIFICITY MITOGEN-ACTIVATED PROTEIN KINASE KINASE 6 (PTHR24361:SF40)	

HUMAN|HGNC = 13890|UniProtKB = Q96J02	ENSG00000078747	E3 ubiquitin-protein ligase Itchy homolog; ITCH; ortholog	E3 UBIQUITIN-PROTEIN LIGASE ITCHY HOMOLOG (PTHR11254:SF66)	Ubiquitin-protein ligase(PC00234)
HUMAN|HGNC = 8827|UniProtKB = Q96FA3	ENSG00000197329	E3 ubiquitin-protein ligase pellino homolog 1; PELI1; ortholog	E3 UBIQUITIN-PROTEIN LIGASE PELLINO HOMOLOG 1 (PTHR12098:SF4)	
HUMAN|HGNC = 592|UniProtKB = P98170	ENSG00000101966	E3 ubiquitin-protein ligase XIAP; XIAP; ortholog	E3 UBIQUITIN-PROTEIN LIGASE XIAP (PTHR10044:SF115)	Protease inhibitor(PC00191)
HUMAN|HGNC = 12028|UniProtKB = P14625	ENSG00000166598	Endoplasmin; HSP90B1; ortholog	ENDOPLASMIN (PTHR11528:SF54)	Hsp90 family chaperone(PC00028)
HUMAN|HGNC = 15842|UniProtKB = Q96RT1	ENSG00000112851	Erbin; ERBIN; ortholog	ERBIN (PTHR45752:SF47)	
HUMAN|HGNC = 3573|UniProtKB = Q13158	ENSG00000168040	FAS-associated death domain protein; FADD; ortholog	FAS-ASSOCIATED DEATH DOMAIN PROTEIN (PTHR15077:SF9)	
HUMAN|HGNC = 13607|UniProtKB = Q9UKB1	ENSG00000072803	F-box/WD repeat-containing protein 11; FBXW11; ortholog	F-BOX/WD REPEAT-CONTAINING PROTEIN 11 (PTHR44129:SF4)	
HUMAN|HGNC = 1144|UniProtKB = Q9Y297	ENSG00000166167	F-box/WD repeat-containing protein 1A; BTRC; ortholog	F-BOX/WD REPEAT-CONTAINING PROTEIN 1A (PTHR19854:SF16)	G-protein-coupled receptor(PC00021)
HUMAN|HGNC = 3757|UniProtKB = O75955	ENSG00000137312	Flotillin-1; FLOT1; ortholog	FLOTILLIN-1 (PTHR13806:SF16)	
HUMAN|HGNC = 3758|UniProtKB = Q14254	ENSG00000132589	Flotillin-2;FLOT2;ortholog	FLOTILLIN-2 (PTHR13806:SF20)	

(continued)

Table 1 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	**Panther protein class**
HUMAN|HGNC = 6570|UniProtKB = O00182	ENSG00000168961	Galectin-9; LGALS9; ortholog	GALECTIN-9 (PTHR11346:SF80)	Cell adhesion molecule(PC00069); signaling molecule(PC00207)
HUMAN|HGNC = 5395|UniProtKB = Q16666	ENSG00000163565	Gamma-interferon-inducible protein 16; IFI16; ortholog	GAMMA-INTERFERON-INDUCIBLE PROTEIN 16 (PTHR12200:SF5)	Transcription factor(PC00218)
HUMAN|HGNC = 5173|UniProtKB = P01112	ENSG00000174775	GTPase HRas; HRAS; ortholog	GTPASE HRAS (PTHR24070:SF385)	Small GTPase(PC00208)
HUMAN|HGNC = 6407|UniProtKB = P01116	ENSG00000133703	GTPase KRas; KRAS; ortholog	GTPASE KRAS (PTHR24070:SF388)	Small GTPase(PC00208)
HUMAN|HGNC = 7989|UniProtKB = P01111	ENSG00000213281	GTPase NRas; NRAS; ortholog	GTPASE NRAS (PTHR24070:SF189)	Small GTPase(PC00208)
HUMAN|HGNC = 5232|UniProtKB = P0DMV8	ENSG00000204389	Heat shock 70 kDa protein 1A; HSPA1A; ortholog	HEAT SHOCK 70 KDA PROTEIN 1A-RELATED (PTHR19375:SF223)	
HUMAN|HGNC = 5233|UniProtKB = P0DMV9	ENSG00000204388	Heat shock 70 kDa protein 1B; HSPA1B; ortholog	HEAT SHOCK 70 KDA PROTEIN 1A-RELATED (PTHR19375:SF223)	
HUMAN|HGNC = 3611|UniProtKB = P30273	ENSG00000158869	High-affinity immunoglobulin epsilon receptor subunit gamma; FCER1G; ortholog	HIGH AFFINITY IMMUNOGLOBULIN EPSILON RECEPTOR SUBUNIT GAMMA (PTHR16803:SF0)	

HUMAN|HGNC = 4983|UniProtKB = P09429	ENSG00000189403	High-mobility group protein B1; HMGB1; ortholog	HIGH MOBILITY GROUP PROTEIN B1 (PTHR13711:SF164)	HMG box transcription factor(PC00024); chromatin/chromatin-binding protein(PC00077); signaling molecule(PC00207)
HUMAN|HGNC = 3373|UniProtKB = Q09472	ENSG00000100393	Histone acetyltransferase p300; EP300; ortholog	HISTONE ACETYLTRANSFERASE P300 (PTHR13808:SF23)	Acetyltransferase(PC00038); chromatin/chromatin-binding protein(PC00077); transcription cofactor(PC00217)
HUMAN|HGNC = 1974|UniProtKB = O15111	ENSG00000213341	Inhibitor of nuclear factor kappa-B kinase subunit alpha; CHUK; ortholog	INHIBITOR OF NUCLEAR FACTOR KAPPA-B KINASE SUBUNIT ALPHA (PTHR22969:SF13)	Non-receptor serine/threonine protein kinase(PC00167)
HUMAN|HGNC = 5960|UniProtKB = O14920	ENSG00000104365	Inhibitor of nuclear factor kappa-B kinase subunit beta; IKBKB; ortholog	INHIBITOR OF NUCLEAR FACTOR KAPPA-B KINASE SUBUNIT BETA (PTHR22969:SF7)	Non-receptor serine/threonine protein kinase(PC00167)
HUMAN|HGNC = 6155|UniProtKB = P05107	ENSG00000160255	Integrin beta-2; ITGB2; ortholog	INTEGRIN BETA-2 (PTHR10082:SF15)	Cell adhesion molecule(PC00069); extracellular matrix glycoprotein(PC00100); receptor(PC00197)
HUMAN|HGNC = 5346|UniProtKB = P32942	ENSG00000076662	Intercellular adhesion molecule 3; ICAM3; ortholog	INTERCELLULAR ADHESION MOLECULE 3 (PTHR13771:SF11)	

(continued)

Table 1 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	**Panther protein class**
HUMAN|HGNC = 6116|UniProtKB = P10914	ENSG00000125347	Interferon regulatory factor 1; IRF1; ortholog	INTERFERON REGULATORY FACTOR 1 (PTHR11949:SF3)	Nucleic acid binding(PC00171); winged helix/forkhead transcription factor(PC00246)
HUMAN|HGNC = 6118|UniProtKB = Q14653	ENSG00000126456	Interferon regulatory factor 3; IRF3; ortholog	INTERFERON REGULATORY FACTOR 3 (PTHR11949:SF1)	Nucleic acid binding(PC00171); winged helix/forkhead transcription factor(PC00246)
HUMAN|HGNC = 6122|UniProtKB = Q92985	ENSG00000185507	Interferon regulatory factor 7; IRF7; ortholog	INTERFERON REGULATORY FACTOR 7 (PTHR11949:SF2)	Nucleic acid binding(PC00171); winged helix/forkhead transcription factor(PC00246)
HUMAN|HGNC = 18873|UniProtKB = Q9BYX4	ENSG00000115267	Interferon-induced helicase C domain-containing protein 1; IFIH1; ortholog	INTERFERON-INDUCED HELICASE C DOMAIN-CONTAINING PROTEIN 1 (PTHR14074:SF14)	
HUMAN|HGNC = 6112|UniProtKB = P51617	ENSG00000184216	Interleukin-1 receptor-associated kinase 1; IRAK1; ortholog	INTERLEUKIN-1 RECEPTOR-ASSOCIATED KINASE 1 (PTHR24419:SF1)	
HUMAN|HGNC = 17020|UniProtKB = Q9Y616	ENSG00000090376	Interleukin-1 receptor-associated kinase 3; IRAK3; ortholog	INTERLEUKIN-1 RECEPTOR-ASSOCIATED KINASE 3 (PTHR24419:SF7)	

HUMAN|HGNC = 17967|UniProtKB = Q9NWZ3	ENSG00000198001	Interleukin-1 receptor-associated kinase 4; IRAK4; ortholog	INTERLEUKIN-1 RECEPTOR-ASSOCIATED KINASE 4 (PTHR24419:SF22)	
HUMAN|HGNC = 9472|UniProtKB = Q99538	ENSG00000100600	Legumain; LGMN; ortholog	LEGUMAIN (PTHR12000:SF3)	Cysteine protease(PC00081)
HUMAN|HGNC = 13299|UniProtKB = Q9BXB1	ENSG00000205213	Leucine-rich repeat-containing G-protein-coupled receptor 4; LGR4; ortholog	LEUCINE-RICH REPEAT-CONTAINING G-PROTEIN COUPLED RECEPTOR 4 (PTHR24372:SF67)	Extracellular matrix protein(PC00102); receptor(PC00197)
HUMAN|HGNC = 6887|UniProtKB = P49137	ENSG00000162889	MAP kinase-activated protein kinase 2; MAPKAPK2; ortholog	MAP KINASE-ACTIVATED PROTEIN KINASE 2 (PTHR24349:SF63)	Non-motor microtubule binding protein(PC00166); non-receptor serine/threonine protein kinase(PC00167)
HUMAN|HGNC = 6888|UniProtKB = Q16644	ENSG00000114738	MAP kinase-activated protein kinase 3; MAPKAPK3; ortholog	MAP KINASE-ACTIVATED PROTEIN KINASE 3 (PTHR24349:SF64)	Non-motor microtubule binding protein(PC00166); non-receptor serine/threonine protein kinase(PC00167)
HUMAN|HGNC = 29233|UniProtKB = Q7Z434	ENSG00000088888	Mitochondrial antiviral-signaling protein; MAVS; ortholog	MITOCHONDRIAL ANTIVIRAL-SIGNALING PROTEIN (PTHR21446:SF6)	
HUMAN|HGNC = 6848|UniProtKB = Q13233	ENSG00000095015	Mitogen-activated protein kinase kinase kinase 1; MAP3K1; ortholog	MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE 1 (PTHR24361:SF414)	

(continued)

Table 1 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	**Panther protein class**
HUMAN|HGNC = 6859|UniProtKB = O43318	ENSG00000135341	Mitogen-activated protein kinase kinase kinase 7; MAP3K7; ortholog	MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE 7 (PTHR46716:SF1)	
HUMAN|HGNC = 1628|UniProtKB = P08571	ENSG00000170458	Monocyte differentiation antigen CD14; CD14; ortholog	MONOCYTE DIFFERENTIATION ANTIGEN CD14 (PTHR10630:SF3)	
HUMAN|HGNC = 6819|UniProtKB = Q9UDY8	ENSG00000172175	Mucosa-associated lymphoid tissue lymphoma translocation protein 1; MALT1; ortholog	MUCOSA-ASSOCIATED LYMPHOID TISSUE LYMPHOMA TRANSLOCATION PROTEIN 1 (PTHR22576:SF29)	Cysteine protease(PC00081)
HUMAN|HGNC = 7562|UniProtKB = Q99836	ENSG00000172936	Myeloid differentiation primary response protein MyD88; MYD88; ortholog	MYELOID DIFFERENTIATION PRIMARY RESPONSE PROTEIN MYD88 (PTHR15079:SF3)	
HUMAN|HGNC = 5961|UniProtKB = Q9Y6K9	ENSG00000269335	NF-kappa-B essential modulator; IKBKG; ortholog	NF-KAPPA-B ESSENTIAL MODULATOR (PTHR31553:SF3)	
HUMAN|HGNC = 7797|UniProtKB = P25963	ENSG00000100906	NF-kappa-B inhibitor alpha; NFKBIA; ortholog	NF-KAPPA-B INHIBITOR ALPHA (PTHR46680:SF1)	

HUMAN|HGNC = 7800|UniProtKB = Q9UBC1	ENSG00000204498	NF-kappa-B inhibitor-like protein 1; NFKBIL1; ortholog	NF-KAPPA-B INHIBITOR-LIKE PROTEIN 1 (PTHR15263:SF1)	
HUMAN|HGNC = 29890|UniProtKB = Q86UT6	ENSG00000160703	NLR family member X1; NLRX1; ortholog	NLR FAMILY MEMBER X1 (PTHR24106:SF152)	Nucleic acid binding(PC00171); serine protease(PC00203); transcription cofactor(PC00217)
HUMAN|HGNC = 7794|UniProtKB = P19838	ENSG00000109320	Nuclear factor NF-kappa-B p105 subunit; NFKB1; ortholog	NUCLEAR FACTOR NF-KAPPA-B P105 SUBUNIT (PTHR24169:SF9)	
HUMAN|HGNC = 7962|UniProtKB = P20393	ENSG00000126368	Nuclear receptor subfamily 1 group D member 1; NR1D1; ortholog	NUCLEAR RECEPTOR SUBFAMILY 1 GROUP D MEMBER 1 (PTHR24082:SF113)	C4 zinc finger nuclear receptor(PC00169); nucleic acid binding(PC00171); receptor(PC00197)
HUMAN|HGNC = 16390|UniProtKB = Q9Y239	ENSG00000106100	Nucleotide-binding oligomerization domain-containing protein 1; NOD1; ortholog	NUCLEOTIDE-BINDING OLIGOMERIZATION DOMAIN-CONTAINING PROTEIN 1 (PTHR24106:SF18)	Nucleic acid binding(PC00171); serine protease(PC00203); transcription cofactor(PC00217)
HUMAN|HGNC = 5331|UniProtKB = Q9HC29	ENSG00000167207	Nucleotide-binding oligomerization domain-containing protein 2; NOD2; ortholog	NUCLEOTIDE-BINDING OLIGOMERIZATION DOMAIN-CONTAINING PROTEIN 2 (PTHR24106:SF64)	Nucleic acid binding(PC00171); serine protease(PC00203); transcription cofactor(PC00217)

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Table 1 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	**Panther protein class**
HUMAN|HGNC = 7966|UniProtKB = Q13133	ENSG00000025434	Oxysterols receptor LXR-alpha; NR1H3; ortholog	OXYSTEROLS RECEPTOR LXR-ALPHA (PTHR24082:SF259)	C4 zinc finger nuclear receptor(PC00169); nucleic acid binding(PC00171); receptor(PC00197)
HUMAN|HGNC = 19353|UniProtKB = Q96ST3	ENSG00000169375	Paired amphipathic helix protein Sin3a; SIN3A; ortholog	PAIRED AMPHIPATHIC HELIX PROTEIN SIN3A (PTHR12346:SF2)	Chromatin/chromatin-binding protein(PC00077); deacetylase(PC00087); transcription factor(PC00218)
HUMAN|HGNC = 8974|UniProtKB = Q8NEB9	ENSG00000078142	Phosphatidylinositol 3-kinase catalytic subunit type 3; PIK3C3; ortholog	PHOSPHATIDYLINOSITOL 3-KINASE CATALYTIC SUBUNIT TYPE 3 (PTHR10048:SF7)	Kinase(PC00137)
HUMAN|HGNC = 8982|UniProtKB = Q99570	ENSG00000196455	Phosphoinositide 3-kinase regulatory subunit 4; PIK3R4; ortholog	PHOSPHOINOSITIDE 3-KINASE REGULATORY SUBUNIT 4 (PTHR17583:SF0)	
HUMAN|HGNC = 1663|UniProtKB = P16671	ENSG00000135218	Platelet glycoprotein 4; CD36; ortholog	PLATELET GLYCOPROTEIN 4 (PTHR11923:SF12)	Receptor(PC00197)
HUMAN|HGNC = 12463|UniProtKB = P0CG47	ENSG00000170315	Polyubiquitin-B; UBB; ortholog	POLYUBIQUITIN-B (PTHR10666:SF165)	Ribosomal protein(PC00202)
HUMAN|HGNC = 12468|UniProtKB = P0CG48	ENSG00000150991	Polyubiquitin-C; UBC; ortholog	POLYUBIQUITIN-C (PTHR10666:SF277)	Ribosomal protein(PC00202)

HUMAN|HGNC = 19102|UniProtKB = O95786	ENSG00000107201	Probable ATP-dependent RNA helicase DDX58; DDX58; ortholog	ATP-DEPENDENT RNA HELICASE DDX58-RELATED (PTHR14074:SF31)	
HUMAN|HGNC = 25942|UniProtKB = Q8IY21	ENSG00000137628	Probable ATP-dependent RNA helicase DDX60; DDX60; ortholog	ATP-DEPENDENT RNA HELICASE DDX60-RELATED (PTHR44533:SF3)	
HUMAN|HGNC = 29517|UniProtKB = Q96C10	ENSG00000108771	Probable ATP-dependent RNA helicase DHX58; DHX58; ortholog	ATP-DEPENDENT RNA HELICASE DHX58-RELATED (PTHR14074:SF7)	
HUMAN|HGNC = 9568|UniProtKB = Q06323	ENSG00000092010	Proteasome activator complex subunit 1; PSME1; ortholog	PROTEASOME ACTIVATOR COMPLEX SUBUNIT 1 (PTHR10660:SF5)	
HUMAN|HGNC = 9569|UniProtKB = Q9UL46	ENSG00000100911	Proteasome activator complex subunit 2; PSME2; ortholog	PROTEASOME ACTIVATOR COMPLEX SUBUNIT 2 (PTHR10660:SF6)	
HUMAN|HGNC = 9570|UniProtKB = P61289	ENSG00000131467	Proteasome activator complex subunit 3; PSME3; ortholog	PROTEASOME ACTIVATOR COMPLEX SUBUNIT 3 (PTHR10660:SF4)	
HUMAN|HGNC = 20635|UniProtKB = Q14997	ENSG00000068878	Proteasome activator complex subunit 4; PSME4; ortholog	PROTEASOME ACTIVATOR COMPLEX SUBUNIT 4 (PTHR32170:SF3)	
HUMAN|HGNC = 9571|UniProtKB = Q92530	ENSG00000125818	Proteasome inhibitor PI31 subunit; PSMF1; ortholog	PROTEASOME INHIBITOR PI31 SUBUNIT (PTHR13266:SF1)	Protease inhibitor(PC00191)

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Table 1 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	**Panther protein class**
HUMAN|HGNC = 9530|UniProtKB = P25786	ENSG00000129084	Proteasome subunit alpha type-1; PSMA1; ortholog	PROTEASOME SUBUNIT ALPHA TYPE-RELATED (PTHR11599:SF12)	Protease(PC00190)
HUMAN|HGNC = 9531|UniProtKB = P25787	ENSG00000106588	Proteasome subunit alpha type-2; PSMA2; ortholog	PROTEASOME SUBUNIT ALPHA TYPE-2 (PTHR11599:SF16)	Protease(PC00190)
HUMAN|HGNC = 9532|UniProtKB = P25788	ENSG00000100567	Proteasome subunit alpha type-3; PSMA3; ortholog	PROTEASOME SUBUNIT ALPHA TYPE-3 (PTHR11599:SF10)	Protease(PC00190)
HUMAN|HGNC = 9533|UniProtKB = P25789	ENSG00000041357	Proteasome subunit alpha type-4; PSMA4; ortholog	PROTEASOME SUBUNIT ALPHA TYPE-4 (PTHR11599:SF13)	Protease(PC00190)
HUMAN|HGNC = 9534|UniProtKB = P28066	ENSG00000143106	Proteasome subunit alpha type-5; PSMA5; ortholog	PROTEASOME SUBUNIT ALPHA TYPE-5 (PTHR11599:SF14)	Protease(PC00190)
HUMAN|HGNC = 9535|UniProtKB = P60900	ENSG00000100902	Proteasome subunit alpha type-6; PSMA6; ortholog	PROTEASOME SUBUNIT ALPHA TYPE-6 (PTHR11599:SF11)	Protease(PC00190)
HUMAN|HGNC = 9536|UniProtKB = O14818	ENSG00000101182	Proteasome subunit alpha type-7; PSMA7; ortholog	PROTEASOME SUBUNIT ALPHA TYPE-7 (PTHR11599:SF40)	Protease(PC00190)
HUMAN|HGNC = 9537|UniProtKB = P20618	ENSG00000008018	Proteasome subunit beta type-1; PSMB1; ortholog	PROTEASOME SUBUNIT BETA TYPE-1 (PTHR11599:SF59)	Protease(PC00190)

HUMAN|HGNC = 9538|UniProtKB = P40306	ENSG00000205220	Proteasome subunit beta type-10; PSMB10; ortholog	PROTEASOME SUBUNIT BETA TYPE-10 (PTHR11599:SF41)	Protease(PC00190)
HUMAN|HGNC = 9539|UniProtKB = P49721	ENSG00000126067	Proteasome subunit beta type-2; PSMB2; ortholog	PROTEASOME SUBUNIT BETA TYPE-2 (PTHR11599:SF6)	Protease(PC00190)
HUMAN|HGNC = 9540|UniProtKB = P49720	ENSG00000277791	Proteasome subunit beta type-3; PSMB3; ortholog	PROTEASOME SUBUNIT BETA TYPE-3 (PTHR11599:SF62)	Protease(PC00190)
HUMAN|HGNC = 9541|UniProtKB = P28070	ENSG00000159377	Proteasome subunit beta type-4; PSMB4; ortholog	PROTEASOME SUBUNIT BETA TYPE-4 (PTHR11599:SF5)	Protease(PC00190)
HUMAN|HGNC = 9543|UniProtKB = P28072	ENSG00000142507	Proteasome subunit beta type-6; PSMB6; ortholog	PROTEASOME SUBUNIT BETA TYPE-6 (PTHR11599:SF46)	Protease(PC00190)
HUMAN|HGNC = 9544|UniProtKB = Q99436	ENSG00000136930	Proteasome subunit beta type-7; PSMB7; ortholog	PROTEASOME SUBUNIT BETA TYPE-7 (PTHR11599:SF42)	Protease(PC00190)
HUMAN|HGNC = 9545|UniProtKB = P28062	ENSG00000204264	Proteasome subunit beta type-8; PSMB8; ortholog	PROTEASOME SUBUNIT BETA TYPE-8 (PTHR11599:SF53)	Protease(PC00190)
HUMAN|HGNC = 9546|UniProtKB = P28065	ENSG00000240065	Proteasome subunit beta type-9; PSMB9; ortholog	PROTEASOME SUBUNIT BETA TYPE-9 (PTHR11599:SF50)	Protease(PC00190)
HUMAN|HGNC = 11968|UniProtKB = Q9BT09	ENSG00000137161	Protein canopy homolog 3; CNPY3; ortholog	PROTEIN CANOPY HOMOLOG 3 (PTHR15382:SF2)	

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Table 1 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	**Panther protein class**
HUMAN|HGNC = 9399|UniProtKB = Q05655	ENSG00000163932	Protein kinase C delta type; PRKCD; ortholog	PROTEIN KINASE C DELTA TYPE (PTHR24356:SF322)	Annexin(PC00050); calmodulin(PC00061); non-receptor serine/threonine protein kinase(PC00167); transfer/carrier protein(PC00219)
HUMAN|HGNC = 9401|UniProtKB = Q02156	ENSG00000171132	Protein kinase C epsilon type; PRKCE; ortholog	PROTEIN KINASE C EPSILON TYPE (PTHR24356:SF159)	Annexin(PC00050); calmodulin(PC00061); non-receptor serine/threonine protein kinase(PC00167); transfer/carrier protein(PC00219)
HUMAN|HGNC = 24489|UniProtKB = Q8ND56	ENSG00000257103	Protein LSM14 homolog A; LSM14A; ortholog	PROTEIN LSM14 HOMOLOG A (PTHR13586:SF2)	RNA-binding protein(PC00031)
HUMAN|HGNC = 13481|UniProtKB = Q9H1C4	ENSG00000110057	Protein unc-93 homolog B1; UNC93B1; ortholog	PROTEIN UNC-93 HOMOLOG B1 (PTHR46744:SF1)	
HUMAN|HGNC = 11283|UniProtKB = P12931	ENSG00000197122	Proto-oncogene tyrosine-protein kinase Src; SRC; ortholog	PROTO-ONCOGENE TYROSINE-PROTEIN KINASE SRC (PTHR24418:SF53)	
HUMAN|HGNC = 14957|UniProtKB = Q14671	ENSG00000134644	Pumilio homolog 1; PUM1; ortholog	PUMILIO HOMOLOG 1 (PTHR12537:SF1)	mRNA processing factor(PC00147); translation factor(PC00223)

HUMAN|HGNC = 14958|UniProtKB = Q8TB72	ENSG00000055917	Pumilio homolog 2; PUM2; ortholog	PUMILIO HOMOLOG 2 (PTHR12537:SF52)	mRNA processing factor(PC00147); translation factor(PC00223)
HUMAN|HGNC = 30908|UniProtKB = Q8WXG1	ENSG00000134321	Radical S-adenosylmethionine domain-containing protein 2; RSAD2; ortholog	RADICAL S-ADENOSYL METHIONINE DOMAIN-CONTAINING PROTEIN 2 (PTHR21339:SF0)	
HUMAN|HGNC = 9829|UniProtKB = P04049	ENSG00000132155	RAF proto-oncogene serine/threonine-protein kinase; RAF1; ortholog	RAF PROTO-ONCOGENE SERINE/THREONINE-PROTEIN KINASE (PTHR44329:SF22)	
HUMAN|HGNC = 30278|UniProtKB = Q14699	ENSG00000131378	Raftlin; RFTN1; ortholog	RAFTLIN (PTHR17601:SF3)	
HUMAN|HGNC = 10019|UniProtKB = Q13546	ENSG00000137275	Receptor-interacting serine/threonine-protein kinase 1; RIPK1; ortholog	RECEPTOR-INTERACTING SERINE/THREONINE-PROTEIN KINASE 1 (PTHR44329:SF6)	
HUMAN|HGNC = 10432|UniProtKB = P51812	ENSG00000177189	Ribosomal protein S6 kinase alpha-3; RPS6KA3; ortholog	RIBOSOMAL PROTEIN S6 KINASE ALPHA-3 (PTHR24351:SF58)	
HUMAN|HGNC = 10434|UniProtKB = O75582	ENSG00000100784	Ribosomal protein S6 kinase alpha-5; RPS6KA5; ortholog	RIBOSOMAL PROTEIN S6 KINASE ALPHA-5 (PTHR24351:SF115)	
HUMAN|HGNC = 19000|UniProtKB = Q6AZY7	ENSG00000168077	Scavenger receptor class A member 3; SCARA3; ortholog	SCAVENGER RECEPTOR CLASS A MEMBER 3 (PTHR24020:SF10)	Receptor(PC00197)

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Table 1 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	**Panther protein class**
HUMAN|HGNC = 10698|UniProtKB = Q92503	ENSG00000129657	SEC14-like protein 1; SEC14L1; ortholog	SEC14-LIKE PROTEIN 1 (PTHR23324:SF51)	
HUMAN|HGNC = 8590|UniProtKB = Q13153	ENSG00000149269	Serine/threonine-protein kinase PAK 1; PAK1; ortholog	SERINE/THREONINE-PROTEIN KINASE PAK 1 (PTHR24361:SF232)	
HUMAN|HGNC = 8591|UniProtKB = Q13177	ENSG00000180370	Serine/threonine-protein kinase PAK 2; PAK2; ortholog	SERINE/THREONINE-PROTEIN KINASE PAK 2 (PTHR24361:SF281)	
HUMAN|HGNC = 8592|UniProtKB = O75914	ENSG00000077264	Serine/threonine-protein kinase PAK 3; PAK3; ortholog	SERINE/THREONINE-PROTEIN KINASE PAK 3 (PTHR24361:SF250)	
HUMAN|HGNC = 11584|UniProtKB = Q9UHD2	ENSG00000183735	Serine/threonine-protein kinase TBK1; TBK1; ortholog	SERINE/THREONINE-PROTEIN KINASE TBK1 (PTHR22969:SF14)	Non-receptor serine/threonine protein kinase(PC00167)
HUMAN|HGNC = 10899|UniProtKB = P63208	ENSG00000113558	S-phase kinase-associated protein 1; SKP1; ortholog	S-PHASE KINASE-ASSOCIATED PROTEIN 1 (PTHR11165:SF24)	
HUMAN|HGNC = 17074|UniProtKB = Q6SZW1	ENSG00000004139	Sterile alpha and TIR motif-containing protein 1; SARM1; ortholog	STERILE ALPHA AND TIR MOTIF-CONTAINING PROTEIN 1 (PTHR22998:SF1)	
HUMAN|HGNC = 27962|UniProtKB = Q86WV6	ENSG00000184584	Stimulator of interferon genes protein; TMEM173; ortholog	STIMULATOR OF INTERFERON GENES PROTEIN (PTHR34339:SF1)	

HUMAN|HGNC = 11858|UniProtKB = O43657	ENSG00000000003	Tetraspanin-6; TSPAN6; ortholog	TETRASPANIN-6 (PTHR19282:SF169)	
HUMAN|HGNC = 18157|UniProtKB = Q15750	ENSG00000100324	TGF-beta-activated kinase 1 and MAP3K7-binding protein 1; TAB1; ortholog	TGF-BETA-ACTIVATED KINASE 1 AND MAP3K7-BINDING PROTEIN 1 (PTHR13832:SF533)	Kinase inhibitor(PC00139); protein phosphatase(PC00195)
HUMAN|HGNC = 17075|UniProtKB = Q9NYJ8	ENSG00000055208	TGF-beta-activated kinase 1 and MAP3K7-binding protein 2; TAB2; ortholog	TGF-BETA-ACTIVATED KINASE 1 AND MAP3K7-BINDING PROTEIN 2 (PTHR46253:SF2)	
HUMAN|HGNC = 30681|UniProtKB = Q8N5C8	ENSG00000157625	TGF-beta-activated kinase 1 and MAP3K7-binding protein 3; TAB3; ortholog	TGF-BETA-ACTIVATED KINASE 1 AND MAP3K7-BINDING PROTEIN 3 (PTHR46253:SF3)	
HUMAN|HGNC = 18348|UniProtKB = Q8IUC6	ENSG00000127666	TIR domain-containing adapter molecule 1; TICAM1; ortholog	TIR DOMAIN-CONTAINING ADAPTER MOLECULE 1 (PTHR47230:SF1)	
HUMAN|HGNC = 12033|UniProtKB = Q13114	ENSG00000131323	TNF receptor-associated factor 3; TRAF3; ortholog	TNF RECEPTOR-ASSOCIATED FACTOR 3 (PTHR10131:SF76)	Signaling molecule(PC00207)
HUMAN|HGNC = 16903|UniProtKB = Q15025	ENSG00000145901	TNFAIP3-interacting protein 1; TNIP1; ortholog	TNFAIP3-INTERACTING PROTEIN 1 (PTHR31882:SF3)	

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Table 1 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	**Panther protein class**
HUMAN|HGNC = 19118|UniProtKB = Q8NFZ5	ENSG00000168884	TNFAIP3-interacting protein 2; TNIP2; ortholog	TNFAIP3-INTERACTING PROTEIN 2 (PTHR31882:SF6)	
HUMAN|HGNC = 11850|UniProtKB = O00206	ENSG00000136869	Toll-like receptor 4; TLR4; ortholog	TOLL-LIKE RECEPTOR 4 (PTHR24365:SF521)	
HUMAN|HGNC = 11851|UniProtKB = O60602	ENSG00000187554	Toll-like receptor 5; TLR5; ortholog	TOLL-LIKE RECEPTOR 5 (PTHR24365:SF525)	
HUMAN|HGNC = 11562|UniProtKB = Q92844	ENSG00000136560	TRAF family member-associated NF-kappa-B activator; TANK; ortholog	TRAF FAMILY MEMBER-ASSOCIATED NF-KAPPA-B ACTIVATOR (PTHR15249:SF0)	
HUMAN|HGNC = 9955|UniProtKB = Q04206	ENSG00000173039	Transcription factor p65; RELA; ortholog	TRANSCRIPTION FACTOR P65 (PTHR24169:SF1)	P53-like transcription factor(PC00253); Rel homology transcription factor(PC00252); nucleic acid binding(PC00171)
HUMAN|HGNC = 9956|UniProtKB = Q01201	ENSG00000104856	Transcription factor RelB; RELB; ortholog	TRANSCRIPTION FACTOR RELB (PTHR24169:SF18)	P53-like transcription factor(PC00253); Rel homology transcription factor(PC00252); nucleic acid binding(PC00171)
HUMAN|HGNC = 24552|UniProtKB = Q3LXA3	ENSG00000149476	Triokinase/FMN cyclase; TKFC; ortholog	TRIOKINASE/FMN CYCLASE (PTHR28629:SF4)	
HUMAN|HGNC = 16276|UniProtKB = Q9C035	ENSG00000132256	Tripartite motif-containing protein 5; TRIM5; ortholog	TRIPARTITE MOTIF-CONTAINING PROTEIN 5 (PTHR24103:SF426)	

HUMAN|HGNC = 11896|UniProtKB = P21580	ENSG00000118503	Tumor necrosis factor alpha-induced protein 3; TNFAIP3; ortholog	TUMOR NECROSIS FACTOR ALPHA-INDUCED PROTEIN 3 (PTHR13367:SF3)	DNA-binding protein(PC00009); cysteine protease(PC00081)
HUMAN|HGNC = 4037|UniProtKB = P06241	ENSG00000010810	Tyrosine-protein kinase Fyn; FYN; ortholog	TYROSINE-PROTEIN KINASE FYN (PTHR24418:SF44)	
HUMAN|HGNC = 6735|UniProtKB = P07948	ENSG00000254087	Tyrosine-protein kinase Lyn; LYN; ortholog	TYROSINE-PROTEIN KINASE LYN (PTHR24418:SF42)	
HUMAN|HGNC = 12446|UniProtKB = Q06418	ENSG00000092445	Tyrosine-protein kinase receptor TYRO3; TYRO3; ortholog	TYROSINE-PROTEIN KINASE RECEPTOR TYRO3 (PTHR24416:SF517)	
HUMAN|HGNC = 12508|UniProtKB = Q9UMX0	ENSG00000135018	Ubiquilin-1; UBQLN1; ortholog	UBIQUILIN-1 (PTHR10677:SF16)	
HUMAN|HGNC = 2584|UniProtKB = Q9NQC7	ENSG00000083799	Ubiquitin carboxyl-terminal hydrolase CYLD; CYLD; ortholog	UBIQUITIN CARBOXYL-TERMINAL HYDROLASE CYLD (PTHR11830:SF15)	Cysteine protease(PC00081); ribosomal protein(PC00202)
HUMAN|HGNC = 25118|UniProtKB = Q96BN8	ENSG00000154124	Ubiquitin thioesterase otulin; OTULIN; ortholog	UBIQUITIN THIOESTERASE OTULIN (PTHR33662:SF2)	
HUMAN|HGNC = 10417|UniProtKB = P62979	ENSG00000143947	Ubiquitin-40S ribosomal protein S27a; RPS27A; ortholog	UBIQUITIN-40S RIBOSOMAL PROTEIN S27A (PTHR10666:SF258)	Ribosomal protein(PC00202)

(continued)

Table 1 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	**Panther protein class**
HUMAN|HGNC = 12458|UniProtKB = P62987	ENSG00000221983	Ubiquitin-60S ribosomal protein L40; UBA52; ortholog	UBIQUITIN-60S RIBOSOMAL PROTEIN L40 (PTHR10666:SF268)	Ribosomal protein(PC00202)
HUMAN|HGNC = 12475|UniProtKB = P62837	ENSG00000131508	Ubiquitin-conjugating enzyme E2 D2; UBE2D2; ortholog	UBIQUITIN-CONJUGATING ENZYME E2 D2 (PTHR24068:SF40)	Ligase(PC00142)
HUMAN|HGNC = 12476|UniProtKB = P61077	ENSG00000109332	Ubiquitin-conjugating enzyme E2 D3; UBE2D3; ortholog	UBIQUITIN-CONJUGATING ENZYME E2 D3 (PTHR24068:SF48)	Ligase(PC00142)
HUMAN|HGNC = 12492|UniProtKB = P61088	ENSG00000177889	Ubiquitin-conjugating enzyme E2 N; UBE2N; ortholog	UBIQUITIN-CONJUGATING ENZYME E2 N (PTHR24068:SF152)	
HUMAN|HGNC = 12494|UniProtKB = Q13404	ENSG00000244687	Ubiquitin-conjugating enzyme E2 variant 1; UBE2V1; ortholog	HCG2044781-RELATED (PTHR24068:SF169)	
HUMAN|HGNC = 20451|UniProtKB = Q8IWB7	ENSG00000085449	WD repeat and FYVE domain-containing protein 1; WDFY1; ortholog	WD REPEAT AND FYVE DOMAIN-CONTAINING PROTEIN 1 (PTHR46189:SF2)	

Table 2 List of known molecules involved in antigen processing and presentation expressed by the human BNB transcriptome in health that may be relevant in physiological cross-talk between the systemic immune compartment and peripheral nerve endoneurium and in peripheral nerve autoimmune disorders

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	Panther protein class
HUMAN|HGNC = 9553|UniProtKB = P62333	ENSG00000100519	26S protease regulatory subunit 10B; PSMC6; ortholog	26S PROTEASE REGULATORY SUBUNIT 10B (PTHR23073:SF31)	Hydrolase(PC00121)
HUMAN|HGNC = 9547|UniProtKB = P62191	ENSG00000100764	26S protease regulatory subunit 4; PSMC1; ortholog	26S PROTEASE REGULATORY SUBUNIT 4 (PTHR23073:SF24)	Hydrolase(PC00121)
HUMAN|HGNC = 9549|UniProtKB = P17980	ENSG00000165916	26S protease regulatory subunit 6A; PSMC3; ortholog	26S PROTEASE REGULATORY SUBUNIT 6A (PTHR23073:SF7)	Hydrolase(PC00121)
HUMAN|HGNC = 9551|UniProtKB = P43686	ENSG00000013275	26S protease regulatory subunit 6B; PSMC4; ortholog	26S PROTEASE REGULATORY SUBUNIT 6B (PTHR23073:SF8)	Hydrolase(PC00121)
HUMAN|HGNC = 9548|UniProtKB = P35998	ENSG00000161057	26S protease regulatory subunit 7; PSMC2; ortholog	26S PROTEASE REGULATORY SUBUNIT 7 (PTHR23073:SF13)	Hydrolase(PC00121)
HUMAN|HGNC = 9552|UniProtKB = P62195	ENSG00000087191	26S protease regulatory subunit 8; PSMC5; ortholog	26S PROTEASE REGULATORY SUBUNIT 8 (PTHR23073:SF12)	Hydrolase(PC00121)
HUMAN|HGNC = 9554|UniProtKB = Q99460	ENSG00000173692	26S proteasome non-ATPase regulatory subunit 1; PSMD1; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 1 (PTHR10943:SF2)	Enzyme modulator(PC00095)

(continued)

Table 2 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	Panther protein class
HUMAN|HGNC = 9555|UniProtKB = O75832	ENSG00000101843	26S proteasome non-ATPase regulatory subunit 10; PSMD10; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 10 (PTHR24126:SF24)	
HUMAN|HGNC = 9556|UniProtKB = O00231	ENSG00000108671	26S proteasome non-ATPase regulatory subunit 11; PSMD11; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 11 (PTHR10678:SF2)	
HUMAN|HGNC = 9557|UniProtKB = O00232	ENSG00000197170	26S proteasome non-ATPase regulatory subunit 12; PSMD12; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 12 (PTHR10855:SF1)	Enzyme modulator(PC00095)
HUMAN|HGNC = 9558|UniProtKB = Q9UNM6	ENSG00000185627	26S proteasome non-ATPase regulatory subunit 13; PSMD13; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 13 (PTHR10539:SF0)	Enzyme modulator(PC00095)
HUMAN|HGNC = 16889|UniProtKB = O00487	ENSG00000115233	26S proteasome non-ATPase regulatory subunit 14; PSMD14; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 14 (PTHR10410:SF5)	Metalloprotease(PC00153); transcription factor(PC00218)

HUMAN|HGNC = 9559|UniProtKB = Q13200	ENSG00000175166	26S proteasome non-ATPase regulatory subunit 2; PSMD2; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 2 (PTHR10943:SF1)	Enzyme modulator(PC00095)
HUMAN|HGNC = 9560|UniProtKB = O43242	ENSG00000108344	26S proteasome non-ATPase regulatory subunit 3; PSMD3; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 3 (PTHR10758:SF2)	Enzyme modulator(PC00095)
HUMAN|HGNC = 9561|UniProtKB = P55036	ENSG00000159352	26S proteasome non-ATPase regulatory subunit 4; PSMD4; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 4 (PTHR10223:SF0)	Enzyme modulator(PC00095)
HUMAN|HGNC = 9563|UniProtKB = Q16401	ENSG00000095261	26S proteasome non-ATPase regulatory subunit 5; PSMD5; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 5 (PTHR13554:SF10)	
HUMAN|HGNC = 9564|UniProtKB = Q15008	ENSG00000163636	26S proteasome non-ATPase regulatory subunit 6; PSMD6; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 6 (PTHR14145:SF1)	
HUMAN|HGNC = 9565|UniProtKB = P51665	ENSG00000103035	26S proteasome non-ATPase regulatory subunit 7; PSMD7; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 7 (PTHR10540:SF7)	Metalloprotease(PC00153)

(continued)

Table 2 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	Panther protein class
HUMAN|HGNC = 9566|UniProtKB = P48556	ENSG00000099341	26S proteasome non-ATPase regulatory subunit 8; PSMD8; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 8 (PTHR12387:SF0)	Enzyme modulator(PC00095)
HUMAN|HGNC = 9567|UniProtKB = O00233	ENSG00000110801	26S proteasome non-ATPase regulatory subunit 9; PSMD9; ortholog	26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 9 (PTHR12651:SF1)	Enzyme modulator(PC00095)
HUMAN|HGNC = 652|UniProtKB = P84077	ENSG00000143761	ADP-ribosylation factor 1; ARF1; ortholog	ADP-RIBOSYLATION FACTOR 1 (PTHR11711:SF308)	
HUMAN|HGNC = 167|UniProtKB = P61163	ENSG00000138107	Alpha-centractin; ACTR1A; ortholog	ALPHA-CENTRACTIN (PTHR11937:SF370)	Actin and actin-related protein(PC00039)
HUMAN|HGNC = 2707|UniProtKB = P12821	ENSG00000159640	Angiotensin-converting enzyme; ACE; ortholog	ANGIOTENSIN-CONVERTING ENZYME (PTHR10514:SF25)	Metalloprotease(PC00153)
HUMAN|HGNC = 43|UniProtKB = Q03518	ENSG00000168394	Antigen peptide transporter 1; TAP1; ortholog	ANTIGEN PEPTIDE TRANSPORTER 1 (PTHR24221:SF249)	Cysteine protease(PC00081); serine protease(PC00203)
HUMAN|HGNC = 44|UniProtKB = Q03519	ENSG00000204267	Antigen peptide transporter 2; TAP2; ortholog	ANTIGEN PEPTIDE TRANSPORTER 2 (PTHR24221:SF428)	Cysteine protease(PC00081); serine protease(PC00203)
HUMAN|HGNC = 554|UniProtKB = Q10567	ENSG00000100280	AP-1 complex subunit beta-1; AP1B1; ortholog	AP-1 COMPLEX SUBUNIT BETA-1 (PTHR11134:SF3)	Membrane traffic protein(PC00150)
HUMAN|HGNC = 555|UniProtKB = O43747	ENSG00000166747	AP-1 complex subunit gamma-1; AP1G1; ortholog	AP-1 COMPLEX SUBUNIT GAMMA-1 (PTHR22780:SF26)	Transmembrane receptor regulatory/adaptor protein(PC00226)

HUMAN|HGNC = 13667|UniProtKB = Q9BXS5	ENSG00000072958	AP-1 complex subunit mu-1; AP1M1; ortholog	AP-1 COMPLEX SUBUNIT MU-1 (PTHR10529:SF257)	Extracellular matrix glycoprotein(PC00100); receptor(PC00197)
HUMAN|HGNC = 560|UniProtKB = P56377	ENSG00000182287	AP-1 complex subunit sigma-2; AP1S2; ortholog	AP-1 COMPLEX SUBUNIT SIGMA-2 (PTHR11753:SF19)	Vesicle coat protein(PC00235)
HUMAN|HGNC = 18971|UniProtKB = Q96PC3	ENSG00000152056	AP-1 complex subunit sigma-3; AP1S3; ortholog	AP-1 COMPLEX SUBUNIT SIGMA-3 (PTHR11753:SF18)	Vesicle coat protein(PC00235)
HUMAN|HGNC = 561|UniProtKB = O95782	ENSG00000196961	AP-2 complex subunit alpha-1; AP2A1; ortholog	AP-2 COMPLEX SUBUNIT ALPHA-1 (PTHR22780:SF4)	Transmembrane receptor regulatory/adaptor protein(PC00226)
HUMAN|HGNC = 562|UniProtKB = O94973	ENSG00000183020	AP-2 complex subunit alpha-2; AP2A2; ortholog	AP-2 COMPLEX SUBUNIT ALPHA-2 (PTHR22780:SF30)	Transmembrane receptor regulatory/adaptor protein(PC00226)
HUMAN|HGNC = 563|UniProtKB = P63010	ENSG00000006125	AP-2 complex subunit beta; AP2B1; ortholog	AP-2 COMPLEX SUBUNIT BETA (PTHR11134:SF9)	Membrane traffic protein(PC00150)
HUMAN|HGNC = 564|UniProtKB = Q96CW1	ENSG00000161203	AP-2 complex subunit mu; AP2M1; ortholog	AP-2 COMPLEX SUBUNIT MU (PTHR10529:SF236)	Extracellular matrix glycoprotein(PC00100); receptor(PC00197)
HUMAN|HGNC = 565|UniProtKB = P53680	ENSG00000042753	AP-2 complex subunit sigma; AP2S1; ortholog	AP-2 COMPLEX SUBUNIT SIGMA (PTHR11753:SF6)	Vesicle coat protein(PC00235)
HUMAN|HGNC = 566|UniProtKB = O00203	ENSG00000132842	AP-3 complex subunit beta-1; AP3B1; ortholog	AP-3 COMPLEX SUBUNIT BETA-1 (PTHR11134:SF10)	Membrane traffic protein(PC00150)

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Table 2 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	Panther protein class
HUMAN|HGNC = 568|UniProtKB = O14617	ENSG00000065000	AP-3 complex subunit delta-1; AP3D1; ortholog	AP-3 COMPLEX SUBUNIT DELTA-1 (PTHR22781:SF12)	Transporter(PC00227)
HUMAN|HGNC = 16608|UniProtKB = Q9ULZ3	ENSG00000103490	Apoptosis-associated speck-like protein containing a CARD; PYCARD; ortholog	APOPTOSIS-ASSOCIATED SPECK-LIKE PROTEIN CONTAINING A CARD (PTHR10454:SF203)	Cysteine protease(PC00081); protease inhibitor(PC00191)
HUMAN|HGNC = 50|UniProtKB = Q9NP78	ENSG00000150967	ATP-binding cassette subfamily B member 9; ABCB9; ortholog	ATP-BINDING CASSETTE SUB-FAMILY B MEMBER 9 (PTHR24221:SF242)	Cysteine protease(PC00081); serine protease(PC00203)
HUMAN|HGNC = 16695|UniProtKB = P51572	ENSG00000185825	B-cell receptor-associated protein 31; BCAP31; ortholog	B-CELL RECEPTOR-ASSOCIATED PROTEIN 31 (PTHR12701:SF15)	Membrane traffic protein(PC00150); transporter(PC00227)
HUMAN|HGNC = 914|UniProtKB = P61769	ENSG00000166710	Beta-2-microglobulin; B2M; ortholog	BETA-2-MICROGLOBULIN (PTHR19944:SF62)	Major histocompatibility complex antigen(PC00149)
HUMAN|HGNC = 168|UniProtKB = P42025	ENSG00000115073	Beta-centractin; ACTR1B; ortholog	BETA-CENTRACTIN (PTHR11937:SF195)	Actin and actin-related protein(PC00039)
HUMAN|HGNC = 1473|UniProtKB = P27824	ENSG00000127022	Calnexin; CANX; ortholog	CALNEXIN (PTHR11073:SF11)	Calcium-binding protein(PC00060); chaperone(PC00072)
HUMAN|HGNC = 1455|UniProtKB = P27797	ENSG00000179218	Calreticulin; CALR; ortholog	CALRETICULIN (PTHR11073:SF16)	Calcium-binding protein(PC00060)
HUMAN|HGNC = 2529|UniProtKB = P07339	ENSG00000117984	Cathepsin D; CTSD; ortholog	CATHEPSIN D (PTHR13683:SF230)	Aspartic protease(PC00053)

HUMAN|HGNC = 2531|UniProtKB = Q9UBX1	ENSG00000174080	Cathepsin F; CTSF; ortholog	CATHEPSIN F (PTHR12411:SF444)	Cysteine protease(PC00081); protease inhibitor(PC00191)
HUMAN|HGNC = 2537|UniProtKB = P07711	ENSG00000135047	Cathepsin L1; CTSL; ortholog	CATHEPSIN L1 (PTHR12411:SF411)	Cysteine protease(PC00081); protease inhibitor(PC00191)
HUMAN|HGNC = 2545|UniProtKB = P25774	ENSG00000163131	Cathepsin S; CTSS; ortholog	CATHEPSIN S (PTHR12411:SF525)	Cysteine protease(PC00081); protease inhibitor(PC00191)
HUMAN|HGNC = 2092|UniProtKB = Q00610	ENSG00000141367	Clathrin heavy chain 1; CLTC; ortholog	CLATHRIN HEAVY CHAIN 1 (PTHR10292:SF7)	Vesicle coat protein(PC00235)
HUMAN|HGNC = 2090|UniProtKB = P09496	ENSG00000122705	Clathrin light chain A; CLTA; ortholog	CLATHRIN LIGHT CHAIN A (PTHR10639:SF1)	Vesicle coat protein(PC00235)
HUMAN|HGNC = 2577|UniProtKB = P13498	ENSG00000051523	Cytochrome b-245 light chain; CYBA; ortholog	CYTOCHROME B-245 LIGHT CHAIN (PTHR15168:SF0)	
HUMAN|HGNC = 2961|UniProtKB = Q14204	ENSG00000197102	Cytoplasmic dynein 1 heavy chain 1; DYNC1H1; ortholog	CYTOPLASMIC DYNEIN 1 HEAVY CHAIN 1 (PTHR10676:SF314)	Hydrolase(PC00121); microtubule-binding motor protein(PC00156)
HUMAN|HGNC = 2963|UniProtKB = O14576	ENSG00000158560	Cytoplasmic dynein 1 intermediate chain 1; DYNC1I1; ortholog	CYTOPLASMIC DYNEIN 1 INTERMEDIATE CHAIN 1 (PTHR12442:SF34)	Microtubule family cytoskeletal protein(PC00157)
HUMAN|HGNC = 2964|UniProtKB = Q13409	ENSG00000077380	Cytoplasmic dynein 1 intermediate chain 2; DYNC1I2; ortholog	CYTOPLASMIC DYNEIN 1 INTERMEDIATE CHAIN 2 (PTHR12442:SF37)	Microtubule family cytoskeletal protein(PC00157)

(continued)

Table 2 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	Panther protein class
HUMAN|HGNC = 2966|UniProtKB = O43237	ENSG00000135720	Cytoplasmic dynein 1 light intermediate chain 2; DYNC1LI2; ortholog	CYTOPLASMIC DYNEIN 1 LIGHT INTERMEDIATE CHAIN 2 (PTHR12688:SF1)	Enzyme modulator(PC00095); microtubule family cytoskeletal protein(PC00157)
HUMAN|HGNC = 2962|UniProtKB = Q8NCM8	ENSG00000187240	Cytoplasmic dynein 2 heavy chain 1; DYNC2H1; ortholog	CYTOPLASMIC DYNEIN 2 HEAVY CHAIN 1 (PTHR10676:SF352)	Hydrolase(PC00121); microtubule-binding motor protein(PC00156)
HUMAN|HGNC = 24595|UniProtKB = Q8TCX1	ENSG00000138036	Cytoplasmic dynein 2 light intermediate chain 1; DYNC2LI1; ortholog	CYTOPLASMIC DYNEIN 2 LIGHT INTERMEDIATE CHAIN 1 (PTHR13236:SF0)	Microtubule family cytoskeletal protein(PC00157)
HUMAN|HGNC = 2711|UniProtKB = Q14203	ENSG00000204843	Dynactin subunit 1; DCTN1; ortholog	DYNACTIN SUBUNIT 1 (PTHR18916:SF40)	Non-motor microtubule-binding protein(PC00166)
HUMAN|HGNC = 2712|UniProtKB = Q13561	ENSG00000175203	Dynactin subunit 2; DCTN2; ortholog	DYNACTIN SUBUNIT 2 (PTHR15346:SF0)	Microtubule-binding motor protein(PC00156)
HUMAN|HGNC = 2713|UniProtKB = O75935	ENSG00000137100	Dynactin subunit 3; DCTN3; ortholog	DYNACTIN SUBUNIT 3 (PTHR28360:SF1)	
HUMAN|HGNC = 15518|UniProtKB = Q9UJW0	ENSG00000132912	Dynactin subunit 4; DCTN4; ortholog	DYNACTIN SUBUNIT 4 (PTHR13034:SF2)	
HUMAN|HGNC = 24594|UniProtKB = Q9BTE1	ENSG00000166847	Dynactin subunit 5; DCTN5; ortholog	DYNACTIN SUBUNIT 5 (PTHR46126:SF1)	
HUMAN|HGNC = 2974|UniProtKB = P50570	ENSG00000079805	Dynamin-2; DNM2; ortholog	DYNAMIN-2 (PTHR11566:SF23)	Hydrolase(PC00121); microtubule family cytoskeletal protein(PC00157); small GTPase(PC00208)

HUMAN|HGNC = 15476|UniProtKB = P63167	ENSG00000088986	Dynein light chain 1, cytoplasmic; DYNLL1; ortholog	DYNEIN LIGHT CHAIN 1, CYTOPLASMIC (PTHR11886:SF52)	Enzyme modulator(PC00095); microtubule family cytoskeletal protein(PC00157)
HUMAN|HGNC = 24596|UniProtKB = Q96FJ2	ENSG00000264364	Dynein light chain 2, cytoplasmic; DYNLL2; ortholog	DYNEIN LIGHT CHAIN 2, CYTOPLASMIC (PTHR11886:SF35)	Enzyme modulator(PC00095); microtubule family cytoskeletal protein(PC00157)
HUMAN|HGNC = 26077|UniProtKB = Q8TCQ1	ENSG00000145416	E3 ubiquitin-protein ligase MARCH1; MARCH1; ortholog	E3 UBIQUITIN-PROTEIN LIGASE MARCH1 (PTHR45981:SF1)	
HUMAN|HGNC = 18173|UniProtKB = Q9NZ08	ENSG00000164307	Endoplasmic reticulum aminopeptidase 1; ERAP1; ortholog	ENDOPLASMIC RETICULUM AMINOPEPTIDASE 1 (PTHR11533:SF156)	Metalloprotease(PC00153)
HUMAN|HGNC = 29499|UniProtKB = Q6P179	ENSG00000164308	Endoplasmic reticulum aminopeptidase 2; ERAP2; ortholog	ENDOPLASMIC RETICULUM AMINOPEPTIDASE 2 (PTHR11533:SF239)	Metalloprotease(PC00153)
HUMAN|HGNC = 5398|UniProtKB = P13284	ENSG00000216490	Gamma-interferon-inducible lysosomal thiol reductase; IFI30; ortholog	GAMMA-INTERFERON-INDUCIBLE LYSOSOMAL THIOL REDUCTASE (PTHR13234:SF8)	Reductase(PC00198)

(continued)

Table 2 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	Panther protein class
HUMAN|HGNC = 10535|UniProtKB = Q9Y6B6	ENSG00000152700	GTP-binding protein SAR1b; SAR1B; ortholog	GTP-BINDING PROTEIN SAR1B (PTHR45684:SF2)	
HUMAN|HGNC = 4886|UniProtKB = Q30201	ENSG00000010704	Hereditary hemochromatosis protein; HFE; ortholog	HEREDITARY HEMOCHROMATOSIS PROTEIN (PTHR16675:SF172)	
HUMAN|HGNC = 3611|UniProtKB = P30273	ENSG00000158869	High-affinity immunoglobulin epsilon receptor subunit gamma; FCER1G; ortholog	HIGH AFFINITY IMMUNOGLOBULIN EPSILON RECEPTOR SUBUNIT GAMMA (PTHR16803:SF0)	
HUMAN|HGNC = 4963|UniProtKB = P30511	ENSG00000204642	HLA class I histocompatibility antigen, alpha chain F; HLA-F; ortholog	HLA CLASS I HISTOCOMPATIBILITY ANTIGEN, ALPHA CHAIN F (PTHR16675:SF187)	
HUMAN|HGNC = 4932|UniProtKB = Q31612	ENSG00000234745	HLA class I histocompatibility antigen, B-73 alpha chain; HLA-B; ortholog	HLA CLASS I HISTOCOMPATIBILITY ANTIGEN, B-73 ALPHA CHAIN (PTHR16675:SF248)	
HUMAN|HGNC = 1697|UniProtKB = P04233	ENSG00000019582	HLA class II histocompatibility antigen gamma chain; CD74; ortholog	HLA CLASS II HISTOCOMPATIBILITY ANTIGEN GAMMA CHAIN (PTHR14093:SF17)	Protease inhibitor(PC00191)

HUMAN|HGNC = 4934|UniProtKB = P28067	ENSG00000204257	HLA class II histocompatibility antigen, DM alpha chain; HLA-DMA; ortholog	HLA CLASS II HISTOCOMPATIBILITY ANTIGEN, DM ALPHA CHAIN (PTHR19944:SF50)	Major histocompatibility complex antigen(PC00149)
HUMAN|HGNC = 4935|UniProtKB = P28068	ENSG00000242574	HLA class II histocompatibility antigen, DM beta chain; HLA-DMB; ortholog	HLA CLASS II HISTOCOMPATIBILITY ANTIGEN, DM BETA CHAIN (PTHR19944:SF65)	Major histocompatibility complex antigen(PC00149)
HUMAN|HGNC = 4938|UniProtKB = P20036	ENSG00000231389	HLA class II histocompatibility antigen, DP alpha 1 chain; HLA-DPA1; ortholog	HLA CLASS II HISTOCOMPATIBILITY ANTIGEN, DP ALPHA 1 CHAIN (PTHR19944:SF64)	Major histocompatibility complex antigen(PC00149)
HUMAN|HGNC = 4940|UniProtKB = P04440	ENSG00000223865	HLA class II histocompatibility antigen, DP beta 1 chain; HLA-DPB1; ortholog	HLA CLASS II HISTOCOMPATIBILITY ANTIGEN, DP BETA 1 CHAIN (PTHR19944:SF46)	Major histocompatibility complex antigen(PC00149)
HUMAN|HGNC = 4942|UniProtKB = P01909	ENSG00000196735	HLA class II histocompatibility antigen, DQ alpha 1 chain; HLA-DQA1; ortholog	HLA CLASS II HISTOCOMPATIBILITY ANTIGEN, DQ ALPHA 1 CHAIN (PTHR19944:SF59)	Major histocompatibility complex antigen(PC00149)

(continued)

Table 2 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	Panther protein class
HUMAN|HGNC = 4947|UniProtKB = P01903	ENSG00000204287	HLA class II histocompatibility antigen, DR alpha chain; HLA-DRA; ortholog	HLA CLASS II HISTOCOMPATIBILITY ANTIGEN, DR ALPHA CHAIN (PTHR19944:SF63)	Major histocompatibility complex antigen(PC00149)
HUMAN|HGNC = 4953|UniProtKB = Q30154	ENSG00000198502	HLA class II histocompatibility antigen, DR beta 5 chain; HLA-DRB5; ortholog	HLA CLASS II HISTOCOMPATIBILITY ANTIGEN, DR BETA 4 CHAIN-RELATED (PTHR19944:SF56)	Major histocompatibility complex antigen(PC00149)
HUMAN|HGNC = 4948|UniProtKB = P01911	ENSG00000196126	HLA class II histocompatibility antigen, DRB1-15 beta chain; HLA-DRB1; ortholog	HLA CLASS II HISTOCOMPATIBILITY ANTIGEN, DR BETA 4 CHAIN-RELATED (PTHR19944:SF56)	Major histocompatibility complex antigen(PC00149)
HUMAN|HGNC = 3621|UniProtKB = P55899	ENSG00000104870	IgG receptor FcRn large subunit p51; FCGRT; ortholog	IGG RECEPTOR FCRN LARGE SUBUNIT P51 (PTHR16675:SF3)	
HUMAN|HGNC = 6150|UniProtKB = P06756	ENSG00000138448	Integrin alpha-V; ITGAV; ortholog	INTEGRIN ALPHA-V (PTHR23220:SF4)	
HUMAN|HGNC = 6160|UniProtKB = P18084	ENSG00000082781	Integrin beta-5; ITGB5; ortholog	INTEGRIN BETA-5 (PTHR10082:SF26)	Cell adhesion molecule(PC00069); extracellular matrix glycoprotein(PC00100); receptor(PC00197)

HUMAN|HGNC = 5344|UniProtKB = P05362	ENSG00000090339	Intercellular adhesion molecule 1; ICAM1; ortholog	INTERCELLULAR ADHESION MOLECULE 1 (PTHR13771:SF9)	
HUMAN|HGNC = 6323|UniProtKB = Q12840	ENSG00000155980	Kinesin heavy chain isoform 5A; KIF5A; ortholog	KINESIN HEAVY CHAIN ISOFORM 5A (PTHR24115:SF317)	Microtubule-binding motor protein(PC00156)
HUMAN|HGNC = 6387|UniProtKB = Q07866	ENSG00000126214	Kinesin light chain 1; KLC1; ortholog	KINESIN LIGHT CHAIN 1 (PTHR45783:SF7)	
HUMAN|HGNC = 20716|UniProtKB = Q9H0B6	ENSG00000174996	Kinesin light chain 2; KLC2; ortholog	KINESIN LIGHT CHAIN 2 (PTHR45783:SF2)	
HUMAN|HGNC = 17060|UniProtKB = Q92845	ENSG00000075945	Kinesin-associated protein 3; KIFAP3; ortholog	KINESIN-ASSOCIATED PROTEIN 3 (PTHR15605:SF2)	Microtubule-binding motor protein(PC00156)
HUMAN|HGNC = 6388|UniProtKB = P52732	ENSG00000138160	Kinesin-like protein KIF11; KIF11; ortholog	KINESIN-LIKE PROTEIN KIF11 (PTHR24115:SF105)	Microtubule-binding motor protein(PC00156)
HUMAN|HGNC = 6391|UniProtKB = Q14807	ENSG00000079616	Kinesin-like protein KIF22; KIF22; ortholog	KINESIN-LIKE PROTEIN KIF22 (PTHR24115:SF462)	Microtubule-binding motor protein(PC00156)
HUMAN|HGNC = 6392|UniProtKB = Q02241	ENSG00000137807	Kinesin-like protein KIF23; KIF23; ortholog	KINESIN-LIKE PROTEIN KIF23 (PTHR24115:SF467)	Microtubule-binding motor protein(PC00156)
HUMAN|HGNC = 20226|UniProtKB = Q9ULI4	ENSG00000066735	Kinesin-like protein KIF26A; KIF26A; ortholog	KINESIN-LIKE PROTEIN KIF26A (PTHR24115:SF407)	Microtubule-binding motor protein(PC00156)

(continued)

Table 2 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	Panther protein class
HUMAN|HGNC = 6318|UniProtKB = O00139	ENSG00000068796	Kinesin-like protein KIF2A; KIF2A; ortholog	KINESIN-LIKE PROTEIN KIF2A (PTHR24115:SF486)	Microtubule-binding motor protein(PC00156)
HUMAN|HGNC = 6319|UniProtKB = Q9Y496	ENSG00000131437	Kinesin-like protein KIF3A; KIF3A; ortholog	KINESIN-LIKE PROTEIN KIF3A (PTHR24115:SF472)	Microtubule-binding motor protein(PC00156)
HUMAN|HGNC = 6320|UniProtKB = O15066	ENSG00000101350	Kinesin-like protein KIF3B; KIF3B; ortholog	KINESIN-LIKE PROTEIN KIF3B (PTHR24115:SF744)	Microtubule-binding motor protein(PC00156)
HUMAN|HGNC = 6321|UniProtKB = O14782	ENSG00000084731	Kinesin-like protein KIF3C; KIF3C; ortholog	KINESIN-LIKE PROTEIN KIF3C (PTHR24115:SF734)	Microtubule-binding motor protein(PC00156)
HUMAN|HGNC = 9472|UniProtKB = Q99538	ENSG00000100600	Legumain; LGMN; ortholog	LEGUMAIN (PTHR12000:SF3)	Cysteine protease(PC00081)
HUMAN|HGNC = 6656|UniProtKB = Q9UIQ6	ENSG00000113441	Leucyl-cystinyl aminopeptidase; LNPEP; ortholog	LEUCYL-CYSTINYL AMINOPEPTIDASE (PTHR11533:SF42)	Metalloprotease(PC00153)
HUMAN|HGNC = 4975|UniProtKB = Q95460	ENSG00000153029	Major histocompatibility complex class I-related gene protein; MR1; ortholog	MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I-RELATED GENE PROTEIN (PTHR16675:SF241)	
HUMAN|HGNC = 40|UniProtKB = P08183	ENSG00000085563	Multidrug resistance protein 1; ABCB1; ortholog	MULTIDRUG RESISTANCE PROTEIN 1 (PTHR24221:SF251)	Cysteine protease(PC00081); serine protease(PC00203)

HUMAN|HGNC = 10907|UniProtKB = P49279	ENSG00000018280	Natural resistance-associated macrophage protein 1; SLC11A1; ortholog	NATURAL RESISTANCE-ASSOCIATED MACROPHAGE PROTEIN 1 (PTHR11706:SF52)	Cation transporter(PC00068)
HUMAN|HGNC = 7660|UniProtKB = P14598	ENSG00000158517	Neutrophil cytosol factor 1; NCF1; ortholog	NEUTROPHIL CYTOSOL FACTOR 1-RELATED (PTHR15706:SF6)	
HUMAN|HGNC = 7661|UniProtKB = P19878	ENSG00000116701	Neutrophil cytosol factor 2; NCF2; ortholog	NEUTROPHIL CYTOSOL FACTOR 2 (PTHR15175:SF3)	Oxidase(PC00175)
HUMAN|HGNC = 16390|UniProtKB = Q9Y239	ENSG00000106100	Nucleotide-binding oligomerization domain-containing protein 1; NOD1; ortholog	NUCLEOTIDE-BINDING OLIGOMERIZATION DOMAIN-CONTAINING PROTEIN 1 (PTHR24106:SF18)	Nucleic acid binding(PC00171); serine protease(PC00203); transcription cofactor(PC00217)
HUMAN|HGNC = 5331|UniProtKB = Q9HC29	ENSG00000167207	Nucleotide-binding oligomerization domain-containing protein 2; NOD2; ortholog	NUCLEOTIDE-BINDING OLIGOMERIZATION DOMAIN-CONTAINING PROTEIN 2 (PTHR24106:SF64)	Nucleic acid binding(PC00171); serine protease(PC00203); transcription cofactor(PC00217)
HUMAN|HGNC = 16398|UniProtKB = Q9BXW6	ENSG00000141447	Oxysterol-binding protein-related protein 1; OSBPL1A; ortholog	OXYSTEROL-BINDING PROTEIN-RELATED PROTEIN 1 (PTHR10972:SF53)	

(continued)

Table 2 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	Panther protein class
HUMAN|HGNC = 1663|UniProtKB = P16671	ENSG00000135218	Platelet glycoprotein 4; CD36; ortholog	PLATELET GLYCOPROTEIN 4 (PTHR11923:SF12)	Receptor(PC00197)
HUMAN|HGNC = 2535|UniProtKB = P09668	ENSG00000103811	Pro-cathepsin H; CTSH; ortholog	PRO-CATHEPSIN H (PTHR12411:SF572)	Cysteine protease(PC00081); protease inhibitor(PC00191)
HUMAN|HGNC = 9568|UniProtKB = Q06323	ENSG00000092010	Proteasome activator complex subunit 1; PSME1; ortholog	PROTEASOME ACTIVATOR COMPLEX SUBUNIT 1 (PTHR10660:SF5)	
HUMAN|HGNC = 9569|UniProtKB = Q9UL46	ENSG00000100911	Proteasome activator complex subunit 2; PSME2; ortholog	PROTEASOME ACTIVATOR COMPLEX SUBUNIT 2 (PTHR10660:SF6)	
HUMAN|HGNC = 9570|UniProtKB = P61289	ENSG00000131467	Proteasome activator complex subunit 3; PSME3; ortholog	PROTEASOME ACTIVATOR COMPLEX SUBUNIT 3 (PTHR10660:SF4)	
HUMAN|HGNC = 20635|UniProtKB = Q14997	ENSG00000068878	Proteasome activator complex subunit 4; PSME4; ortholog	PROTEASOME ACTIVATOR COMPLEX SUBUNIT 4 (PTHR32170:SF3)	
HUMAN|HGNC = 9571|UniProtKB = Q92530	ENSG00000125818	Proteasome inhibitor PI31 subunit; PSMF1; ortholog	PROTEASOME INHIBITOR PI31 SUBUNIT (PTHR13266:SF1)	Protease inhibitor(PC00191)
HUMAN|HGNC = 9530|UniProtKB = P25786	ENSG00000129084	Proteasome subunit alpha type-1; PSMA1; ortholog	PROTEASOME SUBUNIT ALPHA TYPE-RELATED (PTHR11599:SF12)	Protease(PC00190)

HUMAN|HGNC = 9531|UniProtKB = P25787	ENSG00000106588	Proteasome subunit alpha type-2; PSMA2; ortholog	PROTEASOME SUBUNIT ALPHA TYPE-2 (PTHR11599:SF16)	Protease(PC00190)
HUMAN|HGNC = 9532|UniProtKB = P25788	ENSG00000100567	Proteasome subunit alpha type-3; PSMA3; ortholog	PROTEASOME SUBUNIT ALPHA TYPE-3 (PTHR11599:SF10)	Protease(PC00190)
HUMAN|HGNC = 9533|UniProtKB = P25789	ENSG00000041357	Proteasome subunit alpha type-4; PSMA4; ortholog	PROTEASOME SUBUNIT ALPHA TYPE-4 (PTHR11599:SF13)	Protease(PC00190)
HUMAN|HGNC = 9534|UniProtKB = P28066	ENSG00000143106	Proteasome subunit alpha type-5; PSMA5; ortholog	PROTEASOME SUBUNIT ALPHA TYPE-5 (PTHR11599:SF14)	Protease(PC00190)
HUMAN|HGNC = 9535|UniProtKB = P60900	ENSG00000100902	Proteasome subunit alpha type-6; PSMA6; ortholog	PROTEASOME SUBUNIT ALPHA TYPE-6 (PTHR11599:SF11)	Protease(PC00190)
HUMAN|HGNC = 9536|UniProtKB = O14818	ENSG00000101182	Proteasome subunit alpha type-7; PSMA7; ortholog	PROTEASOME SUBUNIT ALPHA TYPE-7 (PTHR11599:SF40)	Protease(PC00190)
HUMAN|HGNC = 9537|UniProtKB = P20618	ENSG00000008018	Proteasome subunit beta type-1; PSMB1; ortholog	PROTEASOME SUBUNIT BETA TYPE-1 (PTHR11599:SF59)	Protease(PC00190)
HUMAN|HGNC = 9538|UniProtKB = P40306	ENSG00000205220	Proteasome subunit beta type-10; PSMB10; ortholog	PROTEASOME SUBUNIT BETA TYPE-10 (PTHR11599:SF41)	Protease(PC00190)
HUMAN|HGNC = 9539|UniProtKB = P49721	ENSG00000126067	Proteasome subunit beta type-2; PSMB2; ortholog	PROTEASOME SUBUNIT BETA TYPE-2 (PTHR11599:SF6)	Protease(PC00190)

(continued)

Table 2 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	Panther protein class
HUMAN|HGNC = 9540|UniProtKB = P49720	ENSG00000277791	Proteasome subunit beta type-3; PSMB3; ortholog	PROTEASOME SUBUNIT BETA TYPE-3 (PTHR11599:SF62)	Protease(PC00190)
HUMAN|HGNC = 9541|UniProtKB = P28070	ENSG00000159377	Proteasome subunit beta type-4; PSMB4; ortholog	PROTEASOME SUBUNIT BETA TYPE-4 (PTHR11599:SF5)	Protease(PC00190)
HUMAN|HGNC = 9543|UniProtKB = P28072	ENSG00000142507	Proteasome subunit beta type-6; PSMB6; ortholog	PROTEASOME SUBUNIT BETA TYPE-6 (PTHR11599:SF46)	Protease(PC00190)
HUMAN|HGNC = 9544|UniProtKB = Q99436	ENSG00000136930	Proteasome subunit beta type-7; PSMB7; ortholog	PROTEASOME SUBUNIT BETA TYPE-7 (PTHR11599:SF42)	Protease(PC00190)
HUMAN|HGNC = 9545|UniProtKB = P28062	ENSG00000204264	Proteasome subunit beta type-8; PSMB8; ortholog	PROTEASOME SUBUNIT BETA TYPE-8 (PTHR11599:SF53)	Protease(PC00190)
HUMAN|HGNC = 9546|UniProtKB = P28065	ENSG00000240065	Proteasome subunit beta type-9; PSMB9; ortholog	PROTEASOME SUBUNIT BETA TYPE-9 (PTHR11599:SF50)	Protease(PC00190)
HUMAN|HGNC = 4606|UniProtKB = P30101	ENSG00000167004	Protein disulfide-isomerase A3; PDIA3; ortholog	PROTEIN DISULFIDE-ISOMERASE A3 (PTHR18929:SF191)	
HUMAN|HGNC = 10697|UniProtKB = P55735	ENSG00000157020	Protein SEC13 homolog; SEC13; ortholog	PROTEIN SEC13 HOMOLOG (PTHR11024:SF2)	Membrane trafficking regulatory protein(PC00151)
HUMAN|HGNC = 10701|UniProtKB = Q15436	ENSG00000100934	Protein transport protein Sec23A; SEC23A; ortholog	PROTEIN TRANSPORT PROTEIN SEC23A (PTHR11141:SF7)	G-protein modulator(PC00022)

HUMAN|HGNC = 10703|UniProtKB = O95486	ENSG00000113615	Protein transport protein Sec24A; SEC24A; ortholog	PROTEIN TRANSPORT PROTEIN SEC24A (PTHR13803:SF1)	Vesicle coat protein(PC00235)
HUMAN|HGNC = 10704|UniProtKB = O95487	ENSG00000138802	Protein transport protein Sec24B; SEC24B; ortholog	PROTEIN TRANSPORT PROTEIN SEC24B (PTHR13803:SF4)	Vesicle coat protein(PC00235)
HUMAN|HGNC = 10705|UniProtKB = P53992	ENSG00000176986	Protein transport protein Sec24C; SEC24C; ortholog	PROTEIN TRANSPORT PROTEIN SEC24C (PTHR13803:SF5)	Vesicle coat protein(PC00235)
HUMAN|HGNC = 10706|UniProtKB = O94855	ENSG00000150961	Protein transport protein Sec24D; SEC24D; ortholog	PROTEIN TRANSPORT PROTEIN SEC24D (PTHR13803:SF6)	Vesicle coat protein(PC00235)
HUMAN|HGNC = 17052|UniProtKB = O94979	ENSG00000138674	Protein transport protein Sec31A; SEC31A; ortholog	PROTEIN TRANSPORT PROTEIN SEC31A (PTHR13923:SF23)	Vesicle coat protein(PC00235)
HUMAN|HGNC = 30266|UniProtKB = Q96NA2	ENSG00000167705	Rab-interacting lysosomal protein; RILP; ortholog	RAB-INTERACTING LYSOSOMAL PROTEIN (PTHR21502:SF7)	Nucleic acid binding(PC00171)
HUMAN|HGNC = 30278|UniProtKB = Q14699	ENSG00000131378	Raftlin; RFTN1; ortholog	RAFTLIN (PTHR17601:SF3)	
HUMAN|HGNC = 9759|UniProtKB = P61026	ENSG00000084733	Ras-related protein Rab-10; RAB10; ortholog	RAS-RELATED PROTEIN RAB-10 (PTHR24073:SF483)	
HUMAN|HGNC = 9766|UniProtKB = P51159	ENSG00000069974	Ras-related protein Rab-27A; RAB27A; ortholog	RAS-RELATED PROTEIN RAB-27A (PTHR24073:SF511)	

(continued)

Table 2 (continued)

Gene ID	Mapped IDs	Gene name/gene symbol	Panther family/subfamily	Panther protein class
HUMAN|HGNC = 9772|UniProtKB = Q13637	ENSG00000118508	Ras-related protein Rab-32; RAB32; ortholog	RAS-RELATED PROTEIN RAB-32 (PTHR24073:SF862)	
HUMAN|HGNC = 16519|UniProtKB = Q9BZG1	ENSG00000109113	Ras-related protein Rab-34; RAB34; ortholog	RAS-RELATED PROTEIN RAB-34 (PTHR24073:SF468)	
HUMAN|HGNC = 9774|UniProtKB = Q15286	ENSG00000111737	Ras-related protein Rab-35; RAB35; ortholog	RAS-RELATED PROTEIN RAB-35 (PTHR24073:SF933)	
HUMAN|HGNC = 9778|UniProtKB = P20337	ENSG00000169213	Ras-related protein Rab-3B; RAB3B; ortholog	RAS-RELATED PROTEIN RAB-3B (PTHR24073:SF396)	
HUMAN|HGNC = 9781|UniProtKB = P20338	ENSG00000168118	Ras-related protein Rab-4A; RAB4A; ortholog	RAS-RELATED PROTEIN RAB-4A (PTHR24073:SF450)	
HUMAN|HGNC = 9784|UniProtKB = P61020	ENSG00000111540	Ras-related protein Rab-5B; RAB5B; ortholog	RAS-RELATED PROTEIN RAB-5B (PTHR24073:SF555)	
HUMAN|HGNC = 9786|UniProtKB = P20340	ENSG00000175582	Ras-related protein Rab-6A; RAB6A; ortholog	RAS-RELATED PROTEIN RAB-6A (PTHR24073:SF421)	
HUMAN|HGNC = 9788|UniProtKB = P51149	ENSG00000075785	Ras-related protein Rab-7a; RAB7A; ortholog	RAS-RELATED PROTEIN RAB-7A (PTHR24073:SF556)	
HUMAN|HGNC = 30273|UniProtKB = Q92930	ENSG00000166128	Ras-related protein Rab-8B; RAB8B; ortholog	RAS-RELATED PROTEIN RAB-8B (PTHR24073:SF22)	

HUMAN|HGNC = 11276|UniProtKB = O15020	ENSG00000173898	Spectrin beta chain, non-erythrocytic 2; SPTBN2; ortholog	SPECTRIN BETA CHAIN, NON-ERYTHROCYTIC 2 (PTHR11915:SF325)	
HUMAN|HGNC = 11566|UniProtKB = O15533	ENSG00000231925	Tapasin; TAPBP; ortholog	TAPASIN (PTHR23411:SF5)	Immunoglobulin receptor superfamily(PC00124)
HUMAN|HGNC = 30683|UniProtKB = Q9BX59	ENSG00000139192	Tapasin-related protein; TAPBPL; ortholog	TAPASIN-RELATED PROTEIN (PTHR23411:SF7)	Immunoglobulin receptor superfamily(PC00124)
HUMAN|HGNC = 11785|UniProtKB = P07996	ENSG00000137801	Thrombospondin-1; THBS1; ortholog	THROMBOSPONDIN-1 (PTHR10199:SF78)	
HUMAN|HGNC = 9956|UniProtKB = Q01201	ENSG00000104856	Transcription factor RelB; RELB; ortholog	TRANSCRIPTION FACTOR RELB (PTHR24169:SF18)	P53-like transcription factor(PC00253); Rel homology transcription factor(PC00252); nucleic acid binding(PC00171)
HUMAN|HGNC = 12731|UniProtKB = P42768	ENSG00000015285	Wiskott-Aldrich syndrome protein; WAS; ortholog	WISKOTT-ALDRICH SYNDROME PROTEIN (PTHR23202:SF35)	Actin family cytoskeletal protein(PC00041)
HUMAN|HGNC = 910|UniProtKB = P25311	ENSG00000160862	Zinc-alpha-2-glycoprotein; AZGP1; ortholog	ZINC-ALPHA-2-GLYCOPROTEIN (PTHR16675:SF198)	

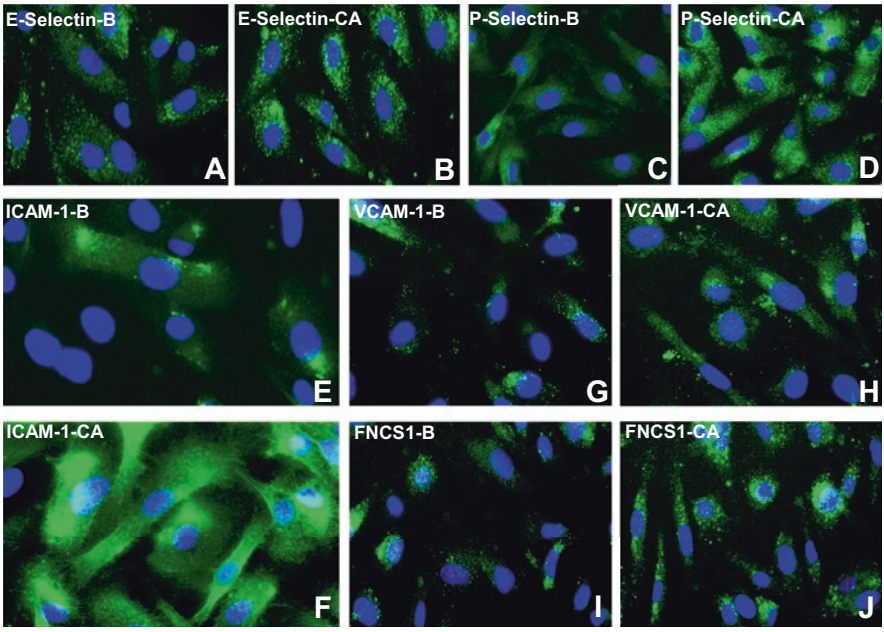

Fig. 4 Composite indirect fluorescent digital photomicrographs showing cellular adhesion molecule expression by confluent primary human endoneurial endothelial cells under basal and physiological cytokine-activated states in vitro (B indicates expression under basal culture conditions; CA indicates expression following cytokine activation with 10 U/mL TNF-α and 20 U/mL IFN-γ for 24 hours) A, C, E, G and I indicate cellular adhesion molecule expression under basal cultures conditions, while B, D, F, H and J indicate upregulated expression following physiological cytokine stimulus in vitro

Structural and Functional Changes at the BNB Associated with Autoimmune Disorders

Increased permeability of or leukocyte trafficking at the human BNB, commonly cited as "BNB breakdown," has been pathologically associated with peripheral nerve autoimmune disorders, with a paper reporting downregulation of BNB tight junction protein claudin-5 and translocation of ZO-1 by immunohistochemistry in sural nerve biopsies of patients with chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), without change in occludin expression [21]. It is important to recognize that claudin-5 was also expressed on epineurial macrovessels that do not form the restrictive tight junctions [21], as well as immature endoneurial microvessels during development [23], calling to question its role in mediating restrictive junction barrier function in human peripheral nerves. Importantly, this commonly held viewpoint implies that the human BNB is relatively passive during autoimmune disorders affecting peripheral nerves.

Recent data demonstrating the complexity of the restrictive junction components and possible redundancy of tight junction-forming molecules involved in the human BNB [22] suggest that downregulation of a single tight junction-forming molecule or reduction in TEER or increase in solute permeability demonstrated in vitro following administration of sera from GBS or CIDP patients [56–58] may be an insufficient structural or functional change at the human BNB in vivo during autoimmune disorders. In support of this, physiological cytokine stimulus of confluent primary human endoneurial endothelial cells grown on transwell inserts with TNF-α and IFN-γ over a 100-fold range did not alter TEER in vitro [55]. Ultrastructural examination of endoneurial microvessels within the inflammatory milieu from patients with GBS and CIDP demonstrates intact electron-dense intercellular tight junctions, with similar electron-dense contacts between infiltrating leukocytes and endothelial cells (Fig. 5) [59, 60]. These observations should provide the impetus for further studies to better understand biologically relevant structural and functional alterations at the human BNB during peripheral nerve autoimmune disorders relative to healthy nerves.

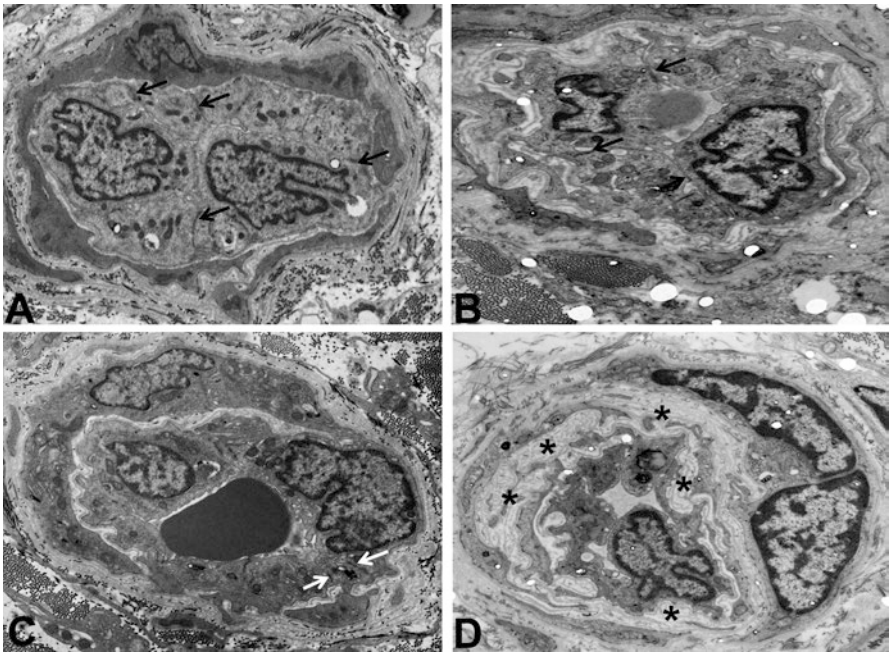

Fig. 5 Composite digital electron ultramicrographs demonstrating intact electron-dense intercellular tight junctions (solid black arrows) between endoneurial endothelial cells within the inflammatory milieu in a GBS (**a**) and CIDP (**b**) patient sural nerve biopsy, with electron-dense intercellular contacts observed between infiltrating leukocytes and endothelial cells (**c**, white arrows) and endoneurial microvessel basement membrane duplication (**d**, black asterisk)

Endoneurial microvessel basement membrane thickening/duplication (Fig. 5d) has been described in association with CIDP and peripheral nerve vasculitis (which typically affects epineurial arteries or arterioles and rarely involves endoneurial capillary-like vessels with resultant endoneurial ischemia) [60–62]. The functional implications of the basement membrane alterations are undetermined; however, this may reflect an adaptive or maladaptive response to chronic and persistent endothelial cell/pericyte pro-inflammatory cytokine exposure or hypoxia/ischemia as a compensatory or reactive means of maintaining BNB functional integrity.

BNB Endothelial-Leukocyte Interactions in Immune-Mediated Neuropathies

While it is unresolved whether systemic immune system activation (e.g., by infections, minor surgery or trauma) with primary attack of peripheral nerves and nerve roots (through the process of "molecular mimicry") [63, 64] or endogenous activation of the innate immune system in peripheral nerves (e.g., by viruses) [65] with secondary selective adaptive immune system activation in genetically susceptible individuals is responsible for tissue-specific autoimmunity, or whether suspected circulating polyclonal anti-myelin protein, anti-axonal nodal protein, and anti-ganglioside or anti-glycolipid autoantibodies can cross the human BNB in vivo, a pathologic hallmark of autoimmune neuropathies is the infiltration of subpopulations of hematogenous leukocytes in peripheral nerves and nerve roots, commonly demonstrated in situ on patient nerve biopsies [61].

In GBS and CIDP, leukocyte infiltration is associated with demyelination, axonal degeneration, or both. In peripheral nerve vasculitis, leukocyte infiltration is associated with vascular wall infiltration, transmural vasonecrosis, and endoneurial ischemia. In HIV-associated distal sensory polyneuropathy (DSP), although not considered an autoimmune neuropathy, clusters of leukocytes are also seen within the endoneurium, associated with axonal loss. Since endoneurial microvessels that form the BNB provide the main route of entry for hematogenous leukocytes from circulation into the endoneurium, leukocyte-endothelial cell interactions are important in the pathogenesis of peripheral nerve autoimmune disorders. In support of this, hematogenous leukocytes interacting with the endoneurial microvessels that form the BNB have been observed in untreated patients with GBS, CIDP, and HIV-DSP in situ (Fig. 6).

Using a flow-dependent leukocyte-BNB trafficking model in vitro, untreated GBS, CIDP, and HIV-DSP patient peripheral blood mononuclear leukocytes (PBMLs) firmly adhere to the surface of confluent primary endoneurial endothelial cells and undergo paracellular transmigration at higher rates that normal healthy donor PBMLs in vitro [53, 55], supporting the notion that leukocyte trafficking at the BNB is pathogenically relevant to autoimmune peripheral neuropathies and potentially HIV-DSP.

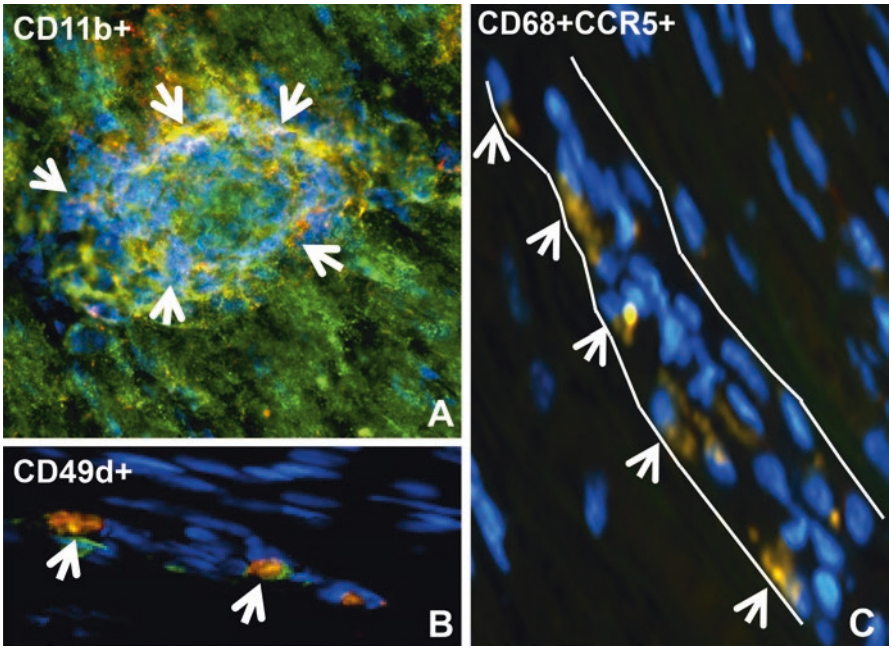

Fig. 6 Composite digital indirect fluorescent photomicrographs showing interaction between hematogenous leukocytes and endoneurial microvessels in GBS (**a**; CD11b+), CIDP (**b**; CD49d+), and HIV-DSP (**c**; CD68+ CCR5+) patient sural nerve biopsies (yellow cells shown with white arrows). S100β+ myelinating Schwann cells associated with axons (green) are also depicted in **a**. The outline of an endoneurial microvessel in longitudinal section is shown with the white lines in **c**

Subpopulation Leukocyte Infiltration in Immune-Mediated Neuropathies

The major challenges in definitively ascertaining the phenotypic characteristics of infiltrating leukocytes in autoimmune neuropathies include disease heterogeneity, the scarcity of pathologic patient biopsies for large-scale comparative analyses, the frequent analysis of sural nerves that may be partially involved in the disease process but practically safer to biopsy in patients rather than clinically and electrophysiologically affected motor nerves, the paucity or multifocal nature of inflammatory infiltrates reducing the likelihood of detecting pathogenic leukocytes in small specimens, and the selection and ascertainment biases intrinsic to immunohistochemistry studies.

The expression of HLA class II molecules, interleukin 1-beta (IL-1β), IFN-γ, TNF-α, CCL2, CXCL10, and ICAM-1 on endoneurial endothelial cells has been described in peripheral nerve biopsies of GBS patients. Similarly, HLA-DR, interleukin-2 (IL-2), IFN-γ, TNF-α, CXCL10, and ICAM-1 have also been expressed at the human BNB in situ in CIDP patient nerve biopsies at higher levels compared to

control nerves, supporting the notion that local activation of the adaptive immune response at the BNB may be pathogenically significant in GBS and CIDP [66–76]. In a single study, chemokine receptors CCR1 and CCR5 were demonstrated on endoneurial macrophages with CCR2, CCR4, and CXCR3 expressed on infiltrating T lymphocytes in GBS and CIDP patient sural nerve biopsies [76]. Another study demonstrated increased numbers of CCR2+ mononuclear cells in GBS patient nerve biopsies [69].

Guided by in vitro observations implying a role for leukocyte integrin CD11b (also known as α_M-integrin or Mac-1)-ICAM-1 interactions in mediating pathogenic leukocyte trafficking at the human BNB under hydrodynamic forces mimicking in vivo capillary flow rates [55], expression of clusters of infiltrated CD11b+ leukocytes interacting with endoneurial endothelial cells that accumulate within untreated GBS patient sural nerve biopsy endoneurium has been shown (Fig. 7) [59]. Similarly, CD49d+ (also known as α_4-integrin or very late antigen-4) mononuclear leukocytes in CIDP patient sural nerve biopsy endoneurium [53] and CCR5+ and CD11d+ (also known as α_D-integrin) mononuclear leukocytes in untreated HIV-DSP patient sural nerve biopsies have been demonstrated (Fig. 6), consistent with a prior report indicating a predominance of CCR5-dependent and macrophage tropic HIV-1 virus based on sequence analysis and evaluation of infectious recombinant viruses containing peripheral nerve-derived C2V3 sequences in autopsied sural and peroneal nerves in decedent HIV+ individuals [77].

Peripheral nerve vasculitis is typically associated with leukocyte infiltration of epineurial macrovascular endothelium walls, rather than direct involvement with endoneurial microvessels that form the BNB. However, strong expression of HLA class I and class II molecules on affected vascular endothelial cells has been described, typically associated with prominent CD4+ and fewer CD8+ T lymphocytes and CD68+ macrophages. CD22+ B lymphocytes and CD16+ natural killer cells are less commonly observed in vasculitic neuropathy than T lymphocytes and macrophages. T lymphocyte infiltrates in vasculitic neuropathy are heterogeneous based on T-cell receptor Vβ utilization, similar to descriptions in CIDP, supporting the polyclonal nature of these conditions [74, 75, 78–81].

Expression of CD58 (also known as lymphocyte function-associated antigen-3; a cell adhesion molecule typically expressed on antigen-presenting cells such as macrophages and binds to CD2 on T lymphocytes) and CD86 (a protein expressed on antigen-presenting cells that provides costimulatory signals necessary for T-cell activation and survival) on affected vascular endothelial cells have also been described, with the former also expressed by Schwann cells [75]. Variable focal expression of hypoxia-inducing factors (HIFs), HIF-1α, HIF-1β, and HIF-2α, as well as VEGF, VEGFR, and erythropoietin receptor was seen on endoneurial microvessels in a small percentage of nerve biopsies from patients with vasculitic neuropathy at higher rates than control sural nerve biopsies [82, 83].

Recent work elucidating the normal adult BNB transcriptome provides molecular targets putatively involved in cross-talk between the innate (Table 1) and adaptive (Table 2) immune responses in peripheral nerves. Validation of these proposed molecules and their associated signaling networks, as well as future single cell tran-

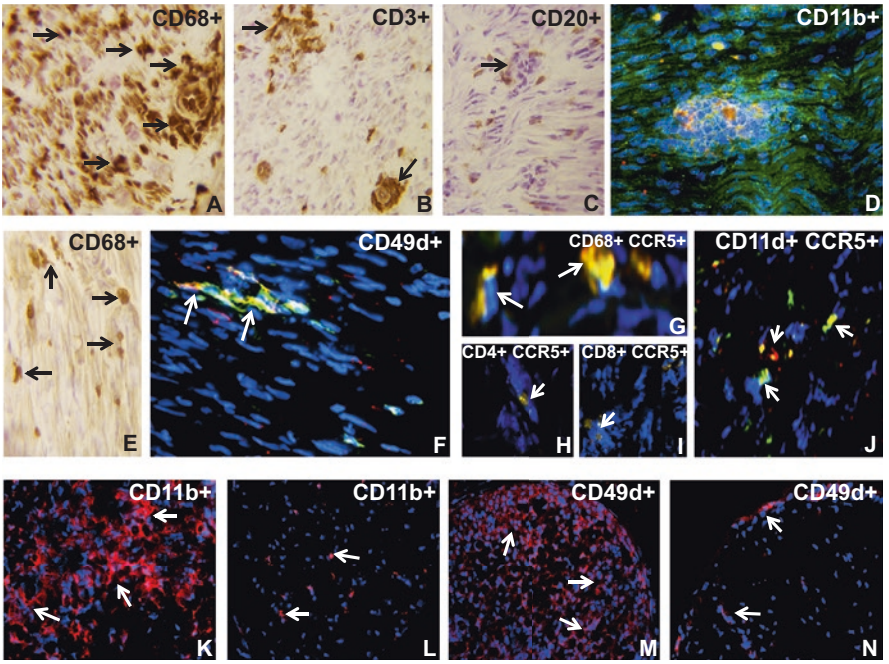

Fig. 7 Composite digital indirect fluorescent photomicrographs depicting subpopulations of hematogenous leukocytes that have infiltrated into sural nerve endoneurium in untreated GBS (**a**–**d**)-, CIDP (**e**, **f**)-, and HIV-DSP (**g**–**j**)-affected patients, the sciatic nerves of representative murine GBS (**k**, **l**) and CIDP (**m**, **n**) animal models, and the effect of targeted molecular inhibition in the mouse models. Clusters of infiltrated monocytes/macrophages (**a**), T lymphocytes (**b**), B lymphocytes (**c**), and CD11b+ leukocytes in a region of demyelination (**d**; green depicts S100β+ myelinating Schwann cells associated with axons) are shown in GBS patients, and clusters of infiltrated monocytes/macrophages (**e**) and CD49d+ leukocytes (**f**) are shown in CIDP patients, with CCR5+ monocytes/macrophages (**g**), CD4+ T lymphocytes (**h**), CD8+ T lymphocytes (**i**), and CD11d+ leukocytes (**j**) shown in HIV-DSP patients. The sciatic nerve of an untreated severe EAN-affected mouse shows intense endoneurial infiltrates of CD11b+ leukocytes (**k**) with a significant reduction in infiltrates seen in another mouse treated with a function-neutralizing rat anti-mouse CD11b monoclonal antibody (**l**). The sciatic nerve of an untreated SAPP-affected mouse shows intense CD45+ leukocyte infiltrates (**m**) that are significantly reduced in another mouse treated with a fibronectin-connecting segment 1 peptide (**n**) early in the disease course. Examples of infiltrated leukocytes are depicted with either black or white arrows in the photomicrographs

scriptomics and proteomics studies, could provide avenues to more comprehensively elucidate molecular changes at the human BNB in situ and characterize the different infiltrated leukocyte subpopulations associated with specific peripheral nerve autoimmune disorders required to better understand the pathogenesis of these conditions and also understand how HIV-infected leukocytes could gain access into peripheral nerves. The ultimate goal is to devise targeted efficacious molecular therapies for autoimmune neuropathies and prevent the development of consequential chronic neuropathic pain.

Animal Models and Targeted Inhibition of Pathogenic Leukocyte Trafficking

Despite the limitations of autoimmune neuropathy animal models and species differences in BNB function and the inflammatory cascade [84, 85], experimental observations made in representative animal models guided by data derived from human in situ leukocyte-BNB interactions in autoimmune neuropathies could provide further insights into the pathogenesis of these disorders and the adaptive or pathological changes that occur at the BNB during autoimmunity. Animal models could also aid dissect the mechanisms by which the systemic immune system engages with peripheral nerves and nerve roots during normal physiologic states and the earliest signaling pathways associated with tissue-specific autoimmune disorders.

Experimental autoimmune neuritis (EAN, an established model of GBS) in the Lewis rat implicated important roles of CD11a (also known as α_L-integrin or lymphocyte function-associated antigen-1) in disease induction [86] and CCL3 and partially CCL2 in pathogenic leukocyte trafficking [87]. Pharmacologic blockade and germline gene knockout of CCR2 (expressed by monocytes/macrophages and a subset of T lymphocytes which most commonly binds to CCL2) ameliorated disease in a severe murine EAN model associated with markedly attenuated leukocyte trafficking into the sciatic nerves [9], while germline CCR5 knockout did not modulate disease in a less severe murine EAN model associated with compensatory increase in sciatic nerve CCL4 and CXCL10 expression [88]. Integrin blockade with a depleting function-neutralizing rat anti-mouse CD11b monoclonal antibody administered after clinically discernible disease onset was efficacious in the severe murine EAN model (Fig. 7) [59], providing further insight into the molecular determinants of pathogenic leukocyte trafficking in acute autoimmune neuropathies in vivo.

Chronic relapsing EAN animal models have been employed to understand CIDP pathogenesis; however, these models are generally limited by variable disease onset and severity. A severe murine chronic demyelinating neuritis model has been established in the autoimmune disease-susceptible CD86 (also known as B7-2)-deficient non-obese diabetic mouse strain, known as spontaneous autoimmune peripheral polyneuropathy (SAPP) that recapitulates features of severe CIDP [89, 90]. In this model associated with a cell and humoral autoimmune response to myelin protein zero [91], peptide blockade of fibronectin connecting segment 1 (which serves as an endothelial counterligand for CD49d or α_4-integrin) ameliorated disease to a similar magnitude as functional neutralizing rat anti-mouse monoclonal CD49d and VCAM-1 antibodies, associated with reduced leukocyte infiltration into the sciatic nerves (Fig. 7) [53], providing further insight into the molecular determinants of pathogenic leukocyte trafficking in chronic autoimmune neuropathies in vivo.

Future Directions

The human BNB, formed by endoneurial microvascular endothelial cells, is a critical interface hypothetically essential to the cross-talk between components of the systemic immune system and peripheral nerves and nerve roots in health during normal immune surveillance and in disease states that manifest as autoimmune neuropathies. The molecular determinants and signaling pathways responsible for hematogenous leukocyte interaction with and trafficking across the human BNB in health and disease are incompletely understood, with advances being made using a near-physiological flow-dependent leukocyte-endothelial cell trafficking model and animal models of peripheral nerve autoimmune disorders, critically supported by observational in situ data obtained from human peripheral nerve biopsies. Applying bioinformatics analyses to transcriptomic and proteomic data derived from normal and pathologic peripheral nerves at the batch or single cell level to establish biologically relevant networks/signaling pathways could accelerate our knowledge of the essential structural and functional characteristics of the human BNB in health, alterations, or adaptations in autoimmune disorders and aid discover molecular targets for disease-specific therapeutic modulation in this group of disorders that takes into account the unique biology of the BNB and the peripheral nervous system.

Acknowledgments and Funding Special thanks to past and current employees of the Shin J Oh Muscle and Nerve Histopathology Laboratory, the University of Alabama at Birmingham, for processing human tissue and generating histopathology slides from which digital photomicrographs are shown and current and past members and collaborators of the Neuromuscular Immunopathology Research Laboratory (NIRL) for digital photomicrographs and ultramicrographs of human cells and tissues and mouse tissues. Work described from the NIRL was supported by National Institutes of Health Grants R21 NS073702 (2011–2014), R21 NS078226 (2012–2015), R01 NS075212 (2012–2018), and a Creative and Novel Ideas in HIV Research Subaward P30 AI27767 (2012-2015), as well as institutional support from the Department of Neurology, the University of Alabama at Birmingham. The content is solely the responsibility of the author and does not necessarily represent the official views of the National Institutes of Health.

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Multi-actions of Microglia

Célestine Brunois and Laurence Ris

Abstract Microglia designate the innate immune cells of the central nervous system (CNS). Their morphology is closely related to their function, from the highly ramified resting phenotype in the healthy brain to the amoeboid-like morphology of the activated typical state of pathological conditions. Indeed, microglial cells act as resident macrophages of the brain in order to respond to injury or pathogens. Recent studies have underlined the function of microglia in physiological conditions, especially via the secretion of several cytokines which have an important impact on synaptic plasticity and cognition. We will discuss the origin, the discovery, and the different activation states of microglia. We will also review the current knowledge about the functions of microglia during CNS development, immune surveillance and their implication in neuronal networks and synaptic plasticity in both physiological and pathological conditions. Microglia could represent a genuine potential therapeutic target in the context of neuroimmune diseases.

Keywords Resident macrophages · Cytokines · Synaptic plasticity · Neuroinflammation · Phagocytosis

Abbreviations

^{11}C-PK11195	^{11}C-1-(2-chlorophenyl)-N-[^{11}C]methyl-N-(1-methylpropyl)-3-isoquinoline carboxamide
AD	Alzheimer's disease

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_9

AMPA	A-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
APC	Antigen-presenting cell
Arg1	Arginase 1
ATP	Adenosine triphosphate
Aβ	Amyloid beta
BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor
CaMK	Ca^{2+}/calmodulin-dependent protein kinase
CCL	Chemokine ligand
CCR	Chemokine receptor
CD	Cluster of differentiation
CNS	Central nervous system
COX-2	Cyclooxygenase-2
CSF-1	Colony-stimulating factor-1
CX3CL1	C-X3-C chemokine ligand 1
CX3CR1	C-X3-C chemokine receptor 1
DAMPs	Damage-associated molecular patterns
DAP12	DNAX-activating protein of 12 kDa
DC	Dendritic cell
EAE	Experimental autoimmune encephalitis
EGFP	Enhanced green fluorescent protein
ERK	Extracellular-signal-regulated kinase
GABA	Γ-Aminobutyric acid
GAD65	Glutamate decarboxylase 65
Gal-1	Galectin-1
GDNF	Glial cell-derived neurotrophic factor
GFP	Green fluorescent protein
GluR2	GluR2 subunit of AMPA receptor
Iba1	Ionised calcium-binding adapter molecule 1
IdU	5-iodo-2′-deoxyuridine
IFN-γ	Interferon gamma
IGF-1	Insulin-like growth factor-1
IL	Interleukin
iNOS	Inducible nitric oxide synthase
iPSCs	Induced pluripotent stem cells
LPS	Lipopolysaccharide
LTP	Long-term potentiation
Ly6C	Lymphocyte Antigen 6C
MAPK	Mitogen-activated protein kinase
M-CSF	Macrophage colony-stimulating factor
MHC class II	Major histocompatibility complex class II
MS	Multiple sclerosis

MW151	4-Methyl-6-phenyl-3-(4-pyrimidin-2-ylpiperazin-1-yl)pyridazine (minozac)
NADPH	Nicotinamide adenine dinucleotide phosphate
NFKB	Nuclear factor kappa B
NGF	Nerve growth factor
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
NR2B	NR2B subunit of NMDA receptor
P2RY12	Purinergic receptor P2Y 12
PAMPs	Pathogen-associated molecular patterns
PD	Parkinson's disease
PET	Positron-emission tomography
PI3K	Phosphoinositide 3-kinase
PKA	Protein kinase A
PKC	Protein kinase C
PLC-γ	Phospholipase C gamma
PSD-95	Postsynaptic density 95
ROS	Reactive oxygen species
TGF-β	Transforming growth factor beta
TMEM119	Transmembrane protein 119
TNF-α	Tumour necrosis factor alpha
TREM2	Triggering receptor expressed on myeloid cells 2
TrkB	Tropomyosin receptor kinase B
TSPO	Translocator protein

Introduction

Microglial cells represent the innate immune cells of the central nervous system (CNS) and play crucial roles in both physiological and pathological conditions. In the context of neuroimmune diseases, microglia are rapidly activated and represent the first line of defence in the CNS. Microglial cells are known to act as resident macrophages of the brain and are considered to be supervisors in the maintenance of CNS homeostasis. Besides these immune functions, they participate actively in the development and maintenance of neuronal networks. Through their essential role in synaptic circuits, they are modulators of neuronal function and have an important impact on synaptic plasticity and cognition [26, 27, 103]. We review the discovery and origin of microglia, and then discuss more extensively the numerous cellular characteristics and functions of these cells, in both healthy and pathological conditions.

Discovery of Microglia

The contributions from many talented researchers, and new staining techniques' developments, led to the discovery of microglia at the beginning of the twentieth century. At that time, Ramon y Cajal was considered to be one of the world leaders in the field of neuroanatomy. Thanks to his numerous contributions to science, he was awarded the Nobel Prize in Physiology or Medicine, along with the Italian Camillo Golgi, in 1906. Ramon y Cajal was the first to report on neuroglia. He explained that neuroglia preserved neuronal circuits and prevented incorrect contacts [71].

One of his students, Nicolas Achúcarro, was interested in neuroglia and especially in the "rod cells" discovered by Franz Nissl in 1898. These cells contain fatty degeneration products and are visualised around necrotic foci. Achúcarro hypothesised that these "granuloadipose cells" had a phagocytic activity [2]. Later, he acquired and improved Ramon y Cajal's staining techniques to develop his own version [3]. Using a tannin and ammoniacal silver technique, he clearly distinguished two cell types: a first population of phagocytic, non-fibrous and granuloadipose rod cells and a second constituted of fibrous cells with a stellar morphology and vascular end-feet [4]. These two cell types correspond, respectively, to our current knowledge of microglia and astrocytes.

These observations pushed Ramon y Cajal to improve the detection method in order to enhance the visualisation of these cells. He developed the formol uranium nitrate and sublimated gold chloride technique [72]. In 1913, he introduced the term "third element" of the CNS, after neurons and astrocytes. Under this controversial appellation, he described the "third element" as a population of small apolar cells of the white matter with a perivascular and perineuronal location which has a mesodermal origin.

Achúcarro became severely ill and another neuroanatomist, Pio del Rio Hortega, was designated by Ramon y Cajal to take over the research. He carried out modifications to the methods designed by his two mentors and developed an ammoniacal silver carbonate stain. The latter allowed him to identify two cell types: microglia and interstitial cells (later named oligodendrocytes). He characterised microglia as having a "small, dark nucleus enveloped by scant protoplasm and its ramified expansions adorned with lateral spines" [76–78]. He also proposed that microglia were dynamic, migratory cells, in contrast to astrocytes. Del Rio Hortega's experiments did not support the "third element" theory of Ramon y Cajal, which considered that oligodendrocytes and microglia belong to the same class. Del Rio Hortega's experiments continued and allowed the origin of different cells to be determined. Although all the aforementioned scientists participated in the discovery of microglia, Del Rio Hortega was the first to describe clearly these cells; microglia are also called Del Rio Hortega cells.

Cellular Origin of Microglia

Ontogenesis, Invasion and Proliferation of Microglia

Ramon y Cajal proposed the mesodermic origin of the "third element" [72]. Indeed, the embryogenic origin of microglia is distinct from other cell types (glia and neurons). Our current knowledge in embryology has formally confirmed that primitive macrophages from the yolk sac blood islands colonise the neuroepithelium from E9.0 to E9.5, giving rise to the embryonic microglia in mice. This is especially demonstrated by yolk sac progenitor staining before E7.5 [25]. These cells use the blood circulation to reach their location in the absence of the blood-brain barrier (BBB) at this stage of development. These precursors are observed near the fourth ventricle at E13 in mice. In humans, progenitors are detected at 13 weeks of gestation, and ramified microglia appear 8 weeks later. Well-differentiated microglia are detected close to term, at 35 weeks. Microglia proliferate and colonise the whole CNS until adulthood. Nonetheless, beyond this mesodermic origin, it is also known that peripheral macrophages can enter the brain parenchyma in the context of inflammatory conditions [25, 39].

Lifetime and Turnover of Microglia

To shed light on the implication of microglia in brain function, a comprehension of their turnover capacity is necessary. In homeostatic conditions, microglial density remains stable and is maintained by highly dynamic turnover and apoptosis [10]. Indeed, microglia maintain their self-renewing capacity over the entire lifespan. Whereas it is well established that microglia are not replenished by infiltrating macrophages in the healthy brain, their lifetime remains a matter of debate.

The Cx3Cr1^{CreER}:IDTR system was developed by Bruttger et al. (2015) to understand the origin and the self-renewal capacity of microglia [15]. Microglial repopulation appears after 5 days of depletion, and exclusively comes from a pool of internal resident cells. This phenomenon is independent of infiltration of peripheral cells but requires IL-1 signalling. However, under pathological conditions, infiltration of monocyte-derived macrophages is triggered [6]. Askew et al. (2017) estimated microglial turnover at about 95 days in mice and showed that an average of 0.69% of microglia is in S phase at a given time, more than previously indicated in a study by Lawson et al. (1992) with a turnover rate of approximately 0.05% [48]. In another recent study, Füger et al. (2017) showed that neocortical resident microglia had a median lifetime of about 30 months and that approximately half of the microglial population survived the mouse's lifespan [23]. The turnover can increase in neurodegenerative diseases, such as in the APPPS1 model [23].

It remains difficult to infer microglia dynamic from animals to humans that have different lifespans, and which generally live in pathogen-free conditions.

Higher rates (2%) of turnover have been suggested in humans, leading to an estimation of 100 cycles over 80 years of life [10]. Réu et al. (2017) showed that a large part of microglia in the healthy human cortex were renewed at a median rate of 0.08% per day and had an average age of 4.2 years. It represents a lower rate than previously estimated by IdU incorporation (0.14% per day) [73].

All these findings show divergences in estimations of the term of microglia lifetime that may be due to the utilisation of different protocols. Further investigations in both rodents and humans are required.

Similarities and Differences Between Microglia and Macrophages

Microglia are considered to be resident macrophages of the brain and the spinal cord. They are exclusively present in the CNS. In the healthy brain, they ensure the surveillance and protection of their microenvironment. However, under pathological conditions, and especially when the blood-brain barrier (BBB) is damaged, monocyte-derived macrophages can replenish microglia [6]. In this review, these macrophages are called "infiltrating macrophages". Resident and infiltrating macrophages present similitudes and differences in terms of localisation, cellular origin and expression levels of specific markers (Table 1).

Microglia come from yolk sac progenitors while macrophages originate from hematopoietic stem cells [25]. These two cellular types are distinguished by their levels of expression of specific markers. Both express the CX3C chemokine receptor

Table 1 Comparison between resident and infiltrating macrophages

Characteristics	Microglia "Resident macrophages"	"Infiltrating macrophages"
Location	CNS	From periphery to the CNS
Cellular origin	Yolk sac progenitors	Hematopoietic stem cells
Levels of expression		
CX3CR1	Yes (High)	Yes (Low)
CD45	Yes (Low)	Yes (High)
CD11b	Yes	Yes
Iba1	Yes	Yes
CSF-1	Yes	Yes
F4/80	Yes	Yes
CD39	Yes	No
TMEM119	Yes	No
P2RY12	Yes	No
Ly6C	No	Yes
CCR2	No	Yes

Microglia and infiltrating macrophages differ in terms of location, cellular origin and levels of expression of different molecules

1 (CX3CR1) and CD45, but microglia are CX3CR1^{hi}CD45^{lo} and macrophages are CX3CR1^{lo}CD45^{hi} [25, 109]. In addition, both are positive for CD11b, Iba1, CSF-1 and F4/80 markers. However, only microglia express CD39, P2Y purinoceptor 12 (P2RY12) and TMEM119. In contrast, the expression of Ly6C and CCR2 is specific for infiltrating macrophages [5, 25, 39, 81]. Moreover, some comparative studies have allowed infiltrating macrophages CX3CR1^{lo}Ly6C^{+}CCR2^{+} and peripheral monocytes CX3CR1^{hi}Ly6C^{-}CCR2^{-} to be distinguished [24, 109].

Morphology Related to Activation States and Phenotypes of Microglia

Morphology Related to Activation States

The morphology of microglia is intimately related to their functional state. Under physiological conditions, microglia are ramified cells with a small nucleus, regularly extending and retracting these fine processes while the small cellular body remains in place (Fig. 1a). Studies using Iba1-EGFP or CX3CR1^{GFP+} transgenic mice have revealed this exceptional morphology constituted of highly motile processes, which have the capacity to reach and contact other cells of the CNS [35, 38]. This "resting" phenotype is thought to be inactive for a long time. In reality, these cells are not quiescent. They are highly dynamic and are continually scanning the

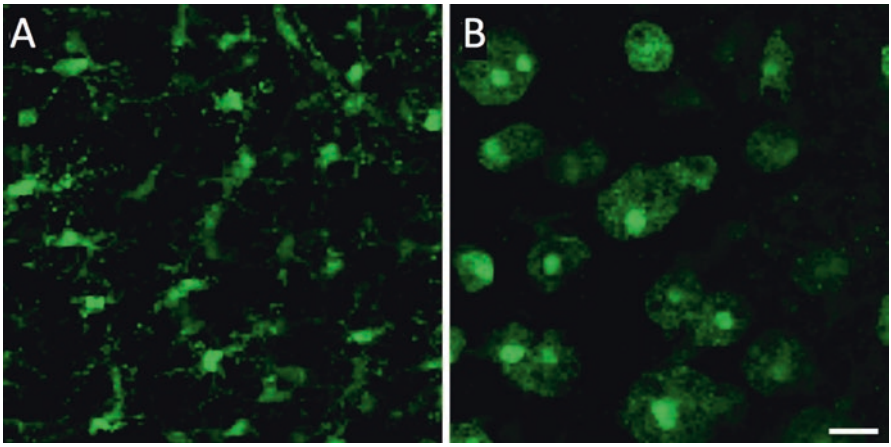

Fig. 1 Morphologies of microglia. Ramified (**a**) and amoeboid (**b**) morphologies of Iba1+ microglia in hippocampal organotypic slices incubated with specific glutamate decarboxylase 65 (GAD65) antibodies. (Courtesy of C. Hampe, University of Washington. Images obtained by confocal microscopy in the Department of Neuroscience of the University of Mons (Belgium). Scale bar: 25 microns)

environment. Indeed, this “resting state” allows them to survey their environment and interact with other cells and synapses. Therefore, they play a central role to ensure CNS maintenance, synapse pruning and CNS functionality [61].

Under pathological conditions such as injury, microglia can be rapidly modified into an “activated” state, characterised by a larger cell body, shorter processes and an amoeboid-like morphology (Fig. 1b). This morphological modification allows them to migrate to damaged sites and play specific roles, such as phagocytosis and antigen presentation. This phenotype is found in pathological conditions, as well as during development in the healthy brain [27, 100].

Microglial activation depends on their receptor expression and is modulated by a very large number of signals, such as cytokines, chemokines, complement system, PAMPs, DAMPs, integrins, immunoglobulins, neurotransmitters and growth factors [9].

Different Phenotypes of Microglial Activation

During the activation of microglia, cells are polarised to a pro-inflammatory (M1 or classically activated) or an anti-inflammatory phenotype (M2 or alternatively activated). The M1 phenotype (CCR7+ CD40+ CD74+ CD86+) is characterised by a high production of pro-inflammatory cytokines, such as TNF-α, IL-1β and reactive oxygen or nitrogen species, which are important for host defence. However, these elements can exacerbate neuroinflammation and provoke tissue damage. The M1 phenotype is mostly observed after stimulation by LPS or IFN-γ [46, 57, 63]. On the other hand, the M2 phenotype (Arg1+ CD206+ CCL22+) has a global anti-inflammatory effect. This large group is subdivided into three categories. First, the M2a phenotype, which is induced by IL-4 and IL-13, downregulates pro-inflammatory mediators and upregulates factors implicated in tissue repair (Arg1, IGF-1). Second, the M2b phenotype, produced after IL-1β or LPS stimulation, secretes high levels of IL-10 and interestingly also several pro-inflammatory cytokines (IL-1β, IL-6, TNF-α). Finally, the M2c phenotype, induced by IL-10 and TGFβ, downregulates the production of several pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α). Thus, the M2a-c subtypes seem to play a protective role and to promote tissue repair [46, 57, 63].

It was initially proposed that microglial cells acquire only one of these phenotypes, but recent studies have demonstrated that at a given time, microglia may simultaneously exhibit M1 and M2 markers and adopt inflammatory and restorative functions. These phenotypes are not permanent. Microglia may alternate from one activation state to another. Thus, cells may have the possibility of providing a large range of immune responses, depending on the stimulus intensity and the activation context [21, 101].

Physiological Role of Microglia in the CNS

Generalities

In terms of functions, it was initially considered that the roles of microglia were restricted to those assigned to macrophages, such as phagocytosis of cell debris. Recent studies demonstrate that microglia are not limited to this role but are also involved in physiological brain functions [94].

Functions

Development and Maintenance of Neuronal Networks

During CNS development, microglia seem to be a key element in the creation of correct neuronal networks by regulating apoptosis and the survival of neurons. The release of diffusible factors and phagocytosis underlie these functions. Microglia phagocytose dead, dying and some living cells in the developing and adult brain. Phagocytosis is indeed an important phenomenon for maintaining brain homeostasis. It avoids the release of toxic and pro-inflammatory elements from apoptotic cells (Fig. 2).

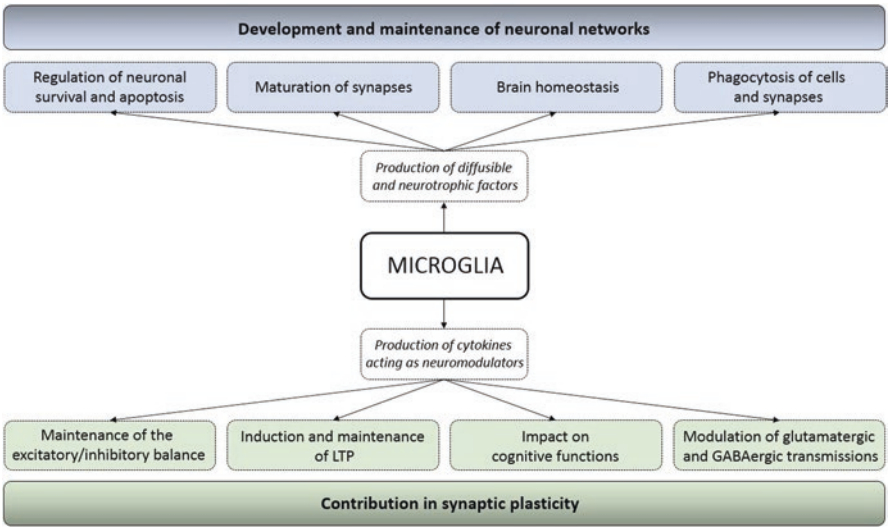

Fig. 2 Physiological effects of microglia. Microglia are implicated in the development and maintenance of neuronal networks through the production of diffusible and neurotrophic factors. Microglia also contribute in synaptic plasticity by their production of cytokines acting as neuromodulators

Microglia are located near apoptotic neurons in order to eliminate cellular debris. They especially participate in apoptosis by the release of superoxide ion by CD11b integrin and DAP12 immunoreceptor after microglial contact with the target neuron. The implication of microglia in programmed cell death has been proven by in vitro studies which have demonstrated that this function was performed without inducing an inflammatory phenotype [98].

Furthermore, microglia are essential in the installation of neuronal networks to promote neuronal survival via the secretion of neurotrophic factors [94]. The experiments of Ueno et al. (2013) showed the importance of IGF-1 in neuronal survival and revealed a decrease in the production of this beneficial factor in CX3CR1-deficient mice. They also demonstrated an increase in apoptosis especially in layer V subcerebral and callosal projection neurons via the inactivation of microglia by minocycline or the transient deletion of microglia in transgenic mice. Microglia are also involved in synaptic pruning during the post-natal period. This process is mediated through the phagocytosis of synapses and can be altered by the loss of CX3CR1 [65].

By activating several signalling pathways (PLC-γ, PI3kinase and MAP/ERK) in neurons via TrkB, brain-derived neurotrophic factor (BDNF) is a crucial mediator in neuronal survival. Although the major source of BDNF is neurons, it can also be secreted by other cell types, such as microglia, being able to promote emergence and maintenance of dendrites and dendritic spines [105]. Indeed, it has been demonstrated that the suppression of microglial BDNF has a negative impact on dendritic spines [66].

Microglia are also involved in the maturation of synapses. It has been observed that microglia establish direct contact with pre- and postsynaptic components. By in vivo two-photon imaging of fluorescent-labelled microglia and neurons, Wake et al. (2009) observed that microglial processes establish direct contacts with neuronal synapses which are neuron activity-dependent and last 4–5 minutes once per hour. After transient cerebral ischemia, it has been shown that the contacts between microglia and synapses are remarkably prolonged (about 1 hour) and are regularly followed by the loss of the presynaptic element [97].

Besides their functions in the development and maturation of neuronal networks, microglia are also implicated in the vascularisation of the CNS. Their role in the vascular network formation is especially demonstrated by a decreased number of vascular branching points in the retina in CSF1-deficient mice which do not have retinal microglia [20, 44]. Rymo et al. (2011) also showed that vascular network is impaired in the genetic absence of microglia and that their angiogenic effect can be restored by microglia supplementation [80].

Contribution in Synaptic Plasticity in the Healthy Brain

In physiological conditions, it has been demonstrated that microglia extend long motile processes able to contact neurons and to modulate their activity. This action is performed without the displacement of microglia soma [51, 52]. Physiological

levels of cytokines are secreted by microglia in the healthy brain. These molecules act as neuromodulators via receptors located at the surface of neurons and play an important role in the neuronal viability and function (Fig. 2).

IL-1β has been identified as a crucial cytokine in the long-term potentiation (LTP) processes under physiological conditions. It allows both the induction and the maintenance of LTP in the hippocampus. IL-1β stimulates the expression of AMPA receptors and induces the phosphorylation of the NR2B subunit of NMDA receptors in hippocampal neurons. The increase in glutamatergic receptor activity promotes calcium entry into neurons, which contributes to the LTP. The liberation of glutamate by the presynaptic element is also fostered in these conditions. Moreover, the activation of NMDA receptors stimulates the insertion of IL-1 receptors in the cell membrane. Spulber et al. (2009) observed an increase of IL-1 expression in the hippocampus during spatial or contextual learning. This cytokine seems to be necessary in the memory processes [86]. An alteration of cognitive functions is detected in animals with a pharmacological inhibition of IL-1 receptors or in IL-1 receptor knock-out mice [11, 29]. Avital et al. (2003) observed a longer latency to reach the platform in the Morris water maze, a decreased freezing in the contextual fear conditioning and no LTP in the dentate gyrus in IL-1 receptor-deficient mice. The administration of IL-1 receptor antagonists during prenatal development has a negative impact on the memory of adult mice [29].

Furthermore, fractalkine (CX3CL1), a chemokine expressed by neurons, might impact on the synaptic plasticity via microglia. Indeed, Sheridan et al. (2013) observed that the activation of CX3CR1 (fractalkine receptor) on microglia induces the release of adenosine, which activates $A2_A$ receptors located on microglia [84]. This activation leads to the liberation of D-serine acting as a co-agonist of NMDA receptors. We can also notice that CX3CR1 knock-out mice present an increase in both IL-1β and TNF-α levels, and associative and spatial memory impairment [79].

TNF-α represents another relevant cytokine with an effect on homeostatic synaptic plasticity [42], which allows the maintenance of the excitatory/inhibitory balance when neuronal activity is either increased or decreased. Its presence is not essential in the LTP process, but its expression ameliorates the excitatory transmission via the insertion of AMPA receptors (without the GluR2 subunit) into the postsynaptic membrane, and the liberation of glutamate by astrocytes and the presynaptic element [88]. It also regulates the inhibitory transmission by downregulating cell-surface level of $GABA_A$ receptors. Thus, TNF-α seems to induce a higher excitatory/inhibitory ratio [70, 88]. Stellwagen and Malenka (2006) proposed that synaptic scaling occurring after a chronic blockade of synaptic transmission in cultured hippocampal slices is mediated by TNF-α [89]. On the other hand, a study performed by Lewitus et al. (2014) revealed that TNF-α promotes the internalisation of AMPA receptors in the mouse dorsolateral striatum causing a decrease in corticostriatal synaptic strength [50].

Finally, Parkhust et al. (2013) showed that, like neuronal BDNF, microglial BDNF seems to modulate the excitatory synaptic transmission [74] and the inhibitory synaptic transmission in the hippocampus [111] or the spinal cord [18] via BDNF-TrkB signalling.

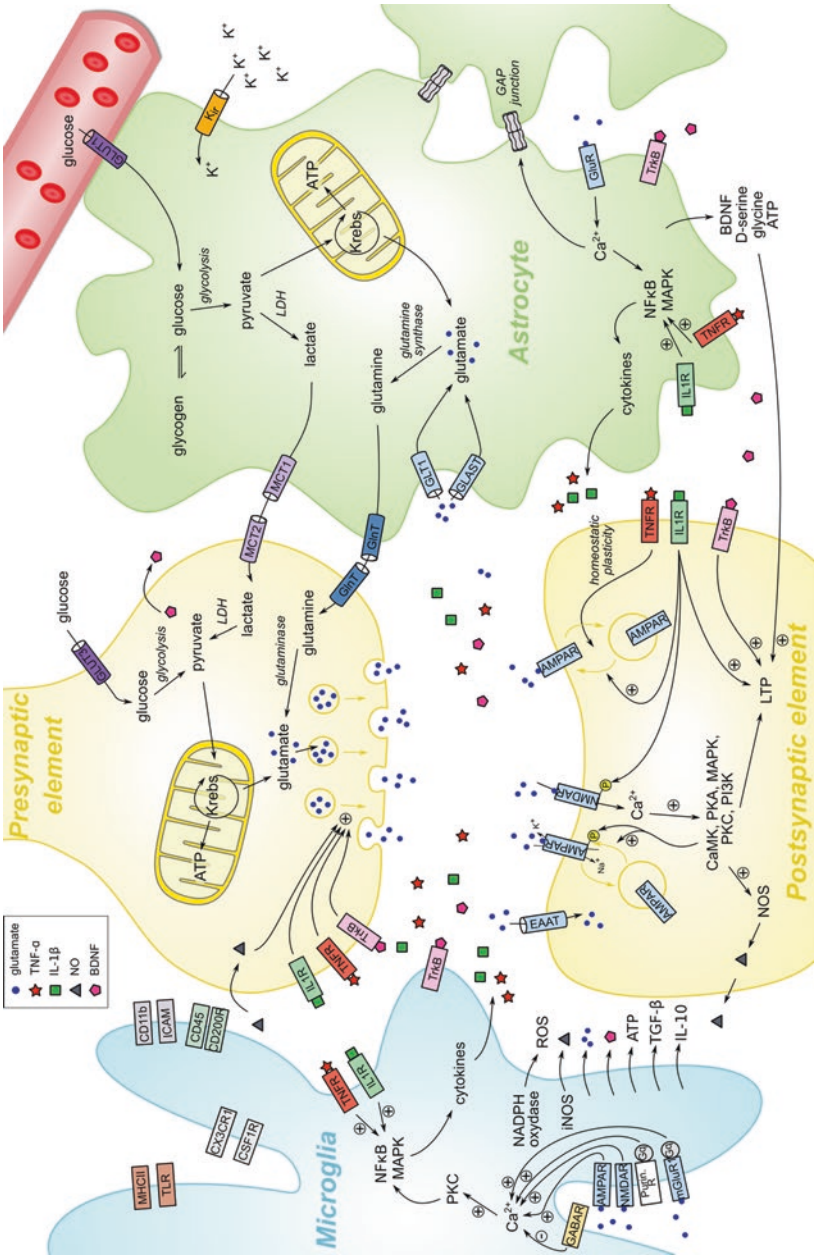

Fig. 3 Schematisation of neuroimmune interactions between neurons, microglia and astrocytes in the synaptic plasticity phenomena. (Modified from a representation created in the Department of Neuroscience of the University of Mons (Belgium))

Involvements of Neuroimmune Interactions Between Neurons, Microglia and Astrocytes in Synaptic Plasticity

Inside the CNS, electrical activity is unidirectionally directed from one neuron (presynaptic element) to another (postsynaptic element) through neurotransmitter release. However, other cell types are involved in the neuronal function, microglia and astrocytes, creating the quadripartite synapse represented in Fig. 3. This schematisation exhibits the numerous neuroimmune interactions and mechanisms involved in synaptic function. First of all, the excitatory glutamate is released by the presynaptic element in the synaptic cleft and reaches both AMPA and NMDA receptors to induce synaptic plasticity phenomena which include activation of several calcium-dependent signalling pathways, such as MAPK, PKC, PKA, PI3K and CaMK. They promote phosphorylation and expression of surface AMPA receptors. From the postsynaptic neuron, NO acts as a retrograde messenger and can stimulate the neurotransmitter release. Moreover, glucose from blood vessels provides an important source of energy for neurons, via astrocytes. A glutamate-glutamine cycle between neurons and astrocytes allows the recapture of extracellular glutamate and its transfer to the presynaptic neuron providing a stock of neurotransmitters. Astrocytes also reduce extracellular potassium levels coming from the neuronal activity through inward rectifying potassium channel, and release other important factors such as BDNF, ATP, glycine and D-serine. In addition, astrocytes exhibit neurotransmitter receptors and GAP junctions to modulate and transmit their activity from an astrocyte to another. In the quadripartite synapse, the last element is microglia, which secrete different cytokines modulating the synaptic transmission. They express numerous molecules such as MHC class II, CD45, CD11b, and receptors (CX3CR1 and CSF1R) to interact with neurons. The neuronal activity also mediates microglial activity through their expression of neurotransmitter receptors. Thus, microglia, and also astrocytes, release pro- and anti-inflammatory mediators which have significant impacts on the synaptic plasticity [42, 74, 79, 88].

Physiopathological Roles of Microglia in the CNS

Generalities

Microglia are well-known for their numerous roles in pathological situations. Cells acquire another morphology, adapted to their specific functions especially in pathological states. Besides their roles in immune defence, microglia, and their secreted molecules, have a multitude of impacts on the neuronal networks and synaptic plasticity in response to pathological events [9].

Functions

Immune Surveillance

In a pathological context, microglia adopt an activated state, express a M1 or M2 polarisation, and secrete high levels of diffusible factors. This activation is characterised by morphological modifications and the acquisition of migration capacity. Cells acquire an amoeboid form and retracted processes. This morphology allows them to migrate in the direction of damaged sites to proliferate and also to phagocyte pathogens and cell debris [63].

M1 and M2 phenotypes have distinct roles. On the one hand, the pro-inflammatory M1 phenotype secretes cytokines, such as TNF-α, IL-1β, IFN-γ, IL-2, IL-6, IL-12, IL-17 and IL-23 and also chemokines and glutamate. They also express highly inducible NO synthase (iNOS), NADPH oxidase and MHC-II molecules. These elements aim to combat infections and eliminate toxic stimuli but can cause secondary damage in the cerebral tissue. Cytokines are implicated in astrogliosis, the activation of T and B lymphocytes, the alteration of BBB integrity, the production of chemokines and the amplification of inflammatory responses. Moreover, the release of glutamate by microglia promotes the excitotoxicity phenomenon. On the other hand, the anti-inflammatory M2 phenotype secretes cytokines (IL-4, IL-10, IL-13 TGF-β) and growth factors (BDNF, GDNF, NGF, IGF-1). They promote tissue repair, homeostasis restoration, and the inhibition of the production of pro-inflammatory cytokines. Growth factors are extremely important in neurogenesis, remyelination and the growth of both axons and dendrites [21, 46, 57, 63, 101].

According to the activation of microglia and the acquisition of these two different phenotypes, cells have both protective and noxious effects. The M1 phenotype releases pro-inflammatory factors, which provide a negative environment for neurons. To counterbalance these detrimental effects, the M2 phenotype brings anti-inflammatory molecules, which play protective and restorative roles in the brain parenchyma. However, as previously mentioned, the M1-M2 dichotomy is an oversimplified classification. Microglia are able to express both M1 and M2 phenotypes and exhibit inflammatory and protective effects [46, 63].

Impacts on Synaptic Plasticity and Induction of Cell Death in the Pathological Brain

Any modification in the CNS homeostasis that implies microglia causes a compensatory mechanism and a neuroinflammation may affect the synaptic plasticity. Thus, during CNS inflammation, activated microglia interact and modify the survival of other cell types such as neurons and have an impact on their functions.

In neuroimmune diseases, excessive stimulation of microglia results in a dramatical increase in cytokine production and a decrease in anti-inflammatory and neurotrophic factors which are susceptible to induce cognitive troubles [108].

Table 2 Comparison between IL-1β and TNF-α effects under physiological and pathological conditions

	Physiological conditions	Pathological conditions
IL-1β	Induction and maintenance of the LTP Enhancement of glutamatergic transmission Stimulation of the expression of AMPA receptors Stimulation of the phosphorylation of NR2B subunit (NDMA receptors)	Cognitive impairment (learning and spatial memory) Neuronal death (direct effects or induction of neurotoxic factor production by astrocytes)
TNF-α	Maintenance of the excitatory/inhibitory balance Enhancement of glutamatergic transmission Improvement of the insertion of AMPA receptors in the postsynaptic element Increase of glutamate liberation (from presynaptic neurons and astrocytes) Regulation of GABAergic transmission	Cognitive impairment (LTP) Neuronal death (direct effects or induction of neurotoxic factor production by astrocytes)

These two cytokines have distinct effects in the healthy and pathological brain

The two pro-inflammatory cytokines, TNF-α and IL-1β, have been extensively studied in both physiological and pathological contexts (Table 2). Pathological levels of TNF-α seem to impair LTP in the CA1 region of the hippocampus in a model of peripheral nerve injury. Surprisingly, LTP is improved at C-fiber synapses in the spinal dorsal horn in this model [54, 56] and in the dentate gyrus after a pre-treatment of hippocampal slices with TNF-α [99]. It has also been shown that the increase in IL-1β levels in a model of cerebral lesion results in learning and spatial memory impairment in rodents [17]. Similar results have been obtained after administration of exogenous IL-1β, and in transgenic mice overexpressing this cytokine [32, 59]. Hippocampal LTP seems also to be impaired in experimental autoimmune encephalomyelitis (EAE) which is an inflammatory model [19]. The same authors describe that LTP inhibition and behavioural alterations were recovered by suppressing microglia activation. Indeed, it is well-known that an activation of both microglia and astrocytes occurs during the course of EAE. The activation of microglia appears even as a major component in EAE pathogenesis [106, 110]. Moreover, TNF-α and IL-1β may cause neuronal death by their direct effects on neurons or by induction of neurotoxic factor production by astrocytes [93]. However, despite all experiments studying TNF-α and IL-1β, today, it remains difficult to generalise the effects of these cytokines on synaptic plasticity. Moreover, damaged neurons can secrete neurotoxic factors exacerbating these phenomena. In activated microglia, cytokines can also induce production of ROS and NO, and an elevated extracellular glutamate concentration by stimulating its liberation from presynaptic neurons and microglia, and by decreasing its recapture by astrocytes. Thus, more than just an alteration of synaptic plasticity, a chronic inflammatory state can be installed which induces important neuronal damages and excitotoxicity leading up to neurodegeneration [95, 108].

Phagocytosis

Like infiltrating macrophages, microglia are phagocytic cells able to eliminate bacteria, dying or dead neurons, and other debris. Phagocytosis is an extremely important phenomenon in neurodegenerative diseases. The phagocytic function of resident macrophages is considered as a protective mechanism, especially in the phagocytosis of myelin debris and axons in MS [43], and amyloid β (Aβ) proteins in the case of Alzheimer's disease (AD) [7]. It has been demonstrated that CX3CR1 knock-out mice present a default in myelin product removal, affecting the axonal integrity and myelin sheaths, and resulting in an inefficient remyelination in MS [47], as observed by electron microscopy imaging of Iba1-positive microglial cells. Moreover, wild-type microglia show an accumulation of myelin debris internalised by endosomes and cholesterol crystals inside their cytoplasm. In contrary, in CX3CR1-deficient mice, microglia were almost lacking endosomes, myelin debris, and cholesterol crystals, suggesting a reduced phagocytic activity. Together, these results suggest that CX3CR1 deficiency has an important impact on the phagocytic activity of microglia [47, 53].

In addition, in CX3CR1 knock-out mice, the expression of TREM2 (triggering receptor expressed on myeloid cells 2), a protein also implicated in the phagocytic activity, is reduced [91]. Downregulation of TREM2 in microglia causes an impaired phagocytosis of apoptotic neurons and an overexpression of pro-inflammatory factors (TNF-α and iNOS). Moreover, in EAE, administration of TREM2-blocking antibodies before EAE induction revealed an exacerbation of the disease severity [68]. In this MS experimental model, it has also been showed that transplantation of myeloid precursors overexpressing TREM2 at the peak of EAE increases myelin debris clearance and anti-inflammatory cytokines production and reduces pro-inflammatory factor expression in the spinal cord [92].

Antigen Presentation

In normal conditions, microglia are the only one's immune cells of the brain parenchyma. However, a small number of T cells, dendritic cells and macrophages can be observed near blood vessels, choroid plexus, circumventricular structures and meninges which are strategical locations to protect brain parenchyma [8, 28].

Neuroimmune diseases such as MS are characterised by T cell infiltration, recruitment of dendritic cells (DC) and macrophages in the CNS. In this situation, development of inflammatory lesions, axonal damages and demyelination may be observed [30, 58]. In addition to MS, T cell infiltration is also observed in post-mortem brain tissue of AD and PD patients [14, 22].

In this context, antigen presentation constitutes an immune process that allows the recognition of fragmented antigens which are presented on antigen-presenting cells (APC) by specific T cell receptors. Dendritic cells are known to be an important cell type for antigen presentation. However, during neuroimmune diseases such as MS and its animal model, the CNS contains other populations of APC. Indeed,

the expression levels of MHC and co-stimulatory molecules revealed the ability to present antigen and indicate the maturation state of different APC. Microglia, expressing both MHC class II and co-stimulatory molecules, are shown to be effective CNS-resident APC to the infiltrating $CD11c^+$ cells [104]. The overexpression of MHC and co-stimulatory molecules by microglia induced by infiltrated Th17 promotes the production of pro-inflammatory cytokines, ROS and complement proteins which enhance neuroinflammation. In addition to the brain parenchyma, meninges also represent an important site of antigen presentation to T cells (CD4+) by microglia and dendritic cells during autoimmune diseases [40]. Moreover, it has been shown that $CD4^+$ T cells have an important impact on PD pathology, especially through MHC class II expression in microglia [75].

Neuroprotection Versus Neurotoxicity

Microglia activation was thought to be a negative phenomenon for a long time. However, a large number of studies revealed their neuroprotective and beneficial effects (Fig. 4). Microglia activation is a well-controlled phenomenon particularly regulated by their interactions with neurons and astrocytes. These cells maintain the process under control and ensure phagocytosis of pathogens and debris clearance, tissue repair, spontaneous disappearance of inflammation and brain homeostasis recovery. Thus, in a normal context, a large proportion of secreted factors by microglia have beneficial effects on the brain. However, in the case of injury or chronic inflammatory disease, a strong elevated activation can appear due to a disruption in the system of regulation or an excessive stimulation. In this situation, microglia maintain a highly activated state which becomes deleterious for the brain

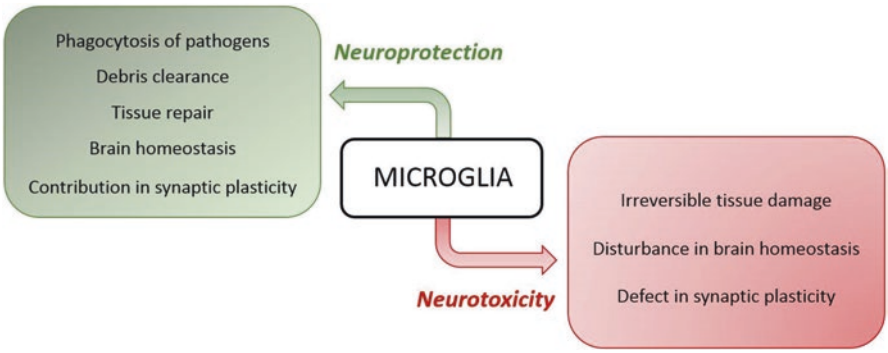

Fig. 4 Neuroprotection versus neurotoxicity. Microglia present either beneficial or deleterious effects in the function of the stimulation context

parenchyma and generates irreversible tissue damage due to the uncontrolled production of pro-inflammatory cytokines and neurotoxic factors. The high expression of these molecules is a detrimental element for the tissue and the synaptic plasticity [13, 29, 95, 108].

Translation from Animal Studies to Humans

The large majority of research on microglia is performed in animal models, in particular on rodents, due to the difficulty to study their human counterparts. Despite the very little research on humans, we know that rodent and human microglia show important similarities, as well as several relevant differences. However, important similarities justify the use of these models, as does the fact that rodent models are largely employed in this domain. Based on this, evaluating the functional impact of molecules in rodent models remains essential, but differences due to species must be taken into account, especially when these characteristics are associated with neurological disorders.

Human and rodent microglia colonise the CNS over an analogous timeline. In both rodents and humans, many factors are similarly expressed, such as Iba1, M-CSF receptor and DAP12. The activation of these cells into M1 and M2 phenotypes also seems to show no difference between species. However, for instance, human microglia seem to have a higher level of IL-10 production. Moreover, the implication of TGF-β1 seems to be more important in mice than in humans, as well as the production of NO. Thus, we understand that there is a strong need to interpret results obtained from animals before extrapolating in human [16, 85, 90].

Recently, in vivo imaging of microglia performed by positron emission tomography (PET) has been revealed as a new tool to evaluate the participation of activated microglia in neuroimmune diseases. Indeed, neuroinflammation observed in human or in in vivo models is now largely studied by PET scanning and particularly by the detection of the translocator protein (TSPO) radioligand ^{11}C-PK11195. TSPO is a protein located in the outer mitochondrial membrane which is normally expressed in healthy cells and is highly expressed in reactive cells, such as microglia, macrophages and astrocytes, which are key elements in neuroinflammation. This exciting technique allows the pathology progression to be followed and the efficacy of treatments tested in neuroinflammatory diseases to be evaluated. The technique is largely used in multiple sclerosis (MS), Parkinson's disease (PD), Alzheimer's disease (AD) and Huntington's disease [69, 96]. Recently, Horti and collaborators (2019) developed a new PET radiotracer. This (^{11}C)CPPC ligand is specific for CSF1R which is only expressed by microglia and infiltrating macrophages, and is upregulated under inflammatory conditions. This PET ligand resolves well-known limitations from TSPO such as detection of astrocytes and endothelial cells beyond microglia [36].

One exciting alternative method to investigate human microglia is the recent technology of induced pluripotent stem cells (iPSCs). Patient-derived iPSCs repre-

sent a renewable source of specific cells to investigate their functions in both healthy and pathological conditions [1]. Microglia-like cells exhibit numerous features of microglia such as expression of specific markers (CX3CR1, Iba1, CD45 and CD11b), ramified or amoeboid morphology, production of cytokines, and phagocytosis function [1, 31, 60, 64]. Thus, the generation of microglia-like cells from iPSCs allows the study of their implication in neurological diseases, such as AD [1].

Microglia as a Potential Therapeutic Target and Methods to Counterbalance Their Activation

Glucose is a key element for microglial function and activation. Recently, the expression of high levels of glucose transporter (GLUT1) in activated microglia has been revealed in a study of Wang and collaborators (2019) under physiopathological conditions. They showed that glucose uptake in microglia is supported mainly by this transporter. In a pathological context, microglia seem to upregulate the expression of GLUT1 to promote glucose uptake and anaerobic glycolysis. Therefore, blocking GLUT1 could represent a new approach to control microglial activation and to increase glucose availability to neurons [102].

Some therapeutic methods aim to deactivate or modulate the M1 phenotype. Indeed, galectin-1 (gal-1) is able to modulate the M1 phenotype, and especially control iNOS and CCL2 expression via p38 and NF-KB signalling pathways. Gal-1 is also known to ameliorate the disease course of EAE [87].

Minocycline is a well-known and characterised molecule inhibiting microglial activation, in particular M1 polarisation. An in vivo study of Kobayashi et al. (2013) showed that the administration of minocycline reduced the expression of M1 phenotype while the M2 phenotype was not affected [41]. However, Scott et al. (2018) revealed that the antibiotic minocycline attenuated chronic microglial activation but increased neurodegeneration in a model of traumatic brain injury. These results suggest that microglial activation could provide beneficial and reparative functions in the chronic phase of traumatic brain injury [82].

One natural product especially known for its anti-inflammatory properties is ginseng. In China, Japan and Korea, it is considered as one of the most precious of all traditional medicinal herbs and is used not only for its anti-inflammatory effects but also for its potential benefits as antioxidant, anti-tumour and anti-fatigue [33, 49]. Ginsenosides, which are molecular components of ginseng implicated in these beneficial effects, are categorised into two different structural groups. These two classes, the 20 (S)-protopanaxadiol and the 20 (S)-protopanaxatriol conformations, include, respectively, $Ra_{1–3}$, $Rb_{1–2}$, Rb_3, Rc, Rd, Rg_3, Rh_2 and Re, Rf, $Rg_{1–2}$, Rh_1 molecules [62]. Experiments on aged mice revealed that the administration of ginsenoside Rg1 improves the spatial memory and upregulates the expression of several synaptic plasticity-associated proteins in the hippocampus, such as synaptophysin, NR1 subunit of NMDA receptors, postsynaptic density protein 95

(PSD-95) and CaMKIIα [107]. Moreover, it has been shown that a ginsenoside Rg1 pretreatment of LPS-induced BV-2 microglial cells may activate the phospholipase C signalling pathway and modulate the expression of TNF-α, IL-1β, iNOS, COX-2 and NF-κB [112]. Ginsenoside Rh3 was also shown to decrease the expression of TNF-α, iNOS and IL-6 [49, 83]. In the same line of evidence, ginsenoside Rh1 inhibited the production of pro-inflammatory cytokines and the expression of iNOS, COX-2, but it increased the expression of an anti-inflammatory cytokine (IL-10) in LPS-stimulated microglia [37]. Rb1 represents another ginsenoside component known to improve spatial learning and memory and to increase cell survival but not proliferation in the hippocampus [55]. It may also present a beneficial role in AD by modulating neurofibrillary tangle formation and tau hyperphosphorylation [101]. This phosphorylation state is also reduced by Rd components, as showed on cultured cortical neurons and AD rats [55]. Together, these results indicate that several ginseng components possess anti-inflammatory effects by modulating the activation of microglia.

Ginseng and minocycline have been simultaneously experimented in a study of Kumar et al. (2014). It was shown that animals exposed to brain traumatic injury and to a rehabilitation period presented a cognitive impairment, revealed by an increase in escape latency and total distance travelled to reach the platform in the Morris water maze. Moreover, animals presented an important increase in oxidative stress and pro-inflammatory markers. However, after a ginseng and minocycline treatment, behavioural troubles and inflammatory factor levels were attenuated. Thus, the combination of low doses of ginseng and minocycline seems to potentiate their beneficial and anti-inflammatory effects [45]. A study performed in humans also demonstrated that AD patients with a high dose of ginseng showed a significant improvement on the Alzheimer's Disease Assessment Scale and Clinical Dementia Rating after 12 weeks of therapy [34].

MW151 is a molecule that suppresses upregulation of pro-inflammatory cytokines but does not block the synthesis of anti-inflammatory cytokines in inflammatory diseases or after injury. It has been demonstrated that MW151 suppresses IL-1β overproduction but does not affect proliferation, migration and phagocytosis of microglia after traumatic brain injury. In an amyloid beta infusion model, the administration of MW151 resulted in a significant suppression of IL-1β production correlated with a practically complete recuperation of cognitive function and a reduction of neuronal dysfunction markers [12]. So, MW151 seems to improve neurological outcomes where pro-inflammatory cytokines are key elements of the physiopathological progression.

Finally, it has been observed that voluntary physical exercise constitutes a natural method to maintain microglia and brain homeostasis. Indeed, exercise induces an increase in neurotrophic factor and anti-inflammatory cytokine production, a reduction of pro-inflammatory cytokine levels and microglial activation. By these multiple beneficial effects, it seems to have a global anti-inflammatory effect in the brain [67].

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Autoimmune Astrocytopathy

Jorge Correale and María I. Gaitán

Abstract Astrocytes are the most abundant and heterogeneous type of glial cell in the Central Nervous System. In addition to their role maintaining physiological conditions stable in the CNS, they are recognized as early and highly active players in immune responses in the CNS, and their dysfunction is believed to contribute to neuroimmune disease.

Perhaps one of the most important discoveries in recent years has been the identification of IgG-NMO, a specific pathogenic antibody directed against water channel aquaporin-4 (AQP4). IgG-NMO has not only made neuromyelitis optica diagnosis easier but has allowed differential diagnoses to be established more clearly and lead to the design of better therapeutic alternatives. Likewise, a novel autoantibody directed against GFAP has been identified as biomarker of a relapsing autoimmune form of meningoencephalomyelitis, responsive to steroids, often associated with tumors. Similarly, in Rasmussen's encephalitis, $CD8^+$ T lymphocytes cause astrocyte apoptosis and loss in affected areas, altering normal neuron function. Reactive astrocytes also play an important role in different CNS infections, not only during acute phases of disease but also long term, and may condition the development of post-infectious sequelae. Finally, multiple mechanisms mediated by astrocytes are known to participate in both the genesis and the progression of MS and in processes of remyelination. Overall, these observations indicate astrocytes actively participate in both pathological and in repair mechanisms, observed in CNS neuroimmune diseases.

Keywords Anti-AQP4 · Anti-GFAP · Astrocytes · Multiple sclerosis · Neuromyelitis optica · Rasmussen's encephalitis

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_10

Abbreviations

AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AQP4	Aquaporin 4
ATP	Adenosine triphosphate
B4GALT5	4-Galactosyltransferase 5
BAFF	B-cell activating factor
BBB	Blood–brain barrier
C1q	Complement component subunit 1q
CNS	Central Nervous System
CNTF	Ciliary neurotrophic factor
CSPGs	Chondroitin sulfate proteoglycans
Cx	Connexin
DAMPS	Danger-associated molecules patterns
EAAT2	Excitatory amino acid transporter 2
EAE	Experimental autoimmune encephalomyelitis
ECM	Extracellular matrix
EPH	Ephrins
Fas-L	Fas ligand
FGF	Fibroblast growth factor
FoxP3	Forkhead box P3
GAG	Glycosaminoglycan
GFAP	Glial fibrillary acidic protein
GLAST	Glutamate/aspartate transporter
GLT-1	Glutamate transporter-1
GluR3	Glutamate receptor 3
GM-CSF	Granulocyte macrophage colony-stimulating factor
GS	Glutamine synthetase
HMGB1	High-mobility box-1
ICAM 1	Intercellular adhesion molecule 1
IFNs	Interferons
iNOS	Inducible nitric oxide synthase
IRF-1	Interferon regulatory factor 1
ISGs	Interferon-stimulated genes
LacCer	Lactosylceramide
LFA-1	Lymphocyte function-associated antigen
LIF	Leukemia inhibitory factor
LPS	Lipopolysaccharide
M-CSF	Macrophage colony-stimulating factor
MMPs	Matrix metalloproteinases
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NG2	Neuron-glial antigen 2
NMDA	N-methyl-D-aspartate
NMO	Neuromyelitis optica

NMOSD	Neuromyelitis optica spectrum disorders
NO	Nitric oxide
$ONOO^-$	Peroxinitrate
OPCs	Oligodendrocyte progenitor cells
PAMPs	Pathogen-associated molecular patterns
PRRs	Pattern recognition receptors
RAGE	Receptor for advanced glycation end products
RE	Rasmussen's encephalitis
RLRs	Retinoic acid-inducible gene-like receptors
S100β	S100 calcium-binding protein
TGF	Transforming growth factor
Th	T helper cell
Tim-3	T cell immunoglobulin and mucin domain 3
TIMPs	Tissue inhibitors of metalloproteinases
TLR	Toll-like receptor
Tr1	Type 1 regulatory T cells
VCAM-1	Vascular cell adhesion protein 1
VLA-4	Very late antigen 4

Introduction

Astrocytes comprise the most abundant population of glial cells in the Central Nervous System (CNS) of mammals. They are crucial for the good health and proper function of the nervous system, as they provide important metabolic and trophic support to neurons [1]. Classic neuroanatomy studies established two groups of astrocytes based on their morphology and location [2]: (a) fibrous astrocytes with small cell bodies associated with myelinated axonal tracts, presenting elongated morphology, and in contact with the nodes of Ranvier [3]; and (b) protoplasmic astrocytes, with more primary processes, as well as a higher degree of branching compared to fibrous astrocytes, located in gray matter [4]. The latter, envelop neuronal synapses through their fine perisynaptic processes, which originate from secondary and tertiary branches [5] and are in direct contact with blood vessels through end-feet [6]. Even though all astrocytes have a number of characteristics in common, physiological and gene expression studies have revealed astrocytes are a far more diverse cell population than was previously believed. Morphologically and functionally different astrocytes populations develop at different times in varying locations [7]. Consequently, astrocytes can no longer be considered a single homogeneous group of cells. Much of this diversity is related to structural and functional interactions with the microenvironment, including neurons on one side and blood vessels on the other, or to pia matter and/or the ventricular space [8]. Two hypotheses have been proposed to explain when this heterogeneity is established: one suggests that individual astrocyte fate and specific characteristics are determined early during the patterning of the neuroepithelium and, the second, that astrocyte

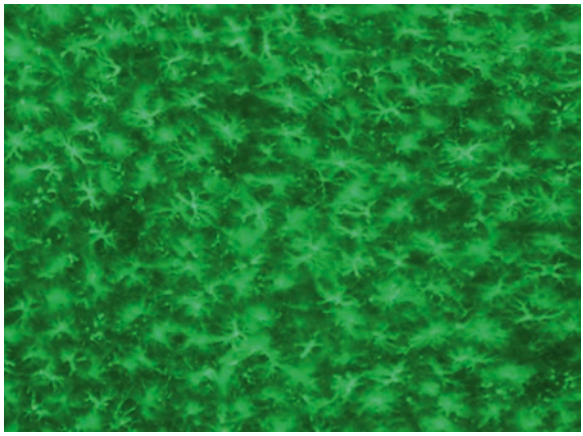

Fig. 1 Immunofluorescence staining with GFAP of mouse cerebral cortex astrocytes. (Courtesy Dr. Javier Ramos)

precursors are generated in the neuroepithelium and specific subtypes determined later, either during migration or at the site where the cells finally reside [9].

Astrocytes have at least two different origins: (i) direct from radial glial cells and (ii) indirect, from a proliferative and migratory population located in the subventricular zone [10–12]. New astrocytes may arise either from proliferation of mature astrocytes or from differentiation of progenitors. Notably, there is little evidence to indicate that mature astrocytes divide in the uninjured brain [13]. By contrast, very active proliferation is associated with scar formation following CNS injury.

Astrocytes can be visualized by immunocytochemical labeling of certain antigens restricted to these cells. Expression of glial fibrillary acidic protein (GFAP) has become the classical marker used to identify astrocytes within the CNS (Figs. 1 and 2). However, expression patterns differ across anatomical regions [14]. Moreover, other CNS-resident cells such as NG2 cells and pericytes also show GFAP positivity [15]. For this reason, several other antibodies directed against intermediate filament proteins, including either cytoplasmic or membrane markers, such as: vimentin, nestin, S100 calcium-binding protein (S100β), glutamine synthetase (GS) or glutamate/aspartate transporter (GLAST) are also commonly used to label normal and reactive astrocytes [14]. However, a significant drawback of current immunohistochemistry techniques is that no reliable markers exist to identify astrocyte subtypes, making it hard to establish whether any given behavior observed corresponds to astrocytes in general, or is characteristic of a particular subtype only.

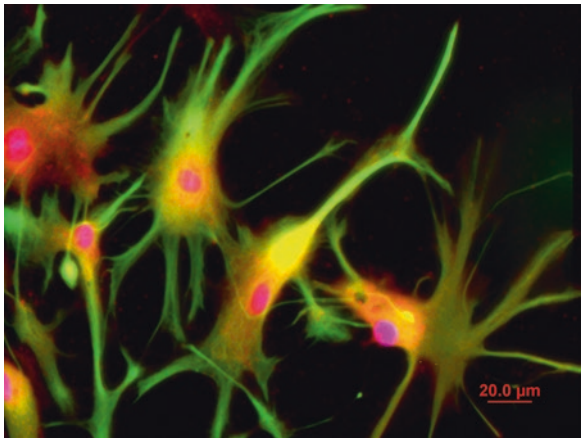

Fig. 2 Immunofluorescent staining of mouse activated astrocytes stained with GFAP (green), and TLRA4 (red). (Courtesy Dr. Javier Ramos)

Biological and Immunological Functions of Astrocytes

Biological Functions

Many key regulatory functions maintaining brain homeostasis have been linked to astrocytes. Astrocytes functionally connected to each other via gap junctions, and to oligodendrocytes via heterotypic gap junctions, form a large syncytium-like glial network. Adjacent astrocytes present homomeric gap junctions at the cytoplasmic level made up of connexins (Cx) 43 and 30, through which molecules such as K^+ and glutamate are dissipated and intracellular Ca^{2+} waves propagated [16].

Astrocytic end-feet processes play a crucial role in the formation and maintenance of the blood–brain barrier (BBB). The BBB is extremely selective and protects the brain from entry of toxic substances and influx of ions such as K^+ and Ca^{2+}, thereby regulating the extracellular environment [17]. Astrocytes also control the dynamics of cerebral blood flow, by increasing oxygen and glucose availability, thereby regulating cell metabolism as a function of changes in neuronal activity [18]. When neuronal activity levels are high, astrocytes also maintain pH levels normal within the CNS, during fluctuations in ion concentrations [19]. Astrocytes are the only cells in the CNS which store glycogen. These deposits may serve as a source of energy under conditions of hypoglycemia, during which neuron energy requirements increase [10]. Because the BBB is impermeable to many lipid-soluble molecules including cholesterol and lipoproteins, synthesis of sterols and lipoproteins by astrocytes is also key, to supply energy to other CNS cells [20]. In addition, astrocytes secrete neurotrophic factors [21] and participate in the synthesis of neurosteroids within the CNS such as allopregnanolone, estrogen, and dehydroepiandrosterone [22], which modulate neuronal excitability, promote remyelination, and dampen proinflammatory responses. Astrocytes also play an active role in both synapsis development and neuronal remodeling, regulating the plasticity of different neural circuits [23].

Roles of Astrocytes in Immune Regulations

In addition to maintaining stable physiological conditions in the CNS, astrocytes are recognized as early and highly active participants in immune responses occurring in the CNS. Innate immune cell responses to diverse stimuli are triggered by an array of pattern recognition receptors (PRRs) that bind to diverse pathogen-associated molecular patterns (PAMPs). PRRs also recognize self molecules, known as danger-associated molecule patterns (DAMPS) including heat shock proteins, double-stranded DNA, and purinergic metabolites [15]. Responses to endogenous host molecules may trigger inflammatory reactions, and therefore participate in the development of autoimmunity.

Astrocytes can mediate innate and adaptive immune responses through several mechanisms. First, they directly affect cell entry to the CNS via the BBB, by regulating the expression of adhesion molecules, particularly VCAM-1 and ICAM-1 that bind to lymphocyte receptors VLA-4 and LFA-1, respectively [24, 25]. In addition, through production of matrix metalloproteinases (MMPs) or of their inhibitors (tissue inhibitors of metalloproteinases (TIMPs), astrocytes may induce increased or decreased BBB permeability.

Second, astrocytes secrete different chemokines such as CCL-2, CCL-5, IP-10, CXCL12, and IL-8, which attract both peripheral immune cells and resident CNS cells (microglia) to lesion sites [26].

Third, astrocytes may affect the number and phenotype of T cells in the CNS. Cytokines produced by astrocytes have the potential of committing T cells to a proinflammatory phenotype (Th1 and Th17) or to a regulatory phenotype ($Foxp3^+$ or Tr1 cells). Under conditions of inflammation, astrocytes express IL-12/IL-23 as well as CD24, favoring the development of Th1 and Th17 cells in the experimental autoimmune encephalomyelitis (EAE) model, thereby affecting its severity [27, 28]. Furthermore, astrocytes drive IL-15 production, which has been observed in MS lesions, and shown to have an important role in the development of encephalitogenic activity of $CD8^+$ T cells [29]. By contrast, astrocytes can also terminate autoreactive T cell activity, through the action of Fas-L, highly expressed on astrocyte end-feet [28] or through interactions between Galectin-9 and its ligand Tim-3, expressed on Th1 and $CD8^+$ T cells [30].

Fourth, in the inflamed CNS, reactive astrocytes may contribute to B cell maturation, survival and proliferation as well as immunoglobulin production by generating B-cell activating factor (BAFF). Likewise, production of IL-6 and IL-15 also contribute to B cell survival [31].

Fifth, astrocytes modulate microglia and macrophage activity through two separate mechanisms: (i) inducing their recruitment into CNS lesions by producing chemotactic factors, and (ii) by secreting GM-CSF, M-CSF and TGF-β, which regulate microglial phagocytosis [32].

Finally, astrocytes can act as antigen-presenting cells (APCs). However, although they express MHC Class I and MHC Class II molecules and are capable of presenting myelin antigens in vitro, controversy persists over their capacity to express

costimulatory molecules such as CD40, CD80, and CD86 in vivo, particularly in humans [33, 34].

Recent studies have demonstrated that different injuries can elicit at least two types of reactive astrocytes. Based on their transcriptome profile, they have been categorized as either "A1" or "A2" [35]. This terminology parallels the "M1" and "M2" nomenclature applied to macrophages and microglia. Transcriptome analysis shows that "A1" neuroinflammatory, reactive astrocytes upregulate many genes previously shown to be destructive for the synapse, therefore exerting a "deleterious" effect. Conversely, ischemia-induced "A2" reactive astrocytes upregulate both neurotrophic factors, promoting neuronal growth and survival, and thrombospondins, promoting synapse repair, suggesting they may "contribute" to neuronal regeneration [35]. It is important to consider that in the same way that microglia have multiple simultaneous reactive profiles, "A1" and "A2" astrocyte phenotypes represent extremes of a continuous spectrum of reactive profiles. Emerging evidence has shown the importance of bidirectional communication between microglia and astrocytes [36]. Both in vitro and in vivo findings have identified a role for activated microglia in inducing "A1" astrocytes, via secretion of IL-1α, TNF-α, and complement component 1q (C1q) [35].

Figure 3 summarizes the main biological and immunological functions of astrocytes.

Neuromyelitis Optica Spectrum Disorders

Neuromyelitis optica (NMO; also known as Devic's disease) is a demyelinating disease of the CNS, preferentially affecting the optic nerve and spinal cord (Fig. 4). Detection of a highly specific and pathogenic serum antibody marker (NMO-IgG), in the serum of these patients [37], further broadened the clinical and neuro-imaging spectrum of NMO. NMO-IgG binds to aquoporin-4 (AQP-4), which is the main channel regulating water homeostasis in the CNS. In 2007, the term NMO spectrum disorders (NMOSD) was introduced to include AQP4-IgG serum-positive patients with limited initial forms of NMO, who were at risk of suffering future attacks. NMOSD is also used to encompass cerebral, diencephalic, and brainstem lesions observed in some NMO patients [38].

Aquaporins, also known as "water channels proteins", are a family of membrane proteins that facilitate trans-membrane water movement [39]. At present, 13 aquaporin isoforms are known, of which AQP4 is the predominant form expressed in rodent brain, although small amounts of AQP1 and AQP9 have also been detected [40]. There are two major isoforms of AQP4 generated by alternative gene splicing, a longer M1-AQP4 isoform and a shorter M23-AQP4 isoform [41]. Electron microscopy studies have established that AQP4 is largely confined to astrocytes and ependymal cells [42]. Microscopically, AQP4 is expressed on the perivascular end-feet of astrocytes throughout the brain, while AQP1 is predominantly found on processes and end-feet of fibrous astrocytes [43].

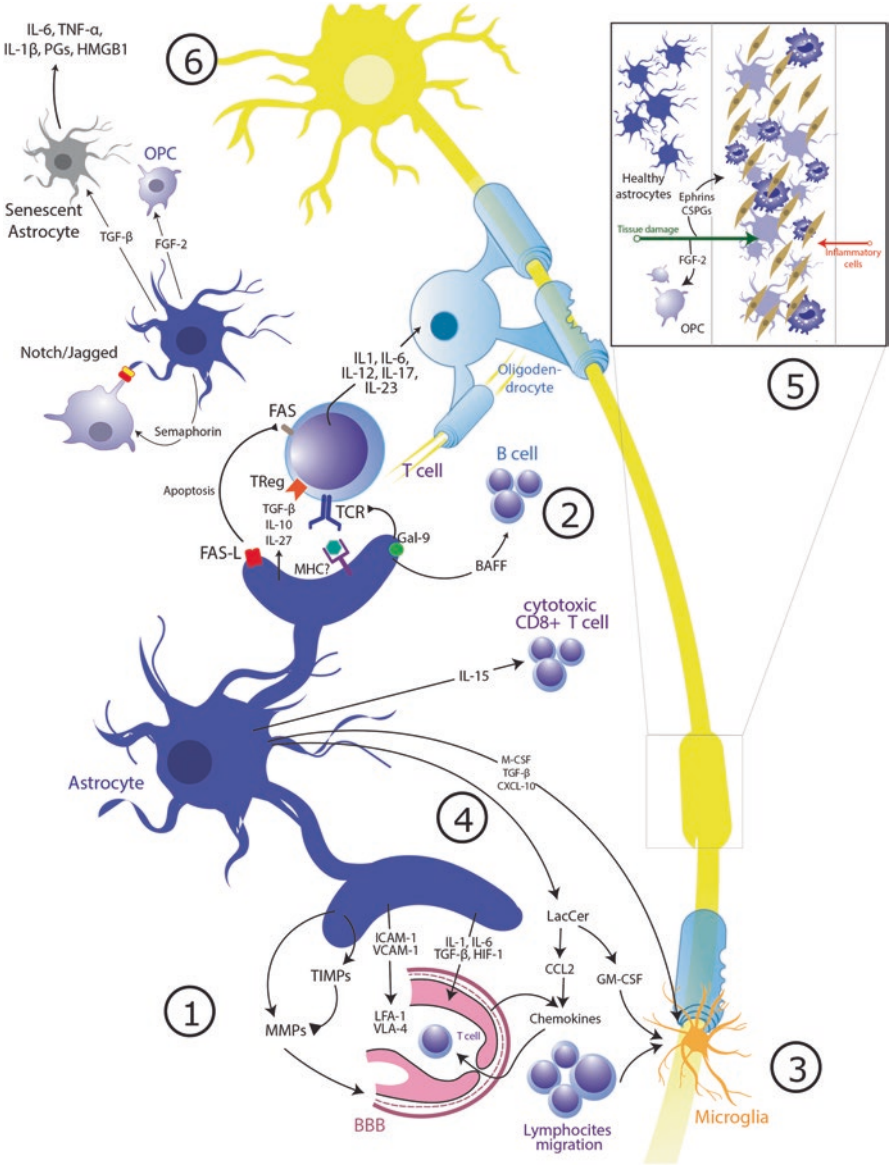

Fig. 3 Several studies have demonstrated diverse roles of astrocytes in lesion development during the course of MS. Activation of astrocytes and loss of end-feet around small vessels are early events in lesion development, associated with loss of BBB function and consequently with CNS inflammation (1). Astrocytes mediate innate immune responses through several mechanisms. They modulate cell entry into the CNS by regulating adhesion molecule expression profiles, particularly of VCAM-1 and ICAM-1 (1). Astrocytes may also affect the number and phenotype of T cells in the CNS, committing T cells to a proinflammatory or regulatory phenotype. By contrast, astrocytes may also terminate T cell response, either by induction of apoptosis, or induction of Galectin-9. Furthermore, production of IL-15 or of BAFF drives immune responses mediated by

Antibodies against AQP4 are generated in the periphery, although why this occurs is not known. Outside the CNS, AQP4 is present in distal renal tubules, gastric parietal cells, muscle fibers, and in the placenta. It is believed that antibodies enter the CNS at the level of the circumventricular organs, which do not present a classic BBB, but express high amounts of AQP4 and extensive vascularization, ideal conditions for contact between serum AQP4-IgG and AQP4-expressing astrocyte foot processes [44].

Once inside the CNS, AQP4-IgG binds to astrocytic AQP4, activating several mechanisms contributing to tissue injury. First, astrocytes produce chemokines such as CCL-5 and IL-8, which facilitate eosinophil and neutrophil infiltration, ultimately responsible for the necrosis frequently observed in advanced NMO lesions [45]. However, it is important to note that eosinophil presence is observed early in NMO lesions, prior even to onset of astrocytic injury and demyelination, suggesting an important role in initial lesion development, through mechanisms preceding chemotactic effects. Eosinophils are also essential for T cell polarization to a Th2 phenotype [46]. Pathogenic T cell generation in the CNS increases BBB permeability, amplifying the inflammatory process and increasing astrocyte destruction. Second, in regions rich in AQP4 M23 isoforms, complement activation occurs, causing further destruction of astrocytes [47]. In regions rich in M1 isoforms, AQP4 is internalized, and while activation of complement is prevented, water transport is altered, causing tissue and myelin vacuolization and/or edema in lesions [47]. Notably, AQP4-IgG may alter several neighboring or coupling components of the astrocyte membrane, like Na^+, K^+-ATPase, affecting not only water regulation but also K^+ homeostasis [48]. This in turn may modify different physiological processes such as membrane potential preservation, or voltage-gated transporter inactivation. Furthermore, AQP4 is functionally coupled to the major excitatory amino acid transporter 2 (EAAT2), and AQP4-IgG depletes astrocyte membranes of EAAT2. This prevents glutamate reuptake, which contributes to astrocyte excitotoxicity and causes oligodendrocyte death, resulting in demyelination [49].

Fig. 3 (continued) cytotoxic $CD8^+$ T cells or by B cells (2). Activated astrocytes secrete different chemokines, which attract both peripheral immune cells and microglia to MS lesions (2, 3). In the EAE model, astrocytes produce LacCer during the chronic phase, leading to induction of GM-CSF and CCL2 genes, and to subsequent microglial activation and monocyte infiltration (4). Astrocytes respond to injuries by forming a glial scar that inhibits remyelination and axonal regeneration. These effects are mediated through secretion of fibroblast growth factor-2 (FGF-2) and of inhibitory extracellular matrix (ECM) molecules, such as chondroitin sulfate proteoglycans (CSPGs) and ephrins (5). Old age adversely affects astrocyte viability and self-renewal capacity, resulting in the generation of senescent and/or dysfunctional cells, evidenced in the form of cell fragmentation (6). Senescent astrocytes appear to be in a state of chronic activation, associated with proinflammatory cytokine and prostaglandins secretion

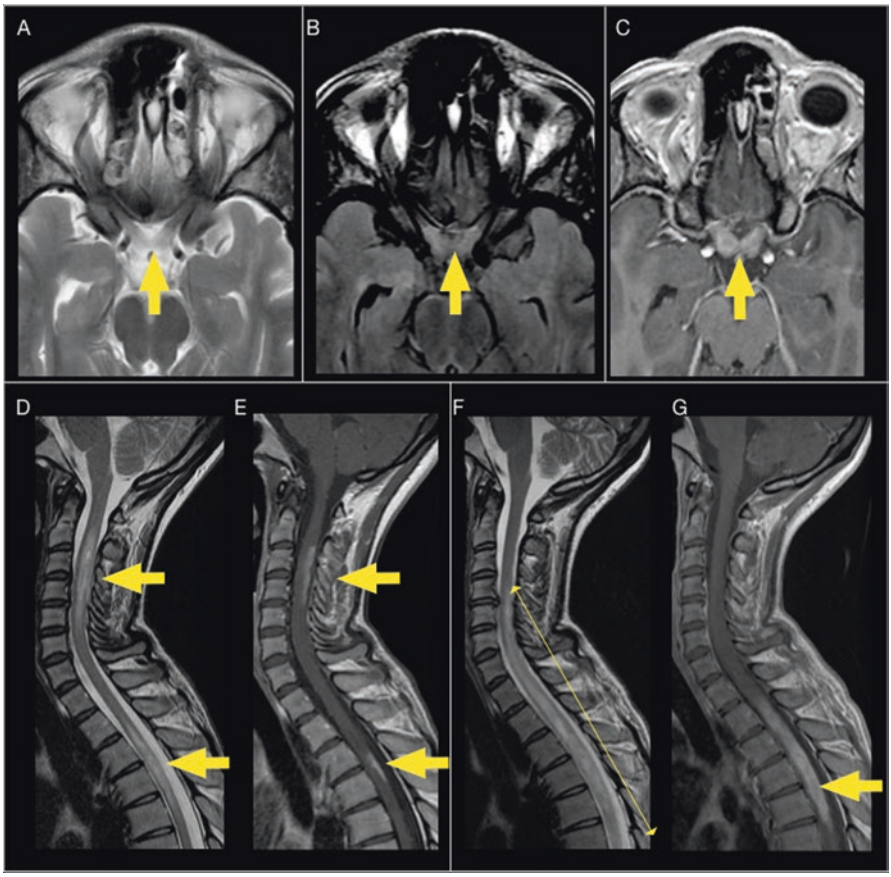

Fig. 4 (**a–c**) Twenty-five-year-old woman who developed AQP4(+) bilateral optic neuritis. (**a**) Axial T2-weighted, and (**b**) T2-FLAIR weighted, arrows are pointing to a hyperintense lesion of the chiasm and bilateral posterior optic nerve parte. (**c**) Extensive area of gadolinium-enhancement in the bilateral posterior part of the optic nerve/chiasm. (**d–g**) Forty years old woman with recurrent longitudinal extensive transverse myelitis (LETM) AQP4(+). (**d**) Sagittal T2-weighted, arrows point to a cervical and a thoracic LETM. (**e**) T1-weighted post contrast, arrows showing enhancement of same lesions. (**f**) LETM (eight segments), with extensive area of contrast enhancement (**g**)

Autoimmune Glial Fibrillary Acidic Protein Astrocytopathy

Autoimmune encephalitis and encephalomyelitis are inflammatory diseases of the CNS associated with binding of IgG antibodies to intracellular or plasma membrane neuronal cell antigens. Autoantibody detection in serum and in CSF contributes to diagnosis, allowing administration of appropriate treatment [50]. An early publication from the Mayo Clinic reported presence of autoantibodies to GFAP in patients with well-defined clinical meningoencephalomyelitis [51], which caused unique immunofluorescent staining patterns when placed on sections of mouse nervous

system. In this model, immunostaining was confined to pia, subpia, midbrain foci, periventricular areas and the rostral migratory stream. Enteric ganglia and nerves with mucosal penetrating filaments also showed prominent immunoreactive elements in the periphery. Along the spinal cord, immunoreactive filaments were prominent around the central canal and in gray matter, radiating to the pia. Identification of GFAP as the autoantigen was established using Western blot, on which a 50 kDa protein band was detected, and with mass spectroscopy. Antigen specificity was further confirmed by GFAP-transfected HEK293 cell-based assay [51, 52]. Patients with GFAP-IgGs reacted with mature (α, the predominant intermediate filament protein in adult astrocytes) and immature (δ/ε predominant in neural progenitor cells and immature astrocytes) GFAP isoforms [53].

Two publications from a single center reported on 102 patients in whom the predominant clinical manifestation in 81% of cases was either meningitis, encephalitis, myelitis, meningoencephalomyelitis, or a combination of the above [51, 52]. Their findings, subsequently confirmed by other studies [54, 55], indicated that 88% of patients presented significantly elevated white cell counts in CSF, and 54% showed elevated oligoclonal band numbers in CSF exclusively, confirming the inflammatory nature of the syndrome. Although these changes may also occur as a result of meningoencephalitis secondary to infection [56, 57] or neoplasms [51, 52], anti-GFAP antibody presence helps to distinguish between these alternative etiologies and alerts to the existence of an immune-mediated, steroid-responsive syndrome. Likewise, striking abnormalities on MRI, particularly intense periventricular radial linear enhancement, mimicking immunofluorescence-binding patterns observed in mouse brain tissue were also found [51, 52]. In some patients, there was even evidence of intrathecal antibody synthesis. Antibody testing in CSF may therefore be more sensitive than serum, as is the case with antibodies against the NMDA receptor [58] and the opposite to what has been observed for antibodies against AQP4 in MNOSD [59]. No false-positive results were detected in CSF testing of GFAP-transfected cells. All findings were subsequently confirmed in a prospective 1-year follow-up of 90 patients [56].

The relatively homogeneous neurologic spectrum ascertained in blind screening would indicate that GFAP-specific IgG seropositivity will distinguish autoimmune GFAP meningoencephalitis or meningoencephalomyelitis from other disorders commonly considered in the differential diagnosis, such as infectious, granulomatous, or inflammatory demyelinating disorders; lymphoma; carcinomatosis; and vasculitis [52].

Compelling evidence suggests that autoantibodies binding to extracellular domains of cell-surface-expressed neuronal or glial proteins, like the NMDA receptor or AQP4, have pathogenic potential [60, 61] while antibodies specific to intracellular antigens are not pathogenic, but rather surrogate markers of an underlying cytotoxic, cell-mediated autoimmune response [62]. GFAP is a cytoplasmic protein not accessible to IgG in intact glial cells. Thus, in autoimmune GFAP meningoencephalitis, GFAP-derived peptides on the plasma membrane presented by MHC Class I molecules and up-regulated on inflamed meningeal astrocytes are plausible targets for attack by cytotoxic-T cells [51]. In line with this concept, GFAP-peptide

specific cytotoxic T cells were shown to be pathogenic in a mouse model of autoimmunity [63]. Furthermore, pathology specimens from four patients revealed extensive inflammation, with prominent perivascular B and T cell infiltrates, and loss or decreased expression of GFAP antigens, further emphasizing the specificity of this autoimmune disorder [64].

As observed in other autoimmune diseases [50], patients with autoimmune GFAP meningoencephalomyelitis have other associated autoimmune disorders or autoantibodies, particularly NMDA receptor and anti-AQP4 autoimmunity [51, 52, 65]. Systemic neoplasms, most often ovarian teratomas, were found in 34% of GFAP-specific IgG-positive patients [52]. These findings contrast with an 18% tumor incidence in patients with GFAP-negative serology from the same center [66] and are consistent with a possible paraneoplastic origin of GFAP autoimmunity. In this context, cancer screening, appropriate for age, sex and risk factors is recommended for GFAP-specific IgG positive patients [51]. These observations acquire even more relevance when anti-GFAP-IgG is accompanied by both anti-NMDA receptor and anti-AQP4 antibody presence. In these circumstances, the positive predictive value observed for teratomas was high (71%) [52].

Astrocytes During Infectious Diseases of the Central Nervous System

Infectious agents can cross the BBB through several mechanisms including a paracellular route, transcytosis, receptor hijacking or infected leukocytes [67]. After entering brain parenchyma, they are met by astroglial cells. Astrocytes express several receptors for PAMPs, and can recognize different infectious agents. After infection, astroglial cells can either mount an astrogliotic defensive response, which is associated with neuroinflammation, or undergo pathological remodeling which may mediate pathological progression or generate a reservoir for infection.

Many bacteria which cause brain infections interact with astroglia, (e.g., *Streptococcus pneumonia*, *Neisseria meningitidis*, and *Borrelia burgdorferi*), triggering astroglia reactivity. This activation results in significant increase in proinflammatory cytokine secretion including IL-6, TNF-α, IL-8, CXCL-1, and CXCL-10. In this context, astroglial reactivity exerts a defensive role. Indeed, in mice lacking GFAP, spread of infection and neurological damage is exacerbated [68]. Likewise, bacterial infections can result in down-regulation of connexins, decreasing syncytial connectivity between astroglial cells. This decrease in gap junction connections may reflect a defense mechanism limiting the spread of infection [69].

Certain parasites such as *Toxoplasma gondii* show affinity for astroglial cells, and can activate them. Production of proinflammatory cytokines, particularly IL-6, limits infection progress [70]. Similarly, astrocytes can modify the course of cerebral malaria caused by *Plasmodium falciparum*. In early stages of infection, TNF-α

production by astrocytes can limit the course of the disease, while in later stages, loss of astrocytes and their protective function can cause BBB failure and spread of infection [71].

Neurotropic viral infections of the CNS often elicit serious chronic impairment. Although antiviral lymphocytes and myeloid cells recruited are generally efficient and successful in clearing viral pathogens, their actions may impact resident CNS cells, altering their functional and morphological characteristics. Astrocyte activation and its effects on the immune response have been implicated in the pathogenesis of different viral infections, as well as in the persistence of neurological complications following acute infections.

Toll like receptors (TLRs) and retinoic acid-inducible gene-like receptors (RLRs) are examples of PPRs activated by viral PAMPs. TLRs and RLR activation contribute to neuronal damage, maintaining glial activation and generating different cytokines such as type I interferons (IFNs) [72]. Type I IFNs along with other innate cytokines including IL-6, TNF-α, and IL-1β regulate BBB integrity through different mechanisms, including activation of MMP-9, and regulation of Rho GTPases [73]. Furthermore, viral proteins can alter potassium channels and glutamate uptake by astrocytes resulting in BBB breakdown and increased penetration of viruses and/or infiltrating immune cells [74].

As occurs with microglia, astrocytes participate in innate immune responses via PRR detection of viral PAMPS, Nod-like receptors, C-type lectin receptors and cytokine receptors, promoting interferon-stimulated genes (ISGs) contributing to viral infection control by limiting viral replication and inflammatory cell infiltration into the CNS. ISG expression has been associated with increased inflammatory cytokine and chemokine production in astrocytes, including TNF and CXCL-10 [75]. CXCL-10 is considered the principal driver of antibody-secreting cell production, vital for ultimate virus clearance and prevention of persistence [76]. Furthermore, CXCL-10 is an important ligand for CXCR3 on $CD8^+$ T cells, recruitment of which leads to viral infection control. Moreover, both in vitro and animal models of viral infection show increased levels of MHC Class I molecules, which are involved in $CD8^+$ T cell activation [77].

Although astrocyte activation during acute infection is critical for viral control and clearance, persistence of activation has been implicated in long-term neurological impairment after infections. Damage resulting from activated astrocytes causes: increased neuronal cell death and inhibition of proliferation, decreased neurogenesis, altered synaptic transmission, and greater synapse elimination. The main effects of viral infections in astrocytes are illustrated in Fig. 5.

Rasmussen's Encephalitis

Rasmussen's encephalitis (RE) is a rare progressive neurological disorder mostly affecting children and associated with hemispheric atrophy, focal epilepsy, cognitive deterioration and progressive neurological deficit [78] (Fig. 6). Pathology

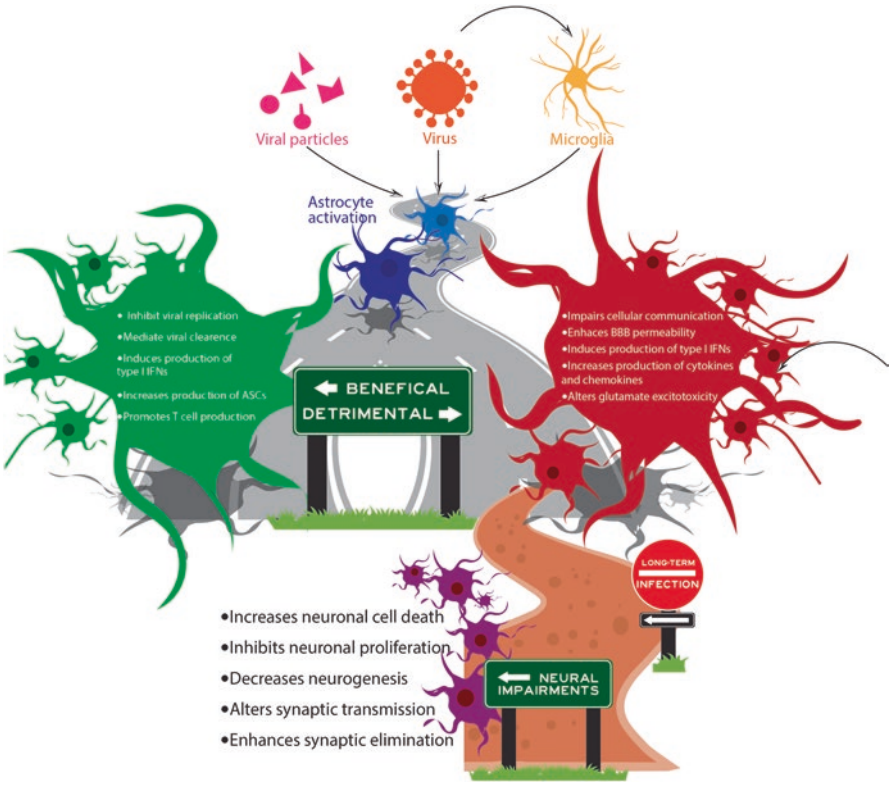

Fig. 5 During viral infections, astrocytes can exert beneficial or deleterious effects. Additionally, chronic accumulation of viral particles within astrocytes can result in neuronal impairments and long-term neurological symptoms. (Adapted from Soung and Klein [129])

findings in RE show lymphocytic infiltrates, microglial nodules, loss of neurons and astrocytes and gliosis in affected hemispheres [79]. Active brain inflammatory lesions contain a large number of T lymphocytes, recruited early within lesions, and corresponding mainly to granzyme B-containing $CD8^+$ T cells, in direct apposition to MHC Class I neurons and astrocytes [79–81]. Several pathophysiological mechanisms have been proposed for this disease. Early studies pointed to different viral infections; however, no conclusive link was ever established. Initial studies showed immunization of rabbits with Glutamate receptor 3 (GluR3) produced a disease resembling RE, and serum samples of patients with RE contained anti-GluR3 antibodies [82, 83]. These antibodies could activate GluR [84] or destroy neurons and astrocytes either by excess stimulation of the receptor or through complement-mediated death [85, 86]. Nevertheless, these findings have not been reproduced by other groups [87, 88]). Later studies demonstrated cytotoxic T cells filled with granzyme B granules present around blood vessels and on the border of lesions in which astrocytes were dead or dying [81]. The major reason explaining the presence of astrocyte-depleted lesions was finding areas specifically lacking GFAP or S100β

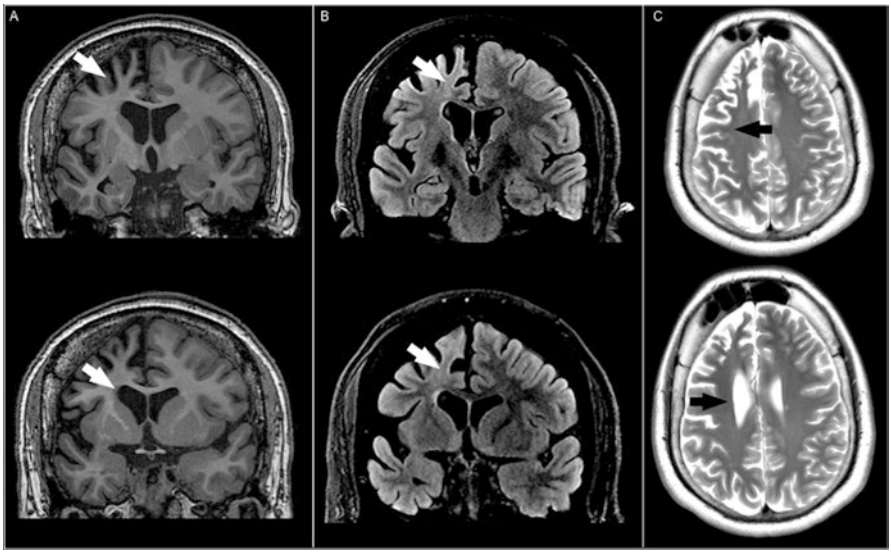

Fig. 6 Twenty-five-year-old man diagnosed with Rasmussen's encephalitis. (**a**) Coronal T1-weighted images showing right hemispheric atrophy, white arrows pointing to widened frontal sulci and right ventricle horn enlargement. (**b**) Coronal T2-flair, white arrows show hyperintensity signal in the white matter. (**c**) Axial T2-weighted, black arrows point to hemispheric atrophy and lateral horn enlargement

protein, with apoptotic astrocytes at the borders. Immunohistochemistry staining also showed astrocyte casapse3 level was upregulated in RE, and GFAP degradation, suggesting GFAP itself is a caspase 3 substrate. Oligodendrocytes and microglial cells were found in normal numbers [81]. Thus, astrocyte degeneration in RE is believed to result from a specific attack by cytotoxic T cells, ultimately causing neuronal dysfunction, seizure induction and finally enhanced neuronal death. Antigen specificity of brain infiltrating lymphocytes, however, remains unknown.

CNS specimens from RE showed clonal T-cell expansions, which support the hypothesis of an antigen-driven, T cell-mediated autoimmune process, as opposed to a random secondary immune response attraction of cells. Notably, these clonal expansions in the brain shared a $CD8^+$ T-peripheral cell repertoire, while no shared expansion was detected in the $CD4^+$ T cell compartment [87]. Interestingly, CD8+ T cell clones stay expanded for over 1 year, possibly fostering sustained survival of pathogenic $CD8^+$ T cell clones, detectable in peripheral blood of RE patients. Exposure to either a CNS-derived autoantigen(s) or persisting viral infection might be the underlying mechanism through which this occurs [87].

Recently, the role of TLRs and of HMGB1 (endogenous high-mobility group box-1) in RE pathogenesis was evaluated [89]. HMGB1 is a DNA-binding protein that participates in nucleosome formation and in the regulation of gene transcription, including proinflammatory gene expression [90, 91]. HMGB1 is secreted by macrophages, natural killer cells, myeloid dendritic cells, and astrocytes in response

to inflammatory stimuli, binding to the receptor for advanced glycation end-products (RAGE) and to TLRs [92]. Astrocytes release HMGB1, which promotes secretion of a specific subset of inflammatory factors, such as MMP-9, cyclo-oxygenase-2 and other chemokines favoring monocyte infiltration [93]. Perhaps HMGB1-TLR-RAGE represents a novel proinflammatory axis which becomes active after brain injury [93]. Further analysis of RE has shown (i) cytoplamsic translocation of HMGB1 in neurons, reactive astrocytes and reactive microglial cells, (ii) increased HGMB1 immunoreactivity in reactive astrocyte cytoplasm, and (iii) intralesional expression of RAGE, TLR4, and TLR2 in reactive astrocytes, neurons, and microglial cells. Overall, these findings provide evidence of a chronic inflammatory state involving these pathways particularly in astrocytes [89, 94].

The Role of Astrocytes in Multiple Sclerosis

Astrocyte-Mediated Exacerbation in Local Neural Inflammation

Astrocytes are increasingly recognized as cells critically contributing to the development of MS lesions. They not only participate at a late post-inflammatory stage by forming a glial scar, but are now considered early active players in lesion pathology [95]. In murine EAE, for example, astrocytes in early lesions show activation before significant immune cell infiltration in the parenchyma is observed [96]. In the same model, astrocyte activation and loss of end-feet around small vessels was recorded at the beginning of lesion development and found to be linked to loss of BBB function, subsequent CNS inflammation, and perivascular edema [95]. Likewise, uptake of damaged myelin by astrocytes, induced chemokine secretion, leading to astrocyte-mediated influx of lymphocytes also during this stage [97]. In addition, BAFF production by reactive astrocytes may contribute to MS pathogenesis by promoting B cell survival and proliferation in the CNS [98]. BAFF expression has also been described in reactive astrocytes adjacent to inflammatory cells expressing the BAFF receptor [31].

Nitric Oxide and Peroxynitrite In most areas where myelin breakdown occurs, activated astrocytes secrete compounds with toxic effects on neurons, axons and oligodendrocytes/myelin, including reactive oxygen and nitrogen species, glutamate and ATP [95]. In rodents, astrocytes stimulated with IFN-γ, IL-17, or LPS induce nitric oxide synthase (iNOS) [99]. Likewise, IL-1β as well as combined treatment with TGF-β plus IFN-γ increases the percentage of astrocyte-secreted nitric oxide (NO), which is one of the most prominent damage-inducing molecules in neurodegeneration [100]. Remarkably, the predominant contribution of NO to excitotoxicity depends on increased superoxide ion O_2^- production, which reacts with NO, forming peroxynitrite ($ONOO^-$) resulting in neuronal necrosis or apoptosis, depending on its concentration [101]. ONOO also inactivates glutamate transporters in astrocytes, directly damaging myelin, oligodendrocytes, and axons [102].

Decreased uptake of glutamate by astrocyte transporters could contribute to abnormal increase of extracellular glutamate levels, which are directly toxic to oligodendrocytes, axons and neurons [103]. Indeed, knockdown of glutamate transporters GLAST and GLT-1, using antisense oligonucleotides, causes neurotoxicity in mice [104]. Excitotoxicity is caused mainly by sustained activation of glutamate receptors and massive subsequent influx of Ca^{++} into viable neurons. Calcium, which is the primary signaling agent involved in excitotoxic injury, enters cells through various mechanisms, but the most important is entrance through ion channels coupled to NMDA receptors and AMPA/kainate glutamate receptors [105, 106]. Studies have shown glutamate can also be toxic to white matter oligodendrocytes and myelin, via mechanisms triggered by AMPA/kainate receptors [107]. Indeed, treatment with glutamate receptor antagonists protects oligodendrocytes from damage, ameliorating EAE [108]. Thus, proper function of glutamate uptake in astrocytes is critical to preclude brain cell damage, and strict regulation of extracellular glutamate levels appears to be a very promising therapeutic strategy to prevent neurodegeneration in MS.

Purine/Pyridine Metabolites Extracellular purine/pyrimidine metabolites are also exogenous signals playing important destructive/protective roles in neuron-to-glia or glia-to-glia communication within normal or injured brain tissue. They activate membrane-bound ionotropic or metabotropic P2 receptors. Astrocytes express various types of metabotropic P2Y, and ionotropic P2X purinoreceptors. Studies in MS lesions have shown preferential expression of P2X7 receptor on astrocytes [109]. Although expression is low in resting human fetal astrocytes, P2X7 is upregulated in response to IL-1β in vitro, and in reactive astrocytes around MS lesions [110]. Functionally, upregulation of P2X7 results in increased responsiveness to ATP, formation of membrane pores, and increased Ca^{++} influx [111]. Furthermore, purinergic signaling through P2X7 receptors stimulates IL-1β-induced upregulation of NO synthase [110]. Thus, activation of the P2X7 receptor in EAE can trigger toxic effects on oligodendrocytes, axons, and neurons through different mechanisms, producing in vivo lesions reminiscent of MS plaques, displaying oligodendrocyte death, demyelination, and axonal damage.

Involvements of Astrocytes in Chronic Stage

Signal Molecules In addition to their role in the initial development of lesions, astrocytes in EAE also contribute to the chronic phase of disease. Deleterious effects of astrocytes are mediated by preferential expression of 4-galactosyltransferases 5 and 6 (B4GALT5 and B4GALT6) [112]. Notably, in human MS lesions, B4GALT6 expressed by reactive astrocytes synthesizes the signaling molecule lactosylceramide (LacCer), the expression of which is significantly increased in CNS during EAE progression. Intraperitoneal administration of LacCer also exacerbates existing signs of EAE. LacCer promotes astrocyte activation in an autocrine manner, via

NF-κB and IRF-1 pathways [112], inducing GM-CSF activating microglia, and CCL2 genes causing infiltration of blood monocytes. Remarkably, inhibition or knockout of B4GALT6 in mice suppresses disease progression, local innate immunity in the CNS and neurodegeneration in EAE, and interferes with human astrocyte activation in vitro [112].

Glial Scar Astrocytes also inhibit remyelination and axon regeneration by forming glial scar. It is important to remember that although some aspects of glial reactivity are likely to be protective, others may contribute to disease progression. Scars are composed primarily of astrocytes; however, in severe lesions, interaction with other cell types including oligodendrocyte progenitor cells (OPCs) and fibromeningeal cells also occurs [113]. Several specific molecular and morphological features have been observed in astrocytes during reactive astrogliosis, both in human disease and in animal models [114], of which upregulation of GFAP, vimentin, nestin, and the less investigated synemin are hallmarks. A number of other molecules, such as TGF-α, ciliary neurotrophic factor (CNTF), LIF, and oncostatin M, trigger astrocyte activation in the rodent brain [115]. It is also conceivable that at least some of these molecules exert effects on astrocytes through other cell types such as microglia, neurons or endothelial cells.

Glial scar rigidity results in inhibition of remyelination and axonal regeneration, both negative effects mediated through different mechanisms. First, astrocytes may be detrimental for remyelination by oversecreting FGF-2 which in turn promotes oligodendrocyte progenitor cell (OPC) proliferation and survival, but prevents maturation [116]. Another molecule that appears to play an important role in preventing OPC maturation is the glycosaminoglycan, hyaluronan, which is found throughout the extracellular matrix (ECM) and in CNS white matter [117]. Hyaluronan is produced by astrocytes, and interacts with CD44, a receptor present on OPCs, astrocytes, and T cells in both MS and EAE CNS tissue [118]. Oligodendrocytes colocalizing with hyaluronan express immature phenotype, and treatment of OPCs with hyaluronan in vitro prevents maturation [119]. Astrocytes release inhibitory ECM molecules known as chondroitin sulfate proteoglycans (CSPGs) in injured areas [120]. CSPGs are a family of molecules characterized by a protein core to which highly sulfated glycosaminoglycan (GAG) chains are attached. Three types of CSPGs are preferentially localized to astrocytes in vivo: neurocan, brevican, and NG2. Neurocan (secreted) and brevican (cell bound) are the major proteoglycans produced by astrocytes in vitro, and both have been shown to inhibit axon growth, following CNS damage [121]. There is clear evidence that CSPGs are produced in excess by astrocytes when they become reactive and that CSPG inhibitory activity depends on the GAG component, as removal of GAG chains from the protein core eliminates inhibition [121, 122]. After injury, CSPGs expression is rapidly upregulated by reactive astrocytes, forming an inhibitory gradient that is highest at the center of lesions and diminishes gradually toward the periphery [123]. Meanwhile, NG2 is most often considered a marker of OPCs in adult CNS tissue. Along the borders of glial scars, NG2+ cells are found in great numbers. While many of these

cells are regarded as OPCs, evidence indicates that NG2+ cells can also become astrocytes in vivo [124]. Therefore, NG2-derived astrocytes may provide inhibitory signals, suppressing axon regeneration. In vitro studies have demonstrated that NG2 inhibits axonal growth, an inhibition that can be overcome by anti-NG2 antibody treatment [125]. CSPG-mediated inhibition could severely affect both cytoskeleton and membrane components of growth cone architecture.

Aside from CSPGs, there are other less studied inhibitory molecules expressed by astrocytes that suppress axonal growth. Ephrins (EPH) and their receptors for example are secreted by normal astrocytes and increased in MS lesions [126]. Evidence indicates that astrocyte-derived ephrins create a basal lamina around areas of injury, contributing to scar formation. Additionally, ephrins induce collapse of the axonal growth cone through activation of axon-bound EPH tyrosine-receptor kinase [127].

It is important to point out the dual role of astrocytes, not only aiding in axonal degeneration and demyelination, but also creating a permissive environment promoting remyelination (Table 1). Astrocyte impact on the pathogenesis and repair of inflammatory processes will therefore be dependent on a number of factors including timing after injury, type of lesion and surrounding microenvironment, as well as interactions with other cell types and factors affecting activation [36, 128].

Table 1 The dual role of astrocytes in the pathophysiology of multiple sclerosis

Deleterious role	Protective/remyelinating role
Recruitment of T cells, macrophages, and microglia cells to CNS lesion Chemokine production Modulation of adhesion molecules (VCAM-1 and ICAM-1) Modulation of BBB integrity (VEGF-A and HIF-1) Secretion of MMPs	Modulation of BBB integrity: secretion of TIMPs
Activation of immune response Secretion of proinflammatory cytokines (IL-1β, IL-6, IL-12, IL-17, IL-23; TNF-α) IL-15-driven cytotoxic activity of CD8+ T cells Production of BAFF contributing to B-cell dependent autoimmunity	Termination of the immune response Induction of apoptosis (Gal 9-Tim-3 interaction) Support differentiation of Treg cells (TGF-β, IL-10, IL-27) Secretion of anti-inflammatory cytokines (IL-10, TGF-β, IL-27) Microglia inhibition (Gal-1)
Inhibition of axonal regeneration Secretion of CSPGs NOGO-NgR-TROY-LINGO interactions Secretion of ephrins	Viability of neurons: secretion of NT-3, BDNF, and CNTF

(continued)

Table 1 (continued)

Deleterious role	Protective/remyelinating role
Secretion of cytotoxic factors: NO, ROS, purinergic metabolites	Prevention of excitotoxicity by glutamate uptake
Inhibition of remyelination Regulation of NG2/OPC migration (glial scar)[a] Secretion of FGF-2 prevents OPC maturation Production of semaphorin 3A produces OPC repulsion Notch/Jagged 1 interaction arrests OPC in immature state	Promotion of remyelination Glial scar formation[a] Modulation of NG2/OPCs survival, proliferation and differentiation into Oligodendrocytes (IL-6, IL-11, LIF, IGF-1, FGF-2) Production of semaphorin 3F producing OPC attraction Myelin breakdown clearance (phagocytosis)[b]
Secretion of LacCer Induces activation of microglia (GM-CSF) Induces chemotaxis of monocytes (chemokine CCL2)	
TGF-β production induces a SASP phenotype	
Release of HMGB1 (secretion of MMP-9, cyclo-oxigenase2 and chemokines	
Antigen-presenting cell function (?)[b]	

BAFF B-cell activating factor, *BBB* blood-brain-barrier, *BDNF* brain derived neurotrophic factor, *CNS* Central Nervous System, *CNTF* ciliary neurotrophic factor, *CSPGs* chondroitin sulfate proteoglycans, *FGF* fibroblast growth factor, *Gal* galectin, *GM-CSF* granulocyte-macrophage colony-stimulating factor, *HIF-1* hypoxia inducible factor-1, *HMGB1* high mobility group box-1, *ICAM-1* intercellular adhesion molecule-1, *IGF-1* insulin growth factor, *LacCer* lactosylceramide, *LIF* leukemia inhibitory factor, *MMPs* metalloproteinases, *NG2* neuron glial antigen, *NgR* NOGO receptor, *NO* nitric oxide, *NT-3* neurotrophin-3, *OPC* oligodendrocyte precursor cells, *ROS* reactive oxygen species, *SASP* senescence-associated secretory phenotype, *Tim* T cell immunoglobulin domain, *TIMPs* tissue inhibitors, *Treg* regulatory T cell, *VCAM-1* vascular adhesion molecule-1, *VEGF-A* vascular endothelial growth factor A

[a]Glial scar can impact both beneficially and detrimentally on surrounding neuronal and non-neuronal cells

[b]Whether antigen-presenting cell function and phagocytosis by astrocytes occurs in vivo under physiological conditions remains unclear

Conclusions and Future Perspectives

Astrocytes are the most abundant and heterogeneous type of glial cell in the CNS. Many key regulatory functions such as maintaining brain homeostasis as well as other specific effects are known to be carried out by astrocytes. In recent years, research has shown they play a critical role in regulating immune responses occurring within the CNS and in providing neurotrophic support. Given the pleiotropic nature of astrocyte function, it is not surprising their dysfunction contributes so importantly to neurological disease. Indeed, in some cases astrocyte dysfunction

can be the primary cause of disease. One of the most important aspects of astrocytes in relation to disease is the fact that these cells can exert protective as well as deleterious effects, i.e., completely divergent properties which may become manifest, depending on injury severity, presence or absence of other signaling molecules in the extracellular milieu, or stage of disease. Adding further complication to this context, particular molecules secreted by astrocytes may cause detrimental effects during one phase of disease and beneficial ones during others. Therefore, targeting astrocytes for treatment of neurological diseases may generate opposing, compensatory, or off-target effects on neurons, or blood vessels, dampening the sought-after benefit. Although potential scope exists for treatment of neurological disorders through astrocyte manipulation, future work will need to consider the complex interactions occurring in vivo between different astrocyte cell populations.

Acknowledgments This work was supported by an unrestricted grant from FLENI. The authors thank Dr. Ismael Calandri for preparation of some figures.

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Genetic Factors in Neuroimmune Diseases

Alessandro Didonna and Ester Cantó

Abstract Neuroimmune diseases consist of a heterogeneous group of neurological disorders characterized by aberrant immune responses against either the central or the peripheral nervous system. Unlike monogenic diseases, neuroimmune disorders do not follow Mendelian patterns of inheritance, and their genetic basis has been elusive for decades. It has been only recently that novel methodologies of analysis, such as the genome-wide association study (GWAS) paradigm, have provided the tools for deciphering the complex genetic architecture proper of these disorders. Indeed, immunogenetic and epidemiological data suggest a polygenic model of inheritance in which the interplay between multiple genetic and environmental factors is crucial for disease risk. Among the different genetic determinants, the major histocompatibility complex (*MHC*) locus accounts for the highest component of genetic risk for the vast majority of neuroimmune disorders, suggesting that dysfunctions in the antigen presentation process likely play a pivotal role in their pathophysiology. However, further studies will be necessary to fully describe the multifactorial nature of such complex diseases and discover all the molecular pathways associated with the different risk variants.

Keywords Neuroimmune diseases · Major histocompatibility complex (MHC) · Human leukocyte antigen (HLA) · Genome-wide association study (GWAS) · Autoimmunity

Introduction

Neuroimmune diseases are a complex group of demyelinating, inflammatory, parainfectious and post-infectious disorders characterized by heterogeneous pathological mechanisms and clinical manifestations, often associated with fundamental

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_11

derangement in immune regulation [1]. Through the years, a considerable effort has been put toward the elucidation of the molecular mechanisms underpinning immune-mediated neurological impairment, but a complete and coherent model of pathogenesis is still missing.

From a genetic standpoint, neuroimmune diseases do not follow Mendelian patterns of inheritance. This explains why standard genetic investigation has failed to highlight any genes convincingly associated with these neurologic conditions. Yet, family studies support the involvement of a genetic component in their etiology. In fact, the risk of developing such disorders is greater in siblings or offspring of affected individuals. Also, the evidence that disease prevalence often varies among ethnic groups further corroborates the notion that specific genetic determinants likely influence the susceptibility to disease. However, the fact that disease concordance is not absolute even in genetically identical monozygotic twins pinpoints at a multifactorial etiology with genetic and environmental factors both acting in concert to determine the total risk.

In the last decade, the advances in genomic research and in DNA analysis technologies have provided for the first time the theoretical and practical tools to start deciphering the genetic makeup of these complex disorders and ultimately gain insight into their pathophysiology. The current working model to explain their heritability is the "common variant-common disease" paradigm. According to this hypothesis, disease susceptibility is the result of the cumulative effects of multiple alleles common in the population (with minor allele frequency or MAF > 5%), each one contributing a small portion to the overall risk [2]. Remarkably, genetic variation in the major histocompatibility complex (*MHC*) locus accounts for the biggest component of the risk for virtually all neuroimmune diseases, rooting their etiology in the aberrant regulation of antigen presentation function. However, other cellular pathways are being discovered as important for pathogenesis. In this chapter, we review the current knowledge about the genetics of this class of disorders, with an emphasis on key findings which have deepened our understanding of the interactions between the nervous and the immune systems in health and disease.

Genomic Approaches for Studying Complex Diseases

In the pre-genomic era, linkage analysis represented the primary tool to map genetic loci of disease genes. This method relies on tracking across generations the co-segregation of specific genetic markers of known chromosomal location with the trait of interest in families with more than one member affected. If both the marker and the gene responsible for that particular trait are located on the same segment of DNA, their co-transmission will be proportional to the physical distance between them, allowing the mapping of the gene [3]. Different types of molecular markers have been developed through the years to saturate the genome and increase the resolution of genetic maps – restriction fragment length polymorphisms (RFLPs) were initially adopted, followed by simple sequence length polymorphisms (SSLPs),

sequence-tagged sites (STSs), and ultimately single nucleotide polymorphisms (SNPs), which represent by far the most abundant form of genetic variation in the human genome [4–7]. Family-based linkage analysis has been extremely successful in identifying genes that contribute to Mendelian disorders with high penetrance and monogenic patterns of inheritance. In contrast, this approach resulted largely inadequate to explain the genetic architecture of complex diseases, urging for new analytical tools to tackle this group of disorders. However, a paradigm shift in the field was made possible only when chip-based platforms capable of genotyping hundred thousand SNPs at affordable prices became commercially available. These new technologies indeed set the stage for the first genome-wide association studies (GWASs), leading genetic research into the genomic era.

GWAS is a hypothesis-free experimental design in which a dense set of SNPs covering the entire genome is tested for association with a specific trait in case-control cohorts of genetically unrelated subjects [8]. The possibility to analyze much larger datasets considerably increases both resolution and statistical power of the association, making GWAS screenings particularly suited for detecting small effect size disease risk loci. Since their introduction, GWASs have helped characterizing several complex diseases for which linkage studies had previously failed [9]. However, it should be noted that this method does not directly address the biological mechanisms underlying the genetic association signals. In fact, GWAS hits are usually proxy SNPs for the real causative variants. This is mainly due to the extensive linkage disequilibrium (LD) of the human genome – a phenomenon describing the non-random statistical association of alleles in physical proximity [10]. LD forces nearby SNPs to be inherited in large blocks (haplotypes), complicating the discrimination of biologically meaningful associations. On the other hand, LD allows reducing genotyping costs as fewer SNPs are needed in order to capture the genetic variation across a specific locus.

In parallel, the Human Genome Project inspired novel DNA sequencing chemistries and analysis tools. These joint efforts resulted in the unprecedented possibility to sequence the whole genome of an individual (or part of it) in a single run, by generating millions of short overlapping reads and using computers to reconstruct the original sequence [11]. In the context of complex genetic diseases, next-generation sequencing (NGS) technologies have been exploited for the analysis of genetic variation that cannot be interrogated by GWAS. This includes structural variants (such as repeated regions and indels) as well as high-impact variants with low-frequency in the population.

The Major Histocompatibility Complex

The major histocompatibility complex (*MHC*) locus encodes a large group of proteins governing both adaptive and innate immune responses in vertebrates. Initially studied for its role in transplantation, the *MHC* locus has been later found involved virtually in all autoimmune diseases. In humans, where it is named human

leukocyte antigen (*HLA*), the locus maps to the short arm of chromosome 6 (6p21.3) and contains approximately 165 protein-coding genes spanning 5 megabases (Mb). From a functional standpoint, *MHC* genes are grouped into three classes which also reflect their clustered genomic organization (Fig. 1). Genes belonging to class I and II encode cell surface glycoproteins involved in antigen presentation while class III genes specify several critical mediators of inflammation [12].

The *HLA* class I cluster comprises the highly polymorphic classical genes *HLA-A*, *HLA-B*, and *HLA-C* as well as the less polymorphic non-classical *HLA-E*, *HLA-F*, and *HLA-G*. Both classical and non-classical proteins exist as heterodimers with the invariant β2-microglobulin chain on the membrane of all nucleated cells in the organism. Their main function is to present endogenous antigens (such as peptides from virus infected or neoplastic cells) for recognition by cytotoxic $CD8^+$ T cells through T cell receptor (TCR) binding. Additionally, they can be sensed by killer cell immunoglobulin-like receptors (KIRs) on natural killer (NK) cells, and by leukocyte immunoglobulin-like receptors (LILRs) on monocytes [13]. Similar to class I, the class II cluster contains three pairs of α- and β-chain classical genes (*HLA-DP*, *HLA-DQ*, and *HLA-DR*) and two pairs of the non-classical ones (*HLA-DM* and *HLA-DO*). Unlike the ubiquitous class I molecules, classical II proteins form αβ heterodimers only on the membrane of professional antigen-presenting cells (APCs) such as B cells, macrophages, and dendritic cells, where they display pathogen-

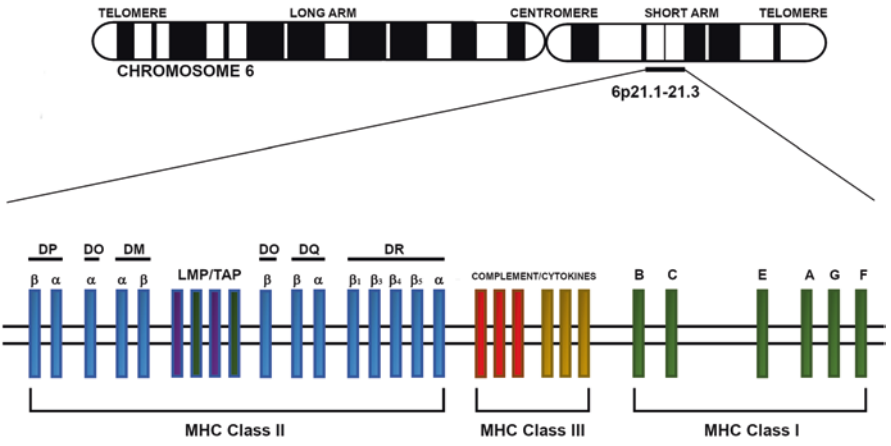

Fig. 1 Genomic structure of the human *MHC* locus. Schematic map of the principal genes forming the three *MHC* clusters on chromosome 6. The class I cluster is the most distal and comprehends the classical genes *HLA-A*, *HLA-B*, and *HLA-C* and the non-classical *HLA-E*, *HLA-F*, and *HLA-G*. The class II cluster is instead the most proximal to the centromere and includes the classical genes *HLA-DQ*, *HLA-DP*, and *HLA-DR* and the non-classical *HLA-DM* and *HLA-DO*. This region also includes genes involved in the processing and presentation of antigen to the immune system, such as low molecular weight polypeptide 2 and 7 (*LMP2* and *LMP7*) and transporter associated with antigen processing protein 1 and 2 (*TAP1* and *TAP2*). Lastly, the class III cluster is located in between the other two and contains genes encoding complement proteins (C2, C4, and factor B) as well as cytokines such as tumor necrosis factor α (TNFα) and lymphotoxin β (LTβ)

derived exogenous antigens to $CD4^+$ T cells. Non-classical HLA II molecules are not expressed on the cell surface but they instead enable peptide exchange and loading of classical II molecules in the endosomal compartment [14]. Lastly, class III cluster contains genes encoding components of the complement cascade (C2, C4A, and C4B), cytokines such as tumor necrosis factor α (TNFα), and other non-immune-related proteins such as heat shock proteins [15].

Both class I and II genes are the most polymorphic loci in the human genome, with the highest degree of variation concentrated at the level of the peptide-binding pocket. Almost 20,000 alleles have been identified to date and the number is likely to increase as the sequencing and typing technologies progress. According to the standard nomenclature, each *HLA* allele is defined by a unique number corresponding to up to four sets of digits separated by colons (such as *HLA-A∗02:04* or *HLA-DRB1∗15:03*). The first digits before the colon indicate the type, which often corresponds to the serological antigen carried by an allotype. The next two digits are used instead to list the subtypes differing in the amino acid sequence (http://hla.alleles.org/nomenclature/index.html).

Several *HLA* alleles have been found implicated in the predisposition toward the vast majority of neuroimmune diseases. Complex association patterns including intricate allelic hierarchical lineages and cis/trans haplotypic effects have been described. Protective effects have been documented as well. Hereafter, we will detail the principal genetic associations for both common and rare disorders, inside and outside the *MHC* locus.

Multiple Sclerosis

Multiple sclerosis (MS) is an autoimmune disease affecting the central nervous system (CNS), characterized by lymphocyte infiltration in the brain parenchyma, focal demyelination, gliosis, and variable grades of axonal degeneration [16]. MS clinical course typically starts with episodic inflammatory relapses followed by complete or partial recovery (relapsing-remitting MS or RR-MS). Over time, in the majority of the afflicted individuals, it evolves into a progressive phase dominated by irreversible deterioration of both motor and cognitive functions as a consequence of neurodegenerative processes (secondary progressive MS or SP-MS). However, up to 15% of MS patients show a progressive course from disease onset, without experiencing initial relapses and remissions (primary progressive MS or PP-MS) [17].

MS affects almost 2.5 million of people worldwide and represents the first cause of neurological disability among young adults, with women being more affected than men. Its prevalence varies with geography and ancestry. People of northern European descent living in northern Europe and North America show the highest susceptibility to the disease, with a lifetime risk of approximately 0.1–0.2%. In contrast, MS is less common in Asian countries and native populations across the Americas and Oceania, and almost not existent in African blacks [18]. Similar to other autoimmune diseases, cases of MS cluster in families [19]. Siblings of affected

individuals have 2–4% risk of developing the disease while the risk in monozygotic twins increases up to 30%; spouses and adoptees hold instead the same risk of general population [20–22]. Altogether, this epidemiological evidence undisputedly recognizes the genetic basis of the disease and provides a solid rationale for genetic research in MS.

HLA Alleles in MS

The *MHC* locus represents the most prominent genetic determinant connected to MS susceptibility. The first evidence of association was found in the 1970s, when the class I alleles *HLA-A∗03* and *HLA-B∗07* were demonstrated to be enriched in MS patient lymphocytes by means of serological methods [23, 24]. Subsequent investigations have independently confirmed the *HLA* association in either RR-MS or PP-MS cohorts with various sample sizes. Moreover, improvements in *HLA* typing eventually revealed that the class I alleles initially identified were part of an extended haplotype in which the class II *HLA-DRB1* locus is the pivotal signal [25]. In particular, the allele *HLA-DRB1∗15:01* shows the strongest association in European populations, with an average odds ratio (OR, a common statistical measure of effect size) of 3.08. The association with *HLA-DRB1∗15:01* explains up to 10.5% of the genetic variance underlying risk and follows an additive model, with clear dose responses to 0, 1, and 2 copies of the risk allele [26].

In addition to *HLA-DRB1∗15:01*, other *HLA* alleles have been proposed through the years for association with MS risk. However, a systematic investigation across the region has remained challenging due to the complex structure of the *MHC* locus and the pervasive LD. It was only through the coordinated efforts of multiple research groups worldwide that the fine mapping of the association within the whole *MHC* region was possible. For example, a study of the International Multiple Sclerosis Genetics Consortium (IMSGC) using GWAS SNP data from 5,091 cases and 9,595 controls identified 11 statistically independent signals in the region: four risk (*HLA-DRB1∗15:01*, *HLA-DRB1∗03:01*, *HLA-DRB1∗13:03*, *HLA-DRB1∗04:04*) and two protective alleles (*HLA-DRB1∗04:01* and *HLA-DRB1∗14:01*) were mapped to the *HLA-DRB1* locus; one risk allele to the *HLA-DPB1* locus (*HLA-DPB1∗03:01*); one protective allele to the *HLA-A* locus (*HLA-A∗02:01*); one risk and one protective allele to the *HLA-B* locus (*HLA-B∗37:01* and *HLA-B∗38:01*, respectively); and one tagging a region in the class III cluster in between MHC class I polypeptide-related sequence B (*MICB*) and leukocyte-specific transcript 1 (*LST1*) genes [27]. A follow-up study highlighted two additional risk alleles (*HLA-DRB1∗08:01* and *HLA-DQB1∗03:02*) and two novel protective associations (*HLA-B∗44:02* and *HLA-B∗55:01*). Moreover, the first evidence of pairwise epistatic interactions was reported between the class II alleles *HLA-DQA1∗01:01–HLA-DRB1∗15:01* and *HLA-DQB1∗03:01–HLA-DQB1∗03:02* [28]. More recently, the last GWAS meta-analysis performed by the IMSGC on 47,351 MS subjects and

68,284 controls, confirmed the prior *HLA* variants and extended the association map to uncover a total of 32 statistically independent effects within the *MHC* locus [29].

The vast majority of MS genetic research has been carried out on cohorts of European ancestry, given the higher incidence of the disease in this ethnicity and the relative facility to collect larger datasets. However, a number of studies have been conducted on other ethnic groups. Despite their lower statistical power, such investigations have been important to gain insights into *HLA* risk variants specific for those populations. For instance, *HLA-DRB1∗04:05* is associated in Japanese population with a clinical variant of MS characterized by earlier age of onset and reduced severity [30]. *HLA-DRB1∗15:01* is still the top risk allele in Japanese individuals without *HLA-DRB1∗04:05* as well as in Han Chinese population [31]. The allele *HLA-DRB1∗09* instead confers protection in both Japanese and Chinese populations [32, 33]. In African Americans, *HLA-DRB1∗15:03* and *HLA-DRB1∗04:05* represent secondary risk variants after *HLA-DRB1∗15:01* whereas *HLA-DRB1∗11:01* and *HLA-DRB1∗04:01* are protective alleles along with class I *HLA-A∗02* [34, 35].

Non-MHC Variants in MS

The GWAS paradigm has been instrumental not only to refine MS association with specific *HLA* alleles, but also to identify risk variants outside the *MHC* locus. In the pre-GWAS era, although several loci showed a suggestive association with the disease, none of them reached formal statistical significance. Only in 2007, in concomitance with the first GWAS conducted by the IMSGC on 931 family trios (patients with MS and unaffected parents), the first two non-*MHC* loci exceeded the genome-wide threshold set *a priori* to $P < 10^{-8}$. They were the previously reported interleukin-7 receptor α (*IL7RA*) locus and a newly identified region containing the interleukin-2 receptor α (*IL2RA*) gene, both displaying odd ratios around 1.2 [36]. After these encouraging results, several GWASs and meta-analyses with increasing numbers of subjects have been carried out, allowing the detection of progressively smaller effects. Among them, the collaborative 2011 GWAS between the IMSGC and the Welcome Trust Case Control Consortium 2 (WTCCC2) employed nearly 10,000 cases and extended the list of genome-wide significant MS loci to 52 [26]. The subsequent study adopted instead a custom genotype array design and detected additional 48 risk variants, screening over 80,000 cases [37]. The most recent and largest meta-analysis from the IMSGC included over 40,000 MS patients and was able to identify 200 autosomal risk variants outside the *MHC* and one chromosome X variant, with ORs as small as 1.05 [29]. Functional annotation of the genes mapping at the susceptibility loci has revealed a significant overrepresentation in immunologically relevant genes. This confirms that MS is, at its core, a disease of the immune system.

Altogether, the *HLA* and non-*HLA* variants so far identified account only for 20–30% of the total heritability, while a substantial part of it still remains unex-

plained. This so-called "missing heritability" may be due to gene–gene and gene–environment interactions or to rare variants with high penetrance. In this regard, a recent whole-exome sequencing effort by the IMSGC on 32,367 MS cases and 36,012 controls detected seven low-frequency coding variants in six genes outside the *MHC* locus: galactosylceramidase (*GALC*), tyrosine kinase 2 (*TYK2*), perforin 1 (*PRF1*), interferon-inducible double-stranded RNA-dependent protein kinase activator A (*PRKRA*), NLR family pyrin domain containing 8 (*NLRP8*), and histone deacetylase 7 (*HDAC7*) – collectively, these rare variants explain as much as 5% of MS heritability [38].

Functional Interpretation of MS Risk Loci

The first mechanistic explanation for MS genetic susceptibility was the discovery that the risk SNP rs6897932 alters the levels of soluble and membrane-bound isoforms of interleukin-7 receptor α by disrupting a splicing acceptor site in exon 6 of *IL7RA* gene [39]. A similar mechanism was later described for the risk SNP rs1800693 which drives the skipping of exon 6 in the gene encoding tumor necrosis factor receptor super family 1A (*TNFRSF1A*). In this case, the alternative splicing event produces a novel soluble form of the receptor with the ability to block TNFα signaling in immune cells – a phenomenon that resembles the exacerbating effects of TNF-blocking drugs on MS course [40].

A few other risk variants have been convincingly characterized at the functional level [41]. Overall, this experimental evidence suggests a "transcriptional hypothesis" where MS risk is principally driven by aberrant expression of a restricted set of genes. However, our picture of the molecular mechanisms underlying MS susceptibility still remains superficial and further investigation is needed to fill the gaps in our knowledge about the specific biological functions affected upon disease.

Neuromyelitis Optica

Neuromyelitis optica (NMO) is an autoimmune inflammatory disorder of the CNS characterized by severe demyelination and axonal loss that predominantly target the optic nerve and spinal cord. Initially classified as a subtype of MS, it has been considered a separate entity for a number of years. One of the main features that separates NMO from MS is the presence of circulating IgG1 autoantibodies against aquaporin 4 (AQP4) in about 80% of NMO patients [42]. AQP4 is a water channel that is primarily expressed in the CNS by astrocytes, but it is also found in other organs such as the kidneys and the gastrointestinal and respiratory tracts. NMO affects principally women (85% of the cases) and its prevalence varies between 0.52 and 10 for each 100,000 individuals in the population, depending on their ethnicity and geographical region [43, 44]. In general, people of Northern European are

considered at a lower risk as compared to people from West Indies and Asia. Around 3% of patients show a familial form of the disease [45].

HLA Alleles in NMO

Although phenotypically similar, the genetic overlap between NMO and MS is limited, corroborating the notion that they are two different diseases. For instance, the main MS risk allele *HLA DRB1∗15:01* was not found associated with NMO susceptibility [46]. Other *HLA* alleles have been instead linked to disease risk in different ethnic groups. In populations with higher NMO prevalence such as Japanese and southern Han Chinese, *HLA-DPB1∗05:01* seems to be the main predisposing allele while *HLA-DRB1∗09:01* confers protection [33, 47, 48]. Other risk alleles in Asian populations include *HLA-DPB1∗03:01*, *HLA-DRB1∗12* and *HLA-DRB1∗16:02* [32, 33, 49]. Conversely, *HLA-DRB1∗03:01* is the main risk allele in Europeans, Brazilian mulattos, and Afro-Caribbeans [50]. A recent GWAS in a European ancestry cohort has replicated the association with *HLA-DRB1∗03:01* but only in those patients positive for AQP4 autoantibodies [51]. The same study also identified a SNP downstream *HLA-DQA1* (rs28383224) as the top signal in all NMO patients, regardless of their anti-AQP4 antibody status [51].

Non-MHC Alleles in NMO

Given the central role of AQP4 autoantibodies in NMO pathogenesis, the possible contribution of genetic variation in the *AQP4* gene has been subjected to intense investigation. However, no *AQP4* genetic variants have been convincingly associated with NMO susceptibility [52, 53]. Subsequent studies have instead highlighted suggestive association with several immune-related genes including cluster of differentiation 58 (*CD58*), Fc receptor-like 3 (*FCRL3*), interleukin 7 (*IL7*), interleukin 7 receptor alpha (*IL7RA*) and interleukin 17 (*IL17*) [54–57]. Additionally, a recent whole-genome sequencing screening has identified a significant association in AQP4 antibody-seropositive NMO patients with copy number variation (CNV) in the genomic locus annotated for the complement component 4 (*C4*) genes [51].

Behçet's Syndrome

Behçet's syndrome (BS) is a rare systemic vasculitis disorder of unknown origin. It typically manifests with acute inflammatory attacks resulting in oral and genital ulcers as well as skin and ocular lesions. Less frequently, the disease also targets the cardiovascular system, the gastrointestinal tract, and the central nervous system.

Neuro-BS occurs in 5–10% of patients and can affect brainstem or basal ganglia causing meningoencephalitis (parenchymal subtype) or can be characterized by cerebral venous thromboses (non-parenchymal subtype) [58]. The prevalence of BS varies between less than 1 and 20 for each 100,000 individuals depending on the geographical region, with a significant presence in Asian countries 30°–40° north of the Equator from the Mediterranean to Japan [59]. Familial aggregation of BS has been reported in 18% of a Turkish cohort, especially in juvenile patients [60].

HLA Alleles in BS

The class I HLA-B51 antigen represents the stronger genetic marker for BS and its association with disease has been consistently confirmed in independent studies across different ethnic groups. Its contribution to overall BS risk has been estimated to be around 20% with an OR of 5.78 [61]. Among all the known HLA-B51 subtypes, *HLA-B∗51:01* has been found the major risk allele associated with BS in all the populations studied [62–65]. Although the association of HLA-B51 with BS has been known since the 1970s, the exact mechanism by which it contributes to the disease is still not fully clear. However, studies in transgenic animals suggest this molecule may be responsible for the neutrophil hyperfunction observed in BS patients [66].

Several other *HLA* alleles have been proposed as additional risk factors for BS but their level of confidence is generally low due to small sample size and the strong LD with *HLA-B∗51:01*. Only approaches encompassing conditioning analysis or employing *HLA-B∗51* non-carrier cohorts have been able to identify truly independent associations. They include either risk alleles such as *HLA-A∗26*, *HLA-B∗15*, *HLA-B∗27* and *HLA-B∗57* or protective alleles like *HLA-A∗03* and *HLA-B∗49* [67, 68].

Non-HLA Alleles in BS

A number of GWASs have been conducted on BS, resulting in several hits outside the *MHC* region. Among them, the interleukin 10 (*IL10*) locus and the intergenic region between interleukin 23 receptor (*IL23R*) and interleukin 12 receptor β2 (*IL12RB2*) have been cross-validated in two independent large GWASs in distinct populations [69, 70]. Notably, these genes encode cytokines involved in the regulation of the immune response – IL-23 is a heterodimeric cytokine that promotes Th17 cell development and induces the production of proinflammatory cytokines; IL10 is instead a potent suppressor of inflammatory cytokine production and inhibits T cell and NK cell activation [71, 72].

A subsequent GWAS in 1,209 Turkish BS patients identified novel associations with loci containing the genes C-C motif chemokine receptor 1 (*CCR1*), signal transducer and activator of transcription 4 (*STAT4*), and killer cell lectin-like receptor C4 (*KLRC4*). Two exonic SNPs in the endoplasmic reticulum aminopeptidase 1 (*ERAP1*) genes were also found associated with BS risk following a recessive model. Interestingly, an epistatic interaction between *ERAP1* and *HLA-B∗51* was also detected [73]. Additional risk loci have been reported in smaller GWASs or candidate gene studies such as the GTPases of immunity-associated protein (*GIMAP*), TNF alpha-induced protein 3 (*TNFAIP3*), fucosyltransferase 2 (*FUT2*), and interleukin 12 subunit alpha (*IL12A*) [74–77]. Rare nonsynonymous risk variants in *IL23R*, toll-like receptor 4 (*TLR4*), nucleotide-binding oligomerization domain-containing protein 2 (*NOD2*), and familial Mediterranean fever gene (*MEFV*) have been also identified in a recent targeted resequencing effort [78]. Although the molecular mechanisms by which these genes cause BS are not well understood, their functions suggest that both the innate and adaptive arms of the immune system concur to disease susceptibility.

Guillain–Barré Syndrome

Guillain–Barré syndrome (GBS) is a peripheral neuropathy that causes acute neuromuscular failure. It is usually preceded by an infection that later evolves into an autoimmune response targeting peripheral nerves and their spinal roots, characterized by autoantibody production, complement activation as well as T cell reactivity [79]. This phenomenon suggests that molecular mimicry between microbial and nerve antigens may be the driving force of GBS. The prevalence of the disease calculated in the latest studies on European populations is estimated to be between 0.8 and 1.9 for each 100,000 persons, with rare reports of familial cases [80, 81].

HLA Alleles in GBS

Considering that infections represent a plausible environmental trigger for GBS, the involvement of the *MHC* locus in the etiology of the disease has been early postulated and many efforts have been undertaken to experimentally verify this connection. However, the vast majority of the studies have dismissed any associations between *HLA* alleles and GBS risk, including a recent meta-analysis which investigated the role of *HLA-DQB1* polymorphisms in Caucasian and Asian populations [82]. Although *HLA-DQB1* is not a general susceptibility factor, it may influence disease trajectory since higher frequencies of the *HLA-DQB1∗05:01* allele have been reported in a cluster of GBS patients with a severe phenotype [83]. A subgroup-specific effect was also reported for *HLA-DRB1∗07:01* as this allele seems to increase the risk of GBS only in those patients with preceding infection [40].

Interestingly, associations with the KIR inhibitory pairs KIR-2DL2/HLA-C2 and KIR-3DL1/HLA-Bw4-T have been also described, supporting a possible contribution of NK cell dysregulation to GBS pathogenesis [84].

Non-HLA Alleles in GBS

In addition to *HLA*, other immune genes have been evaluated for possible associations with GBS susceptibility. Among them, one of the most investigated is TNFα – a cytokine involved in the response against bacterial infections. Several studies have shown that a polymorphism in the *TNF* promoter region (−308 G > A) increases TNFα serum levels and is associated with GBS risk [85, 86]. These results have been confirmed in a recent meta-analysis [87]. Following a similar mechanism, two exonic polymorphisms enhancing the expression of interleukin 17 (*IL17*) and intercellular adhesion molecule 1 (*ICAM1*) genes have been also found associated with GBS [88].

Another class of immune molecules that aroused interest in the context of GBS are the immunoglobulin G Fc gamma receptors (FcγRs). These proteins are essential for host defense as they confer potent cellular effector functions to the specificity of IgGs [89]. Three FcγR subclasses exhibit biallelic functional polymorphisms that determine efficacy of the cellular immune response (FcγRIIa: R131-H131; FcγRIIIa: 158V-158F; FcγRIIIb: NA1-NA2). A number of studies suggested that the R131-H131 polymorphism could be involved in GBS risk and severity [90–92]. However, subsequent investigations were not able to confirm such association [93].

Myasthenia Gravis

Myasthenia gravis (MG) is a relatively rare autoimmune disease characterized by fluctuating weakness and fatigability of skeletal and extraocular muscles. MG shows a high level of clinical and biological heterogeneity, usually associated with the presence of autoantibodies against proteins of the post-synaptic membrane at the neuromuscular junction. The majority of these autoantibodies are directed against the acetylcholine receptor (AChR) (present in about 85% of patients), but muscle-specific kinase (MuSK) and lipoprotein-related protein 4 (LRP4) are also targets [94]. The prevalence of MG is around 3–30 per 100,000 individuals, depending on the geographic region [95]. A few cases of familial MG have been documented as well [96, 97].

HLA Alleles in MG

The association between the *MHC* locus and MG is well established and several class I and II *HLA* alleles have been found enriched in different patient subgroups, possibly explaining the wide spectrum of MG clinical manifestations. Among them, the extended haplotype HLA-A1~HLA-B8~HLA-DR3~HLA-DQ2 (also known as ancestral haplotype AH8.1) has been consistently associated with early-onset MG (EOMG) in Caucasians, while the haplotype HLA-B7~HLA-DR2 seems to be more prevalent in the MG patients with late onset (LOMG) [98, 99]. More recent investigations in different European cohorts have also highlighted *HLA-DRB1∗15:01*, *HLA-DQB1∗05:02*, and *HLA-DRB1∗16* as additional risk alleles for LOMG; *HLA-DRB1∗13:01* was instead shown to exert protective effects for both EOMG and LOMG [100, 101]. Notably, the HLA-DR3 and HLA-DR7 serotypes exert opposite effects on MG phenotypes – the former being positively associated with EOMG and negatively with LOMG and the latter showing the opposite trend [102]. In Asian populations, an association with HLA-DR9 was reported in both Chinese and Japanese cohorts [103, 104].

Different GWASs have been carried out on GM, confirming known associations and finding novel ones. The first GWAS on 649 EOMG cases of European descent replicated *HLA-B∗08* as the strongest risk allele for this subgroup [105]. A subsequent GWAS by the same group on LOMG cases found three new different peaks of association corresponding to *MHC* class II, *HLA-A* and *MHC* class III, while *HLA-DQA1∗05:01* resulted protective [106]. In contrast, a GWAS on both subgroups identified two independent signals at *HLA-DQA1* for EOMG and LOMG, respectively [107].

In addition to disease onset, antibody-specific GM subgroups have shown distinct genetic patterns as well. The most consistent finding is the association between the *HLA-DQ5* serotype and MuSK antibody-positive MG patients, which was replicated in four independent studies [108–111]. An association with *HLA-DRB1∗14* and *HLA-DRB1∗15* was also detected in a Turkish cohort of anti-MuSK MG patients [110].

Non-HLA Alleles in MG

A number of associations outside the *MHC* locus have been identified through candidate gene studies and GWASs, some offering clues to address MG pathogenesis. For example, the first GWAS in EOMG confirmed the previous association with a missense coding variant (rs2476601) in protein tyrosine phosphatase non-receptor type 22 (*PTPN22*) and reported the new association with another nonsynonymous SNP (rs2233290) in the TNFAIP3-interacting protein 1 (*TNIP1*) gene [105]. Interestingly, PTPN22 exerts immunomodulatory functions and the same missense SNP found in MG is associated with susceptibility to multiple autoimmune diseases

[112]. TNIP1 works instead as an inhibitor of the NFκB pathway and its ablation also leads to autoimmunity in animal models [113].

The GWAS on LOMG cases detected with genome-wide significance only the zinc finger and BTB domain containing 10 (*ZBTB10*) locus and reported suggestive associations for *PTPN22* and TNF receptor superfamily member 11a (*TNFRSF11A*) [106]. The latter was independently replicated in the GWAS on combined MG subgroups, which also identified cytotoxic T lymphocyte-associated 4 (*CTLA4*) as an additional risk locus for all MG patients [107]. Other candidate genes associated with MG susceptibility include galectin 1 (*LGALS1*), fork head/winged-helix transcription factor 3 (*FOXP3*), cathepsin L2 (*CTSL2*), *TNF*, interleukin 4 receptor α (*IL4RA*), interleukin 10 (*IL10*), interleukin receptor 2β (*ILR2B*), and muscle nicotinic acetylcholine receptor α-subunit (*CHRNA1*) [114–120]. For several of these genes, risk variants map to their promoter regions, suggesting transcriptional dysregulation as a possible mechanism of action. This is the case of *CHRNA1*, a gene of particular interest as its product is the major target of MG autoantibodies. The risk SNP rs16862847 (−478 A > G) was indeed demonstrated to alter *CHRNA1* promoter activity by disrupting the binding site for the transcription factor interferon regulatory factor 8 (IRF8) [121].

Other Neuroimmune Diseases

In this section, we will group those neuroimmune disorders whose genetic characterization is less developed. This is mainly due to their rarity and to the limited number of available studies. They include autoimmune encephalitis (AE), chronic inflammatory demyelinating polyneuropathy (CIDP), and stiff-man syndrome (SMS).

Genetics of AE

AE refers to an inflammatory process of the CNS mediated by autoantibodies against neuronal epitopes. The most common targets of AE autoantibodies include cell surface and synaptic proteins such as leucine-rich glioma-inactivated 1 (LGI1) or the NMDA and AMPA receptors [122]. On the basis of a few small studies showing enrichment in specific *HLA* alleles, a complex etiology has been postulated for this class of brain disorders [123, 124]. However, no clear environmental or genetic risk factors have been associated with AE risk for long time. Only recently, a GWAS with 1,194 controls and 150 patients with anti-NMDAR or anti-LGI1 AE has identified for the first time a strong association with the class II haplotype *HLA-DRB1∗07:01~HLA-DQA1∗02:01~HLA-DQB1∗02:02* for anti-LGI1 AE, and with the class I allele *HLA-B∗07:02* for anti-NMDAR AE. Outside the *MHC* region, suggestive associations were also found for anti-LGI1 AE with the

doublecortin-like kinase 2 (*DCLK2*) locus and with a cluster of zinc-finger genes of unclear biological function [125].

Genetics of CIDP

CIDP is a neuropathy closely related to GBS, which is often considered its acute counterpart. CIDP etiology is autoimmune, mediated by humoral and cellular responses against Schwann cell antigens in the peripheral nervous system (PNS). However, unlike GBS, no infectious agent has been consistently linked with initiation of disease. In its typical form, CIDP clinical manifestation is characterized by a slowly progressive onset and symmetrical, sensorimotor involvement [126].

A number of candidate genes have been studied for their possible involvement in CIDP pathology. Early investigations reported an enrichment in the HLA-Aw30, HLA-B8, and HLA-Dw3 antigens among CIDP patients [127]. Significant associations were also highlighted with *HLA-DRB1∗13* in a cohort of Tunisian patients, and with *HLA-DRB1∗15* in CIDP patients positive for anti-neurofascin 155 (NF155) antibodies [128, 129]. Interestingly, a recent study has found that the frequency of the combination between KIR-3DL1 and its ligand HLA-Bw4l is greater in CIPD than controls, raising the possibility of NK cell function being an important factor for disease pathogenesis [130]. In addition to *HLA* genes, an association with the alpha-1 antitrypsin (*SERPINA1*) type M3 allele has been reported [131].

Genetics of SMS

SMS is a rare disorder of the CNS characterized by progressive rigidity of the truncal muscles, superimposed spasms, and an exquisite sensitivity to external stimuli. SPS pathology is associated with the presence of high titers of autoantibodies against the enzyme glutamic acid decarboxylase (GAD), which cause synaptic dysfunctions in GABAergic neurons by blocking GABA synthesis [132]. An early study highlighted a strong association with class II allele *HLA-DQB1∗02:01* while the presence of *HLA-DQB1∗06:02* or other *HLA-DQB1∗06* alleles may be associated with a reduced prevalence of diabetes among patients with SMS [133].

Classic Neurodegenerative Diseases

The GWAS unbiased approach has radically changed our understanding of several brain disorders which had been thought for decades to be purely neurodegenerative. This is the case of common proteinopathies caused by the misfolding and aggregation of specific proteins such as Aβ and tau in Alzheimer's disease (AD), or

α-synuclein in Parkinson's disease (PD) [134]. In addition to non-*MHC* loci, a number of *HLA* alleles have been found associated with these diseases, pinpointing at the possible involvement of immune-mediated processes in their pathophysiology.

HLA Alleles in AD

Although suggestive associations with different class I and II *HLA* alleles (such as *HLA-A∗02* and *HLA-DRB1∗03*) had been proposed through the years, the first convincing connection between the *MHC* locus and AD was obtained in a meta-analysis of four GWASs of European ancestry adding a total of 17,008 cases and 37,154 controls. A noncoding variant (rs9271192) tagging the *HLA-DRB5–HLA-DRB1* locus was found associated with late-onset AD risk, and the same results were independently replicated in the Northern Han Chinese population [135, 136]. Interestingly, this SNP may function as an expression quantitative trait locus (eQTL) for *HLA* in the brain. In fact, rs9271192 is associated with increased levels of *HLA-DRB1* transcript in the temporal cortex and cerebellum [137]. More recently, a fine-mapping effort on 5,919 AD cases and 5,771 controls of European origin has identified the extended haplotype *HLA-A∗03:01~HLA-B∗07:02~HLA-DRB1∗15:01~HLA-DQA1∗01:02~HLA-DQB1∗06:02* as a risk factor for patients negative for apolipoprotein (*APOE*) ε4 allele – *APOE* is the principal susceptibility locus in AD. Additionally, the class I haplotype *HLA-A∗03:01~HLA-B∗07:02* was found correlated with higher cerebrospinal fluid (CSF) amyloid levels, while the class II haplotype *HLA-DRB1∗15:01~HLA-DQA1∗01:02~HLA-DQB1∗06:02* was associated with faster cognitive decline in a dose-dependent fashion [138].

HLA Alleles in PD

The first study to highlight an association between the *MHC* locus and PD risk was a GWAS on 2,000 cases and 1,986 unaffected controls of European ancestry which reported a noncoding variant (rs3129882) in *HLA-DRA* as the top peak [139]. A subsequent meta-analysis of five GWASs on American and European cohorts identified another significant association with an intronic SNP (rs32588205) in *HLA-DRB5* [140]. Attempts to replicate these findings have generated conflicting results, possibly reflecting the less polymorphic nature of *HLA-DRA* and the low diffusion of the *HLA-DRB5* allele across the population. In this regard, a case-control study on ethnically homogeneous French cohorts found an association with rs660895 within the highly polymorphic *HLA-DRB1*, which was argued to represent a more legitimate candidate than the previously reported alleles [141]. However, a more recent investigation on the structural and regulatory variants in the *MHC* locus has shown that rs3129882 and the closely linked SNPs rs9268515 and rs2395163

remained significant irrespective of *HLA* alleles. Considering that rs3129882 and rs2395163 are both eQTLs for *HLA-DR* and *HLA-DQ*, the authors suggested that *HLA* gene expression might influence PD pathology [142].

Concluding Remarks and Future Perspectives

A decade of GWASs has proven the efficacy of this methodology in decoding the complex genetic architecture of several immune-mediated neurological diseases. A number of genetic loci, inside and outside the *MHC* region, are now known to be associated with disease susceptibility or disease trajectory (Table 1). Coordinated efforts involving multiple research groups worldwide and the creation of multicenter consortia have been instrumental for collecting sufficiently large datasets to reach adequate statistical power and analyze the genome with increasing resolution. In the future, it will be important to extend such winning strategy also to the investigation of the rarer disorders, which traditionally suffer from limited sample size.

In parallel, it will be crucial to translate this growing amount of genetic data into biologically meaningful information. However, interpreting GWAS statistical associations at the functional level is not an easy task and necessarily requires an experimental follow-up. This is because, in addition to the confounding effects of LD, the vast majority of GWAS hits map to the regulatory regions of the genome (promoters, enhancers, silencers, and other transcription factor binding sites), which are often located several Mb away from their real targets. As clearly seen in MS, most risk variants are believed to trigger disease by affecting the expression of determined genes rather than damaging the functionality of their protein products.

To pursue this aim, it will be first necessary to refine the association within established risk loci in order to identify the most plausible candidate risk variants for subsequent functional studies. Fine-mapping efforts employing batteries of genetic markers saturating the regions of interest will serve the scope. In addition, transancestral studies exploring the association in population characterized by different LD patterns could be extremely informative to narrow down the association signals. In recent times, with the accumulation of publicly available datasets from "omics" screenings, systems biology approaches have been also adopted to gain functional insights into genetic associations. One of the first attempts in this direction took advantage of large protein interaction networks (PIN), showing that the proteins encoded by genes mapping at MS risk loci are more likely to physically interact as they belong to the same or related pathways [143]. More sophisticated approaches integrate functional data coming from different sources in order to score all the SNPs in a given locus for their regulatory potential and identify their most biologically plausible targets. In this regard, there is a constant need of novel analytical methods with the capability of handling multiple layers of big data with increasing size.

Lastly, a new generation of in vivo models is required to validate the best candidate variants in a complex biological environment. To this end, the most recent tools

Table 1 Principal genetic associations with neuroimmune diseases

Disease	*HLA* alleles	Non-*MHC* loci	References
Multiple sclerosis (MS)	**Risk:** *HLA-DRB1∗15:01*, *HLA-DRB1∗03:01*, *HLA-DRB1∗13:03*, *HLA-DRB1∗04:04*, *HLA-DRB1∗08:01*, *HLA-DPB1∗03:01*, *HLA-DQB1∗03:02*, *HLA-B∗37:01*, *HLA-DRB1∗04:05, HLA-DRB1∗15:03* **Protective:** *HLA-A∗02:01*, *HLA-B∗44:02*, *HLA-B∗55:01*, *HLA-B∗38:01*, *HLA-DRB1∗09*, *HLA-DRB1∗11:01*, *HLA-DRB1∗04:01*, *HLA-DRB1∗14:01*	**Risk:** >200 variants including *IL7RA* and *IL2RA*	[26, 27, 29, 30, 32–37]
Neuromyelitis optica (NMO)	**Risk:** *HLA-DPB1∗05:01*, *HLA-DPB1∗03:01*, *HLA-DRB1∗12*, *HLA-DRB1∗16:02*, *HLA-DRB1∗03:01*, *HLA-DQA1* **Protective:** *HLA-DRB1∗09:01*	**Risk:** *CD58*, *FCRL3*, *IL7*, *IL7R*, *IL17*	[32, 33, 47–50, 54–57]
Behçet's syndrome (BS)	**Risk:** *HLA-B∗51:01*, *HLA-A∗26*, *HLA-B∗15*, *HLA-B∗27*, *HLA-B∗57* **Protective:** *HLA-A∗03*, *HLA-B∗49*	**Risk:** *IL10*, intergenic region between *IL23R* and *IL12R*, *CCR1*, *STAT4*, *KLRC4*, *ERAP1*, *GIMAP*, *TNFAIP3*, *FUT2*, *IL12A*	[62–65, 67–70, 73–77]
Guillain–Barré syndrome (GBS)	**Risk:** *HLA-DRB1∗07:01*	**Risk:** *TNF*, *IL17*, *ICAM1*	[40, 85–88]
Myasthenia gravis (MG)	**Risk:** *HLA-B∗08*, HLA-A1~HLA-B8~HLA-DR3~HLA-DQ2 haplotype, *HLA-DRB1∗15:01*, *HLA-DQB1∗05:02*, *HLA-DRB1∗16*, HLA-B7~HLA-DR2 haplotype, *HLA-A*, *HLA-DQA1* **Protective:** *HLA-DRB1∗13:01*, *HLA-DQA1∗05:01*	**Risk:** *PTDN22*, *TNIP1*, *TNFRSF11A*, *ZBTB10*, *CTLA4*, *LGALS1*, *TNF*, *FOXP3*, *CTSL2*, *IL4RA*, *IL10*, *ILR2B*, *CHRNA1*	[98–101, 105–107, 114–120]
Autoimmune encephalitis (AE)	**Risk:** *HLA-DRB1∗07:01~HLA-DQA1∗02:01~HLA-DQB1∗02:02* haplotype, *HLA-B∗07:02*	**Risk:** *DCLK2*	[125]
Chronic inflammatory demyelinating polyneuropathy (CIDP)	**Risk:** HLA-Aw30, HLA-B8, HLA-Dw3, *HLA-DRB1∗13*, *HLA-DRB1∗15*	**Risk:** *SERPINA1*	[127–129, 131]

(continued)

Table 1 (continued)

Disease	*HLA* alleles	Non-*MHC* loci	References
Stiff-man syndrome (SMS)	**Risk:** *HLA-DQB1∗02:01*	–	[133]
Alzheimer's disease (AD)	**Risk:** *HLA-DRB5–HLA-DRB1* locus, *HLA-A∗03:01~HLA-B∗07:02~HLA-DRB1∗15:01~HLA-DQA1∗01:02~HLA-DQB1∗06:02* haplotype	**Risk:** *ABCA7*, *APOE*, *BIN1*, *CLU*, *CR1*, *CD2AP*, *EPHA1*, *MS4A6A–MS4A4E*, *PICALM*, *INPP5D*, *MEF2C*, *NME8*, *ZCWPW1*, *PTK2B*, *CELF1*, *SORL1*, *FERMT2*, *SLC24A4*, *CASS4*	[135, 138]
Parkinson's disease (PD)	**Risk:** *HLA-DRA*, *HLA-DRB5*	**Risk:** *MAPT*, *SNCA*, *BST1*, *GAK*, *LRRK2*, *ACMSD*, *STK39*, *MCCC1–LAMP3*, *SYT11*, *CCDC62–HIP1R*	[139, 140]

for genomic editing such as the CRISPR/Cas9 technology have provided unprecedented opportunities to model human diseases, allowing the precise modification of the host genome at the single nucleotide level as well as the humanization of whole genomic fragments [144]. Single or multiple variants can now be routinely inserted in almost any genetic background and their functional impact can be assessed in more physiological conditions as compared to traditional transgenic models. The possibility to introduce long sequences of exogenous DNA into the recipient genome also facilitates the study of long-range effects of putative regulatory variants.

In summary, after decades of unsuccessful attempts, we have finally started characterizing the genetic factors underlying neuroimmune diseases, fueled by new methods of genetic investigation. In the post-genomic era, the greatest challenge will be to fit such genetic associations into a coherent biological framework. This will allow a deeper understanding of the cellular pathways that are dysregulated upon disease and will likely provide novel targets for developing more effective therapeutic strategies.

Acknowledgments AD holds a Marilyn Hilton Award for Innovation in MS Research from the Conrad N. Hilton Foundation (#17323). The work was also supported by FISM-Fondazione Italiana Sclerosi Multipla Senior Research Fellowships Cod. 2014/B/1 and Cod. 2017/B/3 to AD and financed or co-financed with the "5 per mille" public funding.

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General Principles of Immunotherapy in Neurological Diseases

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Abstract Immunotherapy has changed the prognosis and outcome of many neuroimmunological diseases. In neurology, Immunotherapy aims to suppress or modulate the immune system. Due to the heterogeneity of immunological diseases, not all of the therapeutics are equally suited for different disorders. Thus, it is of importance to understand the pathophysiological and immunological background of the underlying disease as well as the mode of action of the various therapeutic agents. The aim of this chapter is to give an overview on the fundamental principles of the immune system. Selected diseases are presented to show the variety of the respective pathophysiological concepts. The last part describes the immunotherapies that are frequently used in neuroimmunological diseases with the mode of action and effects on the immune system. This chapter is addressed to clinicians who treat neuroimmunological disorders and shall facilitate the decision to find the right drug for the right patient.

Keywords Neuroimmunology · Immunotherapy · Innate immune system · Adaptive immune system · Hematopoietic stem cell transplantation · Anti-CD20 antibodies · Alemtuzumab · Glucocorticosteroids · Mycophenolate mofetil · Azathioprine · Cyclophosphamide · Tocilizumab · Cladribine · Dimethyl fumarate · Teriflunomide · IVIg · Plasma exchange · Interferon beta · Glatiramer acetate · Tocilizumab · Natalizumab

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_12

Introduction

Scientific progress in neurology and especially in neuroimmunology has revolutionized the management of neuroimmunological disorders. The emergence of immunotherapeutics has changed the course of disorders. Whereas in general, immunotherapy is defined as a therapy that aims to stimulate or suppress the immune system to fight infection or disease [1], in neuroimmunological diseases immunotherapy aims to reduce the inflammatory processes. Glucocorticosteroids [GCS] have been used in autoimmune diseases and in neuroimmunological diseases since the 1940s of the last century [2]. Since the approval of interferon beta [INF-ß] for the treatment of multiple sclerosis [MS] almost 25 years ago [3], tremendous changes have been observed in the treatment regimes used in neuroimmunological disorders [4]. Nowadays, a dozen drugs are approved for the treatment of MS [5]. With the emergence of agents with specific points of targets, a shift from unspecific to specific therapy has been achieved. Currently, these immunotherapeutics are used in plenty of neuroimmunological disorders [4].

At the beginning of this chapter, the fundamentals of the immune system will be briefly summarized (part I). Part II will deal with autoimmunity and basic pathophysiological pathways in selected disorders. Part III will summarize approved immunotherapeutics that are utilized in neurology. Fundamental knowledge of the immune system is required to understand the principles of immunotherapy. The chapter will close with concluding remarks.

Part I. Fundamentals of the Immune System

Two basic strategies in the immune defense can be differentiated: the innate and the adaptive immune response. The innate system is established at birth, whereas the adaptive immune system will “adapt” over time to various pathogens. The innate immune system is fast reacting. Its defense spectrum is broad, but not specific. This is in contrast to the adaptive immune system with specific defense mechanisms [6].

Main components of the innate immune system are barriers (skin, gastrointestinal tract, respiratory airways, nasopharynx, eyes, blood-brain barrier [BBB], and mucous membranes), phagocytes, dendritic cells, mast cells, natural killer [NK] cells, and complement [7]. Components of the innate immune system derive from a common myeloid progenitor [colony-forming unit – granulocyte, erythrocyte, monocyte/macrophage, megakaryocyte (CFU-GEMM) [8]] with exception of NK cells [9], whereas cells of the adaptive immune system have a common lymphoid progenitor as the origin. Progenitor cells are ascending from hematopoietic stem cells in the bone marrow. Figure 1 gives an overview of the development of the various immune cells. The differentiation of B cells takes place in the bursa equivalent and secondary lymphoid organs such as gut-associated lymphoid tissue [GALT] in humans, whereas T cells egress and differentiate in the thymus [6].

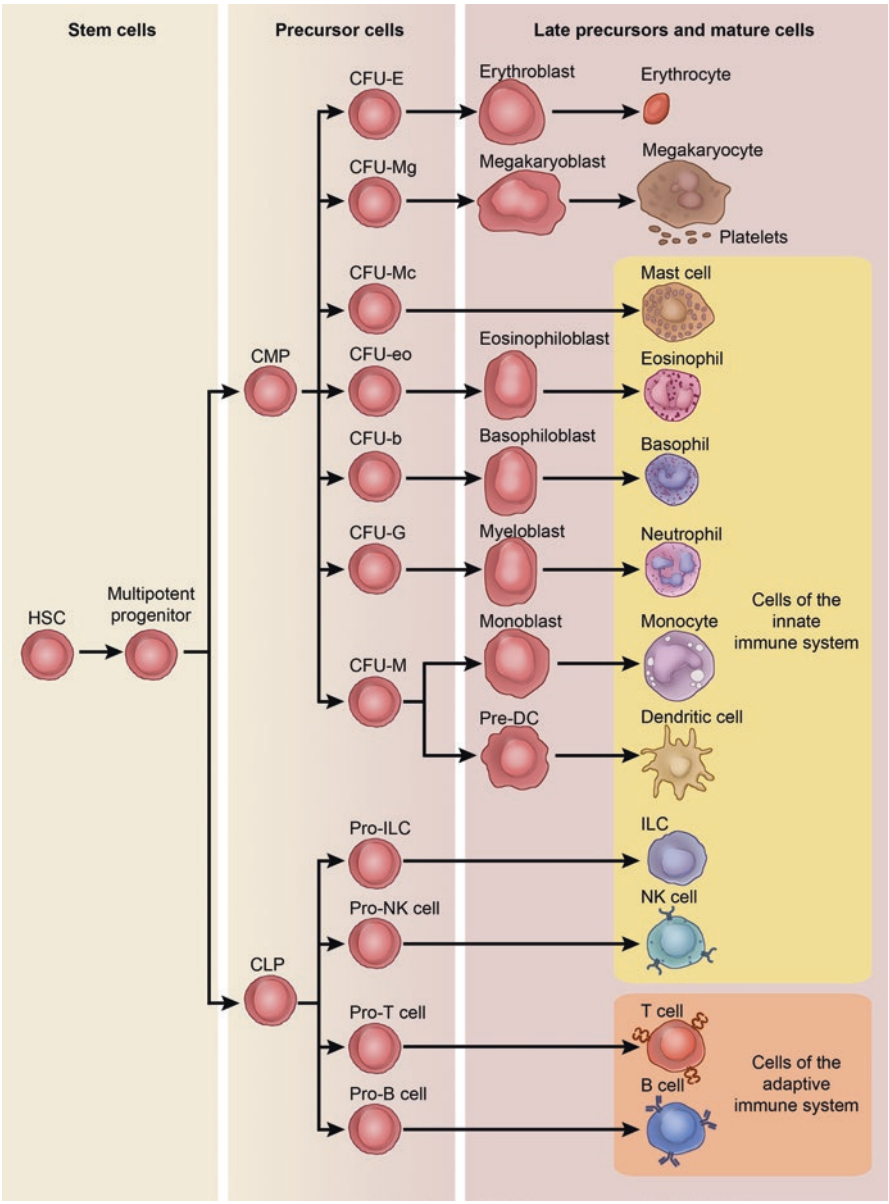

Fig. 1 Overview of the development of immune cells. Hematopoietic stem cells (HSC) differentiate, via multipotent progenitor cells, into progenitors of the myeloid lineage (CMP) and progenitors of the lymphoid lineage (CLP). The progenitor cells specialize further into mature cells. Most cells of the innate immune system belong to the myeloid lineage, but innate lymphoid cells (ILC) and natural killer (NK) cells arise from the lymphoid lineage

Besides its tasks as the first line of defense, the innate immune system activates the adaptive immune system. NK cells are part of the innate immune system [10], whereas NKT cells possess as type I NKT cells properties of the innate immune system and as type II NKT cells share similarities with adaptive immune cells [11]. The complement system needs further activation by cytokines and antibodies [10]. Thus, it is a good example for the links between innate and adaptive immune system.

The first step for provoking the adaptive immune system is the activation of antigen-presenting cells [APC]. Lymphocytes are activated by antigens leading to clones of antigen-specific cells that are responsible for the acquired immunity. Through rearrangements of B and T cell receptors [BCR and TCR] and antibodies, the acquired immune system creates an enormous diversity [12]. One of the main tasks of the acquired immune system is the differentiation of self- from non-self. In response to pathogenic (non-self) antigens, specific antibodies will be released from B cells: IgA, IgD, IgE, IgG, and IgM. Antibodies are circulating in the blood of patients, thus they are part of the humoral immune system [6]. Those antibodies neutralize pathogens, catalyze phagocytosis, and activate complement. T cells belong to the cell-mediated defense strategy. Cytotoxic T cells (CD8+ T cells) recognize pathogenic antigens expressed on infected cells, leading to their cell death. Other parts are T helper cells [Th] (CD4+ T cells). Th cells are activated by antigen presentation via APCs. Th cells do not have phagocytic or cytolytic characteristics, still they mediate the immune response and activate the further immune cascade [13]. Whereas Th1 and Th2 cells might activate the immune system, regulatory T cells [Tregs] suppress the immune system. Th cells are an important player in balancing the immune reaction. T and B cells release cytokines leading to the activation of macrophages, and further activation of B or T cells. Cytokines, however, belong to the humoral immune response. After the activation of B and T cells, memory B and T cells will evolve [14]. This is the consequence of clonal expansion of lymphocytes. Memory cells outlive the pathogenic antigen and are responsible for the specific and fast response to a second exposure to the antigen. This is called immunological memory [7]. Figures 2 and 3 give an overview of the development of B and T cells.

Both immune strategies comprise parts of the cell-mediated and humoral immunity. In the literature, the classification as humoral immune response is based on the release of interleukins and cytokines. Components of the cell-mediated immune system are phagocytes (macrophages, granulocytes, dendritic cells), lymphoid cells, mast cells, NK cells, and T cells. The humoral immune response comprises the complement system and interleukins released from components of the innate immune or adaptive system. The cell-mediated immune system comprises especially the T cells, whereas the humoral immunity is particularly based on B cells [6, 7]. Figure 4 gives an overview of the different T cell subtypes, their major immune functions, and respective proliferation/activation pathways [15–21].

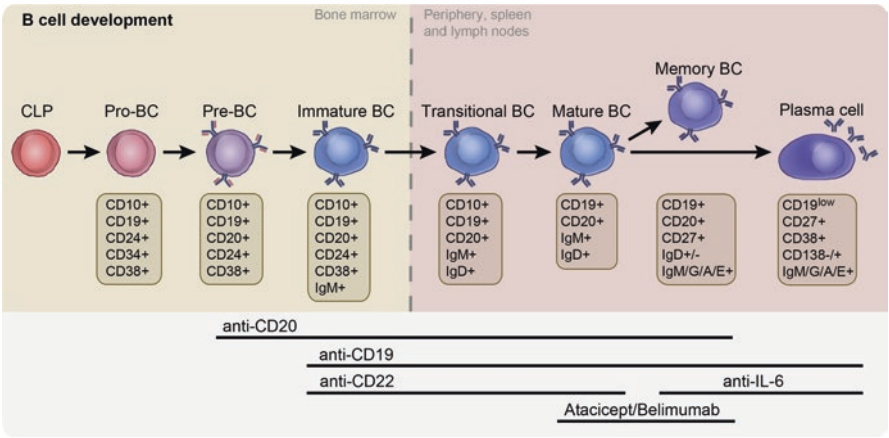

Fig. 2 Overview of the development of B cells and of therapeutic agents that target B cells in distinct stages. In the bone marrow, common lymphoid progenitor cells (CLP) differentiate into pro-B cells (Pro-BC). Once they express a precursor B cell receptor, they become pre-BC. The first IgM-expressing B cells are called immature BC. They migrate into the periphery, spleen or lymph nodes as transitional BC. Upon antigen recognition with the help of T cells and cytokine signaling, mature BC proliferate and differentiate into memory BC or plasma cells

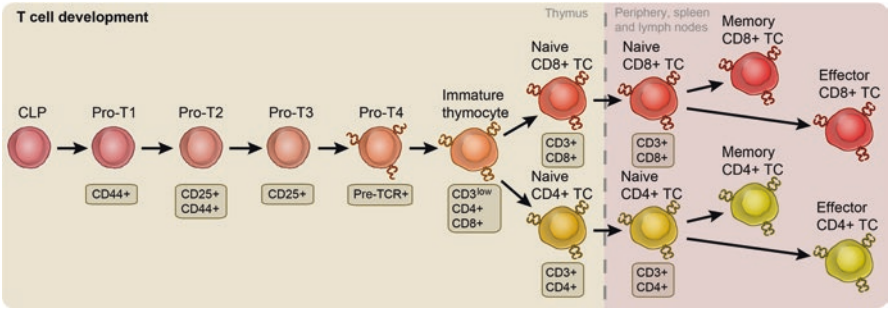

Fig. 3 Overview of the development of T cells. In the thymus, common lymphoid progenitor cells (CLP) develop, via various progenitor-T cell stages, into double-positive immature thymocytes that express the T cell receptor. Upon differentiation into single-positive cells, either naive CD8+ TC or naive CD4+ TC, they migrate into the periphery, spleen, and lymph nodes. Upon recognition of major histocompatibility complex-bound antigens and cytokine signaling, naive cells specialize into memory TC and effector TC

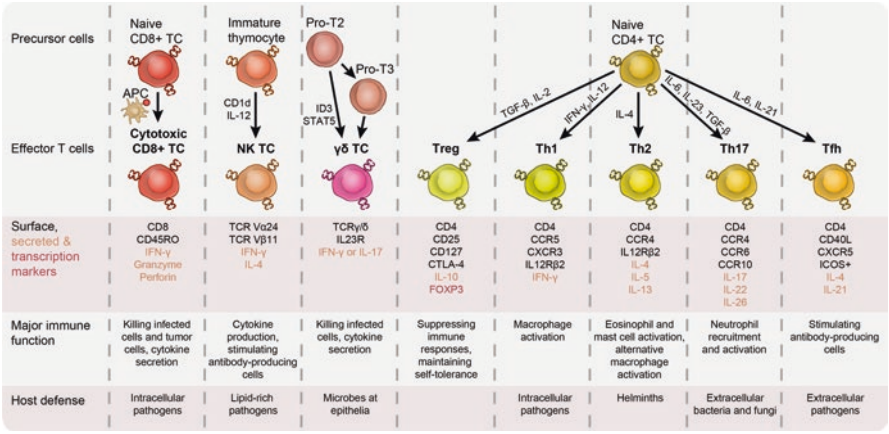

Fig. 4 Overview of effector T cells and their major immune functions. Most T cell (TC) subsets that are distinguished develop from naive CD4+ or CD8+ TC that express the αβ T cell receptor (TCR). Natural killer (NK) TC develop from an earlier stage of αβ TCR-positive thymocytes. TC that express the γδ TCR are called γδ TC and they develop separately from the Pro-T2 or Pro-T3 stages. The various effector TC are characterized by distinct markers, and they mediate distinct immune functions in the defense against different pathogens. Tfh, follicular helper T cells

Part II. Pathophysiology of Neuroimmunological Disorders

In neuroimmunological diseases, autoimmune processes are mostly responsible for the reaction of the immune system against the host. In this case, the immune system attacks components of the central nervous system [CNS] or peripheral nervous system [PNS]. For most of the diseases, e.g., MS, the cause is not clear, but in others, an underlying malignancy, as in paraneoplastic disorders, is the triggering event. An imbalance of regulatory and inflammatory cells is the basis for the autoimmune reaction.

Autoimmunity

Autoimmunity refers to an immune response against cells and tissue of the host (autoantigens). Immunological tolerance is a key factor for that self-antigens are recognized and no immunological response is triggered. Central and peripheral tolerance prevents reactivity to self-antigens. If an immune system is tolerant to antigens, infections may occur, but in case of impaired tolerance, autoimmunity can be the consequence. Tregs, especially CD4 + CD25 + Foxp3+ Tregs, control autoreactive T and B cells [12]. Besides Tregs, also mesenchymal stem cells [MSC], interleukin-10 [IL], and cytotoxic T-lymphocyte-associated antigen 4 [CTLA-4] are players in the elimination of autoreactive cells [22]. Furthermore, intracellular

communication networks can be dysfunctional and result in excessive cytokine, chemokine, and antibody release [12]. Each of these checkpoints is of importance in autoimmunity and a breakdown of these mechanisms will result in autoimmune processes [23].

Central immune tolerance prevents naive T cells with self-antigens and recognizes autoreactive cells. Autoreactive T cells are normally eliminated by negative selection in the thymus, and also in lymphoid organs. Likewise, autoreactive B cells are eliminated in the spleen or in lymph nodes [12, 24]. Autoreactive T cells that are not eliminated in the thymus are subject to further mechanisms to protect from self-reactivity (by deletion, suppression, and clonal anergy). The importance of the autoimmune regulator (AIRE) in the elimination of self-reactive T cells has been elucidated. Mutations in the AIRE gene can result in autoimmune disorders [25].

Immune reactions in autoimmune diseases are specific (adaptive immune system). Autoimmunity may be organ-specific or systemic (like diabetes mellitus on the one side, and systemic lupus erythematodes on the other side). Environmental and genetic factors as well as the sex influence the susceptibility to autoimmune diseases. Genetic variations seem to be important in autoimmune processes that relate to immunoglobulins, TCRs, and the major histocompatibility complex [MHC]. Especially the HLA-alleles HLA B27, DR2, DR3, DR4, and DR5 are crucial [26]. However, discordance rates between monozygotic twins vary from 11% in SLE to 67% for psoriasis [27]. Classical studies in MS showed that the concordance rate is between 14% [28] and 50% [29]. A French study showed that up to 30% of twins (independent of zygosity) have signs of clinical or radiological affection [30]. More recent studies suggest that the familial risk for MS is lower than previously assumed [31]. The fact that the concordance rate is about 20% in a recent study [32] shows that besides genetic factors, environmental factors including infections, diet, stress, aging, drugs, and chemicals trigger epigenetic alterations [27].

Furthermore, infections, caused by viruses – of special importance are viruses in the genesis of autoimmunity and for the development and maintanance of autoreactive cells [33–35] – and bacteria, hormones, cytokines and genetic variations might trigger autoimmune responses. Epitope spreading, bystander activation, and polyclonal activation of B and T cells are of importance in this context [36]. The association of the immune response to self-antigens may be caused by pathogens by molecular mimicry. An immune response to antigens might be sustained due to molecular mimicry with similarities between exogenous and endogenous epitopes [12]. Self-reacting B and T cells that are not eliminated by apoptosis (non-self) can cause immune (self) reactions. Autoimmune diseases show abnormal clonally expanded T cells and antibody production; thus, an antigen-driven process is assumed. Self-antigens and exogenous antigens that are able to induce autoimmune processes are of utmost interest.

Some organs are privileged regions: the BBB protects the CNS from the migration of autoreactive lymphocytes. The CNS is one of these privileged regions, but by far not absolutely privileged [37]. Antigens may migrate to the CNS and induce an immune reaction. Some of the epitopes that may induce an immune reaction within the CNS have been investigated in animal models, e.g., experimental autoimmune

encephalitis [EAE]. Myelin-derived antigens such as myelin basic protein [MBP] and myelin oligodendrocyte glycoprotein induce EAE. Their pathogenic role in human disease has been well established [24, 38]. Damage to host cells is the consequence of autoreactive antibodies, complement reactions, or local inflammations.

Immune tolerance also plays a key factor in pregnancy. Alloimmunity, an immune response to non-self-antigens from members of the same species, can occur if immune tolerance is lost during pregnancy. This might explain why the disease course for most autoimmune diseases is different during pregnancy [39, 40]. To give an overview on the broad range of neuroimmunological disorders, we present five distinct disorders with varying pathomechanisms.

Multiple Sclerosis

MS is an immune-mediated and neurodegenerative disease. More women than men are affected. The etiology of MS is not elucidated although environmental factors, such as infections, and genetic factors are discussed [41]. An autoimmune genesis is assumed. Autoreactive T and B cells escape from negative selection in the thymus [42, 43]. In the lymph node, Th cells are activated by APC in the presence of autoantigens or similar non-self-antigens. The activated T cells egress the lymph nodes and circulate in the peripheral blood [44]. Integrins facilitate cell-matrix-adhesion as well as the rolling and entering of leukocytes through the inflamed endothelium of the BBB [45]. Autoreactive lymphocytes are thus able to penetrate the BBB, enter the CNS, and trigger inflammatory reactions. Cytokines and chemokines are released. Autoreactive cells clonally expand intrathecally and further inflammatory cells are recruited to the CNS [46]. Besides T cells, also the importance of B cells is suggested by the presence of oligoclonal bands and B cells in the cerebrospinal fluid [CSF] of patients [47, 48] and the treatment response to B cell depletion therapy [49, 50]. So far, the triggering antigen has not been elucidated [51]. Cell-mediated and humoral immune responses are of importance in the pathophysiology of MS.

Neuromyelitis Optica Spectrum Disorder

In contrast to MS, in neuromyelitis optica spectrum disorders [NMOSD], a distinct antibody targeting water-channel aquaporin 4 [52] has been shown to be specific for NMO in a majority of patients [53] and responsible for disease progression [52, 54]. After the detection of NMO-IgG, NMOSD can now be distinguished from MS, and is considered a separate disease entity. Passive transfer of human NMO-IgG in EAE led to severe neurological disability and has proven the pathogenic potential in animals [55]. Based on these results and on clinical experience with B cell depletion treatment [56], humoral autoimmunity is thought to be responsible for disease exacerbations.

Autoimmune Encephalitis

Autoimmune encephalitis is a heterogeneous group of immune-mediated disorders leading to a wide range of neurological symptoms including movement disorders, epileptic seizures, and cognitive impairment [57]. Psychiatric symptoms like limbic encephalitis are frequently reported [58]. An association with malignancies is described but not exclusively limited to an existing tumor [59]. Onconeural (intracellular) antibodies (e.g., anti-Yo, Hu, Ri, CV2, MA2) that are usually released in the presence of an existing malignancy can be differentiated from antibodies against surface antigens (e.g., anti-NMDAR, LGI1, CASPR2, IGLON5, AMPAR, GlyR) that are found in patients with varying rates of coexisting malignancies [59]. Whereas most of the antibodies against surface antigens seem to be of pathological relevance, this has not been proven for onconeural antibodies. In patients with onconeural antibodies neurodegeneration is assumed to be more driven by T cells [60]. Coexisting tumors triggering immune responses, e.g., ovarian teratomas in anti-NMDAR encephalitis, have to be removed and treated. Besides humoral immune responses, also cell-mediated mechanisms are seen.

Guillain-Barré Syndrome

Guillain-Barré syndrome [GBS] is an inflammatory demyelinating neuropathy. In up to 70% of the patients, anamnesis reveals an infection up to 6 weeks prior to neurological onset [61]. It is considered a post-infectious disorder, most frequently following gastrointestinal or respiratory infections [62]. Molecular mimicry is suggested, and, for instance, epitopes of *Campylobacter jejuni* are similar to the gangliosides of the peripheral nerve membrane [63]. It is an antibody-mediated disease, and antibodies targeting GQ1B, GD1a, and GM1 can be found in a subset of patients of GBS or their variants [61]. Humoral immune responses are the main players in the pathophysiological concepts.

Myasthenia Gravis

Myasthenia gravis [MG] is an autoimmune disorder leading to neuromuscular dysfunctions. Myasthenia syndromes are a group of disorders that are antibody mediated [64]. In most cases (up to 80% of the patients), antibodies against acetylcholine receptor [AchR] can be found (Ig subclass IgG1, IgG3) leading to complement reactions and AchR crosslinking and internalization. Muscle-specific kinase [MuSK] antibodies are of IgG4 class (and less IgG1 to IgG3) and can be detected in a subset of patients who are negative for antibodies against AchR [65]. In addition, also antibodies against lipoprotein receptor 4 [LRP4] can be found [64]. A failed

negative T cell selection in the thymus seems to be causative for the immune processes [65]. Humoral immune aspects seem to be the main players, but links to the innate immune system (complement) and cell-mediated immune responses are observed.

Part III. Overview of Immunotherapies in Neurology: Mode of Action

Immunotherapy in neuroimmunological disorders aims to reduce the inflammatory processes. It is of importance to understand the pathophysiological mechanisms of diseases, and to have background knowledge of the therapeutic agents. Agents that have either been approved or commonly used for the treatment of neurological disorders are presented. Agents exert their effects by cell-mediated or humoral pathways, or by a combination of them. The mode of action is presented for all drugs in the following section. Therapeutic agents are presented in alphabetic order (see Table 1).

Table 1 Overview of biological effects, main application areas and most common dosages of immunotherapies in neurological diseases

Substance	Mechanism of action	Innate	Adaptive	Utilized for		
Anti-CD20	B cell depletion		+	AIE, MG, MS, NMOSD, vasculitis	i.v.	Various dosage regimens, 600 mg every 6 months (ocrelizumab)
ALT	Lymphocyte depletion, repopulation of lymphocytes		+	MS	i.v.	First year: 12 mg on five consecutive days Second year: 12 mg on three consecutive days
aHSCT	Lymphoid and myeloid cells↓, repopulation of immune system	+	+	MG, MS		
AZA	Lymphocytes ↓ myeloid cells ↓	+	+	AIE, CIDP, GBS, MG, MS, NMOSD	p.o.	2–3 mg/kg daily
CLAD	Lymphocytes ↓		+	MS	p.o.	3.5 mg/kg over 2 years Two cycles consisting of two treatment weeks
CYC	Lymphocytes ↓; TH1 → TH2 shift		+	AIE, MS, NMOSD, vasculitis	i.v.	600 – 1600 mg/m^2 every 4–8 weeks

(continued)

Table 1 (continued)

Substance	Mechanism of action	Innate	Adaptive	Utilized for		
DMF	Nrf2↑, antioxidant proteins ↑, neuroprotection ↑, BBB migration ↓, TH1 → TH2 shift, proinflammatory macrophage activation ↓	+	+	MS	p.o.	240 mg twice daily
ECU	Complement ↓	+		MG, NMOSD	i.v.	900 mg weekly (induction) 1200 mg biweekly (maintenance)
GA	Tregs ↑, TH1 → TH2 shift, competition for MHC	+	+	MS	s.c.	20 mg daily or 40 mg thrice weekly
GCS	Lymphocyte activation↓, BBB migration ↓, macrophage activation ↓	+	+	Universal	i.v.	Relapse treatment (MS): 0.5–1 g (2 g in case of escalation) for 3–5 days
IFN-ß	Proinflammatory lymphocyte activation ↓, anti-inflammatory activation↑, lymphocyte migration↓	+	+	MS	i.m./ s.c.	22–44 mcg thrice weekly[a] 250 mcg every other day[b] 30 mcg weekly[c] 125 mcg biweekly[d]
IVIg	Lymphocyte activation ↓, cytokine production ↓, complement activation ↓, macrophage activation↓	+	+	AIE, CIDP, GBS, MG, MS	i.v./ s.c.	Acute treatment 2 g/kg Maintenance treatment: 0.2/kg monthly
MMF	Lymphocyte proliferation ↓, macrophage activation ↓	+	+	AIE, CIDP, GBS, MG, MS, NMOSD	p.o.	750–3000 mg/day
MTX	Lymphocyte and macrophage proliferation↓; antigen presentation↓, antibody production↓, proinflammatory cytokine secretion↓	+	+	MS	i.v.	12 mg/m^2 every 3 months
NTZ	Lymphocyte migration↓		+	MS	i.v.	300 mg every 4 weeks
PLEX/ IAD	Antibodies↓, complement↓		+	AIE, GBS, MG, MS, NMOSD	i.v.	

Table 1 (continued)

Substance	Mechanism of action	Innate	Adaptive	Utilized for		
S1P	Lymphocyte egress from lymphoid organs ↓, cytotoxicity ↓, remyelination ↑		+	MS	p.o.	0.5 mg daily
TERI	Lymphocyte proliferation ↓		+	MS	p.o.	7 or 14 mg daily[e]
TOC	IL6↓	+	+	Giant cell arteritis, NMOSD	i.v.	6–8 mg/kg monthly

Abbreviations: *anti-CD20* anti-CD20 antibodies (rituximab, ocrelizumab, ofatumumab), *ALT* alemtuzumab, *aHSCT* autologous hematopoietic stem cell transplantation, *AZA* azathioprine, *CLAD* cladribine, *CYC* cyclophosphamide, *DMF* dimethyl fumarate, *ECU* eculizumab, *GA* glatiramer acetate, *GCS* glucocorticosteroids, *IFN-ß* interferon beta, *IVIg* intravenous immunoglobulins, *MMF* mycophenolate mofetil, *MTX* mitoxantrone, *NTZ* natalizumab, *PLEX/IAD* plasma exchange/immune adsorption, *S1P* sphingosine-1-phosphat receptor modulator (fingolimod, siponimod), *TERI* teriflunomide, *TOC* tocilizumab

Route of administration: *i.m.* intramuscular, *i.v.* intravenous, *p.o.* orally, *s.c.* subcutaneous

Disorders: *AIE* autoimmune encephalitis, *CIDP* chronic inflammatory demyelinating polyneuropathy, *GBS* Guillain-Barré syndrome, *MG* myasthenia gravis, *MS* multiple sclerosis, *NMOSD* neuromyelitis optica spectrum disorders

[a]IFN-ß-1a s.c., [b]IFN-ß-1b, [c]IFN-ß-1a i.m., [d]Pegylated-IFN-ß-1a s.c., [e]7 and 14 mg approved in the United States, 14 mg approved in EU

Anti-CD20 Antibodies

The chimeric monoclonal antibody rituximab and the humanized monoclonal antibody ocrelizumab bind to the CD20 antigen on B cells. Rituximab is approved for rheumatological and hematological disorders, but not for any neurological disorder [4], although its use in neuroimmunological disorders is common. Ocrelizumab, however, is the first CD20 antibody approved for neurological disorders (primary progressive and relapsing MS). Complement-dependent cytolysis [CDC] and antibody-dependent cell-mediated cytolysis [ADCC] lead to the depletion of B cells [66]. Whereas rituximab acts more via CDC, ocrelizumab's effects are more driven by ADCC. Currently, a full human CD20 monoclonal antibody – ofatumumab – is tested in MS (NCT02792231, NCT03249714) [67, 68]. The route of administration – intravenously for rituximab and ocrelizumab, and subcutaneously for ofatumumab – and administered doses seem to be of importance. In patients with rheumatological disorders, it has been shown that low doses are sufficient to achieve a complete B cell depletion; however, B cell repopulation was earlier as compared with high doses [69, 70]. Experimental data suggest that subcutaneously administered agents target not only circulating B cells but also B cells sequestered in lymph nodes, whereas intravenously administered CD20 antibodies show more effects on circulating B cells and sequestered ones in the spleen. Moreover, repopulated B cells from the bone marrow show again pathogenic activation [70]. Depletion of B

cells effects the production of antibodies, but in the short run, the roles of B cells as APC and in the secretion of proinflammatory cytokines (IL-6, TNF, lymphotoxin-α, granulocyte macrophage-colony stimulating factor [GM-CSF]↓) needed for activation of other immune cells – such as T cells but also Tregs – are assumed to be more important. B cells also secrete anti-inflammatory cytokines; thus, their depletion will especially be effective in the presence of "pathogenic" B cells [71]. Thus, B cells are of utmost importance in immune responses and will have effects on humoral and cell-mediated immune responses.

Alemtuzumab

Alemtuzumab is a humanized IgG1-monoclonal antibody and utilized in the treatment of hematological malignancies and in MS patients. It binds the CD52 antigen on the surface of most mature lymphocytes – especially B and T cells – and to a lower extent of monocytes and macrophages [72]. The effects of alemtuzumab are dependent on the expression of CD52 on the various subtypes. Whereas on B and T cells CD52 is highly expressed, expression is lower on NK cells and basophils [73, 74]. The binding to CD52 leads to rapid and long-lasting depletion of B and T cells via ADCC and CDC [75]. After 7 days, almost complete depletion of CD4+ T cells has been observed [76]. Additionally, besides these immunosuppressive effects, immunomodulation during the repopulation of lymphocytes contributes to the long-lasting effects [75]. Time of repopulation differs for the various subsets of immune cells. Tregs, TGF-β1-, IL-10-, and IL-4-producing cells expand within the CD4+ subset during repopulation, whereas the ratio of Th1 and Th17 cells is decreased [76]. Levels of monocytes are restored by month 3, whereas repopulation of B cells takes longer but shows an overshoot at month 12, and CD8+ T cells will restore by year 3 and CD4+ T cells by year 5 [77, 78]. Secondary autoimmune disorders are reported in patients treated with alemtuzumab. The causes have not been elucidated in detail so far. However, in animal studies, it was shown that depletion of CD52+ B cells was less marked in lymphoid organs than in peripheral blood. After depletion, B cells will repopulate more quickly, but in the absence of Tregs cells. Thus, their regulatory effects on B cell differentiation are missing [79]. It has been shown that alemtuzumab has effects on dendritic cells in MS patients [80]. Alemtuzumab affects the innate and adaptive immune system and cell-mediated and humoral immune responses. The long-lasting effects may mirror a rebalancing of the immune system.

Autologous Hematopoietic Stem Cell Transplantation

Besides treatments that suppress or modify the immune system, autologous hematopoietic stem cell transplantation [aHSCT] aims to alter the immune system. It has been tested in MS patients with severe disease course [81, 82]. Bone marrow

transplantation has been used for some hematological malignancies, but also non-malignant diseases including autoimmune disorders [83]. Hematopoietic progenitor cells – hematopoietic stem cells [HSC] – shall reestablish the immune system with non-autoreactive immune responses. aHSCT, implemented in MS, mean that HSC are taken from the patient himself prior to eradication of immune cells (autologous) [81, 82, 84].

HSC are mobilized into the peripheral blood with granulocyte colony-stimulating factor [G-CSF]. In a second step, CD34+ cells are derived from peripheral blood of patients, collected and cryopreserved. Eradication of all autoreactive lymphocytes is essential to establish long-lasting effects [85]. High-dose immunosuppression – the eradication of all autoreactive lymphocytes and memory cells – is also called the conditioning regimen [86] and is followed by aHSCT [87]. For immunosuppression, various therapeutics or combinations of them may be used. In MS, cyclophosphamide [CYC], busulfan, and rabbit antithymocyte globulin [86], carmustine, cytarabine, and melphalan [82] are utilized. After the immune system in the patient has been eradicated, HSC are re-infunded. A reset of the immune system is aimed [82]. Clinical response in highly aggressive MS was good. Side effects are common, and deaths have been reported in the follow-up period [82, 86].

The aims of aHSCT in MS are manifold: Depletion of (autoreactive) lymphocytes, and induction of a more tolerant immune system without clonal expansion of pathogenic T and B cells. Indeed, the TCR repertoire is changed in MS patients. Long-lasting effects have been reported, thus supporting the theoretical concepts. It is important that pathogenic lymphocytes are eradicated during the aHCST procedure. For reaching lymphocytes beyond the BBB, therapeutics that are being able to cross the BBB might be more efficacious. CD4+ memory cells repopulate and reach baseline values within 18–24 months, and CD8+ cells repopulate within the first months with the predominance of memory cells. B cells will reach baseline values within 9 months [87]. The increased presence of small DNA circles in T cells called T cell receptor excision circle [TREC] suggests altered thymic output with a new CD4+ T cell population. Increased numbers of circulating CD4+/CD25high/FoxP3+ Tregs are reported; maybe the consequence of a thymic renewal [87]. Central memory T cells are replaced by naive T cells with a more tolerant and less autoimmune profile [88]. The numbers of Tregs increase and reach normal values in MS patients compared with controls. Proliferation of tolerant B cells was reported. Secondary autoimmunity is less compared to alemtuzumab. Proinflammatory T cells are decreased, the Treg repertoire is expanded. Similarly, a shift from proinflammatory to anti-inflammatory cytokines was demonstrated [87].

The innate and adaptive immune systems are affected by aHSCT. Humoral and cell-mediated immune responses are modified. It is an intervention with the aim of resetting the immune system. However, severe (life-threatening) side effects may occur.

Azathioprine

Azathioprine is an immunosuppressive drug, and it is used in a wide range of autoimmune disorders such as MG, MS, and chronic inflammatory demyelinating polyneuropathy [CIDP] [89–92]. Its mode of action is based on the disruption of DNA and RNA by inhibiting purine synthesis [89] leading to decreased levels of white blood cells. This decrease is responsible for the immunosuppressive effects [93]. 6-Mercaptopurine – a metabolite of azathioprine – is an atypical nucleotide and interferes with DNA and RNA synthesis [94] of B and T cells [95, 96]. Apoptosis of activated T cells and monocytes is observed, as the synthesis of the mitochondrial transmembrane molecule B cell lymphoma-extra large [Bcl-xL] is blocked. Side effects include infections, nausea, vomiting, hair loss, and changes in the composition of the blood (anemia and bone marrow suppression are often reported) [97]. Azathioprine is cancerogenic and hematological malignancies have been reported during and after treatment [98, 99]. Co-medication with allopurinol should be avoided as the risk for agranulocytosis is increased. The enzyme thiopurine S-methyltransferase [TPMT] is of importance in the mode of action of azathioprine. In patients with a genetic deficiency for TPMT, the risk for severe leukopenia is increased [100]. Additionally, the common missense variant in NUDT15, rs116855232, should be tested, as it is strongly associated with leukopenia in azathioprine-treated patients [101]. Azathioprine has effects on both the innate and the adaptive immune systems. Humoral as well as cell-mediated immune responses are modified.

Cladribine

Cladribine [2-chloro-2′-deoxyadenosine] is approved for the treatment of MS, and it has been used for the treatment of hairy cell leukemia [5]. It is a nucleoside adenosine, but is prevented from degradation through adenosine deaminases by substitution of a chlorine atom in the purine ring [102]. Consequently, it accumulates intracellularly. There it is phosphorylated and metabolized into its active form: 2-Chloro-2′-desoxyadenosintriphosphate. The phosphorylation is dependent on the ratio of certain enzymes – desoxycytidine kinase, adenosine-monophosphate kinase and nucleoside-diphosphate kinase [103]. The ratio is responsible for its preferential activation in peripheral B and T lymphocytes. Other immunological cells are less affected [104, 105]. As nucleoside analogue, it interferes with DNA synthesis leading to cell death. It leads to the rapid depletion of B and T cells with more depletion of B cells compared with T cells (more pronounced effects for CD4+ than for CD8+ cells) [106]. Repopulation is more rapid for B cells than for T cells. Less pronounced effects are observed for NK cells, neutrophils, and thrombocytes. Long-lasting effects seem to be mediated by a slight recovery of memory Tregs [107]. In vitro inhibition of proinflammatory cytokine secretion was reported [108]. In

contrast to other immunotherapies, such as mycophenolate mofetil [MMF] and azathioprine, cladribine is administered in cycles, thus the immune system reconstitutes over time [106]. Cladribine is able to cross the BBB and will reach concentrations in the CSF 25% of those in the plasma [109]. Effects on the adaptive and to a lesser extent on the innate immune system have been reported.

Cyclophosphamide

CYC has been used for decades in the treatment of malignancies and autoimmune disorders. It is an alkylating agent, and its active metabolite – phosphoramide mustard – triggers its effects [110]. Immunosuppressive and immunomodulatory effects are associated with CYC treatment. Inter- and intrastrand crosslinking induces cell death. It predominantly affects rapidly dividing cells [111]. Cell-mediated as well as humoral immune responses are affected with a rapid decrease in T and B lymphocyte counts and reduced IgG production [112]. Besides apoptosis, immunomodulatory effects have been observed. A shift to type Th2 immune response has been reported with increased levels of IL-4, -5, -10. IL-12 release from monocytes is also decreased [111]. Depleting effects on the innate immune system are less prominent, although some grade of depletion has been reported for granulocytes, but not for monocytes [113]. However, at low doses, CYC might even work as immunostimulator [110]. CYC is able to cross the BBB [114]. This may contribute to its effects in progressive MS [115], but also has been suggested as the main effect for positive results in autoimmune encephalitis [116]. Reported side effects include bladder cancer, hemorrhagic cystitis, and transient myelosuppression [111].

Dimethyl Fumarate

Dimethyl fumarate [DMF] has been used in psoriasis for years. It has been approved for relapsing forms of MS. DMF has immunomodulatory and antioxidant properties. Proliferation of autoreactive T cells is inhibited. DMF is highly lipophilic, penetrates into cells and reacts with thiols [117, 118] to monomethyl-fumarate.

DMF activates the nuclear factor erythroid 2-related factor 2 [Nrf-2] pathway leading to immunomodulatory and anti-inflammatory properties. Neuroprotective properties may also be the result of the Nrf-2 activation. Nuclear factor kappa-light-chain-enhancer of activated B cells [NF-kB]-related cellular responses are inhibited by DMF [119]. Antigen presentation is modified, leading to less activated T cells. The absolute count of lymphocytes in the blood is decreased, but not all subpopulations are affected equally. Effects on CD4+, CD8+, effector and memory T cells, Th17 cells, and B cells are more prominent [120]. The frequency of circulating $CD56^{bright}$ NK cells, Th2 cells, and Tregs is increased [121–124]. Proinflammatory cytokine production is reduced. A shift to Th2 immune responses is observed.

Cytokine release of B cells is altered, and apoptosis of B and T cells is induced [119, 124, 125]. Effects on cells within the CNS are seen with a decreased inflammatory cytokine/chemokine production (IL-6, CXCL10, CCL2) [126]. The active metabolite is able to cross the BBB and may exert additional effects within the CNS [127]. Cases of progressive multifocal leukoencephalopathy [PML] have been described under treatment of DMF [5].

Eculizumab

Eculizumab is a monoclonal antibody targeting C5 protein of the complement system, inhibiting the activation of the membrane attack complex (C5b-9) [128]. By doing so, complement-mediated immune reactions are halted [129]. Based on the results from a phase III trial [130], it has been approved for patients with generalized MG with antibodies against AChR and who were therapy-refractory under conventional treatment [131, 132]. The significantly increased risk for meningococcal infections has to be taken into account [129], and vaccination prior to treatment is obligatory [131]. It has also been investigated in GBS and NMO [133].

Fingolimod

Fingolimod is approved for disease modification of MS [5]. Fingolimod is a sphingosine-1-phosphate receptor [S1P] modulator preventing lymphocytes from egressing the lymph nodes. Five S1P receptors are distinguished. S1P-1 is mainly involved in immune mechanisms [134]. Fingolimod targets S1P-1, 3, 4, 5 [135]. Adverse effects like lowering of the heart rate are explained by the receptors targeted by fingolimod. Viral infections in patients treated with fingolimod were more often seen than compared with placebo-treated patients [136, 137]. Fingolimod is an analogue of sphingosine and it is phosphorylated by sphingosine kinases (mostly 2) into fingolimod-phosphate. S1P receptors are internalized after binding and by these means certain lymphocyte populations are kept within the lymph nodes. Especially, CD4+ and CD8+ naive lymphocytes, central memory T cells and B cells are retained in the secondary lymphoid organs [134, 136, 137]. APCs are shifted into less immunogenic properties [138]. Fingolimod has effects on the composition of B and T cells with reduced levels of memory B cells but increased levels of naive B cells. Elevated levels of memory conventional T and Tregs are reported [139, 140]. These changes may contribute to the disease-modifying effects in MS. Effects on peripheral blood subpopulations show great variability between patients [140, 141]. Fingolimod is able to cross the BBB and stimulates the repair process of glial cells. The effects on glial cells, neurons, and endothelial cells with S1P receptors on their surface are not well understood [142–144].

Currently, derivates of fingolimod that target fewer S1P receptors are in development.

Glatiramer Acetate

Glatiramer acetate [GA] is an immunomodulating drug and has been approved for the treatment of relapsing MS and has now been used for decades in MS. In contrast to other discussed therapeutic agents, GA is only used in MS, which is not surprising, when taking into account the mode of action. The chemical structure – a polymer of amino acids – resembles the amino acid composition of MBP and was developed to induce EAE [5]. EAE could not be induced, but surprisingly GA suppressed the development of EAE [145].

The mode of action is not elucidated in detail, but GA has effects on the cell-mediated and humoral immune responses. GA binds to receptors on APC, and by doing so, prevents other antibodies directed against MBP from binding by TCR and BCR antagonism. GA binds to class II MHC molecules and inhibits T cell responses to myelin antigens [146, 147]. As a consequence, the numbers of myelin-reactive T cells are decreased and those of Tregs are increased, also through activation of transcription factor FoxP3 [148]. GA affects T cells, also macrophages and microglia, and anti-inflammatory responses are seen. Secretion of TNFα and cathepsin-B is decreased. GA reduces the numbers of B cells, plasmablasts, and memory B cells [149]. A shift from Th1 to Th2 immune function is reported. Th2 cells may cross the BBB and secrete anti-inflammatory cytokines [150]. GA modifies the release of brain-derived neurotrophic factor [BDNF] [150, 151]. Moreover, cytokine release from B cells is modulated into more anti-inflammatory properties [147]. Although GA only shows moderate efficacy, it is still widely used in MS patients, due to its good safety profile [5].

Glucocorticosteroids

GCS are hormones that possess anti-inflammatory properties. Their anti-inflammatory effects have been discovered in the 1940s. It is estimated that 1% of the adult population of the United Kingdom receives oral GCS treatment at any time [152]. They have been widely used in autoimmune diseases such as MS, CIDP, and rheumatological disorders [2]. GCS bind to the GCS receptor, thereby triggering the expression of anti-inflammatory proteins [153]. The receptor is a hormone-activated transcription factor [154] and downregulates the expression of proinflammatory proteins. Besides these genomic effects, nongenomic effects are responsible for efficacy. These nongenomic effects are based on the activation of signaling pathways (e.g., PI3K, TCR signaling), resulting in reduced inflammation [2, 153, 155]. Genomic anti-inflammatory effects are established by the increased release of Annexin-1, secretory leukoprotease inhibitor [SLPI], mitogen-activated kinase phosphatase-1 (MAKP-1), inhibitor of NF-kappa B (NF-κB), and glucocorticoid-induced leucine zipper protein (GILZ). NF-κB interacts with co-activator molecules such as cyclic AMP response element binding protein [CBP] and leads to reduced

release of inflammatory cytokines, chemokines, adhesion molecules, inflammatory enzymes, proteins, and receptors [155]. Moreover, lipocortin-1 is synthetized in response to GCS and suppresses phospholipase A2 as well as cyclooxygenase/PGE isomerase (COX-1 and COX-2) [154, 155]. IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-11, IL-12, IL-13, IL-16, IL-17, and IL-18 and TNF-α and GM-CSF are reduced in expression in response to GCS. The levels of chemokines such as IL-8, but also macrophage inflammatory protein 1-α, and monocyte chemoattractant protein [MCP1, 3, 4] are decreased [155]. As adhesion molecules are decreased, GCS inhibits the rolling, adhesion, and activation of neutrophils at endothelia. This effect is based on the reduced expression of endothelial leukocyte adhesion molecule 1 [ELAM-1] and intercellular adhesion molecule 1 [ICAM-1] [156], and activated lymphocytes are prevented from migration to inflammatory spots.

Nongenomic effects are mainly responsible for the rapid effects of GCS treatment [2]. Binding to membrane-bound receptors leads at least in high doses to apoptosis. These effects on apoptosis of T cells have been shown in humans [157]. Reduced stability of mRNA is induced by GCS. Some of the inflammatory proteins are regulated by stable mRNA. As a consequence, inflammatory proteins expression is rapidly broken down [155].

In summary, GCS have a broad range of effects. Humoral as well as cell-mediated immune responses are affected. Effects are seen on B and T cells. Cell-mediated immune effects are reduced by inhibition of proinflammatory cytokines (i.e., IL-2, IFN-ϒ) and upregulation of anti-inflammatory cytokines. Cell death of T cells is seen at least at high doses. Effects on the humoral immunity are established by the apoptosis of B cells. Additional effects on immunosuppression on B cells are mediated via the inhibition of NF-κB, a key transcriptional factor for cytokines and proteins involved in the immune response.

Interferon Beta

INF-ß preparations (interferon beta-1a, interferon beta-1b) have been the first approved immunomodulatory agents in MS in the 1990s. They mediate antiviral, antiproliferative, and immunomodulatory effects [158]. Interferons are cytokines and act as signaling proteins. INF-ß belongs to type I interferons. The mode of action is not completely elucidated.

Recombinant INF-ß binds to type I interferon receptors on cells inducing a downstream of proinflammatory pathways. The effects are mediated through Janus kinases/signal transducer and activator of transcription protein signaling pathways [JAK/STAT] [159]. T cell activation is decreased via reduced antigen presentation. In addition, IFNß has effects on CCR7 expression on T cells. Some CCR7 T cells are preferentially shifted to secondary lymphoid organs, and thus prevented from entering the CNS. The number of activated T cells is decreased by inhibition of costimulatory processes. A shift from Th1 to Th2 immune responses is observed. The release of chemokines is altered with increased levels of anti-inflammatory

cytokines and chemokines (IL-10, IL-4 ↑; IL-12, IFNϒ, TNFα, MMP-9, osteopontin ↓). The number of $CD56^{bright}$ NK cells is expanded. They release IL-10 and may also show anti-inflammatory properties [159]. TNF-related apoptosis-inducing ligand [TRAIL] genes are increased leading to apoptosis of T cells [159]. The IL-12/IL-10 ratio is significantly altered to a more anti-inflammatory cytokine milieu. Treatment with INF-ß has led to a decrease of Th17 cells in peripheral blood [160].

There are also effects on B cells. The expression of BAFF is increased. Nevertheless, anti-inflammatory effects – neurotrophic cytokines are secreted from B cells under IFNß therapy but also increased levels of naive B cell and decreased levels of plasmablasts and memory B cells – may outweigh effects from B cell activation. However, due to its effects on BAFF, INF-ß preparation should be avoided in NMOSD [161, 162]. INF-ß seems to alter the composition of MMPs leading to less migration of leukocytes into the CNS [86]. Soluble VCAM-1 is increased under IFNß therapy, thus competitive blocking with endothelial bound adhesion molecules might be the consequence. Beyond a modulation of the BBB, interferon pathways lead to a more anti-inflammatory cytokine release from activated microglia. In addition, activation of macrophages is reduced. In summary, the effects of INF-ß are specific with the pathophysiology of MS. The safety profile of the drug is good. Increased liver enzymes and thyroid level abnormalities have been reported. In a subset of patients, neutralizing antibodies to INF-ß may lead to ineffectiveness [5].

Intravenous Immunoglobulins

Immunoglobulin therapy is widely used in medicine and in autoimmune disorders [163]. They are used in neuroimmunological diseases such as GBS and its variants, where its use seems to be as effective as plasma exchange [PLEX]. They are intravenously administered. However, recently subcutaneously administered immunoglobulin therapy has become available [164]. Intravenous immunoglobulins [IVIg] are used in CIDP, MG, and multifocal motor neuropathy and in M paraprotein-associated neuropathy, inclusion body myositis, and autoimmune encephalitis as well as in MS under special circumstances [165–168]. IVIg are polyclonal human IgG, purified from human plasma. As they are human proteins, anaphylactic and allergic reactions may occur. In response to this therapy, aseptic meningitis and headache have also been reported as side effects [163, 166]. Its mode of action is not elucidated in detail, but multifactorial mechanisms are discussed. IgG consist of a constant region F_c and the antigen binding region F_{ab}. Both are important for the effects established by IVIgs [169]. The F_{ab} regions block cellular receptors and neutralize cytokines, complement, and autoantibodies. Immune complexes between autoantibodies and IVIg are formed. Such immune complexes bind to F_c receptors on APCs and promote the release of anti-inflammatory cytokines. Complement activation leads to the removal of autoantibodies. Moreover, there are reports on the interaction between IVIg and activated T cells, and microglia activation with reduced levels of TNFα and IL-10 [170].

The F_c fragment blocks receptors on cells of the innate immune system as well as on B cells [169]. Inhibitory and activating effects are modulated. The lifetime of host IgGs is halved, as it depends on the neonatal F_c receptor [F_cRn]. F_c fragments of immunoglobulins will block them, and elimination of autoantibodies is observed [166]. Upregulation of inhibitory factors on APCs and vice versa downregulation of B cell activating factors [BAFF] are reported [166]. Differences of efficacy have been observed dependent on autoimmune disease [171]. In diseases with a distinct antibody-driven pathology, efficacy of IVIg is more prominent [169]. In conclusion, with IVIg treatment intervention in the humoral immune response is marked. It is a good example of the complexity of immunological networks as effects on the innate and adaptive immune system and on humoral and cell-mediated immune responses are the consequences.

Mitoxantrone

Mitoxantrone belongs to the group of anthracenedione antineoplastic agents and has been used for the treatment of metastatic breast cancer and acute myeloid leukemia. In neurology, it has been used for the treatment of MS. Mitoxantrone is an immunosuppressive drug that suppresses B and T cells [172]. It is a type II topoisomerase inhibitor and disrupts DNA synthesis by intercalation [173]. Lysis and apoptosis of T and B cells are observed [174, 175]. It has anti-inflammatory and immunomodulatory effects. Migration of monocytes is decreased and a shift to Th2 responses has been reported [176]. The number of circulating B cells is decreased by approximately 30% after one infusion. Thus, its main effects may be explained by effects on humoral immunity [177]. Due to its side effects – especially cardiomyopathy [178] – it is only used in a subset of patients [179]. Cumulative mitoxantrone dose should not exceed 140 mg/m^2 body surface [180]. Cell-mediated and humoral immune responses are modified. The adaptive immune system is affected, and the innate immune system at least indirectly because of decreased migration of monocytes to inflammatory spots.

Mycophenolate Mofetil

MMF is a prodrug, which has to be metabolized to mycophenolic acid [MPA]. MPA inhibits inosine-5′-monophosphate dehydrogenase [IMPDH], thereby blocking synthesis of guanosine-5'-monophosphate [GMP] from inosine-5'-monophosphate [IMP]. Proliferation of B and T cells is dependent on the synthesis of purines [181]. Whereas most other cell types can use an alternative way of purine synthesis, MMF predominantly inhibits proliferation of lymphocytes [182] and consequently the production of antibodies. In addition, it causes decreased expression of glycoproteins and adhesion molecules that are needed for the recruitment of monocytes and

lymphocytes to the sites of inflammation [183, 184]. Activated lymphocytes are decreased by necrotic cell death [182]. Further effects on Tregs and Th1 cells have been described [182]. Maturation of dendritic cells is also suppressed. Thus, activation of T cells is inhibited [185]. MMF has been used for rheumatological diseases [90, 92, 186] and in neurology for MG [185]. MMF has effects on the innate and adaptive immune system; cell-mediated and humoral immune responses are modified.

Natalizumab

Natalizumab is approved for relapsing forms of MS. Natalizumab is a humanized IgG4 monoclonal antibody. It targets the α4-chain of integrin [α4ß1-integrin], also known as very late activating antigen-4 [VLA-4]. Binding of VLA-4 to VCAM-1 and fibronectin [FN] is blocked. These adhesion molecules are upregulated in inflammatory processes. Integrins are cell surface glycoproteins enabling adhesion, rolling and migration through the BBB. It prevents (autoreactive) lymphocytes from migrating in the CNS. Consequently, inflammatory processes are decreased within the CNS. In peripheral blood, CD4+, CD8+, and CD19 cells are expanded under treatment of natalizumab. However, the peripheral CD4/CD8 ratio remains within the normal range [187]. CSF findings under treatment of natalizumab show that the ratio within the CNS is significantly altered [188]. This may explain the increased risk for PML under treatment of natalizumab [5]. Natalizumab is also used in Crohn's disease [189]. Effects on the adaptive immune system establish efficacy.

Plasma Exchange

PLEX has been utilized in immunological diseases for decades [190]. During PLEX, the blood of the patients is separated, exchanged with donor plasma (containing albumin), and afterward re-infunded [191]. By doing so, inflammatory components are removed. It is currently used in GBS, CIDP, polyneuropathy with monoclonal gammopathies of undetermined significance, autoimmune encephalitis, MG, fulminant demyelinating CNS disease, chronic or secondary MS, relapses in MS, Sydenham chorea, and acute obsessive-compulsive disorder and tics in pediatric autoimmune neuropsychiatric disorder associated with group A streptococci [PANDAS] [191, 192].

PLEX removes autoantibodies and immune complexes in autoimmune disorders, thus having effects on the humoral immune system, whereas effects on the cell-mediated immune system are not that obvious. Total numbers of circulating lymphocytes are not generally affected, although the frequency of Th cells was shown to be decreased and those of T suppressor cells to be increased after PLEX. Clinical effects were not impressive in T cell-driven disorders [190]. In contrast to PLEX,

immunoadsorption only resolves immunoglobulins from the plasma of patients, thus other components stay in the patient [193]. In patients with side effects resulting from PLEX, immunoadsorption is an adequate substitute [194].

Teriflunomide

Teriflunomide is approved for the treatment of relapsing forms of MS. It is the active metabolite of leflunomide. It inhibits the mitochondrial enzyme dihydroorotate dehydrogenase [DHODH] affecting pyrimidine synthesis, and interfering with the de-novo synthesis of uridine monophosphate [UMP]. Consequently, DNA synthesis is inhibited. Reproduction is impaired in rapidly dividing cells such as highly proliferating T and B lymphocytes. This pathway explains side effects such as hair thinning. Resting T cells are not affected by teriflunomide as they do not need DHODH for synthesis. A shift to Tregs and a reduction of clonal diversity of CD4+ cells is reported [195]. Teriflunomide is able to cross the BBB [195]. Effects on resident cells in the CNS have been observed with decreased microglia proliferation in vitro [196]. The levels of proinflammatory cytokines such as IL-17, TNFα as well as protein tyrosine kinases are reduced. Antibody production from B cells is decreased [197, 198]. The NF-κB pathway is modulated and shows less inflammatory cell responses. Oligodendrocyte differentiation was promoted in vitro [199]. Cell-mediated as well as humoral immune responses are modified.

Tocilizumab

Tocilizumab is used in the treatment of rheumatological disorders. It is a humanized monoclonal antibody targeting IL-6 receptor. IL-6 is a cytokine with proinflammatory and anti-inflammatory properties. It is released by macrophages and supports the proliferation of B cells and it is antagonistic to Tregs [200]. The IL-6 receptor is a type I cytokine receptor found on the surface of B and T cells [201], but also on cells of the innate immune system [202]. Tocilizumab leads to a variety of effects – including decreased expression of adhesion molecules such as ICAM-1 and matrix metalloproteinases [MMP]-2 and MMP-9 as well as inhibition of the signaling pathways MAPK and translocation of the transcription factor NF-κB [203]. Together with Th17, that are secreting IL-6, IL-6 seems to be of importance in many autoimmune diseases [204]. IL-6 seems to promote the survival of plasmablasts and to be associated with antibody secretion in NMO [205]. Recently, its efficacy has been shown in neuromyelitis optica (NMO) [206, 207]. It has recently been approved for the treatment of giant cell arteritis [208, 209]. Effects have been established on the innate immune system and the adaptive immune system. Humoral immune responses and indirectly cell-mediated one are modified.

Emerging Treatments

Some examples for emerging treatments include atacicept, belimumab, bortezomib, eculizumab, efgartigimod, and satralizumab. These agents are not commonly used, but trials are ongoing, or they are used in certain conditions. Atacicept is a recombinant fusion protein binding to BAFF and a proliferation-inducing ligand [APRIL]. As a consequence, B cell differentiation and survival is inhibited [210, 211]. Surprisingly, increased disease activity was reported for MS patients treated with atacicept [212] mirroring the complexity of B cell immunology. Belimumab is a human monoclonal IgG1 antibody targeting BAFF. Binding to BAFF leads to inhibition of B cell activation [213, 214]. It has been tested in 18 MG patients in a phase II study [215]. Bortezomib is a proteasome inhibitor leading ultimately to cell death of plasma cells. Proteasome activity is blocked in plasma cells, thus proteolytic pathways are inhibited, protein is accumulated in cells and cell death occurs [216]. First promising experiences with this agent have been collected in a patient with (severe) MG [217] and in five patients with therapy-refractory autoimmune encephalitis [218]. Efgartigimod is a human IgG1-derived Fc fragment fusion protein that binds to FcRn leading to decreased levels of IgG (blocking of recycling of IgG and increasing the clearance of IgG) [219, 220]. It is currently tested in a multicenter phase III study in patients with MG (ClinicalTrials.gov Identifier: NCT03669588). Satralizumab is a humanized IgG2 monoclonal antibody targeting IL-6, and thereby preventing plasma cells from maturation [221]. It is currently tested as monotherapy and as add-on therapy in two respective phase III studies in NMO and NMOSD (NCT02073279, NCT02028884).

Conclusion

The availability of a broad range of immunotherapies allows the clinician to treat a variety of immune-mediated neurological diseases. These options go hand in hand with the difficulty to find the right medication for the right disease for the right patient at the right time.

For choosing the right drug, it is necessary to understand its mode of action and its targets. Figure 4 gives an overview on the main immune functions of effector T cells that may be targeted, and Table 1 summarizes therapeutic agents commonly used in neuroimmunological diseases and their effects on the immune system.

Knowing the immunological background of the various diseases is important for choosing the right drug. As most immunosuppressive drugs have modulating effects, and on the other side immunomodulating drugs will have effects on the ability to defend against pathogens, the dichotomy of immunosuppression and immunomodulation is blurring.

The treating clinician should choose the therapeutic agents according to their properties. Are there effects on the innate and/or on the adaptive immune system?

Are the main effects established by cell-mediated or humoral immune responses? Will the active agent reach the CNS and is it able to cross the BBB? What are the known side effects? Do these effects fit with known immune mechanisms of the various disorders?

It is important for the clinician to visualize the various ways the immune system are affected by immunotherapies:

- First, the direct effects of drugs – such as immunosuppression and the risk of (opportunistic) infections.
- Second, the indirect effects of drugs – such as autoimmunity as seen for alemtuzumab. The mechanisms beyond these phenomena are not understood in detail.
- Third, the effects on the complex network of immunological pathways, which are only scarcely understood.

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Part II
Disorders: Diagnosis and Therapies

Postinfectious Immune-Mediated Neurological Diseases

Marios Hadjivassiliou and Panagiotis Zis

Abstract Infection may trigger immune-mediated neurological dysfunction. In some specific examples, the pathogenesis has been clearly delineated, with a detrimental effect of the immune response to infection. This chapter will cover immune-mediated neurological diseases where infection is the antigenic trigger. Postinfectious cerebellitis, Miller Fisher syndrome, acute disseminated encephalomyelitis, vasculitis and Guillain-Barré syndrome (GBS) are discussed, from the clinical presentation to therapies. Presentation may be monophasic (e.g. GBS) or sometimes evolve into a chronic condition (e.g. vasculitis). In some cases, the prognosis is excellent with full recovery. In others, patients will develop permanent neurological deficits. Therapies are often based on steroids, plasma exchange and intravenous immunoglobulins. Despite a favourable clinical course, postinfectious cerebellitis may rarely require surgical decompression due to oedema of the posterior fossa. Treatment should be initiated as fast as possible.

Keywords Postinfectious · Cerebellitis · Vasculitis · Miller Fisher syndrome · Guillain-Barré syndrome · Acute disseminated encephalomyelitis · ADEM

Introduction

The immune system has a critical role in host defence against various infective pathogens. However, under certain circumstances, activation of the immune system by an infection may prove detrimental to the host. This may be the result of a general immune activation secondary to the infection or due to a targeted immune response to a specific host antigen. Most patients who suffer from an acute infection expect a period of convalescence before regaining full health. For a minority, recovery from the symptoms of the infection is followed by a new illness as a result of

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_13

inappropriate activation of the immune system. One assumes that this group of patients may be more susceptible to autoimmunity in general; yet, most of these patients tend to follow a monophasic illness with little chance of recurrence.

In a large prospective cohort study involving 176 patients with postinfectious neurological syndromes, 70% of patients had a monophasic illness [1]. The neurological manifestations occurred within 30 days after vaccination or systemic infection. Factors that predicted poor recovery included older age and more severe initial presentation. Persistent infections may continue to drive the immune response resulting in chronic inflammation and the development of an autoimmune process resulting in a more protracted and ultimately permanently damaged nervous system. It is also likely that patients with genetic susceptibilities to immune regulation may be vulnerable to infection-driven autoimmune diseases. This chapter will cover some postinfectious neurological syndromes including postinfectious cerebellitis, Miller Fisher syndrome, acute disseminated encephalomyelitis, postinfectious vasculitis and Guillain-Barré syndrome (Table 1).

Postinfectious Cerebellitis

Postinfectious cerebellitis (PIC) refers to an immune-mediated ataxia that usually follows a bacterial or viral infection. The term is usually confined to those cases where the cerebellum is exclusively affected, but sometimes, such involvement may also involve the brainstem (rhombencephalitis/brainstem encephalitis), and thus patients may develop brainstem signs in addition to cerebellar signs.

PIC accounts for as much as 0.4% of neurological presentations in children but is less common in adults. The first paediatric case series were published in 1905, and the infective agent was measles, *Bordetella pertussis* and scarlet fever [2]. There have been numerous reports of PIC, predominantly in children, associated with specific infections, commonly viral illnesses. Primary sites of infections are the gastrointestinal and respiratory tracts, such as influenza, parainfluenza, mumps, measles, rubella, poliomyelitis, variola, cytomegalovirus, vaccinia, ECHO, coxsackie, varicella, herpes simplex, herpes zoster, Epstein-Barr virus (EBV) and epidemic encephalitis. PIC has also been associated with bacterial infections such as Bordetella pertussis, typhoid fever, scarlet fever, Q fever, diphtheria, leptospirosis, mycoplasma, Legionnaire's disease and even falciparum malaria parasitaemia.

PIC accounts for up to 50% of all neurological sequelae of varicella infection and is thus very common in children [3]. It is estimated that 0.1% of patients with varicella infection will develop neurological dysfunction [3]. A large series of 73 patients with acute cerebellitis in childhood reported varicella virus as the most common infective agent seen in 26% of patients [4]. In adults, the most common preceding infection was EBV or mycoplasma [5].

Viral cultures of the cerebrospinal fluid are seldom positive and it is thought that the cerebellitis is immunologically mediated. This is supported by isolated involvement of the cerebellum with sparing of other parts of the brain, the presence of

Table 1 Summary of the clinical features of post-infectious neurological diseases

	Postinfectious cerebellitis	Miller Fisher syndrome	Acute disseminated encephalomyelitis	Infection and neurological vasculitis	Guillain-Barré syndrome
Lesion site	Central nervous system				Peripheral nervous system
Infection triggering the autoimmunity	Varicella, herpes simplex (HSV), influenza, parainfluenza, mumps, measles, rubella, poliomyelitis, variola, cytomegalovirus (CMV), Echo virus, coxsackie, Epstein-Barr virus (EBV), Bordetella pertussis, typhoid, scarlet fever, Q fever, diphtheria, leptospirosis, mycoplasma, Legionella pneumophilia, falciparum malaria, parasitaemia, vaccines: varicella, hepatitis B, rabies, meningococcal group C, human papilloma virus	A viral (respiratory) or bacterial (usually *Campylobacter*) infection	Measles, influenza, enterovirus, mumps, rubella, varicella, EBV, CMV, HSV, hepatitis A, coxsackie virus, mycoplasma, borrelia, leptospira, streptococci, measles, mumps, and rubella vaccine	There are several subtypes. For example, in a case of polyarteritis nodosa; hepatitis B and cryoglobulinaemia in the context of hepatitis C, less association with HIV, CMV, varicella and HTLV-1	*Campylobacter jejuni Mycoplasma pneumoniae*, CMV, Zika virus
Clinical manifestations	Cerebellar ataxia Patients may exhibit brainstem deficits	Ophthalmoplegia, cerebellar ataxia and areflexia	Fever, headache, stiff neck and focal neurological dysfunction often with reduced level of consciousness, sometimes also with seizures	focal neurological deficits	Bilateral weakness, ascending and starting distally, Association with various degrees of sensory symptoms, neuropathic pain, burning sensations and autonomic dysfunction
Clinical courses	Mean latency: 9.9 days Self-limiting	Median latency: 8 days Self-limiting	Self-limiting	Infection can reactivate the vasculitis	Usually, 2–3 weeks' latency

(continued)

Table 1 (continued)

	Postinfectious cerebellitis	Miller Fisher syndrome	Acute disseminated encephalomyelitis	Infection and neurological vasculitis	Guillain-Barré syndrome
Evidence for the autoimmunity	Presence of oligoclonal bands Elevation of TNF, IL5, IL2 Antineuronal antibodies	Anti-GQ1b antibody Immunoreactivities in the cerebellar molecular layer	Presence of oligoclonal bands Pathologies similar to experimental allergic encephalomyelitis (infiltration of lymphocytes and macrophages)	Prophylactic antibiotics are effective to avoid reactivation of vasculitis	Antibodies against GM1 and GD1a gangliosides Response to immunotherapies (plasmapheresis and intravenous immunoglobulins (IVIG))

oligoclonal bands and the good prognosis even in cases where no specific treatment was given. In one study describing 12 patients with a self-limiting cerebellar dysfunction following an attack of falciparum malaria, the authors found elevated serum concentrations of tumour necrosis factor, interleukin 6 and interleukin 2 [6]. These levels were much higher whilst the patients were ataxic than during recovery. Similar findings were observed in the patients' cerebrospinal fluid. Another study demonstrated the presence of antineuronal antibodies in PIC following EBV infection [7].

As the course of this illness is on the whole benign, there is very limited information from the post-mortem examination on the neuropathology of this condition. Isolated case reports of fatal cerebellitis are usually due to severe swelling and brain herniation. The swelling is usually confined to the cerebellum but may sometimes be asymmetrical, also known as hemicerebellitis (Fig. 1). T2 signal hyperintensities on MR imaging can be seen affecting the cerebellar cortex. Whilst in the majority of cases such changes may resolve, in some cases, cerebellar atrophy develops thereafter. It is debatable if such cases represent examples of primary autoimmune cerebellar ataxia rather than PIC. The neuropathological findings seen in some of these reports are compatible with an acute meningoencephalitis, whilst other reports are more in favour of a postinfectious immune reaction similar to acute disseminated encephalomyelitis [8]. Such reports need to be treated with caution as they are atypical given the fulminant course which is unlike most of the cases of PIC where full recovery is the norm.

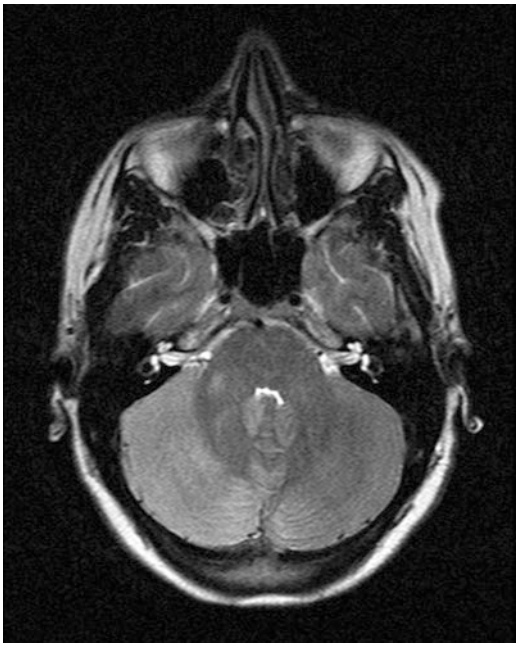

Fig. 1 Asymmetrical swelling of the cerebellum in a 30-year-old patient with acute postinfective cerebellitis

The clinical features in a series of paediatric cases showed remarkable uniformity and were consisted with predominantly gait and lower limb ataxia with a peak incidence at 3 years of age [4]. Thirty four percent of the children had severe ataxia causing inability to walk. Nystagmus was present in 13.7% of the cases. The mean latency from the onset of prodromal illness to the onset of ataxia was 9.9 days (range, 1–43). The recovery period averaged at 2 months with the majority of the patients (88%) making a full recovery. In the adult series the clinical features were very similar to those encountered in children, with the addition of oculomotor disturbances in 73% (broken pursuit being the commonest) [5]. The latency from the onset of prodromal illness to the development of ataxia was longer than what was encountered in children at 3.5 weeks. Complete recovery was observed in the majority of patients and occurred within a mean of 12 weeks. Cerebrospinal fluid examination showed elevation of white cell count, predominantly lymphocytes, in 50% and high protein in 30% of patients. Brain imaging tended to be normal but in few cases demonstrated cerebellar swelling. In those patients where recovery was incomplete, there was cerebellar atrophy evident on MRI. The differential diagnosis includes ADEM, acute labyrinthitis and other immune-mediated ataxias.

There is no evidence to suggest that treatment of the underlying infective agent alters the neurological course. Management is supportive in the form of physiotherapy and occupational therapy during the symptomatic phase of the illness. Rarely, the severity of cerebellar swelling may necessitate posterior fossa decompression to avoid brain herniation.

Miller Fisher Syndrome

In 1956 Miller Fisher reported three patients with clinical features characterised by acute onset of ophthalmoplegia, ataxia and areflexia suggesting that this was a distinct clinical entity [9]. In clinical practice, ataxia and ophthalmoplegia tend to be the dominant presenting feature of Miller Fisher syndrome (MFS) with the neuropathy being mild and axonal. MFS accounts for about 5% of all cases of acute inflammatory polyneuropathies (Guillain-Barré syndrome – see below). The incidence can therefore be estimated to be about 1 per million per year.

In one of the largest reported series, comprising 50 consecutive patients with MFS, a viral (respiratory) or bacterial (usually *Campylobacter*) infection preceded the neurological illness in up to 80% of cases [10]. The median interval between infection and development of neurological symptoms was 8 days. The longest reported interval between prodromal illness and the onset of symptoms is 5 weeks. The initial symptoms consist of diplopia, ptosis, gait ataxia with only minor sensory symptoms. The ophthalmoplegia usually evolves as a symmetrical failure initially of upgaze followed by loss of lateral gaze and last by downgaze [11]. The ataxia is often prominent and disabling. Deep tendon reflexes are depressed or absent in 82% of cases. Cranial nerves other than the oculomotor nerves are involved in more than half the cases (facial 32%, bulbar dysfunction 26%). By 6 months, all patients reported in the above series had made a full recovery, and no deaths were reported.

Nerve conduction studies may be normal, though evidence of peripheral neuropathy (axonal or demyelinating, primarily affecting sensory nerves) is often found. Cerebrospinal fluid protein is often elevated. Imaging of the brain and the cerebellum is usually normal although enhancing lesions visible on magnetic resonance imaging of the brain in the spinocerebellar tracts at the level of the lower medulla have been reported [12]. These lesions disappeared with resolution of the symptoms.

The origin of the ataxia in patients with MFS has always been a source of debate. The original study by Miller Fisher proposed selective involvement of Ia-afferent neurons. Subsequent work suggested that a disparity between proprioceptive information from muscle spindles and kinaesthetic information from joints may be the cause of the ataxia [13]. Direct involvement of the cerebellum has, however, been supported by a number of studies. An FDG-PET study in 10 patients with MFS showed hypermetabolism in the cerebellum and brainstem [14]. A study using sera from patients with MFS demonstrated selective immunocytochemical staining of the molecular layer of the human cerebellum and loss of Purkinje cells has also been reported [15, 16]. MR spectroscopy of the cerebellum during the illness can be abnormal with full resolution after clinical improvement [17].

High titres of anti-GQ1b IgG antiganglioside antibodies have been observed in up to 90% of patients with MFS. The pathogenic impact of this antibody is likely to depend on many factors, including its specificity and the integrity of the blood-nerve barrier. Antibodies to GQ1b cross-react with epitopes contained in the lipo-saccharide of MFS-associated *Campylobacter jejuni* strains suggesting the possibility of molecular mimicry [18]. They remain a useful diagnostic marker for patients with suspected MFS. This is particularly useful in some atypical cases where the ophthalmoplegia is less prominent and bulbar dysfunction predominates. Anti-GQ1b antibodies are associated with more severe damage to sensory rather than motor nerves [19].

Prognosis is usually considered to be excellent, though it may be slow, with a full recovery expected within 6 months. Recurrent episodes have been described but they are rare. MFS is overall a mild and usually self-limiting condition that does not require immunomodulatory treatment. Whilst there are anecdotal reports of response to steroids, intravenous immunoglobulin and plasmapheresis a retrospective study comparing intravenous immunoglobulins, plasmapheresis and no treatment showed no difference in the speed of recovery or the final outcome [20].

Acute Disseminated Encephalomyelitis

Acute disseminated encephalomyelitis (ADEM) is an inflammatory demyelinating disorder of the central nervous system that is usually monophasic and associated with an antecedent or concomitant infection (usually viral). North America epidemiological data in children suggest an estimated incidence of 0.2/100,000 [21]. ADEM accounts for 10% of all known cases of encephalitis. ADEM is more common in children although it can occur at any age. Clear evidence of a preceding infection is usually seen in 2/3 of children and in about half of all adult cases [22].

ADEM can also be triggered by vaccination (postvaccination encephalomyelitis). Viral triggers include infections such as influenza, enterovirus, measles, mumps, rubella, varicella-zoster, EBV, cytomegalovirus, HSV, hepatitis A and coxsackie virus. Bacterial triggers include mycoplasma, borrelia, leptospira and streptococci. Measles infection in particular can result in an acute immune-mediated encephalitis in up to 1 in 1000 children and produces a more severe phenotype. It is the most frequent CNS complication of measles infection. Incidences of ADEM after varicella-zoster and rubella infections are much less common at 1 in 10,000 and 1 in 20,000, respectively [22]. The measles, mumps, rubella vaccine is most commonly associated with postvaccination encephalomyelitis with an incidence of 1–2 per million vaccinations. Still this incidence is less than the 1 in 1000 seen in the context of measles infection.

The onset of ADEM usually occurs in the wake of a febrile prodromal illness or immunisation and is often associated with constitutional signs in addition to the encephalopathy. Thus, patients with ADEM usually present acutely with fever, headache, stiff neck and focal neurological dysfunction often with reduced level of consciousness, sometimes also with seizures. As a result, such patients are usually admitted acutely to medical, infectious diseases or neurology units, initially treated for meningitis or infective encephalitis. What distinguishes ADEM from viral infective encephalitis or other autoimmune encephalitides is the imaging findings of multiple areas of abnormal white matter. These areas are usually found in the subcortical and central white matter but also in the cortical grey-white matter junction of the hemispheres, cerebellum, brainstem and spinal cord. ADEM lesions are usually larger (occasionally resulting in mass effect) and have a tendency to be extensive and symmetrical by comparison to MS lesions. Cerebrospinal fluid shows increased white cells and protein but no evidence of viral or bacterial infection. Over 50% will have evidence of oligoclonal bands but sometimes this is a transient phenomenon unlike what is seen in multiple sclerosis.

The hallmark pathological findings in ADEM are areas of perivenous demyelination with infiltration with lymphocytes and macrophages [23]. These pathological findings are very similar to what is seen in experimental allergic encephalomyelitis (EAE). EAE can be induced experimentally in animals by exposing them to a myelin antigen. The existing evidence therefore suggests that ADEM results from a transient autoimmune response, possibly via molecular mimicry between virus and myelin antigens. Both B and T cell-mediated reactions are responsible for generating CNS inflammatory damage in ADEM [22].

There is no standard treatment for ADEM because it is rare and usually self-limiting. No large clinical trials have been conducted so far. Its immune nature prompts the use of immunosuppression in the form of steroids, plasma exchange and intravenous immunoglobulins. Spontaneous recovery is the rule, usually over a course of weeks to months. The initial presentation is rather severe, but in long term, the outcome is much better when compared to MS. Historically, fatal disease was reported as common, but the current data dispute this. In a series of 150 children with ADEM, no deaths were reported, but in a series of 40 adult patients with ADEM, 2 mortalities were reported possibly suggesting a worse prognosis for adult cases [24].

Infection and Neurological Vasculitis

Vasculitis can affect the nervous system primarily in three possible ways: central nervous system vasculitis that usually takes the form of multiple strokes affecting large or small cerebral arteries; peripheral nervous system vasculitis that usually manifests with mononeuropathy multiplex; granulomatous angitis characterised by granuloma formation affecting brain parenchyma or the meninges. Neurological vasculitis can exist in isolation (e.g. granulomatous angitis of the nervous system or isolated vasculitis of the peripheral nervous system) but more often than not the nervous system is involved in the context of a more systemic disease. Vasculitis can be seen in the context of connective tissue diseases (e.g. primary Sjogren's syndrome, systemic lupus erythematosus, rheumatoid arthritis) or can be primary in the context of ANCA-associated vasculitis or in granulomatous angitis such as Wegener's granulomatosis.

The relationship between infection and systemic vasculitis is complex [25]. Historical evidence for its existence comes from diseases such as syphilitic aortitis and vasculitis in the context of tuberculosis (Fig. 2) [26]. A causal relationship with specific infections has only been established through epidemiological data. Examples include polyarteritis nodosa (PAN) in the context of hepatitis B and cryoglobulinaemia in the context of Hepatitis C [27, 28]. Less robust association between vasculitis and infection exists with HIV, CMV, varicella-zoster and HTLV-1 [29].

In Wegener's granulomatosis, infection can reactivate the vasculitis, and sometimes prophylactic antibiotics are used to avoid such scenario. Cerebral vasculitis

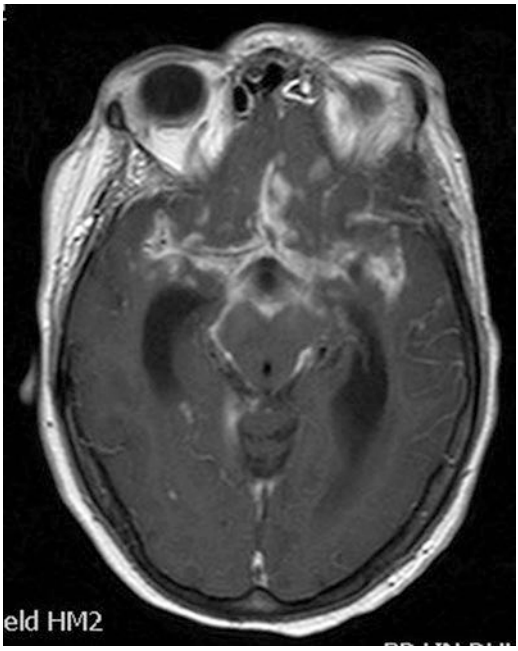

Fig. 2 Vasculitis and perivascular inflammation of the large arteries of the circle of Willis in a patient with TB meningitis. Steroids in addition to anti-TB treatment resulted in complete recovery

can also complicate bacterial meningitis often requiring the use of high-dose steroids in conjunction with appropriate antibiotics. Finally, some bacteria, fungi or parasites can also cause vasculitis mainly by direct invasion of the blood vessels or septic embolisation.

There are a number of immune mechanisms that may result in vasculitis in the context of infection [30]. Molecular mimicry refers to the activation of autoreactive T cells by microbial peptides that have sufficient structural similarity to self-peptides and is thought to be a common immune mechanism. Other mechanisms included the enhanced presentation of autoantigens by antigen-presenting cells that are recruited in the vicinity of the inflammatory site, followed by priming of autoreactive lymphocytes. Bystander activation is another mechanism that refers to the expansion of previously activated T cells at the inflammatory site. Finally, viral infection of lymphocytes may result in enhanced antibody production and the formation of circulating immune complexes.

Recognition of infection as the trigger for some vasculitides is of great importance as the treatment strategies differ from those applied to primary vasculitidies. Effective antimicrobial drugs are mandatory in treating bacterial infections, and antiviral drugs have proven to be effective in the treatment of hepatitis B-related vasculitis [29]. Similarly, treatment of hepatitis C-related cryoglobulinaemic vasculitis involves the use of antiviral drugs for disease control [29]. It is also important to note that unlike primary vasculitides, successful treatment of infection-related vasculitis with elimination of the infective agent is not usually associated with recurrence.

Guillain-Barré Syndrome

Guillain-Barré syndrome (GBS) is an acute, rapidly progressive, polyradiculoneuropathy. The annual incidence of GBS is estimated to be 0.8–1.9 cases per 100.000 people [31]. Based on the incidence rate and life expectancy, the overall estimated lifetime risk of developing GBS for any individual is less than one in 1000 [31].

GBS is usually preceded by a gastrointestinal or other infection (2–3 weeks before the onset of GBS) that induces an abnormal autoimmune response targeting the peripheral nervous system (peripheral nerves and spinal roots). Molecular mimicry between microbial and nerve antigens plays clearly a critical role, at least in the case of *Campylobacter jejuni* infection. However, unwanted autoimmunity does not arise in the vast majority (more than 99%) of patients exposed to infections that can lead to a GBS [32]. Increasing age and male gender are established risk factors for GBS. However, further genetic and environmental factors that affect an individual's susceptibility to develop GBS are still not known.

A variety of clinical phenotypes of GBS exist but all present with rapidly progressive neurological symptomatology of the peripheral nervous system that stabilises within 3–4 weeks of onset. In typical GBS, bilateral weakness is the key presenting complaint. Such weakness is described as ascending, starting distally.

Unlike the chronic axonal neuropathies, where a dying back phenomenon occurs, in GBS, the weakness is usually global, affecting proximal muscles as well [33]. Bilateral facial nerve involvement is not uncommon as the disease progresses. Up to one-third of patients will develop respiratory failure and will need ventilation at an intensive care unit [34].

The majority of patients with GBS experience neuropathic sensory symptoms; however, the degree of sensory involvement varies from none (in pure motor forms of GBS) to very significant. Small fibre dysfunction in GBS is common and manifests with neuropathic pain, burning sensations and autonomic dysfunction [35, 36]. Rarer variants of GBS that affect predominantly facial and bulbar muscles are the pharyngeal-cervical-brachial variant and the Miller Fischer syndrome, which has been described above.

Clinical features needed for the diagnosis of GBS include the progressive weakness and the absent or decreased tendon reflexes particularly in the limbs affected. Typically, a lumbar puncture and cerebrospinal fluid (CSF) analysis will reveal the increased protein content with usually normal CSF white blood cell count. Serological antibody response directed against certain gangliosides can help with the diagnosis. For example, *Campylobacter jejuni* infections are usually associated with a pure motor axonal form of Guillain-Barré syndrome, more severe limb weakness and antibodies against GM1 and GD1a gangliosides [31]. Neurophysiological assessment is needed to confirm the diagnosis and determine the GBS type: Acute inflammatory demyelinating polyradiculoneuropathy (AIDP) is the most common. It is characterised by demyelinating features on nerve conduction studies (NCS), such as slow conduction velocities and conduction block. One of the earliest features is the prolongation of the minimal F-wave latencies, even before the slowing of the conduction velocities, suggesting that the inflammation of the nerve roots occurs earlier in the course of the disease. This may be the reason why patients often complain of radicular sounding pain.

In acute motor axonal neuropathy (AMAN) there is no evidence of sensory impairment and the NCS show reduced compound muscle action potentials (CMAPs). In acute motor and sensory axonal neuropathy (AMSAN), apart from the changes seen in AMAN, there is additional sensory impairment with evidence of reduced sensory nerve action potentials (SNAPs).

Plasmapheresis and intravenous immunoglobulins (IVIG) are currently the only two effective treatments for GBS. Both treatments are equally effective; however, IVIG is usually preferred because of its less invasive nature. Treatment should be initiated as soon as possible after diagnosis to prevent further neural damage [37].

The nature of the preceding infection affects the clinical phenotype and prognosis. For example, *Campylobacter jejuni* infections are usually associated with the AMAN type; these patients generally have a poorer outcome [31]. Other poor prognostic factors include age and intensive care requirement. Mortality in GBS has dropped dramatically over the last years, since effective treatments exist and the provision of intensive care is more widely available. It is estimated that the mortality of GBS is about 10%, with the majority of deaths occurring because of infections, pulmonary embolism and cardiac rhythm disturbances [38].

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Paraneoplastic Neurological Syndromes

Sergio Muñiz-Castrillo and Jérôme Honnorat

Abstract Paraneoplastic neurological syndromes (PNS) are immune-mediated disorders associated with cancer. PNS usually appear in a subacute manner and may affect any level of the nervous system. They generally develop before cancer and PNS recognition leads to cancer diagnosis. Prompt tumor treatment is important to stabilize or improve PNS symptoms. Although PNS may sometimes occur without antibodies, two major groups are identified depending on the antibodies present in the serum or the CSF. Antibodies against intracellular antigens (also called onconeural antibodies) seem to play no direct role in neurological symptoms (with exceptions) but are good markers of cancer, so their detection is very useful to classify a neurological syndrome as paraneoplastic. Although these antibodies are thought to have no pathogenic effect, the immune system still plays a major role, probably mediated by cytotoxic T cells. PNS with onconeural antibodies respond poorly to immunotherapy, with the exception of anti-Ma2 PNS. A second group of antibodies against synaptic and cell surface proteins occur in well-defined neurological syndromes, which are not always PNS and may appear in younger patients without cancer. These antibodies alter the location or function of their antigens, generating the neuronal dysfunction that is the underlying cause of these PNS. Early

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_14

immunotherapy improves prognosis at least in the most frequent disorders (anti-NMDA receptor encephalitis, anti-LGI1 encephalitis). In all PNS, irrespective of the group, appropriate tumor screening should be undertaken. The work-up should include the search for the most common cancers reported in PNS patients according to the age and the associated autoantibodies.

Keywords Paraneoplastic neurological syndromes · Autoimmune encephalitis · Autoantibodies · Onconeural antibodies · Cell surface synaptic antigens · Immunotherapy

Introduction

Paraneoplastic neurological syndromes (PNS) appear in patients with cancer but are not caused by metastases or neoplastic infiltration. They represent "remote" effects of cancer on organs usually not affected by the primary tumor. They may have different causes or mechanisms in other tissues, but PNS are well-defined immune-mediated disorders [1]. This immunological basis is supported by the presence of antibodies or T cells against neuronal antigens that are also expressed by tumor cells, along with inflammatory abnormalities in the CSF and anatomopathological studies [2, 3].

The association with antibodies against neural antigens is the hallmark of PNS, even though seronegativity does not exclude a PNS diagnosis [2]. Two major groups of PNS are recognized depending on the target of these antibodies [2, 3]: (1) Nuclear or cytoplasmic antigens are not accessible to autoantibodies, which are probably not pathogenic but are good markers of cancer. These PNS are thought to be mainly mediated by cytotoxic T lymphocytes, leading to an irreversible neuronal loss with poor response to immunotherapy; (2) cell surface and synaptic antigens can be reached by autoantibodies that disrupt their function or location within the synapse in a reversible manner, with prompt treatment often resulting in better outcomes. Detection of such antibodies is not as useful as those directed against intracellular antigens to establish a PNS diagnosis, as the association with cancer is weaker and other triggers may exist. For example, herpes simplex encephalitis has been reported in N-methyl-D-aspartate (NMDA)-receptor encephalitis [4]. It has been suggested that some PNS related to intracellular antigens involved in synaptic function may have an intermediate pathogeny [5].

PNS may affect any part of the nervous system. Patients may present with multifocal involvement. Some PNS are more specifically associated with a certain antibody and type of cancer, for instance, Lambert-Eaton myasthenic syndrome (LEMS) with antibodies against voltage-gated calcium channels and small cell lung cancer (SCLC), whereas others have a broader spectrum. Neurological symptoms and signs usually appear before cancer in a subacute manner, with PNS diagnosis leading to tumor detection. In order to improve PNS outcome, cancer remission must be achieved. Immunotherapy may also play a role in PNS treatment, especially in those that are antibody-mediated [2, 3, 5].

Epidemiology

No large epidemiological studies concerning PNS are available, but it has been estimated that they affect only 0.01% of patients with cancer [1]. PNS associated with lymphomas are probably more uncommon than among solid cancers [6]. In a multicenter European study lasting 8 years [7], 979 patients with PNS were recruited, without including the more recently described syndromes associated with antibodies against synaptic/cell-surfaces antigens. The most common PNS of the central nervous system was paraneoplastic cerebellar degeneration (PCD; 24.3% of the total), followed by limbic encephalitis (LE; 10%), paraneoplastic encephalomyelitis (PEM; 5.6%) and brainstem encephalitis (5.6%). Sensory neuronopathy was as frequent as PCD (SNN; 24.3%). Other less common PNS of the peripheral nervous system were dysautonomia (5.2%) and LEMS (4.4%). Paraproteinemic neuropathies and myasthenia gravis were excluded. From the same series [7], the types of cancer most frequently associated with PNS were SCLC (38.4%), ovary (10.5%), breast (9.7%), and non-SCLC (7.9%). Anti-Hu (also called antineuronal nuclear antibody 1, ANNA-1), followed by anti-Yo (also called Purkinje cell antibody 1, PCA-1) were the most common antibodies [7, 8].

Recently, the incidence and prevalence of autoimmune encephalitis (AE) have been estimated to be 0.8/100,000 person-years and 13.7/100,000, respectively. These values are relatively similar to those of infectious encephalitis [9]. Twenty-one percent of AE were paraneoplastic [9]. It has been shown that NMDA-R encephalitis is more frequent in young people than viral etiologies [10]. An underlying tumor is detected in nearly 40% of NMDA-R encephalitis [11]. NMDA-R encephalitis is followed in frequency by encephalitis with antibodies against the voltage-gated potassium channel (VGKC) complex [12], which includes leucine-rich glioma-inactivated protein 1 (LGI1) and contactin-associated protein-2 (Caspr2) [13].

Clinical Manifestations

General Clinical Approach

PNS may resemble more common diseases but there are some clues that should raise suspicion. Family or personal history of cancer or autoimmune diseases and smoking are risk factors [2]. Age and sex may also be important. For example, in NMDA-R encephalitis, only 6% of males have an underlying tumor compared to 46% of females [11]. In addition, most of ovarian teratomas (the main tumor associated with NMDA-R encephalitis) occur in patients aged between 12 and 45 years [11]. Anti-Hu antibodies indicate an underlying SCLC in more than 70% of adult patients with PNS [14], whereas 75% of pediatric cases that associate anti-Hu antibodies and neurological disease are non-paraneoplastic [15].

PNS usually develop in an acute or subacute manner, and therefore they must be suspected in case of rapid and/or atypical progression of neurological signs and symptoms. They may affect any level of the central or peripheral nervous system, and involvement of different areas is another characteristic feature but infrequent (<10%) [7]. PNS may manifest as many different neurological syndromes, but those called "classical PNS" (Table 1) are so strongly associated with cancer that diagnosis should lead to tumor screening, even when no antibody is detected [16]. Besides, depending on the clinical presentation, certain antibodies (Table 2) and thus particular cancer (Tables 3 and 4) should be suspected. Therefore, an appropriate clinical classification is an essential first step to PNS diagnosis [5].

Neuromuscular Disorders

Paraneoplastic neuromuscular disorders (NMD) are more common than PNS of the central nervous system, especially if we consider myasthenia gravis and some inflammatory neuropathies that are often excluded from PNS reviews. Many paraneoplastic NMD are clinically identical to those not associated with cancer [17]. Only dermatomyositis, LEMS, chronic gastrointestinal pseudo-obstruction and sensory neuronopathy are specific enough to be considered classical PNS [16].

Table 1 Main clinical syndromes observed in paraneoplastic neurological diseases [16]

	Classical PNS	Nonclassical PNS
Neuromuscular disorders	Subacute sensory neuronopathy Lambert-Eaton myasthenic syndrome Chronic gastrointestinal pseudo-obstruction Dermatomyositis	Necrotizing myopathy Myasthenia gravis Peripheral nerve hyperexcitability Autonomic autoimmune neuronopathy Vasculitic neuropathy Demyelinating neuropathies Polyneuropathies with monoclonal gammopathies Sensorimotor neuropathies Motor neuron diseases
Central nervous system disorders	Encephalomyelitis Opsoclonus-myoclonus Paraneoplastic cerebellar degeneration Limbic encephalitis	Paraneoplastic isolated myelopathy Stiff-person syndrome Brainstem encephalitis Chorea

Table 2 Main associated autoantibodies according the clinical syndrome

Clinical syndrome	Associated antibodies
Myopathy [18]	TIF1 gamma, NXP-2, HMCGR
Neuromuscular junction disorder [25, 38]	muscular Ach-R, VGGC, SOX1
Neuromyotonia/Morvan syndrome [13, 50]	Caspr2, Netrin-1
Autonomic neuronopathy [14, 52, 57]	ganglionic Ach-R, Hu, CV2/CRMP5
Vasculitic neuropathy [60]	None
Polyneuropathy [66, 71]	MAG (gammopathy), Hu, CV2/CRMP5
Sensory neuronopathy [14, 53, 72, 81]	Hu, CV2/CRMP5, amphiphysin
Motor neuron disease [82–84]	Usually none; Hu, Ma2, ANNA2/Ri
Myelopathy [87, 91, 94]	CV2/CRMP5, amphiphysin, GFAP, AQP4
Stiff-person syndrome [96, 100]	Amphiphysin, GAD
PERM [107, 112]	Glycine-R, DPPX
Encephalomyelitis [14, 52, 81, 94, 114]	Hu, CV2/CRMP5, amphiphysin, ANNA2/Ri, GFAP
Opsoclonus-myoclonus [114]	ANNA-2/Ri
Cerebellar degeneration [123, 124]	PCA-1(Yo), Hu, Zic4,Tr/DNER, mGluR1
Brainstem encephalitis [114, 132, 136]	Ma2, Hu, ANNA-2/Ri
Limbic encephalitis [139, 140]	Hu, Ma2, LGI1, GABAb-R, AMPA-R
Encephalitis with movement disorders [137, 183, 184]	NMDA-R, CV2/CRMP5, D2-R

Myopathies

Autoimmune myopathies include dermatomyositis (DM), polymyositis (PM), overlap-myositis, inclusion-body myositis and immune-mediated necrotizing myopathies (IMNM). Among them, DM-PM and IMNM may be associated with cancer [17]. They are clinically characterized by subacute proximal symmetric weakness, with muscle pain, elevated serum creatine kinase, and typical findings of an irritative myopathy on needle electromyography [17, 18]. DM is also defined by skin changes, which can be specific such as Gottron's papules and heliotrope rash, or non-specific such as periungual erythema and calcifications [18]. Prognosis is worse in paraneoplastic DM/PM than in non-paraneoplastic cases [17, 18]. The relative risk of cancer is 1.6 for PM and 5.5 for DM [19]. Different types of cancer are associated with DM and PM, but adenocarcinomas are globally the most common [20]. The risk is higher among males, patients older than 20 years, and within the first year after myositis diagnosis [19]. It remains increased in DM 5 years later, whereas it falls to normal values in PM [20]. Anti-Mi2 and anti-SAE (small ubiquitin-like modifier activating enzyme) antibodies are associated with absence and low prevalence (1–4%) of cancer in DM, respectively [18]. In contrast, anti-TIF1gamma (transcriptional intermediary factor 1 gamma) and anti-NXP2 (nuclear matrix protein 2) have increased malignancy rates in DM. Nearly 60–80% of adults with anti-TIF1gamma and 30% with anti-NXP2 have an underlying cancer. In

Table 3 Main autoantibodies associated with PNS and targeting an intracellular neural antigens. Clinical characteristics of the patients and main tumor associations

Antigen	Clinical syndromes	% paraneoplastic	Associated tumors
ANNA-1/Hu [14, 51]	Autonomic neuronopathy Chronic gastrointestinal pseudo-obstruction Sensory neuronopathy Encephalomyelitis Cerebellar degeneration Limbic encephalitis	>80%	SCLC (>70%) Prostate cancer Gastrointestinal cancer
ANNA-2/Ri [114]	Encephalomyelitis Cerebellar degeneration Brainstem encephalitis Opsoclonus-myoclonus	>80%	Breast cancer Gynecologic cancer Lung cancer
ANNA-3 [115]	Sensory neuronopathy Encephalomyelitis Cerebellar degeneration	>80%	SCLC
PCA-1/Yo [125, 126]	Cerebellar degeneration	>80%	Breast cancer Gynecologic cancer Lung cancer
PCA-2/MAP1B [116]	Encephalomyelitis Cerebellar degeneration	80%	SCLC
Ma2 [132, 134]	Brainstem and limbic encephalitis	>60%	Germ cell testicular tumor
Ma1 and Ma2 [132, 134]	Brainstem encephalitis Cerebellar degeneration	>60%	Non-SCLC
CV2/CRMP5 [52, 87]	Sensory neuropathy Necrotizing myelopathy Encephalomyelitis Chorea Retinopathy and optic neuritis	>80%	SCLC (75%) Thymoma
Amphiphysin [81, 100]	Sensory neuropathy Necrotizing myelopathy Encephalomyelitis Stiff-person syndrome	80%	Breast cancer SCLC
GAD [99, 159]	Stiff-person syndrome Cerebellar ataxia Limbic encephalitis	<10%	Lung cancer Breast cancer Thymoma
AGNA/SOX1 [37]	Lambert-Eaton myasthenic syndrome	>90%	SCLC
Zic4 [117]	Cerebellar degeneration	>90%	SCLC
GFAP [94, 95]	Meningoencephalomyelitis	35%	Ovarian teratoma
Adenylate kinase 5 [172]	Limbic encephalitis without seizures	0%	None

Table 4 Main autoantibodies associated with PNS and targeting cell surface neuronal antigens. Clinical characteristics of the patients and main tumor associations

Antigen	Clinical syndromes	% paraneoplastic	Associated tumors
Ach-R (ganglionic) [57, 59]	Autonomic neuropathy Somatic neuropathy	30%	Adenocarcinomas
Ach-R (muscle) [17, 25]	Myasthenia gravis	10–15%	Thymoma
VGCC [32, 33]	Lambert-Eaton myasthenic syndrome Cerebellar degeneration	50–60%	SCLC
Caspr2 [13, 43]	Neuromyotonia Morvan syndrome Limbic encephalitis	20–25% 50% Usually none	Malignant thymoma
Netrin1-R [50]	Myasthenia gravis Neuromyotonia Morvan syndrome	100%	Malignant thymoma
LGI1 [13, 43]	Limbic encephalitis	10%	Different cancer
NMDA-R [11, 174]	Encephalitis with psychiatric and movement disorders	Up to 50% in young women	Ovarian teratoma
AMPA-R [148, 150]	Limbic encephalitis	50%	Lung cancer Breast cancer Thymoma
GABAb-R [154, 157]	Limbic encephalitis	50–80%	SCLC
GABAa-R [165, 167]	Refractory seizures Status epilepticus	40% of adults Usually none in children	Thymoma
mGluR1 [130, 131]	Cerebellar degeneration	60%	Hodgkin's lymphoma
mGluR5 [163, 164]	Ophelia syndrome	50%	Hodgkin's lymphoma
Glycine-R [107, 108]	PERM Opsoclonus-myoclonus	<10%	Thymoma Hodgkin's lymphoma
DPPX [112, 113]	Encephalitis with CNS hyperexcitability PERM	Just a few patients	B-cells neoplasms
Tr/DNER [128, 129]	Cerebellar degeneration	90%	Hodgkin's lymphoma
AQP4 [91]	Neuromyelitis spectrum disorder	<4%	Lung cancer Breast cancer
D2 [184]	Basal ganglia encephalitis	0%	None
IgLON5 [185]	Sleep disorder, bulbar dysfunction, ataxia, chorea	0%	None

children, the presence of these antibodies is not associated with a higher risk of cancer [18]. Anti-TIF1gamma patients are characterized by severe skin lesions with mild muscular involvement [21]; the pattern is quite the opposite in DM with anti-NXP2, although cutaneous calcinosis is more common [22]. In both conditions, dysphagia is more prevalent than in primary DM [21, 22]. The targets of these antibodies have been related to oncogenesis, directly in the case of TIF1gamma and through the activation of p53 (a well-known tumor suppressor) by NXP2 [18].

IMNM are rare and rapidly progressive immune-mediated myopathies without skin lesions. Unlike DM/PM, little or no inflammation is found in muscle biopsy [17]. Three types of IMNM are identified: anti-SRP (signal recognition particle), anti-HMGCR (3-hydroxy-3-methylglutaryl-coenzyme-A-reductase), and seronegative [18]. The latter two have increased rates of cancer, 11.5% and 21.4%, respectively [23]. No specific type of cancer is associated with IMNM [23]. Prognosis is poorer in paraneoplastic IMNM [23, 24]. Statin exposure may precede the development of anti-HMGCR IMNM, but it is not absolutely necessary [23, 24]. Similarly to antigens of paraneoplastic DM, HMGCR has been related to tumor proliferation and metastasis [18].

Neuromuscular Junction Disorders

Myasthenia gravis (MG) is characterized by fatigable weakness involving ocular and proximal limb muscles. Different autoantibodies against proteins located at the postsynaptic membrane of the neuromuscular junction can be found in MG. Anti-acetylcholine receptor (AchR) antibodies are present in nearly 80% of generalized MG and 50% of ocular MG [25]. Paraneoplastic MG (10–15%) occurs in association with thymoma, and it is almost always generalized with the presence of circulating anti-AchR antibodies [17]. Isolated cases of paraneoplastic MG related to other tumors have been reported [26–28]. Malignant thymomas are more frequent among late-onset MG, whereas thymic hyperplasia is predominant in early-onset MG [25]. High levels of striated-muscle antibodies (titin and ryanodine) are associated with malignant thymoma and with poor prognosis [29–31]. Anti-titin and anti-ryanodine antibodies are detected in 95% and 70% of paraneoplastic MG, respectively. They also occur in nearly 50% of non-paraneoplastic late-onset MG [30, 31]. Thus, they represent more useful biomarkers for tumor in younger patients. Other antibodies present in MG, such as anti-muscle specific kinase (MusK) and low-density lipoprotein receptor 4 (LRP4), are not associated with malignant thymoma [25].

LEMS is defined by the triad of proximal weakness, areflexia, and autonomic dysfunction. Clinical and electromyographic postexercise facilitation are key signs to distinguish LEMS from MG [17, 25]. An underlying cancer is found in 50–60% of LEMS patients. SCLC is by far the most frequent, followed by other lung tumors, lymphomas-leukemias, and prostate carcinomas [32, 33]. LEMS usually precedes cancer diagnosis, with a median interval of 6 months. Nearly 90% of associated

cancers are diagnosed within the first year after LEMS onset [32]. Paraneoplastic LEMS patients are older (approximately 60 years old versus 50), are predominantly male (70% versus 50%), and are more commonly smokers as compared to non-paraneoplastic patients [32, 33]. Clinical presentation is similar, but progression is faster in paraneoplastic LEMS [33]. A prediction score for SCLC in LEMS called DELTA-P (Dutch-English LEMS Tumor Association Prediction) has been developed based on the presence of bulbar involvement, erectile dysfunction, loss of weight $\geqq 5\%$, tobacco use, age $\geqq 50$ years, and Karnofsky score < 70 (one point for each parameter). Scores above 3 points indicate a SCLC risk greater than 80%, reaching 100% when score is 6 [34]. Antibodies against P/Q-type voltage-gated calcium channels (VGCC) are present in 85–90% of all LEMS [25]. Antibodies are pathogenic: they induce VGCC cross-linking and internalization at the presynaptic membrane [25]. Lack of these antibodies is usually related to non-paraneoplastic LEMS [35]. In contrast, anti-glial nuclear antibodies (AGNA) are strongly associated with SCLC [36]. They are directed against SOX, a nuclear protein implicated in neural development, and therefore unlikely play a pathogenic role [37]. Nevertheless, detection of AGNA antibodies is very useful to distinguish LEMS related to SCLC from idiopathic cases, as they show a sensitivity of 67% and a specificity of 95% [38]. They can be also found at lower titers in nearly 30% of SCLC patients with and without other PNS [36–38].

Peripheral nerve hyperexcitability (PNH) are a group of disorders caused by instability of the distal motor axon leading to spontaneous motor activity. They include cramp-fasciculation syndrome, Isaacs' syndrome (also known as acquired neuromyotonia), and Morvan syndrome. PNH are thought to be immune-mediated by its relationship with VGKC-complex antibodies [39]. Cramp-fasciculation syndrome is characterized by cramps and myalgia without weakness. Needle EMG reveals fasciculations and after-discharges evoked by low-frequency repetitive nerve stimulation; 16–24% of patients are anti-VGKC positive. Most cases are non-paraneoplastic, but a few malignant thymomas have been reported [40, 41]. Isaacs' syndrome patients present with generalized muscle stiffness, pseudomyotonia, myokymia, muscle hypertrophy, prominent dysautonomia (hyperhidrosis, tachycardia, postural hypotension, urinary and gastrointestinal symptoms), and neuropathic pain. Fasciculations and myokymic and neuromyotonic discharges can be detected in EMG. Anti-VGKC positivity may be as high as 54% [41–43]. Anti-Caspr2 antibodies are more related to acquired neuromyotonia than anti-LGI1 [13, 43–45]. Isaacs' syndrome may appear with myasthenia gravis (anti-AchR positive) in the same patient, and an underlying tumor is detected in approximately 20–26%. Malignant thymoma is the most common tumor associated with neuromyotonia, followed by lung cancer (especially SCLC) and thyroid and prostate carcinomas [13, 41–43, 46]. Morvan syndrome (MoS) is clinically very close to Isaacs' syndrome, but it also involves the central nervous system. Both affect middle-to-late-aged males more frequently. Along with symptoms and signs of neuromyotonia, MoS patients present weight loss, neuropathic pain, and autonomic disturbances (mainly hyperhidrosis and cardiovascular instabil-

ity). Encephalopathy consists of sleep disorders (insomnia is the commonest) and psychiatric features (confusion, agitation, hallucinations) [13, 43, 47]. Pain may be due to peripheral small fiber involvement and responds better to immunotherapy than to symptomatic treatment [48]. Caspr2 antibodies are found more frequently and at higher titers than LGI1, but they may appear together [43, 47]. As it occurs in Isaacs' syndrome, malignant thymoma (the main associated tumor) and MG are common in MoS patients (40–60% and 30–46%, respectively) [43, 47–49]. Recently, a new antibody directed against Netrin1-receptor has been shown to predict malignant thymoma in neuromyotonia/MoS and MG with a specificity of 100%. Caspr2 antibodies were as specific as Netrin-1R antibodies predicting malignant thymoma in MG (100%) but slightly less in neuromyotonia/MS (70%) [50].

Dysautonomia

Dysautonomia may appear combined with other PNS, as it has been mentioned in LEMS or in acquired neuromyotonia. It can also occur in sensory neuronopathy and encephalomyelitis associated with anti-Hu [14, 51] or anti-CV2/CRMP5 antibodies [52, 53]. Chronic intestinal pseudo-obstruction is the most frequent isolated presentation, but it is also common within other PNS [7]. Patients usually present with weight loss, nausea and vomiting, early satiety, constipation, and abdominal pain. Onset is acute and symptoms progress rapidly. Imaging studies find enteric dilatation without evidence of obstruction [54–56]. Most patients have an underlying SCLC and may be anti-Hu positive [54–56], although association with other onconeural antibodies has been reported [54]. Histopathological studies show myenteric plexus infiltration with plasma cells and lymphocytes, which lead to neuronal degeneration and finally gastrointestinal dysmotility [55].

Autonomic autoimmune neuronopathy or ganglionopathy is a much uncommon disorder. It is characterized by subacute dysfunction of both sympathetic and parasympathetic nervous systems. Common symptoms include orthostatic hypotension, fixed heart rate, anhydrosis, dry mouth, impaired pupillary response, as well as gastrointestinal and genitourinary manifestations [57, 58]. Antibodies against ganglionic acetylcholine-receptor are detected in 50% of patients [57]. Although these antibodies are not specific, high titers are correlated with dysautonomia of autoimmune origin [57–59]. In such cases, autonomic disturbances are often multiple and severe, compared to those with lower titers who present mild autonomic symptoms or none [58, 59]. Ganglionic AchR positivity has been associated with cancer in 30% of cases, mainly adenocarcinomas. Nevertheless, most paraneoplastic patients had low titers and clinical presentation was different from dysautonomia [59]. In a more recent series, only 2 of 95 patients were diagnosed with cancer after detection of ganglionic anti-AchR antibodies, but none had dysautonomia. No paraneoplastic case was identified among 21 autonomic autoimmune ganglionopathy [58].

Vasculitic Neuropathies

Paraneoplastic vasculitic neuropathy is an uncommon disorder that appear mainly in SCLC patients, but it has been also reported in association with lymphomas, lung adenocarcinomas, malignant thymomas, gastrointestinal, and other solid tumors [60–63]. It may account for 15% of all vasculitic neuropathies [60]. It is a non-systemic vasculitis that usually presents in a subacute and progressive manner. Most patients are men older than 50 years [61, 62]. Two phenotypes are recognized: the more frequent asymmetric sensorimotor polyneuropathy and the typical mononeuritis multiplex [60, 61]. Pain is common [61, 63]. Electromyography shows an axonal pattern, and nerve biopsy finds histopathological features of vasculitis [60–63]. No antibody has been detected in isolated vasculitic neuropathies, but erythrocyte sedimentation rate and other inflammatory markers are generally elevated [61, 62].

Paraneoplastic Demyelinating Neuropathies

The relationship between Guillain-Barré syndrome (GBS) and malignancy is controversial. A higher risk of cancer than expected has been shown in GBS [64, 65], although cases do not fulfill current PNS diagnostic criteria [16]. GBS patients with cancer seem to be older, with male predominance, and higher mortality [64, 65]. Hodgkin lymphoma and SCLC are the most frequent reported tumors [66]. Chronic inflammatory demyelinating polyneuropathy (CIDP) has been described in association with lymphomas, mainly non-Hodgkin and particularly those with monoclonal gammopathy [66–68]. Melanoma is the second most common tumor [67]. Some clinical features should raise suspicion of a possible paraneoplastic CIDP: severe ataxia, distal or upper limb predominance, cranial or respiratory involvement, and autonomic dysfunction [67]. In addition, a few cases of neuropathies resembling multifocal motor neuropathy with conduction block have been reported in association with adenocarcinomas and non-Hodgkin lymphoma [69, 70].

Polyneuropathies Associated with Monoclonal Gammopathies

More than 50% of osteosclerotic myelomas are preceded by a CIDP-like polyneuropathy that may be part of the POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal component – IgG or IgA – and skin changes). Compared with classic CIDP, it is usually severe and distal, with prominent ataxia or motor involvement and early axonal changes [66, 71]. Multiple myeloma-associated neuropathies are diverse and of different origins, including secondary amyloidosis [71]. Small fiber involvement is typical of early primary amyloidosis neuropathy, but it usually develops sensory large fiber, motor; and autonomic features in later stages [71]. Monoclonal gammopathy of unknown significance and Waldenström macroglobulinemia can develop a demyelinating, sensorimotor,

chronic, distal polyneuropathy associated with monoclonal IgM against myelin-associated glycoprotein (MAG) [66, 71].

Sensory Neuronopathy and Peripheral Neuropathy Associated with Onconeural Antibodies

Sensory neuronopathy (SNN) is considered as a classical PNS and is the most frequent PNS of the peripheral nervous system [7, 16]. SNN is more commonly associated with cancer compared to other neuropathies. For instance, carcinoma was diagnosed in 47% of SNN, meanwhile in only 10% of CIDP, 4.5% of axonal polyneuropathy, and 1.7% of GBS [72]. The term neuronopathy refers to the location of the primary damage in neuronal cell bodies of the dorsal root ganglia [16]. Along with paraneoplastic SNN, other known causes are cisplatin toxicity, HIV infection and Sjögren syndrome [73]. Diagnostic criteria for paraneoplastic SNN include: subacute onset with modified Rankin score (mRS) $\geqq 3$ before 12 weeks of evolution, onset characterized by numbness and pain with asymmetric distribution, marked proprioceptive loss, involvement of the upper limbs in a non-length-dependent manner, and electrophysiological studies showing severe alteration of sensory nerve action potentials (SNAP) with at least one of them absent [16]. Mild motor involvement may also appear and does not rule out SNN diagnosis [16].

In order to improve SNN diagnosis (regardless of its etiology), a score has been proposed based on the presence of ataxia in the lower or upper limbs (+3.1 points), asymmetrical distribution (+1.7), sensory loss not restricted to the lower limbs at full development (+2), $\geqq 1$ absent SNAP or 3 SNAP <30% of the lower limit in the upper limbs (+2.8), and <2 nerves with abnormal motor nerve conduction studies in the lower limbs; SNN is possible with a score > 6.5 [73]. Sensitivity and specificity have been found to be 95% [74]. In predominant painful paraneoplastic SNN (25%), SNAP may be relatively well preserved, and therefore the score may be less useful [74, 75]. An underlying cancer must be suspected especially in males over 60 years of age and if SNN onset is acute or subacute, only the upper limbs or all four limbs are involved with early pain, and if CSF and motor nerve conduction studies abnormalities are observed [73, 76]. Paraneoplastic SNN accounts for 30–40% of all SNN [73, 76]. It can present with signs and symptoms of involvement of the central nervous system (being part of paraneoplastic encephalomyelitis) and dysautonomic dysfunction [16].

Approximately 90% of paraneoplastic SNN have onconeural antibodies [66, 71, 73, 76]. Anti-Hu is by far the most common [72, 73, 75]. SNN is the predominant presentation of anti-Hu syndrome, but pure SNN (without involvement of other areas of peripheral or central nervous system) is present in only 25% of patients [14]. Autonomic neuropathy occurs in 25% of SNN [14, 51, 77]. Recent series have demonstrated that clinical and/or electrophysiological (sometimes important) motor involvement, symmetrical distribution, and progressive course are not infrequent

[51, 77]. Nerve conduction studies usually show an axonal pattern [51, 77]. Most patients are male, the median age is 60 years, and SCLC is present in >70% [14, 51, 77]. Prostate and gastrointestinal cancers are the second most common tumors [14, 51, 77, 78].

Anti-CV2/CRMP5 (collapsing response-mediator protein 5) antibodies are the second most frequent among SNN patients and may appear in combination with anti-Hu [53, 79, 80]. Sex distribution is more uniform than in anti-Hu patients, but SCLC is again the most common cancer (75%), followed by malignant thymoma (up to 15%) [52, 53, 79]. Initial series reported that anti-CV2/CRMP5 neuropathy was more frequently sensorimotor and with axonal-demyelinating pattern compared to anti-Hu SNN [79, 80]. Upper limb involvement and pain were less common and associated central nervous disorders (mainly cerebellar ataxia) were more typical of CV2/CRMP5 [79]. Nevertheless, in a very recent study, the most common phenotype of anti-CV2/CRMP5 neuropathy was described as a painful, axonal, asymmetric polyradiculoneuropathy with less dysautonomia and more motor electrophysiological involvement than anti-Hu SNN [53]. Prognosis is usually better in anti-CV2/CRMP5 patients than in those with double-positive (CV2/CRMP5 plus Hu) or anti-Hu patients [53, 79].

Many other antibodies against intracellular and cell surface antigens have been related to SNN and peripheral neuropathy, mainly sensorimotor neuropathy with anti-amphiphysin [73, 81] and medium and low titers of anti-ganglionic AchR [59].

Motor Neuron Disease

Paraneoplastic motor neuron disease is controversial, but several cases meeting definite diagnostic criteria for PNS have been described. They are mainly women and the median age is 60 years. Onset is often subacute and lower motor neuron syndrome (also called motor neuronopathy) with predominant upper limb involvement is the most common [82, 83]. No bulbar dysfunction has been reported [82]. Other non-motor neurological features, including subclinical sensory neuronopathy on electrophysiological studies, may be a clue to suspect a paraneoplastic etiology [82, 84]. Some patients have inflammatory abnormalities in the CSF [82, 83, 85]. Paraneoplastic motor neuron disease has been reported in association with Hodgkin's lymphoma [6, 85] and different solid tumors, principally breast cancer [82–84]. Onconeural antibodies have been only described with solid cancers: anti-Hu is the most frequent, followed by anti-Ma2, anti-Ri/ANNA-2, anti-Yo/PCA-1, and anti-CV2/CRMP5 [82–84]. Exclusively motor involvement represents nearly 5% of anti-Hu-associated neuronopathies [51, 77]. Motor neuron disease associated with anti-Ma2 antibodies usually presents with both upper and lower motor neuron signs, and bulbar involvement may occur [86]. It may appear isolated or preceded by typical features of anti-Ma2 syndrome [86].

Paraneoplastic Myelopathy

Isolated paraneoplastic myelopathy is uncommon, less than 1% of all PNS [7]. It occurs more often within multifocal involvement of the peripheral and central nervous system. Isolated myelopathy may have a subacute or insidious course, and is usually severe with significant disability [87, 88]. Median age at onset is approximately 60 years and women are more frequently affected [87, 88]. Lung and breast cancers are the most commonly detected [87, 88]. Nearly 80% of patients have onconeural antibodies, mainly anti-amphiphysin, or anti-CV2/CRMP5 [87]. A few cases of myelopathy with optic neuritis, resembling Devic's disease, have been identified with anti-CV2/CRMP5 [89, 90]. Paraneoplastic neuromyelitis optica spectrum disorder with anti-aquaporin-4 antibodies has been also reported [91–93]. It accounts for less than 4% of all cases [92, 93]. Compared to autoimmune idiopathic patients, those with associated cancer (principally lung and breast) are older and there is greater proportion of men (although women are still more frequently affected) [93]. An underlying tumor should be suspected if there is severe brainstem involvement at onset (nausea, vomiting) and in men older than 45 years presenting with longitudinal extensive transverse myelitis [93]. Finally, among the recently discovered autoimmune glial fibrillary acidic protein (GFAP) astrocytopathy, myelopathy is a common feature, usually in combination with meningitis or encephalitis [94, 95]. Isolated myelitis occurs in nearly 10% and may appear associated with optic neuritis [94, 95]. An underlying cancer is found in approximately 35% of all GFAP astrocytopathies, ovarian teratoma being the most frequent [94, 95].

Stiff-Person Syndrome

Stiff-person syndrome (SPS) is more frequent in women, and the mean age at onset is 35 years [96]. The main symptoms are muscular stiffness with painful muscular spasms. Stiffness is caused by coactivation of agonist and antagonist muscles (which can be shown by electromyography), especially in the trunk and lower limbs, and therefore hyperlordosis and gait disturbances are common complaints. Spasms are often triggered by external sensorial stimuli [96, 97]. Stiff-limb syndrome is the focal form of SPS [97]. Nearly 80% of SPS have antibodies against glutamic acid decarboxylase 65 (GAD65) [96], although recent series have reported 34% of seronegative patients [98]. GAD65-SPS is usually non-paraneoplastic, but it may appear in association with malignant thymoma and breast cancer [99]. Frequency of paraneoplastic GAD65-SPS is unknown, but it is probably less than 6% [99]. In contrast, SPS associated with anti-amphiphysin antibodies is almost always paraneoplastic [81, 100–103]. It has a strong association with breast cancer [81, 100–102], but it has been also described in SCLC [103] and may account for approximately 10% of all SPS [100]. Compared to non-paraneoplastic GAD65-SPS, amphiphysin-SPS patients are even more frequently women, older, with

predominant neck and upper limb involvement, and more electrophysiological abnormalities [100]. Paraneoplastic SPS has also been reported in association with anti-gephyrin and mediastinal carcinoma [104], as well as anti-Ri and lung and bladder cancer [105, 106].

Progressive encephalomyelitis with rigidity and myoclonus (PERM) is part of the SPS spectrum [97]. PERM is mainly associated with glycine-receptor (Gly-R) antibodies [107]. Onset is often subacute, and rigidity and spams are usually preceded or accompanied by sensory or brainstem symptoms. Severe myoclonus, corticospinal signs, cerebellar ataxia, hyperekplexia, and dysautonomic dysfunction are other hallmarks of the disease, which can even lead to death [97, 108]. Encephalopathy and epilepsy are especially common at the peak of illness [108]. Sexes are equally affected and the median age at onset is 50 years [108]. Less than 10% of PERM are paraneoplastic [108]. PERM has been associated with malignant thymoma and Hodgkin's lymphoma [108–111]. Antibodies against dipeptidyl-peptidase-like protein-6 (DPPX) have been identified in a few patients with PERM syndrome [112, 113]. Interestingly, DPPX may also present as an encephalitis with prominent central nervous system hyperexcitability (seizures, myoclonus, agitation, tremor), diarrhea, and weight loss [113]. Most patients with DPPX antibodies have no underlying cancer, but some B-cell neoplasms have been reported [113].

Encephalomyelitis

This term must be used in patients with clinical signs and symptoms of multiple levels of the central and peripheral nervous system, when none of them is predominant. It may include chronic gastrointestinal pseudo-obstruction, sensory neuronopathy, myelitis, cerebellar degeneration, and brainstem or limbic encephalitis [16]. Encephalomyelitis is mainly associated with SCLC or breast cancer and anti-Hu, anti-CV2/CRMP5, anti-amphiphysin, and anti-Ri/ANNA-2 [14, 52, 81, 114]. Multifocal involvement has been also reported in small series of patients with anti-ANNA-3, PCA-2/MAP1B (microtubule associated protein 1B), and Zic4 antibodies [115–117]. SCLC is the most frequent associated cancer in these patients [115–117]. Different combinations of meningoencephalomyelitis also appear in 80% of encephalitis associated with anti-GFAP antibodies [94, 95].

Opsoclonus-Myoclonus Syndrome

This is a rare disease characterized by opsoclonus, which are involuntary, arrhythmic, and multidirectional saccades; and action myoclonus involving limbs, trunk, and head. Onset is acute or subacute [118]. Cerebellar ataxia and encephalopathy may also occur [119]. The two main etiologies are idiopathic (immune-mediated and sometimes thought to be parainfectious) and paraneoplastic [119]. Among

children, 50% of opsoclonus-myoclonus syndrome (OMS) are paraneoplastic, most of them associated with neuroblastoma and below 2 years of age [120]. No autoantibodies are usually detected [120]. Adult-onset OMS may be also paraneoplastic, but its frequency is probably lower [119, 120]. SCLC and breast cancer are the most common associated tumors [119, 121, 122]. Anti-Ri/ANNA-2 is the onconeural autoantibody most related to paraneoplastic adult-onset OMS (especially in breast cancer context), but it is found in less than 20% of patients [114, 121]. Several antibodies against neuronal cell surface antigens have been also reported in a few cases, including anti-glycine-receptor and anti-NMDA-R antibodies [121]. Compared to non-paraneoplastic OMS, paraneoplastic patients are older (mean 60 years), develop encephalopathy more frequently and have poorer prognosis with more relapses [121, 122].

Paraneoplastic Cerebellar Degeneration

This is defined as a pancerebellar syndrome developed subacutely within less than 12 weeks, without radiological evidence of cerebellar atrophy or metastasis [16]. Paraneoplastic cerebellar degeneration (PCD) is severe, disabling, and may be accompanied by non-cerebellar involvement [16]. PCD is the most frequent PNS of the central nervous system [7]. PCD may appear in association with several autoantibodies [123, 124]. Anti-Yo/PCA-1 is the most common, and it is related to gynecological cancers (breast and ovary) [123–126]. Anti-Yo PCD may develop brainstem and corticospinal dysfunction [125, 126]. In contrast to other PNS, anti-Yo PCD is commonly diagnosed in patients with already known cancer [123, 126]. Anti-Hu is the second most frequent antibody, but anti-Hu PCD is usually part of a larger paraneoplastic encephalomyelitis [14, 123]. SCLC is the most common cancer in anti-Hu PCD [123, 124]. PCD in SCLC patients may present with other antibodies, such as those against Zic4, VGCC, and AGNA/SOX [117, 127]. Anti-ANNA-2/Ri are also frequently associated with PCD and may also develop brainstem dysfunction or opsoclonus-myoclonus, and it is usually associated with breast cancer [114, 123, 124]. Anti-Tr/DNER (delta/notch-like epidermal growth factor-related receptor) and anti-mGluR1 (metabotropic glutamate receptor type 1) are two autoantibodies identified in PCD with Hodgkin's lymphoma [128–130], but anti-mGluR1 cerebellar ataxia may be also non-paraneoplastic [131]. As it occurs with anti-Yo patients, those with anti-Tr and anti-mGluR1 always develop cerebellar ataxia, whereas PCD is only present in nearly 18% of anti-Hu patients [123]. Cerebellar ataxia (isolated or not) is also frequent in patients with anti-CV2/CRMP5, anti-amphiphysin, PCA-2, and anti-Ma2 [52, 81, 116, 124, 132]. Anti-GAD cerebellar ataxia is more frequently non-paraneoplastic, but it may appear in association with lung and breast cancer [99]. Approximately 18% of PCD are seronegative [124]. Breast and gynecological cancers are still the most common among women with seronegative PCD, but its relative frequency is lower, whereas the

opposite occurs with lymphomas [124]. Men with seronegative PCD have more commonly non-SCLC and genitourinary cancers than seropositive patients [124].

Brainstem Encephalitis

Brainstem encephalitis (BE) has been widely seen as a typical presentation of PNS associated with anti-Ma antibodies [132]. In fact, most patients (80–90%) develop a combination of BE, limbic encephalitis (LE), and diencephalic involvement [132–135]. BE may present with eye movement abnormalities (especially, vertical gaze paresis), dysarthria, dysphagia, jaw dystonia, or atypical parkinsonism [132]. Hypothalamic dysfunction is mainly characterized by daytime sleepiness and other features resembling narcolepsy, along with abnormal weight gain, hyperthermia, and endocrine abnormalities [132]. Two subgroups of anti-Ma autoimmunity can be distinguished depending on the reactivity of the antibodies. First, patients with only anti-Ma2 antibodies are mainly young men (approximately 35–40 years of age), with predominant LE and testicular germ-cell tumors [132–135]. Secondly, patients with both anti-Ma1 and anti-Ma2 antibodies are older (median age 60 years), with similar distribution of sexes, more brainstem and cerebellar involvement, and association with several different types of cancer, non-SCLC being one of the most common [132–135]. Prognosis is better in patients with only anti-Ma2 antibodies [132–135].

BE is also the predominant feature in 6% of anti-Hu paraneoplastic encephalomyelitis [14]. Isolated anti-Hu BE has also been reported [136]. Unlike anti-Ma BE, which principally involves the mesencephalon, anti-Hu BE affects mainly the medulla: 50% at first evaluation and almost all during the progression of the disease [136]. As it occurs with other anti-Hu PNS, SCLC is the most common cancer [136]. Brainstem involvement is also a prominent feature of anti-Ri/ANNA-2 PNS, reaching 70% [114]. It may also appear with PCA-2, ANNA-3, and CV2/CRMP5 but at much lower rates [52, 115, 116]. Brainstem dysfunction is very frequent (75%) among DPPX autoimmunity, but as mentioned before, it is usually a non-paraneoplastic disorder [113].

Limbic Encephalitis and Related Syndromes

Limbic encephalitis (LE) is one of the most typical PNS that can be defined by subacute development (< 12 weeks) of seizures, short-term memory loss, and psychiatric symptoms suggesting involvement of the limbic system [16]. Several autoimmune encephalitis clinically close to LE have been described, but they have a wider involvement that surpasses the limbic system [137]. Recently, diagnostic criteria for autoimmune LE have been proposed, in order to improve early recognition and to make easier the differential diagnosis (Table 5) [137]. Differential diagnosis includes

Table 5 Diagnostic criteria for autoimmune limbic encephalitis

Possible	Definite
Subacute onset (<3 months) of working memory deficits, altered mental status or psychiatric symptoms	Subacute onset (<3 months) of working memory deficits, seizures or psychiatric symptoms suggesting involvement of the limbic system
At least one of the following: New focal CNS findings Seizures not explained by a previously seizure disorder CSF pleocytosis MRI features suggestive of encephalitis	Bilateral brain abnormalities on T2-FLAIR MRI (or FDG-PET) highly restricted to the medial temporal lobes, and at least one of the following: CSF pleocytosis Epileptic or slow-wave activity on EEG involving temporal lobes
Exclusion of alternative causes	Exclusion of alternative causes
Adapted from Graus et al. [137]	

Definite diagnosis can be also made with the detection of autoantibodies against onconeural or cell-surface antigens, and at least two of : suggestive clinical picture, imaging or other (CSF, EEG) ancillary tests

several disorders such as infectious LE (especially herpes simplex encephalitis, HSE), gliomas, or epilepsy of other etiology [137]. Autoimmune LE and HSE are clinically very similar, but there are some clues that can differentiate them: acute (< 7 days) onset, fever, and aphasia are more suggestive of HSE; meanwhile, psychiatric symptoms support autoimmune LE diagnosis [138]. Nearly 90% of autoimmune LE are positive for either onconeural antibodies or antibodies against cell surface antigens [139, 140]. Some years ago, paraneoplastic and non-paraneoplastic were thought to be approximately equally frequent [139]. Nowadays, due to the description and characterization of novel autoantibodies against neuropil antigens, non-paraneoplastic LE is thought to be more common, especially because anti-LGI1 and anti-Caspr2 LE are usually not related to cancer [3, 5, 140]. GABAb-R (gamma-aminobutyric acid-B receptor) and AMPA-R (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) antibodies follow anti-LGI1 in frequency [140], but nearly 50% of patients with such antibodies have an underlying cancer [3, 5]. Anti-Hu and anti-Ma2 are the most common onconeural antibodies associated with paraneoplastic LE, usually with associated SCLC and testicular germ-cell tumors, respectively [140–142]. Antibody-negative LE is paraneoplastic in 40% of cases, but no main cancer has been identified [140]. The disorder is characterized by prominent cognitive impairment with mild or no seizures, or psychiatric symptoms, compared to antibody-positive LE [140].

Anti-LGI1 LE is more frequent in men and the median age is 60 years [13, 143]. Facio-brachial dystonic seizures (FBDS) are the hallmark of anti-LGI1 LE [144, 145]. They occur in up to 60% of patients, usually preceding the cognitive impairment [144, 145]. FBDS are brief episodes lasting seconds, often triggered by emotions, noises, or movements, and may appear many times a day [144, 145]. They consist of ipsilateral face and arm posturing, sometimes also involving the leg [144, 145]. Sensory aura is common [144, 145]. A contralateral frontal wave can be detected in electroencephalogram before muscle artifacts caused by FBDS [145].

Many other types of seizures have been described in anti-LGI1 LE, usually originated from the temporal lobe, and including cognitive and autonomic symptoms [145, 146]. Paroxysms-dizziness-spells are other specific events of anti-LGI1 LE [45]. Nearly 60% of patients develop hyponatremia, and sleep disturbances appear in approximately 30% [13, 45, 146]. Only 10% of anti-LGI1 LE are paraneoplastic, particularly associated with malignant thymoma [45, 143].

Anti-Caspr2 LE is also more common in men older than 60 years [49]. LE patients with anti-Caspr2 antibodies are usually non-paraneoplastic [45, 49]. Conversely, MoS patients with anti-Caspr2 antibodies usually develop malignant thymoma [45, 49]. Unlike neuromyotonia or MoS, Caspr2-antibodies are present in the CSF of LE patients [49]. Extralimbic involvement may occur, the most common being cerebellar ataxia [49]. Cerebellar ataxia may be permanent or episodic [147].

AMPA-R LE is more frequent in middle-to-late-aged women [148–153]. Seizures seem to be less common than in other LE. Isolated epilepsy onset is rare [149, 150]. By contrast, psychiatric symptoms are frequent, and acute psychosis may be the sole clinical presentation [149–151]. Some patients may present with a more diffuse encephalopathy and even develop a fulminant form with severe brain atrophy and poor prognosis [149, 150, 152]. Approximately 50% of AMPA-R LE are paraneoplastic: lung, thymus, and breast cancer are the most common [148–153].

GABAb-R LE is characterized by prominent seizures and commonly status epilepticus [154–157]. Extralimbic involvement or presentation may also occur, especially cerebellar ataxia [155–157]. SCLC is found in nearly 50% of GABAb-R LE [154–157]. In fact, 70% of LE associated with SCLC and without onconeural antibodies are positive for GABAb-R antibodies [158]. Paraneoplastic patients are often male and older than 60 years of age [154–158].

LE with glutamic acid decarboxylase (GAD) autoantibodies was described as typical of young women, usually non-paraneoplastic and clinically characterized by predominant seizures [159]. Nevertheless, LE has been identified as the most frequent PNS among a recent series of GAD patients, commonly in association with neuronal cell surface autoantibodies (mainly GABAb-R) and lung cancer [98, 158].

Ophelia syndrome was initially reported as a LE associated with Hodgkin's lymphoma [160]. Later, antibodies against metabotropic glutamate receptor 5 (mGluR5) were detected in a few Ophelia syndrome patients and in one patient with a non-paraneoplastic LE [161–163]. A recent series has better defined the clinical profile of encephalitis with anti-mGluR5 antibodies [164]. Most patients are younger than 50 years of age. Psychiatric and cognitive symptoms are the most frequent, followed by sleep disturbances, seizures, altered mental status, and movement disorders [164]. Hodgkin's lymphoma is by far the most common related cancer (80%), but in almost half of the series, no tumor association could be demonstrated [164].

GABAa-R (gamma-aminobutyric acid-A receptor) autoimmunity is defined by a rapidly progressive encephalopathy with refractory seizures that often progress to status epilepticus (50%) [165–167]. Epilepsia partialis continua has been reported [167]. Cognitive, psychiatric, and movement disorders are also common [166, 167]. GABAa-R encephalitis patients are usually young: up to 40% may be under 18 years of age [165, 167]. Children usually do not have an underlying neoplasm; mean-

while, nearly 40% of adult patients have tumors, mainly malignant thymoma [167–169].

DPPX encephalitis is more frequent in middle-aged men [113, 170, 171]. Weight loss and diarrhea precede neurological symptoms in most patients [113, 170, 171]. Onset may be subacute or more frequently progressive for several months [113, 171]. Cognitive and psychiatric disorders are the core of the clinical picture, but central nervous system hyperexcitability is the most typical feature of DPPX encephalitis [113, 170, 171]. It may present as myoclonus, hyperekplexia, tremor, or seizures [113, 170, 171]. Sleep disturbances and brainstem and cerebellar dysfunction are also common [113, 171]. DPPX encephalitis is usually non-paraneoplastic, but a few patients with B-cell neoplasms have been reported [113].

Encephalitis with anti-adenylate kinase 5 antibodies is an uncommon non-paraneoplastic disorder [172, 173]. Patients are often old men with subacute, commonly isolated, cognitive dysfunction [172, 173]. Behavior changes may also appear [172, 173]. Unlike classical LE, no seizures have been reported [172, 173]. Depression, asthenia, and anorexia with weight loss may precede the development of anterograde amnesia [173].

NMDA-R Encephalitis

NMDA-R encephalitis was first reported in young women with ovarian teratoma and a stereotyped clinical presentation characterized by psychiatric symptoms, memory impairment, seizures, and decreased level of consciousness [174]. Later series have expanded the phenotype and described in detail the clinical features [11, 175, 176]. Nearly 80% of NMDA-R encephalitis are women and the median age is approximately 20 years; the disorder is strongly associated with ovarian teratomas (nearly 50% of women) [11, 175, 176]. It is also common in children (up to 37% of patients are under 18 years of age). In children and in patients older than 45 years of age, there is an equal proportion of male and female cases [11]. Tumors other than teratomas (especially carcinomas) are more commonly detected in older patients [11, 177]. Prodromal flu-like symptoms such as headache and fever may appear 2 weeks before the onset of neurological disease [175, 176]. Psychiatric symptoms (such as hallucinations, depression, acute psychosis) and memory loss are the main presenting symptoms in adults, whereas seizures and movement disorders are more frequent in children [11]. Patients older than 45 years of age have less frequently prodromal symptoms and cognitive impairment predominate over seizures [177]. Seizures are also more common as the presenting symptom in men than in women [178, 179]. Nevertheless, most patients develop the full syndrome within the first 4 weeks, regardless of their age [11, 175]. This is defined by six categories of symptoms: abnormal (psychiatric) behavior and cognitive dysfunction; speech dysfunction (pressured speech, verbal reduction, mutism); seizures; movement disorders (orofacial dyskinesias, rigidity, abnormal postures); decreased level of consciousness; and dysautonomia (cardiac dysrhythmias, hyperpirexia, labile blood pressure)

or central hypoventilation [137]. Only 1% remain mono-symptomatic [11]. NMDA-R encephalitis is a severe disease that may need intensive care support in up to 77% of cases [11]. Along with MRI and CSF (see below: diagnosis), EEG may show a typical pattern called "extreme delta brush" in 30% of patients, that can be described as delta activity with beta rhythm superimposed [180]. Diagnostic criteria for NMDA-R encephalitis have been newly proposed (Table 6) [137].

Recently, herpes simplex virus has also been identified as a trigger of NMDA-R encephalitis [4]. Up to a third of HSV encephalitis will develop NMDA-R encephalitis [181, 182]. Herpes simplex PCR in CSF is negative at the time of NMDA-R encephalitis [4, 181, 182]. Young children usually present with choreoathetosis and decreased level of consciousness within the first month after HSV encephalitis [181, 182]. In contrast, older children, adolescents, and adults generally develop behavior and cognitive abnormalities, sometimes in a progressive manner over 6 weeks [181, 182].

Movement Disorders

Movement disorders may appear accompanying other clinical features in PNS, as mentioned before in NMDA-R encephalitis and other limbic and extralimbic encephalitis. Isolated movement disorders as PNS are very uncommon (1%) [183]. Subacute symmetric choreoathetosis is the most frequent presenting form, usually in association with SCLC and anti-CV2/CRMP5 antibodies [183]. Basal ganglia encephalitis with antibodies against dopamine-2 receptor is a non-paraneoplastic

Table 6 Diagnostic criteria for NMDA-R encephalitis

Possible NMDA-R encephalitis can be diagnosed when all the following criteria have been met[a]:
1. Rapid onset (< 3 months) of at least four of the six following major groups of symptoms:
Abnormal (psychiatric) behavior or cognitive dysfunction
Speech dysfunction (pressured speech, verbal reduction, mutism)
Seizures
Movement disorders, dyskinesias, or rigidity/abnormal postures
Decreased level of consciousness
Autonomic dysfunction or central hypoventilation
2. At least one of the following:
Abnormal EEG (focal or diffuse slow disorganized activity, epileptic activity or extrema delta brush)
CSF pleocytosis or oligoclonal bands
3. Exclusion of alternative causes
[a]Alternatively in the presence of three of the major groups of symptoms and systemic teratoma
Definite NMDA-R encephalitis: one or more of the six major groups of symptoms and IgG anti-GluN1 antibodies

Adapted from Graus et al. [137]

disease that appears exclusively in children who develop movement and psychiatric disorders [184]. In contrast, the median age of IgLON5 disease is 65 years [185, 186]. Patients present with a complex sleep disorder, gait instability, chorea-parkinsonism, and bulbar and cognitive dysfunction [185, 186]. Pathology shows taupathy features [184]. No association with cancer has been reported with anti-IgLON5 antibodies [185, 186].

Pathogenesis

PNS are now well clinically defined autoimmune disorders. A role of the immune system is supported by the detection of antibodies or T cells against neuronal antigens that are also expressed by tumor cells, indicating that PNS are the result of a misdirected response of the immune system to cancer, which also explain why many tumors associated with PNS are confined to the primary organ [187–189]. The underlying pathogenesis of PNS is strongly related to the type of accompanying autoantibodies. These can be classified depending on the location of their antigens [187–189].

Antibodies Against Intracellular Antigens

These antibodies target a nuclear or a cytoplasmic antigen that are almost exclusively expressed in the nervous system (Table 7) [189]. They are closely associated with cancer and are therefore also called onconeural antibodies. As intracellular antigens cannot be reached by the antibodies in most cases, they are thought to be non-pathogenic [187–189]. This is supported by passive transfer of anti-Hu antibodies that did not produce neurological disorders in mice; meanwhile, immunization with Hu protein led to antibody synthesis and immune response to tumor, but without PNS [190, 191]. Although they can be detected in patients with cancer without PNS (e.g., 15% of SCLC patients have circulating anti-Hu antibodies), they are more frequently present at higher titers and show intrathecal synthesis in PNS [16, 192, 193]. Along with this humoral response, a strong cellular response has been demonstrated in patients with onconeural antibodies and it is thought to be the main immune effector. Several anatomopathological studies have shown prominent inflammatory infiltrates, particularly of cytotoxic T cells, which lead to neuronal death, explaining the poor response to immunotherapy [187–189]. Furthermore, specific CD8+ T lymphocytes activated against peptides of Hu or Yo proteins have been detected in PNS patients with anti-Hu or anti-Yo antibodies, respectively [194–196]. Recently, new research findings have proposed that loss of immune tolerance to these antigens expressed in tumor cells is important to develop the neurological syndrome, and T CD4+ lymphocytes may also play a major role in PNS pathogenesis [197–199].

Table 7 Characteristics of intracellular onconeuronal antigens [188, 189]

Antibody	Antigen	Cellular location	Function
ANNA1/anti-Hu	Hu proteins (mainly HuD)	Nuclear	RNA-binding proteins
ANNA2/anti-Ri	Nova-1 and Nova-2	Nuclear	RNA-binding proteins
ANNA3	Unknown	Nuclear	Unknown
AGNA	SOX1	Nuclear	Transcription factor
Anti-Ma	Ma1 y 2	Nucleolus	Apoptosis
Anti-PCA1/Yo	CDR2	Cytoplasmic	Transcription factor
Anti-PCA2/ MAP1B	MAP1B	Cytoplasmic	Microtubule-binding protein
Anti-CV2	CRMP5	Cytoplasmic	Signaling of axon guidance and neurite outgrowth
Anti-ZIC4	Zinc finger protein 4	Cytoplasmic	Brain development
Anti-AK5	Adenylate kinase 5	Cytoplasmic	Adenine nucleotide homeostasis
Anti-amphiphysin	Amphiphysin	Cytoplasmic (synapsis)	Synaptic vesicle recycling
Anti-GAD	GAD (mainly GAD65)	Cytoplasmic (synapsis)	Synthesis of GABA from glutamate

Two intracellular antigens related to synapsis have particular characteristics: amphiphysin and GAD65. Amphiphysin is involved in the recycling of synaptic vesicles, whereas GAD65 is the rate-limiting enzyme for the synthesis of GABA and is attached to the membrane of the synaptic vesicles in GABAergic neurons [187–189]. Thus, although intracellular, both can be exposed to antibodies during exo- and endocytosis [187–189]. Passive transfer of anti-amphiphysin IgG has been reported to induce a stiff-person syndrome phenotype in rats [200, 201]. These antibodies were internalized by neurons, disrupting the recycling of inhibitory synaptic vesicles [201]. GAD65-antibodies from SPS have shown to reduce GABA synthesis, and those from GAD65-associated cerebellar ataxia may alter synaptic vesicle exocytosis [202, 203]. Although there is some evidence that antibodies against intracellular synaptic antigens may play a pathogenic role, other studies (including anatomopathological ones) support that the pathogenesis is mediated by a T-cell response [188].

Antibodies Against Cell Surface Antigens

In contrast to patients with associated antibodies targeting intracellular antigens, those with antibodies directed against cell surface antigens develop less frequently cancer and the antibodies are suspected to be pathogenic. First, antigens can be easily reached by the antibodies on the cell surface. Second, most of the associated neurological disorders respond to immunotherapies that remove antibodies, suggesting that neurological dysfunction is reversible and not related to

neuronal cell death [204]. Furthermore, inflammatory infiltrates are mainly composed of B rather than T lymphocytes, and IgG deposits are common [189]. Third, genetic or pharmacological disruption of these antigens often induces similar phenotypes in humans or in animal models, as it has been demonstrated with NMDA-R, LGI1, Caspr2, GABAb-R, or Gly-R proteins [205–210]. Finally, in vitro studies have shown the effects of some autoantibodies on their antigens, usually receptors or proteins related to them, involved in synapse functions (Table 8) [108, 130, 165, 204, 211–217]. In vivo evidence of a direct role of circulating autoantibodies in neuronal dysfunctions only exists for anti-mGluR1 and anti-NMDA-R antibodies [130, 218].

Table 8 Brain effects of autoantibodies recognizing cell surface antigens [188, 189, 204]

Antibody	Antigen	Epitope	In vitro effects
NMDA-R	Ionotropic glutamate receptor	GluN1 subunit	Crosslink and internalization, reducing NMDA-R at synapsis
AMPA-R	Ionotropic glutamate receptor	GluA1 or GluA2 subunits	Crosslink and internalization, reducing AMPA-R at synapsis
GABAa-R	Ligand-gated ion channel	α1 and β3 subunits	Reduction of GABAa-R density
GABAb-R	G protein-coupled receptor	B1 subunit	Receptor antagonist
LGI1	Secreted glycoprotein, interacts with ADAM23 (presynaptic) and ADAM22 (postsynaptic), organizes Kv1.1 potassium channels and AMPA-R	Epitempin and leucine-rich domain	Inhibition of interaction with ADAM
Caspr2	Transmembrane protein, clusters Kv1.1/Kv1.2 potassium channels at juxtaparanodal region	Discoidin and laminin G1 domains	Alteration of gephyrin clusters at inhibitory synaptic contacts
mGluR1	Metabotropic glutamate receptor	Amino-terminal extracellular domain	Reduction of basal activity of Purkinje cells
mGluR5	Metabotropic glutamate receptor	Unknown	Unknown
Gly-R	Chloride channel receptor	α1 subunit	Internalization
DPPX	Membrane glycoprotein, regulated Kv4.2 potassium channels	Unknown	Hyperexcitability of enteric neurons, decrease of DPPX expression in hippocampal neurons
Tr/DNER	Delta/notch-like epidermal growth factor-related receptor	Glycosylated epitopes of the extracellular domain	Unknown

Genetics

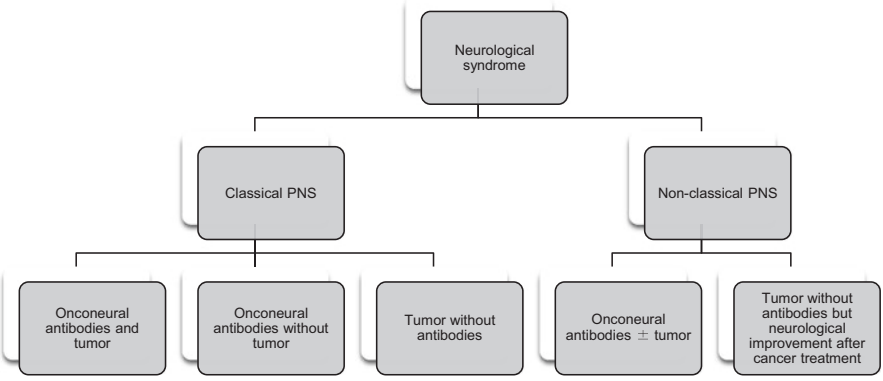
Why some patients with a certain cancer develop PNS and others not may be a result of genetic predisposition. For example, human leukocyte antigen (HLA) serotypes DQ2 and DR3 for anti-Hu PNS, or HLA class II haplotypes DRB1∗13:01-DQA1∗01:03-DQB1∗06:03 for anti-Yo PCD in ovarian cancer have been reported as haplotypes of susceptibility [219, 220]. Although anti-Hu and anti-Yo syndromes are thought to be mainly mediated by T CD8+ lymphocytes, their particular HLA associations (involving major histocompatibility complex (MHC) classes I and II) suggest that CD4+ T cells and humoral response may also be involved in the pathophysiology [219, 220]. Likewise, HLA-DR7 (DRB1∗07:01) is strongly associated with LGI1-encephalitis, a commonly non-paraneoplastic disorder without known trigger [221–223]. MHC class II association is in accordance with the presumed antibody-mediated pathogenesis of LGI1 encephalitis [221]. No tight relationship has been reported so far between HLA and NMDA-R encephalitis [222, 223]. Thus, knowing genetics of PNS may help us to understand their pathogenesis and could be used as biomarkers of the disease.

Diagnosis

Once a neurological syndrome is suspected to be paraneoplastic based on its clinical presentation, ancillary tests such as imaging or CSF studies may help to exclude other possibilities or to reinforce the PNS suspicion [2, 3, 5]. The following step to achieve a correct PNS diagnosis is to establish the presence of a known antibody. Combining the clinical syndrome and the associated autoantibody allows appropriate tumor screening, which should be undertaken as soon as possible [2, 3, 5].

Finally, diagnostic criteria have been defined in order to improve the accuracy of PNS diagnosis [16]. They mainly take into account the clinical syndrome (classical and nonclassical, Table 1) and the type of the antibody (classified as *well-characterized onconeural antibodies*, anti-Hu, Yo, CV2/CRMP5, Ri, Ma2, amphiphysin, SOX1, Tr/DNER, GAD; and *partially characterized onconeural antibodies*, ANNA-3, PCA-2, Zic4, mGluR1) and if a cancer has been detected [16, 224]. According to these three items, PNS diagnosis may be (Algorithm 1) [16, 224]:

1. Definite PNS:
   (a) Classical PNS and cancer within 5 years of the PNS diagnosis.
   (b) Nonclassical PNS that resolves or improves after cancer treatment.
   (c) Nonclassical PNS with onconeural antibodies (irrespective of type) and cancer within 5 years of the PNS diagnosis.
   (d) PNS (irrespective of type) and well-characterized onconeural antibodies, without cancer.



Algorithm 1 Diagnostic criteria for PNS. (Adapted from Graus et al. [16] and Peterson et al. [125])

2. Possible PNS:

   (a) Classical PNS without onconeural antibodies nor cancer, but with high oncological risk (e.g., > 40 years of age and smoker).
   (b) PNS (irrespective of type) with partially characterized onconeural antibodies, without cancer.
   (c) Nonclassical PNS without onconeural antibodies, but cancer within 2 years of the PNS diagnosis.

Imaging

Imaging is often normal in PNS, but it is helpful to exclude other diseases or cancer complications such as metastasis [2]. Nevertheless, there are some exceptions in which neurodiagnostic testing, mainly brain MRI, is important to suspect a PNS disorder or even to diagnose it [2, 3, 5, 137].

Paraneoplastic myelopathy is mainly characterized as longitudinally extensive tract-specific signal changes (up to 65% of patients), resembling Devic's disease (Fig. 1) [87–90]. Lateral columns are the most commonly affected and contrast enhancement may be seen in a half of those with abnormal MRI [87, 88]. Myelopathy associated with GFAP astrocytopathy also presents as a longitudinally extensive myelitis, whereas patients with meningoencephalitis may show a linear perivascular enhancement in brain MRI, extending in a radial manner from the lateral ventricles to the cortex [95].

Limbic encephalitis (irrespective of the autoantibody) is characterized by T2-weighted fluid-attenuated inversion recovery (FLAIR) hyperintensity signal that affects the medial temporal lobes bilaterally (Fig. 2) [15, 49, 132, 137, 140, 141, 148, 154, 156, 173]. Initial MRI may be normal in LGI1 encephalitis, especially

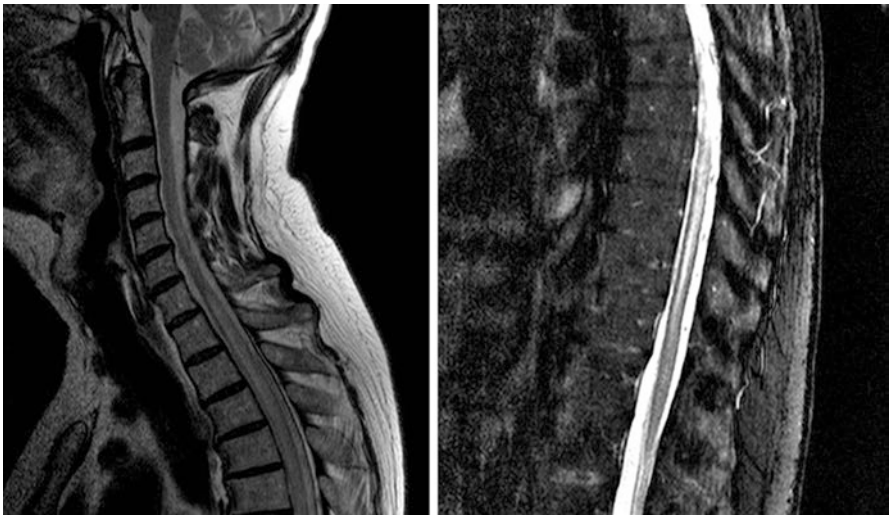


Fig. 1 Cervical (sagittal T2) and thoracic (sagittal STIR) spinal MRI, from a patient with paraneoplastic (lung cancer) myelopathy, showing longitudinally extensive abnormality resembling Devic's disease

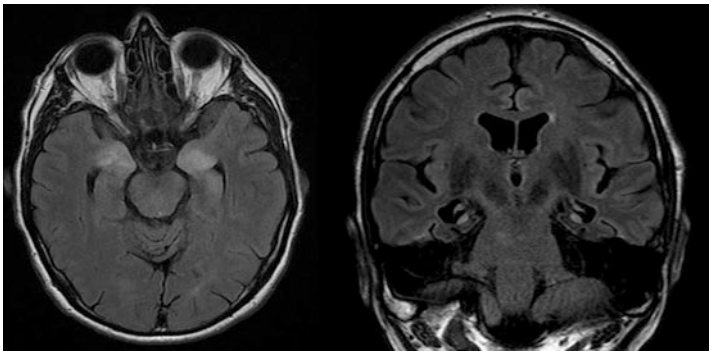


Fig. 2 Axial FLAIR brain MRI from a patient with limbic encephalitis associated with anti-adenylate kinase 5 antibodies, showing bilateral hypersignal in the medial temporal lobes. Coronal FLAIR brain MRI from the same patient 1 year later, showing hippocampal atrophy

when FBDS are the only clinical manifestation, but approximately 75% of patients will show a typical LE MRI, and later half of them will develop hippocampal sclerosis [144–146]. AMPA-R encephalitis may present as LE with typical MRI or as a more diffuse encephalopathy with extralimbic involvement; some patients develop a fulminant form with severe and rapidly progressive brain atrophy (Fig. 3) [149, 150].

Anti-Ma encephalitis generally presents with brainstem, diencephalic, and limbic features, and these are accompanied by prominent MRI abnormalities involving

the symptomatic areas [132–135]. Contrast enhancement is frequent and may mimic lymphoma or glioma tumors [132–135]. GABAa-R encephalitis usually shows a distinctive pattern of severe multifocal, bilateral, cortical, and subcortical lesions, usually asynchronous and without contrast enhancement [165, 167]. NMDA-R encephalitis brain MRI is abnormal in nearly 30% of patients, showing multiple but mild hyperintensity signals that sometimes are transient and may have contrast enhancement [11, 174, 175]. Encephalitis with D2-R antibodies may show basal ganglia abnormalities (Fig. 4) [184].

Positron Emission Tomography with 18F-Fluorodeoxyglucose (FDG-PET)

Recently, FDG-PET has been demonstrated to be more sensitive than MRI to show abnormalities in autoimmune encephalitis [225, 226]. Thus, FDG-PET can be a useful test, especially when MRI is normal, to detect limbic or even extralimbic changes [225, 226]. Mesiotemporal hypermetabolism is the common finding in limbic encephalitis, but it can evolve to hypometabolism when hippocampal atrophy establishes [225, 226]. In NMDA-R encephalitis, hypermetabolism in frontal and temporal regions and hypometabolism in occipital lobes have been described as a characteristic pattern [227].

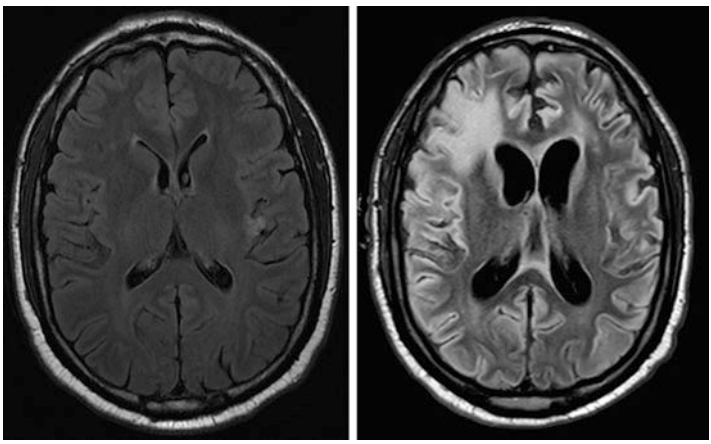


Fig. 3 Axial FLAIR brain MRI from a patient with fulminant AMPA-R encephalitis at onset (left) and 2 months after (right), showing the development of severe and diffuse atrophy

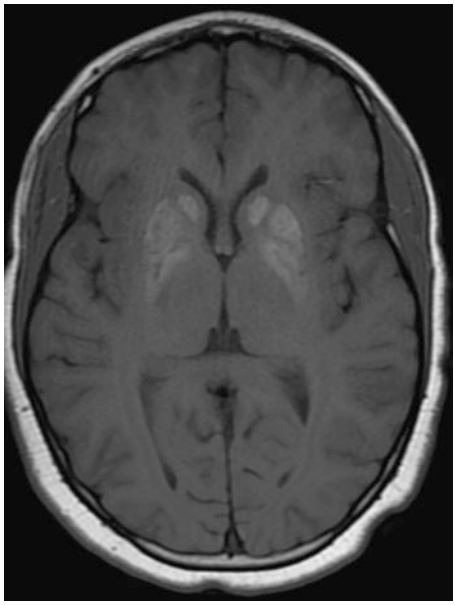


Fig. 4 Axial FLAIR brain MRI from a patient with basal ganglia encephalitis with anti-D2 R antibodies

CSF Abnormalities

More than 90% of PNS associated with onconeural antibodies have CSF abnormalities [228]. They may have lymphocytic pleocytosis, hyperproteinorachia, elevated IgG index, or positive oligoclonal bands (OCB) [228]. Cell count and protein concentration are slightly elevated, usually with median values less than 100 cells/L or mg/dL [3]. OCB may be the only abnormality in nearly 10% of patients, and should therefore never be forgotten [228].

CSF findings are similar for most of the PNS associated with cell surface antibodies, but a normal CSF does not exclude diagnosis as frequency of these findings is quite variable [2, 3]. For example, NMDA-R encephalitis shows CSF abnormalities in 80–90% of patients [11, 175], but LGI1 encephalitis usually presents normal CSF [144–146]. As it occurred in PNS with onconeural antibodies, OCB may appear isolated; for example, in NMDA-R encephalitis, they are detected later in the course of the disease, once pleocytosis has normalized [176].

Antibody Testing

PNS may be not associated with autoantibodies, but their detection in a suitable clinical setting is diagnostic [16]. Thus, antibody investigation is the most important ancillary test in PNS diagnosis. Overall, one clinical syndrome may be associated

with several antibodies, so a panel of tests is generally used. Nevertheless, once a positive result is obtained, it is mandatory to evaluate whether:

1. There is a known relationship between the antibody and the clinical syndrome. Otherwise, the antibody may not be responsible for the PNS, and we should expand the investigation [2, 3, 5].
2. Antibody titers are highly enough to consider the result as specific. Low titers of some antibodies (e.g., anti-Hu) may appear in cancer patients without PNS, and other antibodies (GAD, VGKC) may also appear in healthy population [2, 3, 5].
3. Antibody test has been conducted by an accurate technique on the correct sample:
   (a) Serum is almost always highly sensitive for onconeural antibody testing [2]. In contrast, CSF is usually more sensitive and specific than serum for most of the antibodies against cell surface antigens [2, 3, 5, 137], with probably the exception of LGI1 LE and Caspr2 MoS and neuromyotonia [45, 47, 146]. To avoid doubtful results, it is recommended to always test paired CSF and serum samples [2, 3, 5]..
   (b) The accurate test depends on the type of antibody [137, 229]:
      (i) Tissue-based assays, using indirect immunofluorescence or immunohistochemistry, should be used as a screening method for onconeural and cell surface antibodies (with the exception of anti-GlyR).
      (ii) Immunoblot is the confirmation test for antibodies against intracellular antigens (Fig. 5).

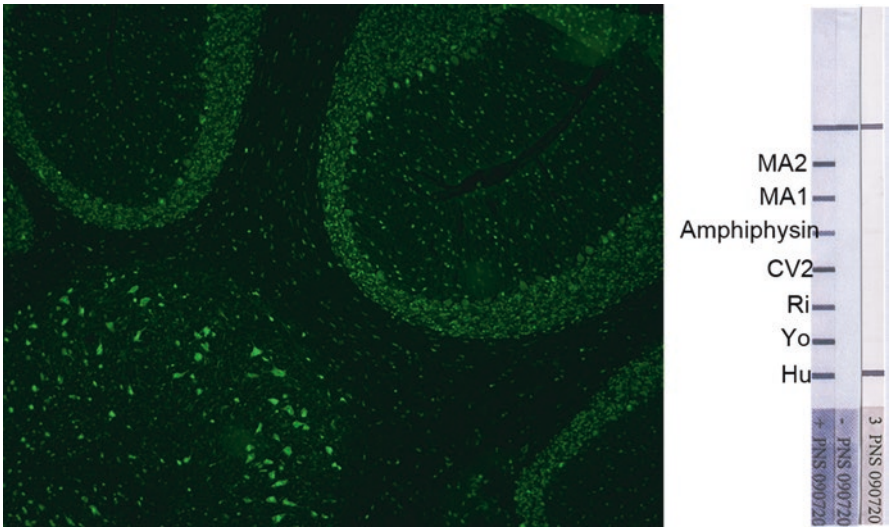


Fig. 5 TBA (rat cerebellum) and dot-blot positive for anti-Hu antibody

(iii) Cell-based assays (transfected HEK cells expressing the antigen) are the confirmation tests for antibodies against cell surface antigens, because their epitopes are usually conformational (unlike linear epitopes of onconeural antibodies) and need to be presented to the antibodies in their native structure (Fig. 6).
(iv) ELISA is the preferred test to quantify antibody titers.

It is currently not recommended to take treatment decisions based on the evolution of antibody titers [5, 137]. Unless some studies have reported changes of anti-NMDA-R antibody titers (especially in CSF) accompanying clinical improvement and relapses, many patients have persistent positivity after clinical recovery [176, 230–232]. Even less evidence exists for the serial evaluation of LGI1-antibody titers [144].

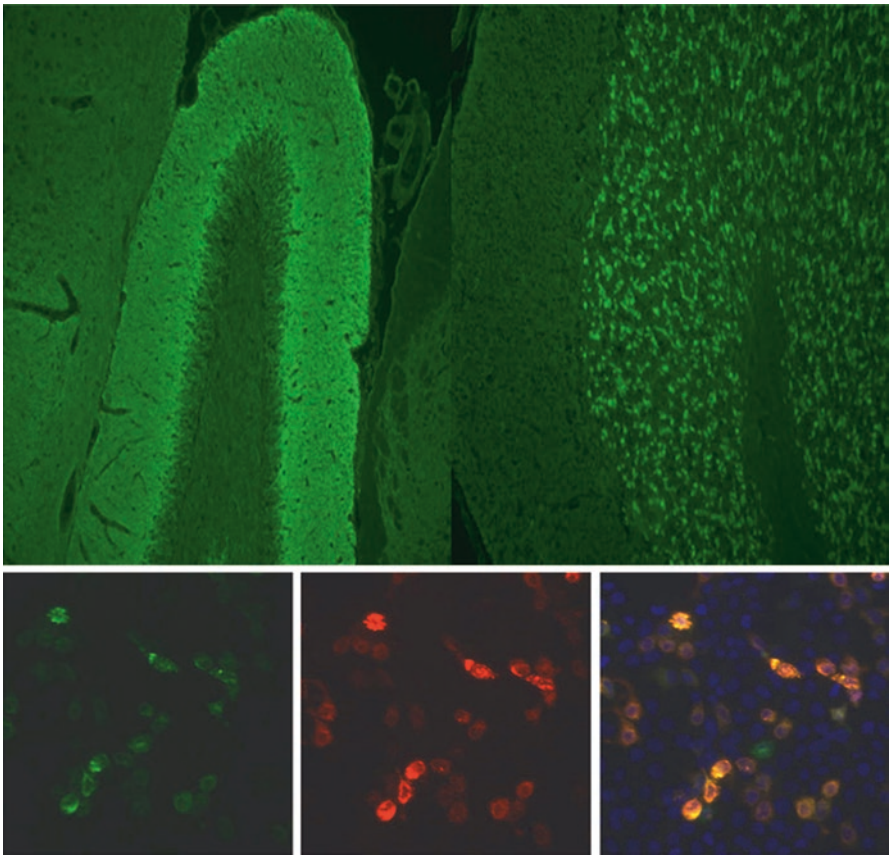


Fig. 6 TBA of rat hippocampus (left) and cerebellum (right) positive for anti-NMDAR antibody. Bottom, CBA: HEK cells expressing recombinant NMDAR subunit 1 (left), after addition of patient CSF with anti-GluN1 IgG (center); and overlay (right)

Tumor Screening

When a PNS is suspected, tumor screening should be promptly undertaken. It is important not only to better establish PNS diagnosis, but also to improve neurological outcome by cancer treatment. The work-up should search for the most common tumors associated with the PNS-antibody combination (Tables 3 and 4), but also taking into account possible symptoms and signs of the underlying neoplasm. If a type of tumor not usually associated with the patient's PNS or antibody is detected, then screening should continue. If PNS appears or deteriorates in a patient with a known cancer, tumor recurrence should be suspected. Ideally, tumor screening should be conducted by a multidisciplinary team [2, 5, 233]. Recommendations according to each type of tumor are the following [5, 233]:

1. SCLC and malignant thymoma: CT thorax followed by FDG-PET or integrated FDG-PET/CT.
2. Breast cancer: mammography, followed by MRI-breast. If negative, FDG-PET/CT.
3. Ovarian teratoma: transvaginal ultrasound (US), followed by MRI-pelvis/abdomen. If negative, CT thorax searching for extra-pelvic teratomas.
4. Ovarian carcinoma: transvaginal US, followed by MRI-pelvis/abdomen or FDG-PET/CT. If negative in post-menopausal women with anti-Yo, exploratory surgery or preventive ovarian removal.
5. Testicular tumors: US, followed by CT of the pelvic region. Biopsy is recommended in men <50 years old with microcalcifications on US.
6. Hodgkin's lymphoma: full-body CT or FDG-PET/CT. Abnormal lymph nodes should be biopsied.

Seronegative PNS may be studied searching for the most common associated cancer according to the clinical picture, and if initial work-up is negative, a total-body FDG-PET is recommended. Adult patients with dermatomyositis should be tested by CT thorax/abdomen, colonoscopy (>50 years old), mammography and pelvis US for women, and testicular US for men [233]. If first screening is negative, screening should be done every 6 months for 4 years in PNS with onconeural antibodies, with the exception of LEMS, in which 2 years is sufficient [2, 233]. In PNS associated with antibodies against cell surface antigens, less frequently related to cancer, screening every 6 months for at least 2 years is recommended [2, 5].

Treatment and Prognosis

Only few clinical trials have been published on PNS treatment and current treatment recommendations are mainly based on retrospective case series and expert opinions. PNS treatment has two principles: tumor removal and immunosuppression [2, 3, 5]. Both of them should be started as soon as possible in order to avoid irreversible

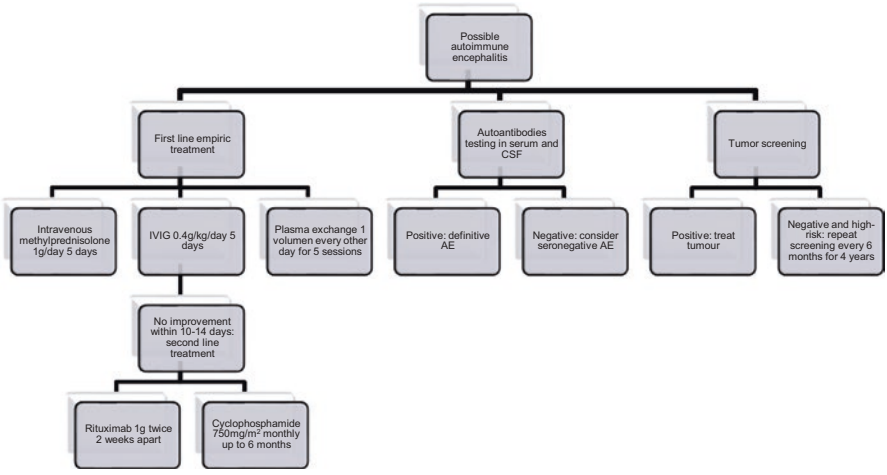
effects caused by neural death. PNS with onconeural antibodies usually have a progressive course, with severe disability at diagnosis and worse response to immunotherapy than PNS with antibodies against cell surface antigens [2, 3, 5].

Immunotherapy used in PNS and related non-paraneoplastic neurological syndromes is usually divided into (1) first-line therapies, including corticosteroids (CC), intravenous immunoglobulins (IVIG), and plasma exchange (PEX), and (2) second-line therapies, mainly rituximab and cyclophosphamide (Table 9). First-line treatments may be used in combination, for example, IVIG and CC. Second-line treatment should be considered if deterioration or no response after 10–14 days (especially in limbic encephalitis); monthly cyclophosphamide generally follows rituximab administration [234, 235]. For autoimmune encephalitis, immunotherapy should be started as soon as diagnosis is suspected, once infectious etiologies have been reasonably excluded; waiting for antibody test results is not recommended (Algorithm 2) [137].

Initial reports described very low rates of improvement among PNS with onconeural antibodies, early cancer treatment being the best predictor of clinical improvement or stabilization [14, 81, 125, 126, 236]. Many patients died because of

Table 9 Main drugs used as immunotherapy in PNS [234, 235]

Drug	Dosage
Methylprednisolone	500–1000 mg/day for 3–5 days
Intravenous immunoglobulins	0.4 g/kg/day for 5 days
Plasma exchange	1 volume every other day for 5 sessions
Rituximab	375 mg/m^2 of body surface every week for 4 weeks, or 1 g twice 2 weeks apart
Cyclophosphamide	1 g (or 750 mg/m^2 of body surface) over one day every month



Algorithm 2 Management of autoimmune encephalitis. (Adapted from Graus et al. [137])

complications of the PNS [14, 125, 126]. In Hu-PNS, mortality was associated with age, greater mRS at diagnosis, multifocal involvement, and absence of treatment [14]. Anti-Hu PNS generally have shorter survival than anti-CV2/CRMP5 PNS [53, 79]. Anti-Ri and anti-Ma PNS usually have better outcomes. Improvement has been reported in up to 70% of anti-Ri patients after treatment (oncological and/or immunotherapy) [114]. Among anti-Ma patients, prognosis is better in young males with only anti-Ma2 antibodies and testicular tumors that respond completely to oncological treatment; rates as high as 100% of stabilization or improvement have been reported in this subgroup [132–135]. Nevertheless, more recent series have shown that immunotherapy may also play a role in classical PNS, especially when it is used promptly (<3 months after PNS onset) and in non-severe disabled patients (mRS <4) [237–239]. High-dose intravenous CC, IVIG, PEX, rituximab, and cyclophosphamide have shown stabilization or improvement in 50–65% of patients with PNS associated with anti-Hu, anti-Yo, and anti-CV2/CRMP5 [53, 237–240].

Most information about PNS with antibodies against cell surface antigens is based on NMDA-R encephalitis experience. Nearly 50% of anti-NMDA-R encephalitis patients do not respond to first-line therapy (IVIG+CC) and need the second one (rituximab+cyclophosphamide) [11]. Most anti-NMDA-R patients improve progressively for 24 months or more, 80% reaching mRS < 2 [11]. Prognosis is better in patients who do not need intensive care, treated early (immunotherapy and tumor removal), and with mild disease within the first month [11]. Relapses may appear in 12% of patients, especially in non-paraneoplastic patients and in those who did not receive second-line treatment for the first episode [11]. LGI1-encephalitis usually responds better to first-line treatment than anti-NMDA-R (80% of patients), particularly to CC [146]. Early immunotherapy may control FBDS (usually refractory to anti-epileptic drugs) and prevent the development of cognitive dysfunction [144]. Relapses are common (up to 35%) and memory deficits may be responsible for impaired mRS scores (67% mRS <2) [146]. Nearly 70% of anti-Caspr2 encephalitis patients improve after immunotherapy, but 40% of them may experience relapses [49]. AMPAR-encephalitis presents a much lower rate of relapses with a similar rate of response, but patients with the fulminant form have a poor prognosis [149, 150]. Anti-GABAbR encephalitis responds to oncological and/or immunological treatment in nearly 50% of patients; relapses are infrequent [154, 156].

Conclusion

PNS are immune-mediated disorders associated with cancer. Onset is often subacute and they may affect any level of the nervous system. They almost always develop before cancer identification, and therefore diagnosis should lead to tumor screening according to the neurological syndrome, the age and the associated antibody. Prompt tumor removal and immunotherapy are important to achieve better outcomes. Two major groups are recognized depending on the associated

antibodies. Antibodies against intracellular antigens (onconeural antibodies) are thought not to be pathogenic but are good markers of cancer. PNS associated with onconeural antibodies are likely to be mediated by cytotoxic T-cells, so they respond to immunotherapy less frequently and usually have worse prognosis. Antibodies against synaptic and cell surface proteins may be detected in several neurological syndromes, with and without cancer. The antibodies alter the location or function of their antigens, generating a neuronal dysfunction that causes the clinical picture. As there is no neural death, at least in the early stages of the disease, immunotherapy can reverse the antibody effects leading to full recovery if treatment is initiated early. Future fields in PNS research include furthering the understanding of pathogenesis (especially the immune mechanisms and the role of genetic predisposition) and to establish treatment protocols based on clinical trials.

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Multiple Sclerosis

Jun-ichi Kira and Noriko Isobe

Abstract Multiple sclerosis (MS) is an inflammatory demyelinating disease that targets myelin in the central nervous system (CNS), with relative sparing of axons. MS affects more than 2.5 million people worldwide and more commonly affects females. MS is prevalent in people of Caucasian descent living in the temperate regions of Europe, North America, and Australia, while it is relatively rare in Asians and Africans, indicating clear racial and geographical differences. Most MS patients initially have a relapsing-remitting phase with a mean age of onset around 30 years of age. This is termed relapsing-remitting MS (RRMS). In its natural course, after 10–20 years, about half of RRMS patients develop a secondary progressive phase with or without superimposed relapses, which is termed secondary progressive MS (SPMS). Approximately 10–20% of MS patients exhibit a relentlessly progressive course from the onset, termed primary progressive MS (PPMS).

MS is assumed to be an autoimmune disease but this is not yet proven. In addition to major effects of human leukocyte antigen (HLA) class II genes (such as *HLA-DRB1*15:01*), genome-wide association studies have revealed many susceptibility genes for MS with modest effect size. The functions of these genes are mostly immune-related, supporting the autoimmune hypothesis. T helper (Th)1/Th17 cell involvement in acute relapse and acute MS lesion formation is supported by perivascular lymphocyte cuffing consisting mainly of $CD4^+$ T cells, increased numbers of T cells showing inter- and intra-molecular epitope spreading against myelin proteins, increased cerebrospinal fluid (CSF) levels of interferon (IFN)γ, interleukin (IL)17 and downstream proinflammatory cytokines, exacerbation of disease following IFNγ administration, and increased percentages of Th1 cells secreting IFNγ and of Th17 cells secreting IL-17 at relapse. Clonal expansion of $CD8^+$ T cells and abundant infiltration of $CD8^+$ T cells suggest a contribution of cytotoxic T cells,

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_15

presumably by acute axonal transection. Although B cell infiltration in the CNS parenchyma is not prominent, ectopic lymphoid follicles that appear to have a close correlation with subpial demyelination are often detected in the meninges. Their existence indicates an involvement of B cells in MS. The efficacy of anti-CD20 monoclonal antibody therapy also supports a B cell contribution to MS, probably through B−T cell interaction and proinflammatory cytokine production. However, no specific autoantibodies for MS have been discovered. Although our understanding of MS pathogenesis has increased remarkably in recent years, its etiology remains to be established. Recently developed disease-modifying drugs (DMDs) can efficiently suppress MS relapse but disability still progresses even with these drugs. Only one DMD is modestly effective for PPMS. The mechanism of the progressive phase remains unknown, and its elucidation and control by novel drugs are major challenges for the future.

Keywords Multiple sclerosis · Demyelination · Magnetic resonance imaging · Epidemiology · Environment · Gene · Neuropathology · Disease-modifying drug

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) that preferentially affects young adults. MS is putatively an autoimmune disease that targets CNS myelin antigens, although this is not yet conclusively proven. The mechanism of MS remains elusive although conspicuous advances from genetic, environmental, and therapeutic studies have provided important clues for deciphering the pathophysiology of MS. Recently developed disease-modifying drugs (DMDs) targeting specific molecules or steps in neuroinflammation are highly efficacious for suppressing MS relapse, and this helps to clarify the inflammatory cascade involved in MS. By contrast, these drugs have no or only modest benefit for the relentless progression of disability in progressive MS. Therefore, a huge unmet medical need still exists for halting the neurodegenerative process and repairing neural damage in MS. This chapter describes the epidemiology, clinical manifestations, pathogenesis, diagnosis, and treatment of MS.

Epidemiology

The concordance rate for MS in monozygotic twins is 30.8%, which is much higher than that of dizygotic twins (4.7%) [1]. This indicates that a genetic contribution is important and that environmental factors play bigger roles than genetic factors in the development of the disease.

Prevalence, Age at Onset, and Sex Ratio

Prevalence of MS varies worldwide from 30–150/100,000 in high-prevalence areas to 5–30/100,000 in medium-prevalence areas and to less than 5/100,000 in low-prevalence areas (Fig. 1) [2]. MS is prevalent in people of Caucasian descent living in the temperate regions of Europe, North America, and Australia/New Zealand, whereas it is relatively rare in Asians and Africans, indicating clear geographical and racial differences [3]. MS prevalence increases with distance from the equator; higher latitude is positively correlated with higher prevalence. In countries having long north-to-south dimensions, like Japan, MS prevalence shows a significant positive correlation with latitude [3]. These findings indicate that the development of MS is influenced by environmental factors related to latitude. The average age at onset for RRMS is approximately 30 years of age, with onset occurring between 15 and 50 years in most patients. However, MS can also develop during childhood or in elderly people. The male to female ratio is about 1:2–4 in relapse-onset MS. By contrast, the average onset of PPMS is later in life (around 40 years old), and the sex ratio is more balanced.

Migration Study Results

Based on migration studies, migration before puberty from high-prevalence areas to low-prevalence areas reduces MS risk, while migration in the reverse direction increases MS risk [4–9]. However, migration study results should be carefully

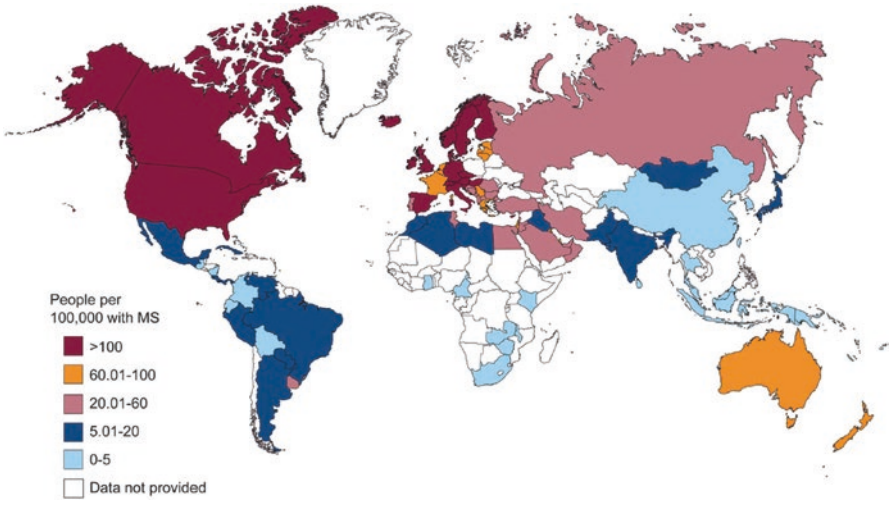

Fig. 1 Geographical distribution of MS prevalence. (The figure is from the Atlas of MS 2013, MS International Federation (http://www.atlasofms.or))

interpreted because migrants may tend to have more genetic admixture, which may increase or decrease MS risk [10]. These observations indicate that the geographical location where one is born and raised until puberty is critical in the occurrence of MS.

Changes in MS Prevalence

MS incidence and prevalence have increased worldwide, especially in women [11–13]. This remarkable increase does not seem to be solely attributable to newer diagnostic criteria or the availability of better diagnostic techniques such as magnetic resonance imaging (MRI). Although MS prevalence in Japan is much lower than in Western countries, it has increased from 1.4/100,000 to 7.7/100,000 over the past 30 years [14]. At the same time, the peak age of onset shifted from the early 30s in 1989 to the early 20s in 2003, while the female-to-male ratio increased from 1.7:1 in 1972 to 2.9:1 in 2003 [14]. In Canada, the female-to-male ratio increases rapidly with advancing birth year [15, 16]. Although the increase in MS prevalence appears to be partly attributable to improved case ascertainment, the worldwide increase in the number of female MS patients [11–13], as well as the younger age of onset in some countries, cannot be fully explained by improved case ascertainment [17]. These observations indicate that MS susceptibility has markedly increased among younger women who have grown up in a Westernized environment, resulting in anticipation of age at onset. It is possible that women are more likely to be exposed to changes in potential MS environmental factors, or that women are more responsive to exposure to environmental factors that have recently changed. Of note, the recent increase of MS incidence has reduced the north-to-south gradient of MS prevalence in some countries [11–13], indicating that the effects of latitude and environmental changes related to modernization may converge in MS disease cascades.

Clinical Manifestations

Clinical Symptoms and Signs

The onset of MS is usually acute or subacute while some patients exhibit insidious onset. A variety of clinical symptoms and signs can develop depending on the sites involved. These symptoms and signs are usually attributable to white matter lesions in the CNS. Sensory impairment, paresthesia, limb weakness, visual impairment, and double vision are common initial manifestations.

Spastic hemiparesis, paraparesis, and quadriparesis are frequent manifestations of pyramidal tract involvement and are accompanied with hyperreflexia, pathological

reflexes, and ankle and patellar clonus. Posterior column and spinothalamic tracts of the spinal cord are also frequently involved, presenting with decreased position sense and vibratory sense, hypesthesia, hypalgesia, thermal hypesthesia, and paresthesia below a horizontal line on the body (sensory level). Romberg's sign is occasionally seen because of posterior column involvement. Lhermitte's sign (an electric shock-like sensation on the back and lower limbs on neck flexion) is occasionally experienced because of cervical posterior column lesions. An unpleasant tightly wrapped sensation (girdle sensation) of the torso and limbs sometimes afflicts MS patients. Demyelinating lesions in the cerebral hemisphere or thalamus often cause sensory impairment of the hemicorpus including the face. Various kinds of pain are also common in MS.

Optic neuritis, manifesting as blurred vision, decreased color perception and visual field defect, is also common in MS. Funduscopic tests show normal (retrobulbar neuritis) or hyperemic disc swelling (papillitis), which is followed by temporal pallor because of papillomacular bundle damage or pallor of the whole optic disc (optic atrophy). Pain on ocular movement is frequently accompanied with optic neuritis.

Limb and truncal ataxia, gaze-evoked nystagmus, and scanning speech are common manifestations of cerebellar damage. Brainstem involvement often presents with diplopia, internuclear ophthalmoplegia, trigeminal neuralgia, facial muscle weakness, facial myokymia, decreased taste sensation, vertigo, tinnitus, hearing impairment, and dysarthria.

Bladder dysfunction is common, and interferes with daily activities. Detrusor hyperreflexia causes urinary urgency, nocturia, and uncontrolled bladder emptying. Detrusor sphincter dyssynergia manifests as difficulty initiating urination, interruption of urination, urinary retention, and overflow incontinence. Most patients exhibit a combination of both types of bladder dysfunction. Both can lead to complications including recurrent urinary tract infections, skin ulceration and infection, nephrolithiasis and, rarely, renal failure. Bowel dysfunction often manifests as constipation, while fecal urgency or incontinence can also occur. Sexual dysfunction, such as decreased libido, impaired genital sensation, diminished vaginal lubrication, and impotence in men, are also frequently seen.

Damage in the cerebrum, including the hippocampus, produces cognitive dysfunction, such as impaired attention, difficulties in executive function, slowness in information processing, and memory impairment in up to 30–50% of MS patients. Mood dysfunctions, such as depression, and euphoria, are encountered in more than half of MS patients. Fatigue is quite common in MS and is often disabling. Patients experience the classical sense of fatigue that is often triggered by little exercise and can be overwhelming.

MS patients frequently show heat sensitivity; elevation of body temperature induces worsening of already present symptoms (Uhthoff's sign), such as visual blurring and limb weakness. Paroxysmal symptoms seen in MS, other than trigeminal neuralgia, are glossopharyngeal neuralgia, hemifacial spasm, and painful tonic spasm (tonic spasm of one or two limb muscles about 1 minute in duration without

consciousness disturbance due to spinal cord lesions). Symptoms and signs derived from gray matter involvement, such as severe cortical dementia, Parkinsonism, severe muscle atrophy, and epileptic seizures are rare in MS.

Clinical Course

Most MS patients initially have a relapsing-remitting phase with a mean age of onset of around 30 years of age. This is termed relapsing-remitting MS (RRMS) (Fig. 2). MS patients who have only one symptomatic episode and do not fulfill the current diagnostic criteria for MS (Table 1) are diagnosed with clinically isolated syndrome (CIS), which has a high probability of eventually developing into MS. In the early course of the disease, complete or substantial recovery occurs over the weeks to months following initial onset, while recovery from relapse later becomes less evident and residual disability accumulates. In a natural course, about a half of RRMS patients develop a secondary progressive phase with or without superimposed relapses at 10–20 years after onset. This phase is termed secondary progressive MS (SPMS) [18]. At this stage, patients suffer from progressive deterioration of neurological function that is unassociated with relapses. The conversion rate from RRMS to SPMS is estimated to have decreased to about 20% in the era of disease-modifying therapy [19]. Approximately 10–20% of MS patients exhibit a relentlessly progressive course from onset, termed primary progressive MS (PPMS). Progressive MS (SPMS and PPMS) preferentially involves distal portions of the pyramidal tracts and the cerebellum, thereby producing a sustained worsening of spastic paraparesis and/or cerebellar ataxia.

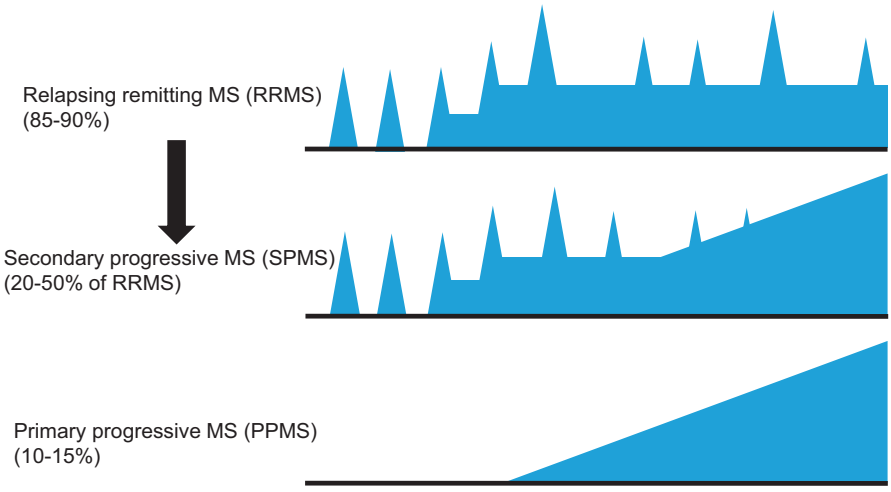

Fig. 2 Representative clinical course of MS

Table 1 The 2017 McDonald criteria for MS diagnosis

For patients with an attack at the onset		
Number of attacks	**Number of lesions with objective clinical evidence**	**Additional data needed for a diagnosis of MS**
≥ 2 clinical attacks	≥ 2	None
≥ 2 clinical attacks	1 (as well as clear-cut historical evidence of a previous attack involving a lesion in a distinct anatomical location)	None
≥ 2 clinical attacks	1	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site *or* by MRI
1 clinical attack	≥ 2	Dissemination in time demonstrated by an additional clinical attack or by MRI *or* demonstration of CSF-specific OCBs
1 clinical attack	1	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MRI Dissemination in time demonstrated by an additional clinical attack or by MRI *or* demonstration of CSF-specific OCBs
For patients with insidious onset		
Clinical evidence	**Additional data needed for a diagnosis of MS**	
1 year of disease progression	*Plus* two out of the three following criteria: ≥ 1 T2-hyperintense lesions characteristic of MS in one or more of the following brain regions; periventricular, cortical or juxtacortical, or infratentorial ≥ 2 T2-hyperintense lesions in the spinal cord Presence of CSF-specific OCBs	

Dissemination in space demonstrated by MRI:

≥ 1 T2 lesions on MRI in at least two out of four MS-typical lesions of the CNS; periventricular, cortical or juxtacortical, or infratentorial, and spinal cord.

Dissemination in time demonstrated by MRI:

Simultaneous presence of gadolinium-enhancing and non-enhancing lesions at any time point or by a new T2-hyperintense or gadolinium-enhancing lesion on follow-up MRI, with reference to a baseline scan, irrespective of the timing of the baseline MRI.

CSF cerebrospinal fluid, *MRI* magnetic resonance imaging, *OCBs* oligoclonal IgG bands

Laboratory Tests

Magnetic Resonance Imaging

MRI is highly sensitive for detecting MS lesions in the CNS. Demyelinating lesions in MS appear as high signal intensity on T2-weighted, fluid-attenuated inversion recovery (FLAIR), and proton-density-weighted images, and as low signal intensity on T1-weighted images. Clinical relapse is often accompanied or even preceded by the emergence of gadolinium-enhanced lesions on T1-weighted images,

indicating disruption of the blood-brain barrier (BBB). Recent 7-T MRI studies clearly showed the presence of vessels in the center of MS lesions [20], which is in accord with the pathological finding that perivascular lymphocytic infiltration is common in active MS lesions. Thus, it is conceivable that clinical relapse is caused by peripheral blood-borne inflammation around the blood vessels. MS lesions in the brain are frequently oriented perpendicular to the lateral ventricular surface (ovoid lesion or Dawson's fingers) (Fig. 3). This configuration of typical MS lesions is explained by the fact that post-capillary venules, from which T cells migrate into the CNS parenchyma, radiate perpendicularly from the lateral ventricles. As gadolinium enhancement of acute lesions disappears within 2–3 months, the coexistence of gadolinium-enhanced (new) and gadolinium-unenhanced (old) lesions on the same MRI scans indicates dissemination in time (a relapsing-remitting course) (Fig. 4) [21]. Although MS lesions rarely expand to more than 3 cm in diameter, such large lesions, resembling a brain tumor, are named tumefactive demyelinating lesions or tumefactive MS (or Marburg variant MS) (Fig. 5). When such lesions contain lamellar structures of alternating layers of demyelination and preserved myelin, a diagnosis of Baló's concentric sclerosis is made (Fig. 5). Low signal intensity areas on T1-weighted images (T1 black hole) reflect demyelination and edema in the acute phase, and irreversible axonal loss in the chronic phase. Thus, accumulation of chronic T1 black holes relates to disability progression. Most brain MRI lesions are asymptomatic. Therefore, MS-like lesions are incidentally discovered in asymptomatic individuals, and such cases are termed radiologically isolated symptoms (RIS) [22]. The prescription of DMDs to patients with RIS is currently a matter of debate.

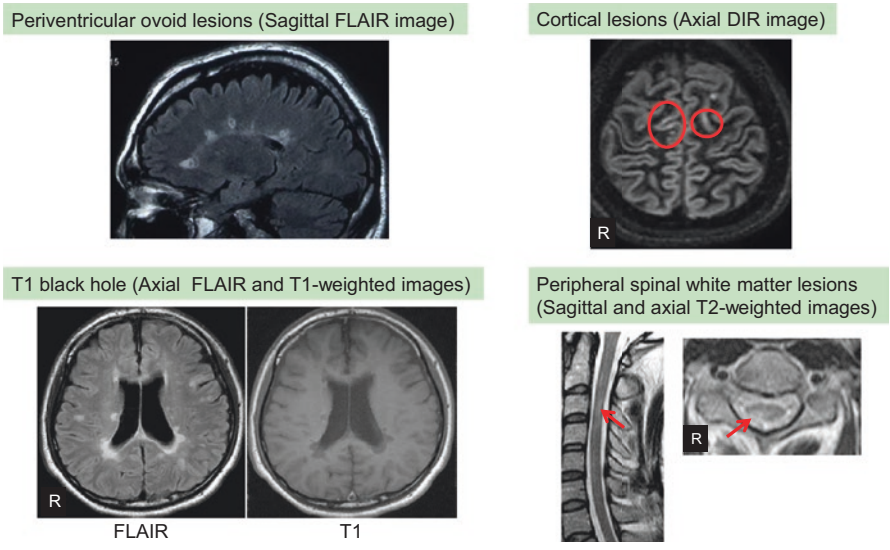

Fig. 3 Examples of MRI lesions suggestive of MS

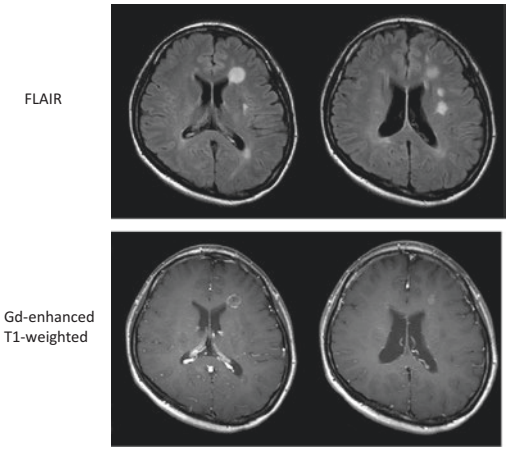

Fig. 4 MRI evidence indicating dissemination in time. Simultaneous presence of gadolinium-enhancing and non-enhancing lesions even in one MRI scan suggests dissemination in time

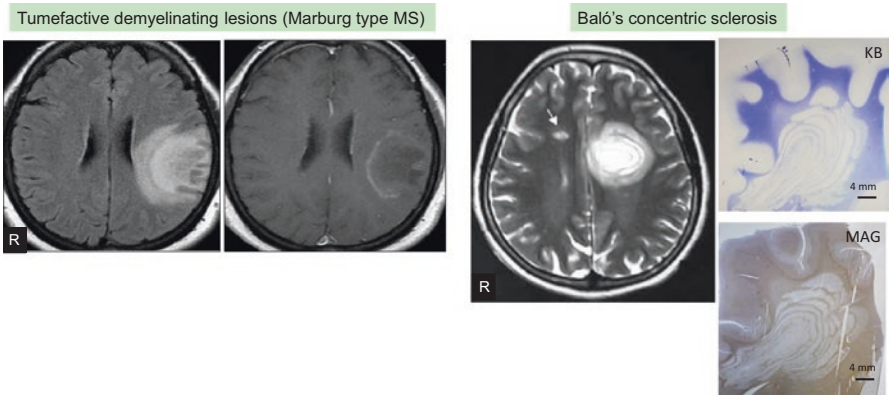

Fig. 5 Tumefactive MS and Baló's concentric sclerosis lesions. Examples of atypical presentation of MS. Tumefactive demyelinating lesions are rarely encountered at the onset of MS and may be followed by a typical relapsing-remitting course of MS. Concentric demyelinating lesions are extremely rare. MRI of Baló's concentric sclerosis lesions is courtesy of Professor Xiaomu Wu (China)

Recent development and application of double inversion recovery imaging enables cortical lesions to be detected much more frequently in MS, which is consistent with the demonstration of abundant cortical demyelinating lesions in autopsied MS brains (Fig. 3) [23, 24]. The presence of such cortical lesions is associated with disability progression and poor prognosis [24, 25]. As the disease progresses, brain volume is successively lost (more than 0.4% per year) [26], resulting in brain atrophy and enlargement of ventricles.

MS lesions in the spinal cord are also detectable by MRI. Typical spinal MS lesions involve the peripheral white matter of the spinal cord and occupy less than half of transverse spinal cord area (Fig. 3). MS preferentially affects the dorsal column of the cervical spinal cord, although the underlying mechanism is yet to be

determined. The length of MS lesions in the sagittal plane is typically fewer than three vertebral segments and is almost always fewer than two vertebral segments. Longitudinally extensive spinal cord lesions spanning three or more vertebral segments indicate neuromyelitis optica spectrum disorders or other inflammatory diseases.

Cerebrospinal Fluid

At acute relapse, CSF shows mild mononuclear pleocytosis (5–50 cells/μl) and normal or mildly elevated protein levels (40–100 mg/dl). As the disease progresses, more plasma cells and B cells infiltrate intrathecally and CSF IgG levels increase. One formula to calculate intrathecal IgG production rates is the IgG index, which expresses the ratio of IgG to albumin in the CSF divided by the same ratio in the serum. An IgG index >0.73 indicates intrathecal IgG synthesis. Isoelectric focusing of CSF can detect two or more oligoclonal IgG bands (OCBs) in the gammaglobulin region in more than 90% of Caucasian MS patients and about 60% of Asian MS patients [3]. OCBs may not be present at onset but may appear later, and the number of OCBs may increase with time. Myelin basic protein (MBP) levels elevate at acute relapse, reflecting myelin destruction. However, increased MBP levels are not specific for MS. Acute destruction of CNS tissues, as a consequence of stroke, for example, may also cause MBP levels in CSF to increase.

Evoked Potentials

EPs are useful in detecting demyelinating lesions in certain pathways of the CNS. EPs include visual evoked potentials (VEPs), somatosensory evoked potentials (SEPs), brainstem auditory evoked potentials (BAEPS), and motor evoked potentials (MEPs), which test visual, posterior column sensory, auditory, and pyramidal pathways, respectively. A marked delay in the latency of a specific EP without a marked decrease of amplitude is suggestive of demyelination in the relevant pathway. EP abnormalities are not specific for MS, and MRI surpasses EPs for detecting asymptomatic lesions and providing evidence of dissemination in space; therefore, the clinical usefulness of EPs is relatively limited, with the exception of VEPs.

Blood Tests

Peripheral blood tests usually show no disease-specific abnormalities in MS. However, it is necessary to rule out other diseases by autoantibody testing of peripheral blood. In particular, anti-aquaporin 4 (AQP4) antibodies for neuromyelitis optica spectrum disorders and anti-myelin oligodendrocyte glycoprotein (MOG) antibodies for anti-MOG antibody disease should be carefully excluded by cell-based antibody assays. These diseases may occasionally present with MS-like

clinical and MRI features but are nevertheless refractory to MS DMDs. In addition, collagen-vascular diseases masquerading as MS should be differentiated by autoantibody testing.

Pathogenesis

MS is thought to be caused by a complex interplay between genetic and environmental factors: genetic factors account for roughly 30% and environmental factors for approximately 70% of MS risk (Fig. 6) [4].

Pathology

White Matter Pathology

MS predominantly involves the CNS white matter where myelin is abundant. The lesions appear as sharply demarcated plaques, within which axons are relatively spared. A number of histological classification systems have been used for MS lesions [27]. The following is a recently proposed, simple classification of MS lesions based on the presence or absence and distribution of macrophages/microglia (inflammatory activity) and the presence or absence of ongoing demyelination (demyelinating activity) (Fig. 7) [28]. *Active lesions* are characterized by macrophages/microglia throughout the lesion area, while *mixed active/inactive lesions* have a hypocellular lesion center with macrophages/microglia limited to the lesion

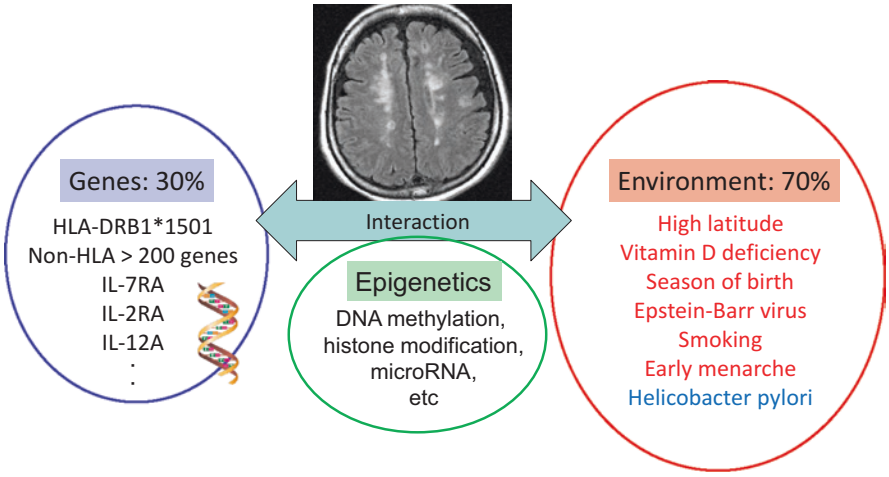

Fig. 6 MS is caused by a complex interplay between genes and the environment

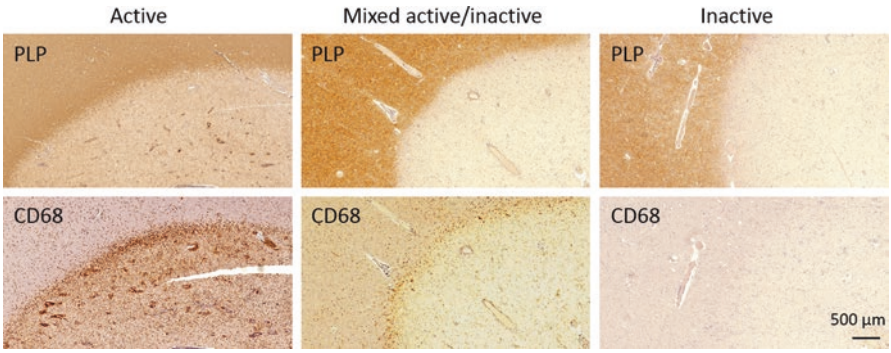

Fig. 7 Active, mixed active/inactive and inactive MS lesions. Recent classification of MS lesions based on the presence or absence and distribution of macrophages/microglia and the presence or absence of ongoing demyelination [28]

border. *Inactive lesions* almost completely lack macrophages/microglia. Active and mixed active/inactive lesions are further subdivided into *demyelinating lesions*, where myelin destruction is ongoing (macrophages contain myelin degradation products), and *post-demyelinating lesions*, where macrophages are still present but the destruction of myelin has ceased (macrophages do not contain myelin degradation products). Active demyelinating lesions are accompanied by perivascular lymphocyte cuffing, mainly consisting of $CD4^+$ T cells, while clonally expanded $CD8^+$ T cells dominantly infiltrate the parenchyma [29].

Remission results from the resolution of acute inflammation, partial remyelination, and redistribution of ion channels along demyelinated axons. Even in normal-appearing white matter, a mild global inflammation characterized by microglial activation and a diffuse low-level T cell infiltration can be seen that is more prominent in SPMS and PPMS than RRMS. In chronic MS plaques, leakage from the BBB is absent, corresponding to a paucity of gadolinium-enhanced lesions in PPMS and SPMS. Accordingly, compartmentalized glial inflammation behind the BBB is postulated as one of the mechanisms for the chronic progressive phase.

Demyelination in the MS brain and spinal cord can be followed by variable remyelination [30–32]. Remyelination is more prominent in early stages of the disease, while chronic lesions have less or no remyelination. Infiltration of fewer inflammatory cells and more remyelination are observed in PPMS brains compared with in SPMS brains [33]. Oligodendroglia are sensitive to oxidative stress because the cells contain a large pool of iron but only have a low capacity antioxidation system [34]. Oligodendroglia are vulnerable to glutamate toxicity and may be damaged by glutamate secreted by activated microglia. However, oligodendroglia progenitor cells (OPCs) exist even in chronic MS lesions [35, 36]. Therefore, failure of remyelination is not attributed to the absence of OPCs but rather to blocked differentiation of OPCs into myelinating oligodendroglia. The differentiation of OPCs into myelinating oligodendroglia can be inhibited by LINGO1 on astrocytes and macrophages [37, 38], PSA-NCAM that is abnormally expressed on demyelinated axons [39], myelin debris [40], and aggregated fibronectin [41].

Acute damage can be detected by the presence of accumulated amyloid precursor protein (APP)-positive spheroids, which reflects impaired axonal transport [42]. APP-positive spheroids are most extensive during the first year after disease onset and decrease with increasing disease duration [43]. The extent of axonal loss correlates well with numbers of $CD8^+$ T cells and macrophages/activated microglia existing in close proximity [43]. Numerous $CD8^+$ T cells that have infiltrated the CNS parenchyma can transect axons, possibly through major histocompatibility complex (MHC) class I-mediated self-antigen recognition [44]. In addition, reactive oxygen and nitrogen species as well as proinflammatory cytokines secreted by these cells may suppress axonal functions and cause mitochondrial damage [45].

Gray Matter Pathology

Gray matter lesions have captured much recent attention because they closely correlate with disability progression. MRI T2 lesion burdens in the white matter only modestly correlate with disability, while double inversion recovery imaging demonstrated that cortical lesions are present from the early stage of RRMS and become more prominent in SPMS [46–49]. Cortical lesion loads and cortical and spinal cord atrophy are significantly associated with clinical progression, whereas white matter atrophy does not correlate with increasing disability [25, 47, 50, 51]. Thus, cortical lesions may play a major role in the development of both physical and cognitive disability [52]. Pathologically, demyelination exists to varying degrees in the cerebral and cerebellar cortex, in the deep gray matter, including the thalamus, basal ganglia, and hypothalamus, and in the spinal cord central gray matter [53, 54]. Frontal and temporal cortices, the cingulate gyrus, and hippocampus are most frequently affected [33], which may explain the correlation between cognitive impairment and cortical pathology. Cortical demyelination does not correlate with severity of underlying white matter lesions [53], indicating that independent mechanisms are involved. Cortical lesions demonstrate increased levels of activated microglia without evident inflammatory infiltrates or significant leakage of plasma proteins, indicating a preserved BBB [33, 53–55]. Meningeal lymphoid follicles consisting of $CD20^+$ B cells and $CD35^+$ dendritic cells are present in approximately 40% of autopsied MS cases and are located predominantly in the deep cortical sulci of the temporal, frontal, cingulate, and insular cortices [56]. In extensive subpial demyelination, increased numbers and activation status of microglia and increased axonal injury and neuronal loss are greatest close to the pial surface [55, 57]. In autopsied meninges from MS patients, expression of proinflammatory cytokines and chemokines, such as IFNγ, TNF, and CXCL13 (B lymphocyte chemoattractant), was upregulated, and similar increases in cytokines/chemokines were also detected in the CSF of MS patients with high levels of cortical gray matter damage at diagnosis [58]. Collectively, secretion of proinflammatory cytokines into the CSF from lymphocytes in the meningeal follicles may cause such cortical demyelinating lesions. Diffuse cortical neuronal loss was also found even in normal-appearing gray matter [55]. Neuronal apoptosis and mitochondrial damage are assumed to be responsible for the neuronal loss [33, 45, 59], while demyelination and neuronal loss may not be directly linked in the gray matter lesions [33].

Glial Pathology

In the white matter, active lesions and mixed active/inactive lesions are accompanied by macrophages and activated microglia. In the cortical gray matter, diffuse microglial activation is present without visible inflammatory infiltrates. In addition to CNS tissue damage and repair, macrophages and resident microglia are thought to play major roles in demyelinating lesion formation through re-stimulation of T cells within the CNS. In the CNS, perivascular and meningeal macrophages act as major antigen-presenting cells to restimulate T cells. Without re-stimulation by relevant antigens, T cells do not invade into the CNS parenchyma via disruption of the glia limitans perivascularis. The recruitment of monocytes/macrophages is mediated by CCL2–CCR2 signaling. Hypertrophic astrocytes in active MS lesions produce CCL2, while its receptor, CCR2, is expressed on monocytes/macrophages [60]. Thus, macrophages play major roles in antigen presentation and tissue destruction. Activated microglia produce numerous proinflammatory cytokines/chemokines, growth factors, reactive oxygen and nitrogen species via oxidative burst and inducible nitric oxide synthase, thereby causing tissue damage. Alternatively, microglia can exert neuroprotective functions by phagocytizing tissue debris and producing neurotrophic substances.

In acute MS lesions, numerous hypertrophic astrocytes with increased expression of glial fibrillary acidic protein (GFAP), vimentin, and nestin are present. Such activated astroglia secrete many proinflammatory cytokines, such as IL1, IL6, IL12, IL15, IL23, IL27, IL33, CCL2 (MCP1), CCL5 (RANTES), CXCL8 (IL8), CXCL10 (IP10), and CXCL12 (SDF1). Moreover, astroglia produce inducible nitric oxide synthase (iNOS), leading to the production of superoxide anions and peroxynitrite, which can damage oligodendrocytes with low antioxidant levels [61, 62]. Astroglia can also produce a variety of growth factors that promote oligodendrocytes to form myelin by influencing OPCs [63, 64]. IL6 and transforming growth factor (TGF)-β produced by activated astrocytes may promote neuroprotection [65]. In chronic MS lesions, astrogliotic scars are formed, which may prevent axonal growth and tissue repair. However, ablation of proliferating astroglia exacerbates experimental autoimmune encephalomyelitis, an animal model of MS, and is associated with a massive infiltration of macrophages and T cells [66], indicating critical roles of astroglia in preventing the expansion of inflammation. These observations indicate that astroglia can play proinflammatory as well as neuroprotective roles in MS.

Genetic Factors

Recent genome-wide association studies (GWASs) have identified more than 200 susceptibility loci for MS with modest effect size. This is in addition to HLA genes, which have major effects on MS susceptibility [67]. Most of these loci are intergenic and have immune-related functions, supporting the autoimmune nature of the disease. In addition, these GWASs found no significant differences in risk genes

between RRMS and progressive MS [68], indicating that these two disease types could be distinct manifestations of the same disease.

In Caucasians of Northern European descent, the *DR15* haplotype (*DRB1*15:01-DQA1*01:02-DQB1*06:02*), especially *HLA-DRB1*15:01*, is most strongly associated with MS risk [69], while the *DR3* (*DRB1*03:01-DQA1*05:01-DQB1*02:01*) and *DR4* (*DRB1*04:05-DQA1*05:01-DQB1*03:01*) haplotypes confer susceptibility to MS in Sardinians [70, 71]. Recent high-resolution mapping of the MHC region in cohorts of European ancestry identified multiple HLA alleles independently associated with MS susceptibility. After *HLA-DRB1*15:01*, the alleles conferring the highest MS risk are *HLA-DRB1*03:01*, *HLA-DRB1*13:03*, and *HLA-DRB1*08:01* from the MHC class II region, while alleles such as *HLA-A*02:01*, *HLA-B*44:02*, *HLA-B*38:01*, and *HLA-B*55:01* from the MHC class I region are reported to be protective [72]. These HLA alleles associated with disease susceptibility also contribute to the disease course. For example, *HLA-DRB1*15:01* is associated with younger age of onset, increased brain white matter lesion volume, reduced normalized brain parenchymal volume, and cognitive impairment [73]. Moreover, as the number of genetic loci discovered to be associated with MS risk has increased, cumulative risk statistics have been developed to quantify the collective effects of disease susceptibility variants in single scores. HLA genetic burdens (HLAGB), calculated according to the roster of MS-associated HLA alleles, correlated well with younger age of onset and atrophy of the subcortical gray matter fraction in female relapsing MS in a cohort of Northern European ancestry [74].

In the Japanese population, *HLA-DRB1*15:01* and *DRB1*04:05* are two major risk alleles for MS (odds ratio, 1.97 and 1.93, respectively). Carriers of the *HLA-DRB1*04:05* susceptibility allele comprised around 40% and those carrying *DRB1*15:01* around 30% of all MS patients in a Japanese MS series [75]. Japanese MS patients with *HLA-DRB1*15:01* have a high frequency of CSF OCBs and a high brain MRI lesion load, similar to that in Western MS patients, while those with *DRB1*04:05* are characterized by an earlier age of onset, milder disability, lower frequencies of brain MRI lesion loads, and lower frequencies of OCBs [75]. Frequency of *HLA-DRB1*04:05* in the general population is high in a few isolated island countries, such as Japan, Sardinia, and Papua New Guinea. It is interesting to note that *HLA-DRB1*04:05* is a susceptibility allele in both Japanese and Sardinian populations, both of which recently demonstrated marked increases in MS incidence [76]. A shift toward a younger peak age at onset was also reported in Sardinia [77]. These observations collectively indicate that recent environmental changes may enhance MS susceptibility in populations carrying certain HLA alleles [14]. By contrast, the frequency of *HLA-DRB1*09:01* is significantly lower in MS patients compared with that in healthy controls [75, 78]. A recent meta-analysis of Chinese populations also indicated that this allele is protective against MS [79]. The *HLA-DRB1*09:01* allele is more frequently observed in Asians than in European ethnic groups (30% of Japanese vs. 1% of Caucasians) [80]. Therefore, the lower prevalence of MS in Asian countries may, in part, be attributable to the relatively high frequency of the *HLA-DRB1*09:01* allele in the region.

Although a substantial proportion of MS heritability is assigned to the MHC region, more than 200 non-MHC MS-associated variants have been identified through international collaborative meta-analyses of GWAS, which included data from more than 47,000 cases and 68,000 controls of European origin [67]. Compared with the effect size of MS-associated HLA alleles (e.g., the effect size of *HLA-DRB1*15:01* in European MS is 3.41–3.92 [72, 74],), the non-MHC risk alleles have a relatively mild effect size with a median value of 1.111, ranging from 1.06 to 2.06 [67]. Although most of the novel MS-associated genetic loci have been found in European populations, some approaches have been made to assess trans-ethnic transferability. European MS variants were generally relevant in African American populations although allelic heterogeneity was observed for some of the loci. Additionally, association studies of European MS variants in Japanese and Indian cohorts replicated the association of a missense SNP in *IL7R* [81, 82], indicating that the functional annotation of variants that contribute to bigger odds ratios (effect size) has an impact on whether the association of the variant is replicated or not.

Environmental Factors

Environmental factors can influence MS susceptibility during three periods: the intrauterine period, during puberty, and around attacks (Fig. 8) [83]. First, as mentioned above, migration before adolescence from a low- to a high-prevalence area increases MS risk, indicating that the childhood environment exerts significant effects on MS susceptibility [4–9]. Second, MS concordance rates are higher in dizygotic twins (5.4%) than in full siblings (2.9%), indicating that a shared intrauterine environment may confer MS risk [84]. The importance of the intrauterine environment is also supported by a well-known month-of-birth effect; in the Northern hemisphere, more MS patients than expected are born in April and May, while fewer are born in October and November [85]. This month-of-birth effect is dependent on the latitude; i.e., it is significant in high-latitude areas but not in low-latitude areas [85]. Because vitamin D is produced mainly in the skin on exposure to sunlight, the month-of-birth effect can be explained by the maternal deficiency of vitamin D during winter pregnancy. As mentioned below, low vitamin D increases MS risk. Third, MS attacks occur two to three-fold more often in association with upper respiratory tract infection than without infection, indicating that non-specific infection can trigger MS relapse [86].

Representative environmental MS risk factors include high latitude, low sunlight exposure, vitamin D deficiency, Epstein-Barr virus (EBV) infection, and smoking (Fig. 6) [4, 87, 88]. Decreased levels of vitamin D have been reported in Western as well as Asian MS patients, and they are related to more severe disability [4, 87–89]. Low sunlight exposure in high latitude areas decreases vitamin D production in the skin. Vitamin D downregulates pathogenic type 1 T helper (Th1) cells and potentiates anti-inflammatory Th2 and regulatory T cells [90]. If vitamin D is not supple-

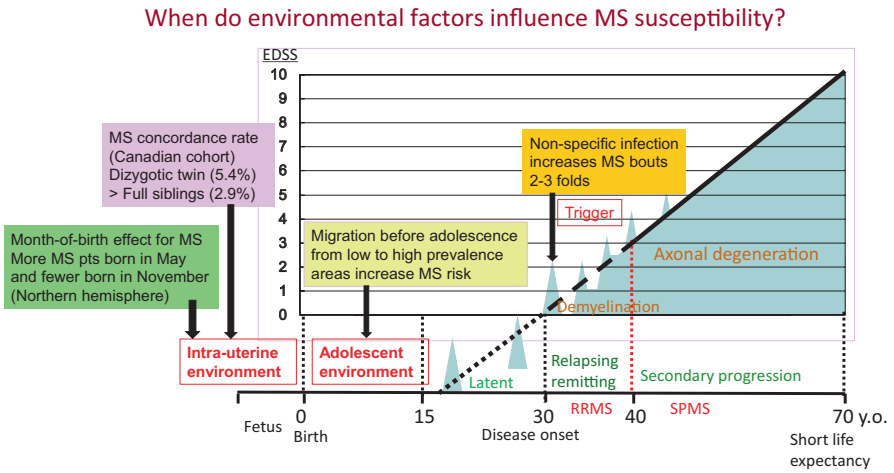

Fig. 8 The timing of environmental factors influencing MS susceptibility. The lifelong clinical course of MS is schematically shown. The vertical axis indicates the expanded disability status scale of Kurtzke (EDSS) scores. (From Kurtzke [152])

mented by diet, for example, by fish, low vitamin D may increase MS risk through Th1 cell over-activation. Vitamin D is not the sole mechanism of the latitude effect because sunlight also affects MS through other mechanisms. However, these three factors are related to each other. Cigarette smoking not only increases the risk for MS but also accelerates the transition from RRMS to SPMS [87, 89]. However, taking snuff is not a risk factor for MS [91], indicating that airway inflammation is important in recruiting autoreactive T cells via pulmonary lymph nodes to the CNS tissues.

The "hygiene hypothesis" argues that improved hygiene in childhood leads to development of not only autoimmune disease but also atopic/allergic inflammation [92]. In line with this hypothesis, it is well known that frequent childhood infections reduce MS susceptibility [93, 94]. More than ten studies performed in various Eastern and Western countries as well as two meta-analyses collectively indicate that the *Helicobacter pylori* (*H. pylori*) infection rate is significantly lower in patients with MS than in healthy controls [95, 96]. *H. pylori* infection occurs in infancy, when the mucosal barrier of the stomach is immature, and persists for life [97]. Thus, the *H. pylori* infection rate reflects sanitary conditions during childhood [98], when MS susceptibility is acquired. The protective effects of *H. pylori* could be explained according to the hygiene hypothesis; repeated childhood infection induces maturation of regulatory immune systems, whereas improved sanitation and the resulting scarcity of childhood infections hamper its development [92]. This subsequently leads to an inability of regulatory immune systems to suppress both autoimmune and allergic inflammation in adulthood [92, 99, 100]. Therefore, improved sanitary conditions in infancy, as reflected by a lower *H. pylori* infection rate, may facilitate the development of MS.

EBV infection is more prevalent in MS patients than in healthy controls in Western countries [101, 102], where a more hygienic environment during childhood predisposes individuals to later EBV infection [103]. This delay in EBV infection increases MS risk, because a history of infectious mononucleosis, a common manifestation of EBV infection in adolescence or adulthood, is associated with occurrence of MS. [100, 103] The risk of MS is extremely low in EBV-seronegative individuals, with an odds ratio of 0.06 [100]. MS susceptibility gene, *HLA-DRB1*15:01*, and EBV infection additively increase MS risk [104]. Individuals with *HLA-DRB1*15:01* have higher anti-EB nuclear antigen 1 (EBNA-1) antibody titers compared with those without the risk allele [105], and a high EBNA IgG titer is associated with increased risk of MS. [100] Some studies report molecular mimicry between EBV proteins and myelin antigens [106, 107].

MS is more often transmitted to the next generation by mothers than fathers. This parent-of-origin effect indicates epigenetic mechanisms, such as DNA methylation and histone deacetylation, to be operative in MS development (Fig. 6) [108]. Major environmental risk factors, such as vitamin D deficiency and EBV infection, are also known to exert epigenetic effects. Characterization of epigenetic factors in MS is ongoing and may help to decipher the mechanism of MS.

Immune Mechanism of MS

T cell involvement in MS is supported by the following findings: (1) an increased frequency of autoreactive T cells showing inter- and intra-molecular epitope spreading against myelin proteins; (2) elevated levels of IFNγ, IL17, and downstream proinflammatory cytokines/chemokines in the CSF at relapse; (3) increased frequency of Th1 cells secreting IFNγ and Th17 cells secreting IL17 at relapse [69, 94, 166]; and finally (4) exacerbation of disease following the administration of IFNγ, a representative Th1 cytokine [109–112]. Myelin antigen-specific $CD4^+$ Th1 and Th17 cells can transfer experimental autoimmune encephalomyelitis to naïve animals; therefore, it is hypothesized that naïve T cells are first sensitized by myelin antigens in the peripheral lymph nodes, such as the deep cervical and hilar lymph nodes, and differentiate into myelin antigen-specific Th1 or Th17 cells in MS. At acute relapse, these peripherally activated Th1 or Th17 cells express increased amounts of adhesion molecules that allow them to pass through the BBB (Fig. 9). Activated T cells can firmly adhere to the surface of vascular endothelial cells lining the BBB via interactions between α4β1 integrin expressed on activated T cells and vascular cell adhesion molecule 1 (VCAM-1) on endothelial cells. These T cells can then egress from postcapillary venules (high endothelial venules), either transcellularly or paracellularly, to reside in the perivascular space (Virchow-Robins space) delineated by the endothelial basement membrane and the glial basement membrane, which is an extension of the subarachnoid space [113, 114]. Antigen presentation by perivascular macrophages to autoreactive T cells is indispensable for these T cells to further invade into the CNS parenchyma across the glia limitans

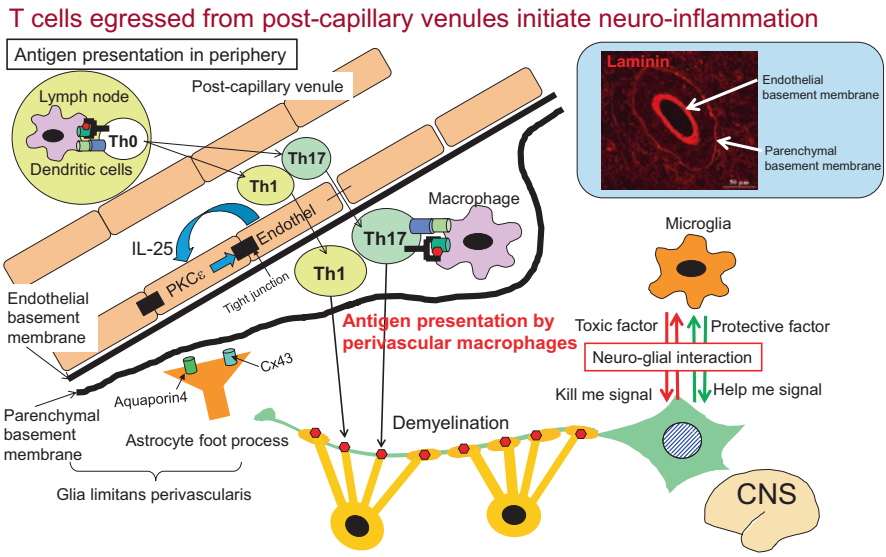

Fig. 9 Hypothesized mechanism of T cell-mediated neuroinflammation in MS. Insert: laminin immunostaining demonstrating the perivascular space between endothelial and parenchymal basement membranes

perivascularis. This promotes the secretion of matrix metalloproteinase-2 and metalloproteinase-9, which disrupt the basement membrane leading to destabilization of astrocyte end-feet anchored to the parenchymal basement membrane [113–115]. Perivascular macrophages continuously repopulated from the peripheral bloodstream can engulf CNS antigens in the perivascular space where myelin antigens are conveyed from the CNS parenchyma via the "glymphatic" system and CSF flow pathway into the subarachnoid space. Once in the CNS parenchyma, T cells secrete proinflammatory cytokines/chemokines, which further recruit effector cells such as macrophages, activated microglia, and neutrophils to destroy parenchymal tissues. However, there is a report describing oligodendroglial apoptosis without lymphocyte infiltration in autopsied cases with very early MS. [116] Whether T cell infiltration is a primary event or secondary to oligodendroglial apoptosis and subsequent microglial activation remains to be elucidated.

B cells are rare in the CNS parenchyma, whereas they exist in the perivascular areas and leptomeninges during all disease stages [57]. Plasma cells are few during the early stages of MS but become increasingly prominent in the CNS with time. As a result, the prevalence of CSF OCBs also increases as disease duration increases [117]. The importance of B cells in MS is directly shown by the fact that anti-CD20 monoclonal antibodies, such as rituximab, ocrelizumab, and ofatumumab, that target B cells but not plasma cells, are highly effective in MS. [118–120] As B cell numbers but not total antibody levels decrease in parallel with a reduction in the number of relapses, B-T cell interactions, such as antigen presentation and proinflammatory cytokine secretion by B cells, are assumed to be the critical step

depressed by anti-CD20 therapy. Immunoglobulin and complement deposits are found in lesions from about 50% of autopsied MS patients [121], suggesting that antibody and complement-mediated myelin phagocytosis might become the dominant mechanism in established MS lesions [122]. The significance of anti-glycolipid antibodies and a recently described autoantibody against KIR4.1, an ATP-sensitive inward rectifying potassium channel expressed in astroglial end-feet and oligodendroglia [123], need further confirmation in large-scale independent cohorts.

However, acute relapses have only a weak effect on disability progression [124]. In both relapse onset (SPMS) and insidious onset (PPMS) patients, a progressive phase was retrospectively found to develop at approximately 40 years of age and to then proceed at a similar rate, irrespective of the initial disease course [125], indicating that common pathogenic mechanisms may underlie clinical disability progression. At the progressive stage of MS, none of the recently developed DMDs, except for the anti-CD20 monoclonal antibody ocrelizumab, are effective, even though they have high efficacy for reducing both annualized relapse rates and new MRI lesions. Thus, the mechanism of the chronic progression of disability may be distinct from that of acute relapse, which is closely associated with BBB disruption induced by peripheral immunocytes. The absence of noticeable peripheral immunocyte-mediated inflammation by contrast-enhanced MRI and neuropathology in progressive MS indicates that compartmentalized glial inflammation behind the BBB and neurodegeneration may play key roles in chronic progressive MS.

Diagnosis

There is still no specific diagnostic test for MS. MS diagnosis is thus based on both spatial (two or more lesions as documented by neurological examination, MRI and EPs) and temporal (two or more episodes of symptoms) evidence of multiplicity and exclusion of other causes of disease. According to the most recent diagnostic criteria (2017 McDonald criteria, Table 1) [21], *cases with ≥ 2 clinical attacks and ≥ 2 lesions* shown by neurological examination can be diagnosed as MS when other diseases are ruled out. *Cases with ≥ 2 clinical attacks but 1 lesion* require an additional attack that implicates a different CNS site or MRI evidence of *dissemination in space* (presence of typical MS lesions in ≥2 regions among four CNS sites, including periventricular, cortical or subcortical, infratentorial, and spinal cord lesions). Cases with only one clinical attack usually require waiting for an additional clinical attack; however, to diagnose MS as early as possible such that DMDs can be administered, the 2017 McDonald criteria allow the second episode to be substituted by MRI evidence of dissemination in time or even by the presence of CSF-specific OCBs. *Cases with 1 clinical attack but ≥ 2 lesions* require *dissemination in time* shown by an additional clinical attack or by MRI (emergence of new T2 lesion or gadolinium-enhanced lesion, or even coexistence of gadolinium-enhanced

and un-enhanced lesions) *or CSF OCBs*. *Cases with one clinical attack and one lesion* require *dissemination in space* shown by an additional clinical attack implicating a different CNS site or by MRI and dissemination in time shown by an additional clinical attack or by MRI *or CSF OCBs*.

In applying the new diagnostic criteria, various conditions mimicking MS (Table 2) should first be carefully excluded by disease-specific tests. As mentioned above, anti-AQP4 antibodies and anti-MOG antibodies should be examined in cases presenting atypical features for MS, such as longitudinally extensive spinal cord lesions, bilateral optic neuritis, horizontal visual field loss, intractable hiccups, marked CSF pleocytosis ($\geq$ 50 cells/μl) and CSF neutrophilia, especially in Asians. In patients with only one clinical attack, acute disseminated encephalomyelitis (ADEM) should be carefully ruled out, because not all lesions are always uniformly contrast-enhanced in ADEM, which may be erroneously interpreted as evidence of dissemination in time. These latest criteria should not be used for suspected pediatric cases of ADEM.

Prognosis

As there is no curative treatment, once patients succumb to MS, the disease persists throughout the patient's life. Average life expectancy is not remarkably shortened and may be about 10 years shorter than that of normal populations [126]. Generally, RRMS patients require assisted walking by 20–25 years after onset and are wheelchair-bound by about 35 years after onset [127]. PPMS shows more rapid progression than relapse onset MS. Essentially, MS is a progressive disease, although several long-term follow-up studies report that 22% of cases were non-progressive after 40 years, and 14% were non-progressive even after 50 years [128]. MS patients with expanded disability status scale of Kurtzke (EDSS) scores $\leq$2–3 after 10 years may be regarded as having benign MS. A benign course is predicted by, being female, a younger age at onset, no motor symptoms at onset, fewer than two relapses in the first years of illness, absence of OCBs, minimal disability 5 years after onset, and low brain MRI lesion loads 5 years after onset [129]. By contrast, poor prognosis is suggested by male sex, older onset age, presence of motor symptoms at onset, presence of cerebellar ataxia, presence of sphincter disturbance, short relapse intervals, frequent relapses in the early course of the disease, residual symptoms from the beginning, multiple functional system involvement, high disability 5 years after onset, a progressive course, high brain MRI lesion load 5 years after onset, presence of brain atrophy and cortical lesions, and presence of spinal cord atrophy [129]. During pregnancy, relapse rates decrease but in the puerperal period (within 3 months after delivery) relapse rates increase [130]. However, overall, the disease course is unaffected by pregnancy [131].

Table 2 Major differential diagnosis of multiple sclerosis

Differential diagnosis	Key features useful for differentiating each disease from MS
Neuromyelitis optica spectrum disorders (NMOSD)	Anti-aquaporin 4 antibodies, longitudinally extensive spinal cord lesions, optic chiasma lesions, area postrema lesions, bilateral hypothalamic lesions, cloud-like enhancement, bright spotty lesions in the spinal cord, absence of MS-like brain lesions
Acute disseminated encephalomyelitis (ADEM)	Monophasic (multiphasic ADEM has encephalopathy), encephalopathy, bilateral white matter lesions (could be asymmetric), deep gray matter involvement, uniform gadolinium enhancement of all lesions (the same disease stage)
Anti-myelin oligodendrocyte glycoprotein (MOG) antibody disease	Anti-myelin oligodendrocyte glycoprotein antibodies, longitudinally extensive spinal cord lesions, sacral spinal cord lesions, focal cortical encephalitis
Primary and secondary CNS vasculitis	Headache, seizure, confusion, stroke-like episodes, microbleeds, intracranial hemorrhage, ischemic lesions, vessel stenosis on angiography, anti-neutrophil cytoplasmic antibodies
Neurosarcoidosis	Cranial nerve involvement, headache, seizure, meningeal enhancement, raised intracranial pressure, peripheral neuropathy, serum and CSF angiotensin-converting enzyme and lysozyme, bilateral hilar lymphadenopathy, CD4/CD8 ratio in broncho-alveolar lavage
Neuro-Behçet's disease	Brainstem symptoms, cognitive impairment, meningoencephalitis, basal ganglia lesions, predominant brainstem lesions, oral and genital ulcers, uveitis, HLA-B51, CSF IL6, CSF pleocytosis, cerebral venous sinus thrombosis
Neuro-sweet disease	Meningoencephalitis, headache, consciousness disturbance, seizure, cognitive impairment, painful erythematous plaques, dermal infiltration of neutrophils on skin biopsy, HLA-B54, HLA-Cw1, CSF pleocytosis, CSF IL6
Connective tissue diseases (e.g., Sjögren syndrome, systemic lupus erythematosus)	Neuropsychiatric symptoms, seizure, ischemic stroke, serum autoantibodies (including anti-nuclear antibody, anti-SS-A/B antibody), systemic organ manifestations, absent CSF OCBs, brain infarct and hemorrhage, peripheral neuropathy
Susac's syndrome	Headache, encephalopathy, visual loss, sensorineural hearing loss, snowball lesions in corpus callosum, leptomeningeal enhancement
Chronic lymphocytic inflammation with pontine periventricular enhancement responsive to steroids (CLIPPERS)	Brainstem and cerebellar symptoms, multiple punctate gadolinium-enhanced lesions in the pons and cerebellum
CNS lymphoma	Headache, raised intracranial pressure, cognitive and consciousness impairment, psychomotor slowing, mass effect, dense enhancement by gadolinium, lymph node swelling, skin rash, ^{18}F-fluorodeoxyglucose (FDG) PET
Cerebral small vessel disease	Stroke, cognitive impairment, focal neurological signs, lacunar infarct, microbleeds, sparing U fibers, diabetes mellitus and other vascular risk factors, absence of CSF OCBs
HTLV-1-associated myelopathy (HAM)/tropical spastic paraparesis (TSP)	Chronic progressive spastic paraparesis, anti-HTLV-1 antibodies in serum and CSF, thoracic spinal cord atrophy on MRI

Table 2 (continued)

Differential diagnosis	Key features useful for differentiating each disease from MS
Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)	Migraine, stroke, dementia, depression, Parkinsonism, lacunar infarct, microbleeds, temporal pole and external capsule lesions, widespread confluent white matter lesions, *NOTCH3* gene mutation, absence of CSF OCBs
Congenital leukodystrophy (adrenoleukodystrophy, metachromatic leukodystrophy, hereditary diffuse leukoencephalopathy with spheroid)	Chronic progressive course, cognitive impairment, peripheral nerve involvement, widespread confluent white matter lesions, CSF protein increase without pleocytosis, absence of CSF OCBs, relevant gene mutation

CSF cerebrospinal fluid, *HTLV-1* human T cell lymphotropic virus type-1, *MRI* magnetic resonance imaging, *OCBs* oligoclonal IgG bands, *PET* positron emission tomography

Treatment

Therapy for MS has three purposes: (1) to decrease the severity of acute relapse and accelerate recovery from acute relapse, (2) to reduce relapse frequency and prevent disability progression by disease-modifying therapy, and (3) to alleviate residual symptoms (symptomatic therapy).

Treatment of Acute Relapse

Acute relapse that limits the activity of daily life is treated with corticosteroids. Generally, intravenous, high dose (1000 mg/day) methylprednisolone (IVMP) for three consecutive days (2–5 days) is administered. A short course of oral corticosteroids (around 1 mg/kg/day) with gradual taper usually follows post IVMP. However, because corticosteroids have no effects on preventing relapse or disability progression, oral corticosteroids should not be continued for a long time, except in atypical cases. IVMP may be repeated once or twice when recovery from acute relapse is insufficient. In cases of corticosteroid-resistant relapse, plasma exchanges can be effective, especially when patients present large demyelinating lesions. For patients with methylprednisolone allergy, intramuscular adrenocorticotropic hormone or plasma exchanges may be beneficial.

Treatment with Disease-Modifying Drugs

Various kinds of DMD effectively reduce relapses in RRMS and are approved for patients with RRMS (Table 3). However, these DMDs mainly target the peripheral immune system and are of little benefit for chronic progression in SPMS and PPMS [132].

Table 3 Widely used disease-modifying drugs for RRMS

Generic name	Route, dose, and schedule	Efficacy on relapse rate	Side effects (rare but serious)
Interferon β-1b	250 μg, SC, every other day	−34%	Influenza-like symptoms, injection site reactions, increased liver enzymes, (liver toxicity)
Interferon β-1a	30 μg, IM, once a week	−32%	Influenza-like symptoms, injection site reactions, increased liver enzymes, (liver toxicity)
Glatiramer acetate	20 mg, SC, every day	−29%	Injection site reactions, post-injection general reaction, lipoatrophy
Dimethyl fumarate	240 mg, PO, twice a day	−51%	Flushing, diarrhea, abdominal pain, lymphopenia (PML)
Fingolimod	0.5 mg, PO, once a day	−52%	Bradycardia and heart conduction block at first dose, lymphopenia, increased liver enzymes (macular edema, generalized herpes zoster infection, herpes simplex encephalitis, PML)
Natalizumab	300 mg, IV, once every 4 weeks	−68%	Hypersensitivity reactions, (PML)

C subcutaneous, *IM* intramuscular, *IV* intravenous, *PML* progressive multifocal leukoencephalopathy, *PO* per oral

DMDs for RRMS

First-line DMDs for RRMS include interferon-beta (IFNβ)-1a and IFNβ-1b, glatiramer acetate (GA), and dimethyl fumarate (DMF) [129, 133]. IFNβ and GA have similar efficacy: about 30% reduction of RRMS relapse rates, and 50% reduction of new and enlarging lesions on MRI. However, about 30% of RRMS patients are non-responders to these injected drugs. These drugs correct a Th1/Th17 shift, suppress antigen presentation and T cell proliferation, and restore immune-regulatory functions. Both drugs have long been used for RRMS without serious adverse events except for rare liver toxicity. Both IFNβ and GA frequently show injection site reactions while IFNβ is commonly associated with influenza-like symptoms on injection. Oral DMF decreases the relapse rate by 50% and new and enlarging MRI lesions by up to 80% [134, 135]. DMF reduces proinflammatory cytokine production, corrects a Th1/Th17 shift, suppresses T cell infiltration into the CNS via activation of the hydroxycarboxylic acid receptor 2 (HCAR2) pathway, and exhibits antioxidant effects via activation of the nuclear factor (erythroid-derived 2)-like 2 (Nrf-2) pathway. DMF frequently causes facial flushing and gastrointestinal symptoms such as diarrhea and abdominal pain, especially within 1 month of initiation of the drug. DMF occasionally produces severe lymphopenia (<500/μl). Sustained lymphopenia is a risk factor for a rare but serious complication of progressive multifocal leukoencephalopathy (PML) mediated by John-Cunningham virus (JCV) (occurrence is about 1:50,000) [136]. For MS patients with mild disease activity, IFNβ, GA, or DMF is reasonable therapy. In people with CIS or RRMS who have not had relapses in the preceding 2 years and do not have active new lesions on

recent MRI, it is possible to closely follow up with serial MRI at least annually for the first 5 years rather than initiating DMDs [133]. If MS patients experience one or more relapses, two or more new MRI lesions, or increased disability on examination over a 1-year period of using the first-line DMD, they are regarded as non-responders [133]. In this case, a direct switch to another first-line DMDs is one option.

When first-line DMDs are ineffective or the patient has highly active disease, second-line DMDs are considered. Commonly used second- to third-line DMDs include fingolimod and natalizumab, while teriflunomid, cladribine, and alemtuzumab are less commonly used [129, 133]. Fingolimod, an oral DMD for RRMS, is an antagonist of sphingosine-1-phosphate receptor 1 ($S1P_1$) and has a unique mechanism of action; it down-modulates $S1P_1$, which is essential for lymphocyte egress from lymph nodes. Thus, fingolimod traps central memory T cells homing to lymph nodes, thereby preventing autoreactive T cells from circulating in the bloodstream. Fingolimod effectively reduces relapse rates 60% and new and enlarging brain MRI lesions 80% [137, 138]. The first dose of fingolimod frequently causes bradycardia and occasionally conduction block via $S1P_1$. Lymphocytopenia is also common, and drug holidays are recommended when lymphocyte counts decrease to <200/μl. Macular edema and liver dysfunction are occasionally encountered in patients taking fingolimod. Fingolimod confers an increased risk of infection and patients may rarely develop generalized varicella zoster infection, herpes encephalitis, or PML (1:12,000) [136, 139]. In comparison with first-line DMFs, suppression of brain atrophy progression has been shown only for fingolimod [140]. Because fingolimod does not deplete autoreactive T cells but just traps them inside lymph nodes, cessation of the drug could induce a rebound phenomenon (flare-up of disease activity).

Natalizumab, an anti-α4β1 integrin antibody, effectively blocks the interaction between vascular cell adhesion molecule 1 (VCAM1) on vascular endothelial cells and very late antigen 4 (VLA4) consisting of α4β1 integrin on lymphocytes, thereby suppressing firm adhesion of T cells on vessel walls. Thus, T cell migration into CNS tissue is highly effectively blocked [141]. Natalizumab suppresses relapse rate 70% and new and enlarging brain MRI lesions 90% [142]. The ability of natalizumab to markedly suppress relapses supports the critical importance of T cell inflammation in the CNS at relapse. However, because natalizumab almost completely blocks T cell migration into CNS tissues, T cell surveillance of the CNS is severely impaired, which allows the occurrence of JCV-mediated PML (4.19/1000) [136]. The risk factors for PML under natalizumab treatment are long-term use (>2 years), prior use of immunosuppressants, and high anti-JCV antibody index (>1.5) [143]. When natalizumab-PML emerges, rapid withdrawal of natalizumab by PE may cause massive infiltration of lymphocytes into the CNS (immune reconstitution inflammatory syndrome; IRIS), resulting in severe tissue destruction and residual disability. When JCV antibody is negative and the patient has highly active disease, natalizumab can be a good choice.

Alemtuzumab is a humanized anti-CD52 monoclonal antibody that causes long-lasting T and B cell depletion. Alemtuzumab markedly reduces relapse rates and prevents new and enlarging brain MRI lesions (>90% decrease), although the drug

can frequently induce autoimmune diseases (>40%), such as Graves' disease, autoimmune thyroiditis, immune thrombocytopenic purpura, and autoimmune hemolytic anemia [144, 145]. Alemtuzumab may be considered for those who have not responded to at least two DMDs. Teriflunomide is a cytostatic drug that inhibits de novo pyrimidine synthesis from carbamoyl phosphate and aspartate by suppressing dihydro-orotate dehydrogenase, thereby limiting rapidly proliferating T and B cells without affecting resting and homeostatically proliferating cells. Teriflunomide has modest efficacy, similar to IFNβ. For early MS, teriflunomide effectively decreased relapse risk or new MRI lesions (around 35% reduction) [146]. Cladribine, a synthetic deoxyadenosine analog, is a cytotoxic drug that depletes T and B cells. Cladribine reduced relapse rates by 60% and decreased MRI brain lesions, while the drug causes lymphocytopenia and increases the risk for infection, such as for tuberculosis and PML [147].

DMDs for Progressive MS

For SPMS patients with active disease, IFNβ is in part beneficial but it is not effective for those without active disease. Recently, siponimod [148] and ozanimod [149], novel $S1P_1$ antagonists, were found to be effective for preventing disability progression in SPMS. These drugs, besides inhibitory effects on lymphocyte egress from secondary lymphoid organs, may directly act on glial cells harboring $S1P_1$, such as microglia and astroglia [150, 151]. For PPMS, only one DMD, ocrelizumab (anti-CD20 humanized monoclonal antibody), significantly decreases disability progression [120]. Ocrelizumab depletes circulating B cells but not plasma cells that do not express CD20. Therefore, interruption of B-T cell interaction, including antigen presentation, and suppression of proinflammatory cytokine secretion from B cells, are assumed to be the mechanisms of ocrelizumab action rather than decreasing autoantibody production. Ocrelizumab reduced the annualized relapse rate by about 50% and prevented new MRI lesions by 95% in RRMS. For PPMS, ocrelizumab decreased clinical disability progression by 24% [120].

Symptomatic Therapy and Management

Spasticity

Spasticity is best managed by a combination of anti-spastic drugs and physiotherapy. Some degree of spasticity is often useful for patients in standing and walking, while an overdose of antispastic drugs may worsen patients' locomotive ability. Baclofen, tizanidine, and gabapentin are used as first-line drugs. Dantrolene is a second-line drug, especially for non-ambulatory patients with spasticity. Benzodiazepines can be helpful, particularly taken as a nighttime dose. To avoid side effects, including weakness, sleepiness, lightheaded sensation, fatigue and

hypotension, it is necessary to gradually increase the doses of these agents. An intrathecal baclofen pump can be tried for oral anti-spastic drug-resistant cases, while repeated local injections of botulinum toxin may be helpful for alleviating focal spasticity.

Sphincter Disturbance and Sexual Dysfunction

For urinary urgency, long-acting anticholinergic drugs or tricyclic antidepressants are useful if there is not excessive urinary retention while nasal desmopressin spray is efficacious for nocturia. An alpha-adrenergic blocker can be used for difficulty with urination and urinary retention. When urinary retention reaches a post-voiding residual urine volume > 100–150 ml, intermittent self-catheterization, or an indwelling or suprapubic catheter may be considered. In some cases, intravesical botulinum toxin injection or sacral electrical stimulation will be useful. Laxatives are used for constipation while fecal incontinence is difficult to treat. Although physiotherapy for the pelvic floor may be helpful, a bowel training program and bowel management permit patients to better manage their lives. For impotence, phosphodiesterase type 5 inhibitors, intrapenile suppositories, or injections of prostaglandin are helpful for men. Sexual dysfunction is common but under-appreciated; therefore, counseling of couples is important.

Fatigue, Mood Disturbance, and Cognitive Impairment

Fatigue due to exercise and work may be improved with rest and naps. Amantadine, modafinil, and fampridine can be helpful for fatigue. Depression can usually be treated with antidepressant medications (selective serotonin reuptake inhibitors, serotonin and norepinephrine reuptake inhibitors, and tricyclics antidepressants) and counseling. Cognitive impairment is very difficult to treat. Donepezil, an acetylcholinesterase inhibitor, and memantine may be useful in some cases, although efficacy has not been confirmed by randomized controlled trials. Cognitive rehabilitation and occupational therapy are also worth doing.

Other Symptomatic Therapies and Management of General Health

Pain is common in patients with MS. Trigeminal and glossopharyngeal neuralgia and other neurogenic pain can be treated with anticonvulsants, such as carbamazepine, pregabalin, gabapentin, and topiramate. Painful tonic spasm is effectively treated with low-dose carbamazepine. Band-like sensations (girdle sensation) is difficult to treat and requires a combination of drugs, such as anticonvulsants, analgesics, and muscle relaxants. Spasticity induces pain in muscles while abnormal posture and gait may accentuate musculoskeletal pain. Depending on the cause, antispastic drugs, analgesics, and antidepressants should be selected. Although

anticonvulsants are applied for persistent numbness, it is hard to resolve. Tremor and ataxia are also difficult to treat and drugs for essential tremor such as beta-adrenergic blockers and clonazepam have only limited efficacy. Use of wrist weights may reduce action tremor in the hand or arm in some patients. 4-Aminopyridine and a sustained release form, dalfampridine, may improve power, endurance, and ambulation in some patients.

Discontinuation of tobacco smoking and supplementation of vitamin D are recommended. Exercise programs as tolerated are also useful for improving mood, fatigue, ambulation, and pain. In disabled patients, prevention of pressure sores and contractures, as well as nutrition and avoiding aspiration, are important in daily care.

Conflict of Interest Statement Jun-ichi Kira has received consultancy fees, speaking fees and/or honoraria from Novartis Pharma, Mitsubishi Tanabe Pharma, Boehringer Ingelheim, Teijin Pharma, Takeda Pharmaceutical Company, Otsuka Pharmaceutical, Astellas Pharma, Pfizer Japan, and Eisai Co. Dr. Isobe is supported by a grant from JSPS KAKENHI (Grant No. 18 K07529), and received grant support from Mitsubishi Tanabe Pharma, Osoegawa Neurology Clinic, Bayer Yakuhin, Ltd., and Japan Blood Products Organization.

Funding This study was supported in part by a Health and Labour Sciences Research Grant on Intractable Diseases (H29-Nanchitou (Nan)-Ippan-043) from the Ministry of Health, Labour, and Welfare, Japan, by the "Practical Research Project for Rare/Intractable Diseases" from the Japan Agency for Medical Research and Development (AMED, 17ek0109115h0003), Japan, by a research grant from the Japanese Multiple Sclerosis Society, by a research grant from the Japan Intractable Disease Research Foundation, by "Glial Assembly" Grants-in-Aid for Scientific Research on Innovative Areas (MEXT KAKENHI Grant Number 25117012) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by a Grant-in-Aid for Scientific Research (A) (JSPS KAKENHI Grant Number 16H02657) from the Japan Society for the Promotion of Science, Japan.

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Neuromyelitis Optica Spectrum Disorder

Samira Saadoun, Vincent T. W. Chang, and Marios C. Papadopoulos

Abstract Neuromyelitis optica (NMO) is an autoimmune disease of the central nervous system, also known as Devic's syndrome, that typically manifests with optic neuritis and transverse myelitis and, like other antibody-mediated autoimmune diseases, primarily affects women. Most NMO cases are caused by a circulating autoantibody termed NMO-IgG or AQP4-IgG that targets the astrocytic water channel protein aquaporin-4 (AQP4). Some NMO cases are caused by a circulating autoantibody against the myelin oligodendrocyte glycoprotein termed MOG-IgG. A small proportion of NMO cases, termed seronegative NMO, are not associated with an autoantibody. AQP4-IgG binds extracellular conformational epitopes on AQP4, activates complement, which in turn causes inflammatory cell infiltration, demyelination and pan-necrosis. Acute NMO exacerbations are treated with methylprednisolone or plasmapheresis. Some drugs used to treat multiple sclerosis, such as interferon beta and natalizumab, exacerbate NMO. Maintenance treatment options include prednisolone, mycophenylate, mitoxantrone, cyclophosphamide, azathioprine, rituximab, tocilizumab and eculizumab. The discoveries of AQP4-IgG and MOG-IgG have shown that NMO is a distinct entity from multiple sclerosis with fundamentally different pathophysiology and treatment.

Keywords AQP4-IgG · Aquaporin-4 · MOG-IgG · Myelin oligodendrocyte glycoprotein · Neuromyelitis optica spectrum disorders · NMO-IgG · Optic neuritis · Transverse myelitis

Introduction

Neuromyelitis optica (NMO) is a rare, antibody-mediated autoimmune disorder of the central nervous system (CNS) that primarily affects the optic nerves and the spinal cord. We discuss the history of NMO, the seminal discovery of NMO-IgG,

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_16

the concepts of seronegative NMO and NMO spectrum disorders, as well as the epidemiology and clinical manifestations of NMO. We then review the cellular mechanisms that are responsible for the pathogenesis of NMO. Finally, the diagnostic criteria, clinical features as well as current and future treatments will be covered.

History of NMO Whilst this field of research has seen great advancements in the past two decades, following the discovery of the NMO-IgG autoantibody, its history dates back to the early nineteenth century. In 1804, Antoine Portal, first physician to Louis XVIII and founding president of the Académie Nationale de Médecine, reported spinal cord inflammation coupled with visual loss in a patient without brain pathology; this is the oldest known account of its kind in Western literature [1]. Following that report, various clinicians recorded similar cases, including the Genoese physician Giovanni Battista Pescetto in 1844, the British physician Christopher Mercer Durant in 1850 and the British neurologist Jacob Augustus Lockhart Clarke in 1862 [1]. In 1894, the name *neuromyélite optique* was given to this syndrome characterized by acute myelitis and optic neuritis by the French neurologist Eugène Devic; Devic's contribution to the identification of this disease is still recognized since NMO is also termed *Devic's disease*.

NMO-IgG Though Devic insisted that NMO was a disease in its own right, over the years, NMO had been regarded by most neurologists as a variant of multiple sclerosis (MS) and has been treated as such [2]. In 2004, Vanda Lennon and colleagues at the Mayo Clinic identified a serum autoantibody, termed NMO-IgG, that was present in NMO patients but was absent from those with MS. After testing serum samples of North American and Japanese patients with suspected NMO and MS, they detected the autoantibody in 73% of those diagnosed with NMO as well as 46% high-risk candidates for NMO; importantly, NMO-IgG was not found in any patient with classic MS or other miscellaneous autoimmune/paraneoplastic neurological disorder [3]. The discovery of NMO-IgG revolutionized our understanding of NMO and conclusively established NMO as distinct entity from MS. In 2005, a water channel protein expressed in the end-feet of astrocytes, termed aquaporin-4 (AQP4), was identified as the target of NMO-IgG and thus NMO-IgG also became known as AQP4-IgG [4]. A subsequent study showed that AQP4-IgG is IgG_1 subclass and, therefore, after binding to AQP4, AQP4-IgG may activate complement via the classical pathway causing plasma cell membrane lysis [5]. Though initially the emphasis was on the inflammation and demyelination as the two characteristic histological features of NMO, the discovery of AQP4 as the target protein, which is expressed in astrocytes, but not neurons or oligodendrocytes, established NMO as an astrocytopathy. The sites of AQP4 expression in the CNS are shown in Fig. 1a. There is now evidence that AQP4-IgG binds at these sites including astrocyte foot processes, subependymal astrocytes and glia limitans and activates complement [6, 7].

Since AQP4-IgG plays a key role in NMO pathogenesis, it became important to revisit the diagnostic criteria so as to incorporate AQP4-IgG. Beginning in 2006, a diagnosis of NMO required the following: optic neuritis, acute myelitis and at least

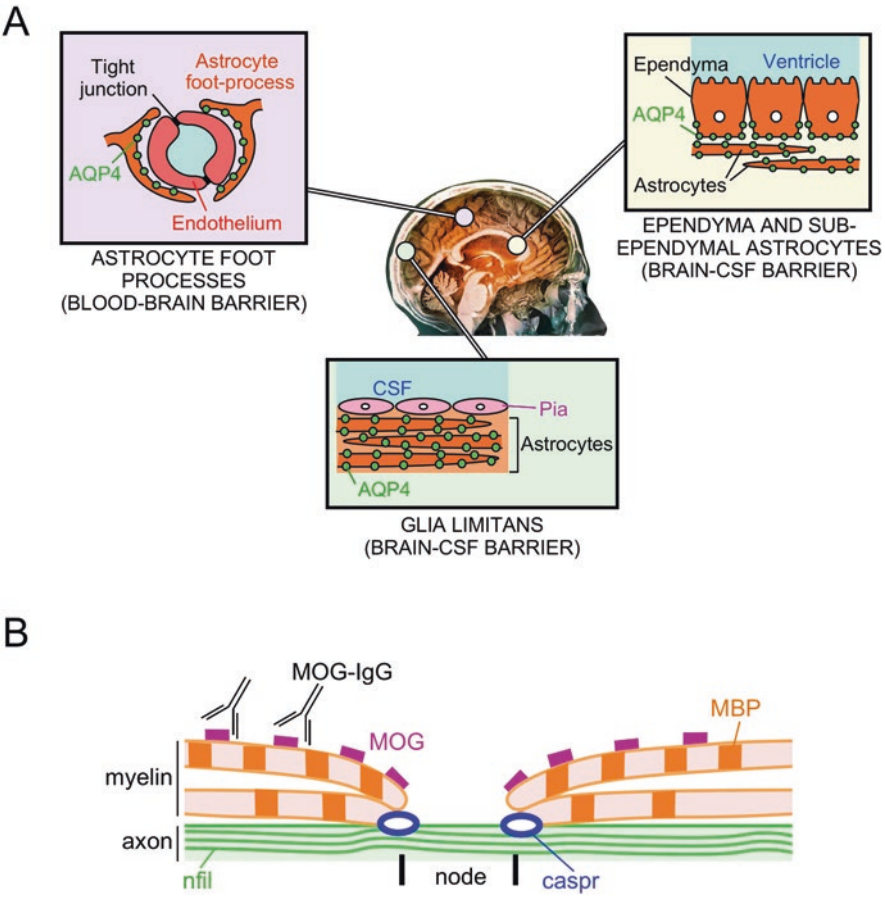

Fig. 1 Sites of expression of AQP4 and MOG in the CNS. (**a**) AQP4 (green circles) is expressed in astrocytes foot processes (blood-brain barrier), the basolateral surface of the ependymal and subependymal astrocytes (brain-CSF barrier) and glia limitans astrocyte processes (brain-CSF barrier). (**b**) MOG (purple) is expressed on the surface of myelin in the central nervous system. Other myelin proteins include MBP (myelin basic protein). The myelin is anchored to the axon at the node of Ranvier by caspr

two of three supportive criteria (contiguous spinal cord MRI lesion extending over three or more vertebral segments known as longitudinally extensive transverse myelitis (LETM); brain MRI not meeting the criteria for MS; AQP4-IgG seropositive status) [8]. The diagnostic criteria for NMO have since been updated and will be discussed later in this chapter. Since 2006, the pathogenicity of AQP4-IgG has been firmly established [5, 9–11]. It thus became clear that AQP4-IgG$^+$ patients with incomplete or atypical NMO presentations are no different from patients fulfilling the 2006 diagnostic criteria. To incorporate these incomplete or atypical patients who are AQP4-IgG$^+$, the term NMO spectrum disorder (NMOSD) is now preferred, rather than NMO, as the name of the disease [12].

Seronegative NMO Some NMO patients do not have circulating AQP4-IgG; their disease is termed seronegative NMO. Serum antibodies against the oligodendrocyte protein myelin oligodendrocyte glycoprotein (MOG) have been detected in some seronegative NMO patients [13–16]. MOG is a transmembrane glycoprotein situated on the cell surface of oligodendrocytes that plays a key role in the myelination of CNS neurons including the adhesion of myelin fibres, the modulation of oligodendrocyte microtubule stability and the regulation of the interaction between the immune system and myelin by the complement pathway [17]. The sites of MOG expression in the CNS, which are targets of MOG-IgG, are shown in Fig. 1b. The discovery of AQP4-IgG and MOG-IgG suggests that other, as yet unidentified, autoantibodies may be pathogenic in some cases of AQP4-IgG$^-$ and MOG-IgG$^-$ NMO.

Epidemiology

Due to the rare incidence of NMO, the relative paucity of good-quality population studies, the variable diagnostic accuracy of AQP4-IgG detecting tests, the misdiagnosis of NMO for MS and vice versa and the variability of NMO between populations, it is difficult to produce exact epidemiological figures. In a review of several studies, NMO prevalence rates from 38 countries ranged from 0.05 per 100,000 in Kenya and Iraq to 4.4 per 100,000 in Denmark [18]. Though it had previously been assumed that NMO was more prevalent in Asians and Afro-Caribbeans than Caucasians, recent studies have suggested that prevalence in Caucasians may be higher than previously reported [18]. It is now well-established that up to 80–90% of NMO patients are female, which is characteristic of antibody-mediated autoimmune diseases [19]. Several risk factors for NMO have been proposed, including previous history of head trauma, low dairy, seafood, fruit and vegetable intake, and low heavy physical activity [20], though the validity of these studies is questionable.

Clinical Manifestations

NMO typically presents with optic neuritis and transverse myelitis [12]. The optic neuritis causes visual loss often associated with eye pain when moving the eyes. In NMO, optic neuritis is often bilateral or rapidly sequential. Transverse myelitis typically causes symmetric limb weakness, loss of sensation and loss of voluntary urinary bladder and anal sphincter control. Symptoms that suggest brainstem lesions include intractable nausea, vomiting or hiccups, due to involvement of the area postrema, excessive daytime somnolence or narcolepsy, due to involvement of the midbrain, as well as neuro-endocrine abnormalities due to involvement of the hypothalamus. Limited forms of the disease (forme fruste) have also been described including isolated unilateral optic neuritis or isolated transverse myelitis.

NMO association with other autoimmune diseases About 35–50% of NMO patients exhibit clinical signs or laboratory findings of other organ-specific or systemic autoimmune disorders (e.g. systemic lupus, autoimmune thyroid disease, or Sjögren's syndrome) [21]. A link between myasthenia gravis (MG) and NMO has received particular attention. Given the rarity of MG and NMO, their coexistence is unlikely to be coincidental. A retrospective study concluded that MG precedes NMO in most cases, often by more than a decade, and that a history of thymectomy poses a risk factor for later development of NMO [22]. Familial existence is more common than would be expected from its prevalence in the general population [23] and association between the HLA-DRB1∗03 and HLA-DPB1∗05 allele groups with NMO has been demonstrated [24, 25]. These findings suggest that genetic factors may play a role in the manifestation of NMO, though the contribution of genetics is relatively minor.

NMO outside the CNS AQP4 is expressed in peripheral organs outside the CNS such as the kidney (collecting duct principal cells, basolateral membranes of distal tubules), skeletal muscle (sarcolemma of fast-twitch type II fibres), stomach (basolateral membranes of parietal cells) and placenta (syncitiotrophoblast mid-gestation) [26–30]. Though AQP4-IgG binds AQP4 in peripheral organs [4], AQP4-IgG-mediated cell damage outside the CNS is rare. No cases of gastric or renal inflammation associated with NMO have been reported and there are only isolated reports of NMO myopathy confirmed by muscle biopsy [31, 32]. One study found elevated serum creatinine kinase in only 0.4% of AQP4-IgG$^+$ NMO patients [33]. Two mechanisms have been proposed to explain why the CNS is so vulnerable to AQP4-IgG-mediated damage, but these AQP4-expressing peripheral organs are largely spared. One possibility is that circulating AQP4-IgG binds the peripheral AQP4-expressing cells but fails to activate the complement, whereas in the CNS, AQP4-IgG binding readily causes complement-mediated damage. This is supported by immunohistochemical studies showing that astrocytic end-feet in the brain lack the complement regulators CD46, CD55 and CD59 and are thus vulnerable to AQP4-IgG and complement-mediated damage [34]. In contrast, the skeletal muscle, stomach and kidney co-express at least one or more of these regulators with AQP4 thus inhibiting complement activation following AQP4-IgG binding, in the peripheral organs [34]. Another, less likely possibility is that the supramolecular aggregation of AQP4 is greater in peripheral organs compared with the CNS, thus allowing more AQP4-IgG to bind near each other, in turn facilitating complement activation [35].

NMO and pregnancy Since the majority of NMO patients are young females, it is important to explore how NMO affects pregnancy. Studies of patients from the UK, Japan and Mexico show high miscarriage rate, of 13–50%, in women with NMO compared with the general population [36]. Women who develop NMO and have other autoimmune disorders are at higher risk of pre-eclampsia. In humans and mice, AQP4 is not found in the sperm or the female reproductive tract, but is transiently expressed in the human and mouse placental syncitiotrophoblast mid-gestation [30]. In a mouse passive transfer study done mid-gestation, AQP4-IgG

induced miscarriage by binding placental AQP4 and activating the classical complement pathway as evidenced by C5b-9 deposits in the plasma membrane of placental syncytiotrophoblast, which resulted in leucocyte infiltration leading to placental necrosis and subsequent foetal death [30]. These findings suggest that high placental vulnerability to AQP4-IgG may be at mid-gestation, whereas at conception and in the early and late gestational phases, pregnancy may not be affected by AQP4-IgG. Several studies showed that the relapse rate of NMO is elevated in the three months after pregnancy [36–42]. A French study of 124 patients suggested that epidural analgesia and breastfeeding do not influence disease activity and have no effect on NMO exacerbation [43]. The high miscarriage rate in NMO women and the possibility of placentitis and the higher NMO relapse rate postpartum suggest that close monitoring and immunosuppressive treatment are required. Currently, there are no guidelines on the treatment of NMO in pregnancy. Before producing guidelines, further research is required to determine the effect of AQP4-IgG and of anti-NMO immunosuppressive medication on the foetus and neonate. With regard to pregnancy, the US Food and Drug Administration labels drugs as Category A (no evidence of risk to human foetuses), Category B (no evidence of risk to animal foetuses), Category C (harmful to animal foetuses but unknown in humans), Category D (harmful to human foetuses) and Category X (harmful to both human and animal foetuses) [44]. Methylprednisolone and rituximab are classified as category C for pregnancy purposes; thus these treatments should only be continued if potential benefits outweigh risks. Azathioprine and mycophenolate are category D and should be suspended in pregnancy. Since the frequency of relapses increases postpartum, immediate start of prophylactic treatment is advised [44].

Pathogenesis

Aquaporin-4 Aquaporins are integral membrane proteins that selectively facilitate the osmotic flow of water via passive transport [45]. Of the 13 known aquaporins, AQP4 is the most abundant in the CNS where it is expressed in the perimicrovessel astrocyte foot processes, glia limitans and ependyma [46], i.e. at the borders between the CNS and major fluid compartments such as blood and cerebrospinal fluid. In addition to allowing water movement into and out of the brain and spinal cord, AQP4 plays key roles in enhancing astroglial cell migration during glial scar formation [47], in modulating neuronal excitability and in determining the size of the extracellular space [48–51]. AQP4 comprises four monomers, each with six helical transmembrane domains and two short helical segments surrounding an aqueous pore [52]. AQP4 has two isoforms, produced by alternative splicing: *M1*, a full-length isoform with translation initiation at Methionine-1, and *M23*, a shorter isoform with translation initiation at Methionine-23 [53]. A key functional difference between these isoforms is that M23 forms supramolecular arrays termed orthogonal arrays of particles (OAPs). M1 isoforms do not form OAPs on their own, but do so when be co-assembled with M23 and the size of the arrays depends on the M23:M1

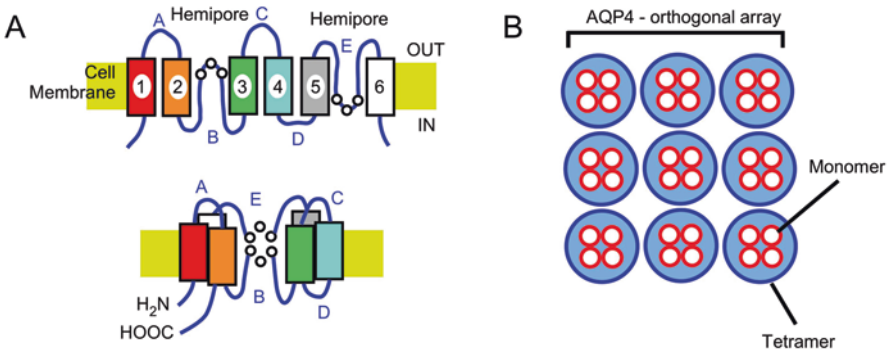

Fig. 2 Structure of AQP4. (**a**) *(Top)* AQP4 has six membrane spanning domains (1–6) linked by aminoacid loops A–E. There is a hemipore between 2 and 3 and another hemipore between 5 and 6. *(Bottom)* The membrane spanning domains, loops, and hemipores assemble to form an AQP4 monomer. (**b**) Four AQP4 monomers are linked to form an AQP4 tetramer. Tetramers assemble into higher-order structures termed orthogonal arrays

ratio [53]. AQP4-IgG binds more avidly to cells expressing M23 than M1, which suggests that AQP4-IgG preferentially targets OAPs [2]. AQP4-IgG recognizes conformational epitopes involving the extracellular AQP4 loops A, C and E [54]. The structure of AQP4 is shown in Fig. 2.

Complement AQP4-IgG is IgG_1 subclass, which can activate complement, and binds AQP4 extracellular conformational epitopes, i.e. immunolabels nonpermeabilized cultured AQP4-expressing cells [5]. The presence of AQP4 OAPs allows multiple AQP4-IgG to bind in close proximity, thus leading to complement activation. Samira Saadoun showed that, in mouse brain, passive transfer of AQP4-IgG produces lesions only when co-injected with human complement, since human IgG_1 does not effectively activate mouse complement. The mouse NMO lesions had the characteristic features of human NMO lesions including loss of AQP4 and GFAP expression, indicating astrocyte death, demyelination, inflammatory cell infiltration and perivascular deposition of the activated complement components C5b-9 [11]. Though both complement-dependent (CDC) and antibody-dependent cell-mediated (ADCC) cytotoxicities feature in NMO pathogenesis [55], CDC is thought to be the principal mechanism. CDC is significantly enhanced in AQP4 channels assembled as OAPs and thus cells expressing M23-AQP4 are more prone to AQP4-IgG-mediated destruction than M1-AQP4 expressing cells [56, 57]. ADCC involving natural killer cells is not dependent on OAP formation and thus occurs equally in M23- and M1-expressing cells [57], though the role of natural killer cells in NMO is unclear. In a clinical trial of 14 AQP4-IgG^+ women with aggressive NMO, eculizumab, a monoclonal IgG that neutralizes the complement protein C5, reduced attack frequency and stabilized or improved neurological disability. The therapeutic efficacy of eculizumab also supports the essential role of complement activation in NMO pathogenesis.

CNS inflammation At perivascular astrocytic end-feet, activation of the classical complement pathway leads to a cascade of downstream effects [2]. Complement causes astrocyte lysis by deposition of the C5b-9 membrane attack complex channels into the astrocyte plasma cell membrane. Activated complement recruits and activates neutrophils, eosinophils and macrophages. In a passive transfer NMO mouse model, the first cells to enter the CNS are neutrophils and eosinophils, which infiltrate the perivascular (Virchow-Robin) spaces and glia limitans; these granulocytes damage the CNS, as the neutrophils release elastase and the eosinophils release basic granule proteins such as eosinophil-derived neurotoxin, MBP, eosinophil peroxidase and eosinophil cationic protein [7]. In a mouse NMO model, the neutrophil elastase inhibitor sivelestat [58] and the anti-eosinophil histamine antagonist cetirizine [59] reduced AQP4-IgG-mediated brain damage. Macrophages generate local proteases, cytokines and free radicals, resulting in pan-necrosis, i.e. the destruction of white and grey matter including not only astrocytes but also neuronal axons and oligodendrocytes [7]. Since oligodendrocytes are responsible for the myelination of axons in the CNS, their loss explains the demyelinating feature of NMO. Though demyelination is a major histological feature of NMO, it is not the primary pathological event. Axonal destruction ultimately leads to neuronal cell death [7]. Since AQP4 facilitates astrocyte migration, destruction of AQP4 impairs the formation of a glial scar that normally provides the barrier that excludes circulating leucocytes from entering the CNS [60, 61]. Figure 3 summarizes key events responsible for AQP4-IgG$^+$ NMO pathogenesis.

AQP4-IgG production The triggers responsible for AQP4-IgG production are unclear. One possibility is infection-induced cross-reactivity between human AQP4 and bacterial proteins such as aquaporin-Z [62] or Clostridium perfringens adenosine triphosphate-binding cassette transporter permease [63]. Some reports proposed an association between SLE and NMO, even suggesting a direct causal link between SLE onset and AQP4-IgG production [64, 65]. Some NMO is paraneoplastic: NMO has been described in the setting of several cancer types, with thymoma and breast

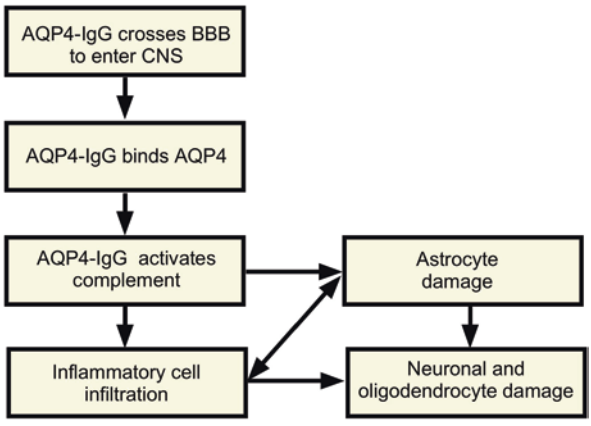

Fig. 3 AQP4-IgG$^+$ NMO pathogenesis. Summary of key events from entry of AQP4-IgG into the CNS to cell death

carcinoma the most common [66]. AQP4 is abnormally expressed at high levels in certain tumours [66], perhaps prompting the patients' immune response against the tumour to generate IgG that binds tumoural AQP4.

AQP4-IgG crossing the blood-brain barrier AQP4-IgG is produced in the periphery and enters the CNS secondarily as evidenced by the high blood:CSF AQP4-IgG ratio [67]. How does AQP4-IgG cross the blood-brain barrier (BBB) to access AQP4 in the CNS? One route for CNS entry is through the microvasculature of the circumventricular organs (CVOs, i.e. area postrema, subfornicial organ, pituitary gland), which express high levels of AQP4 and where the microvascular endothelium lacks tight junctions. The CVO lack BBB allowing AQP4-IgG to cross into the brain from the bloodstream. This explains why NMO lesions are frequently found in the periaqueductal brainstem and hypothalamus thus causing diencephalic symptoms or area postrema symptoms with intractable nausea and vomiting [68]. The involvement of CVOs in NMO is now recognized in the 2015 NMO diagnostic criteria, which are summarized in Table 1. Another proposed route for AQP4-IgG entry from the periphery into the CNS is by direct transit across the BBB; this route requires autoantibodies against glucose-regulated protein 78 to open the BBB [68] or interleukin-6 production by astrocytes that signals BBB endothelial cells to decrease BBB function [69, 70]. AQP4-IgG does not have a direct effect on the endothelial cells to permeabilize the BBB [70], which explains why some patients may have circulating high titres of AQP4-IgG without developing CNS lesions [71]. The frequent involvement of the spinal cord in AQP4-IgG-mediated damage cannot be explained by a lack of blood-spinal cord barrier; perhaps another as yet unidentified event occurs to transiently open the blood-spinal cord barrier and allow the AQP4-IgG to cross from the bloodstream into the cord.

MOG-IgG NMO MOG proteins, whilst only making up <0.5% of the myelin sheath in the CNS, are highly immunogenic [17]. There are major differences between the pathophysiology of AQP4-IgG- and MOG-IgG-mediated NMO. The hallmark of AQP4-IgG NMO is astrocyte damage, with secondary oligodendrocyte damage; on the other hand, astrocyte damage seems to be absent in MOG-IgG cases [17]. Myelin basic protein (MBP), but not the astrocyte marker GFAP (glial fibrillary acidic protein), is elevated in patients with MOG-IgG when compared to those with AQP4-IgG [72]. This finding suggests that the main mechanism of MOG-IgG-induced CNS damage is oligodendrocyte damage rather than astrocyte damage. Classified under 'seronegative NMO', the MOG-IgG patients possess a different phenotype from those with AQP4-IgG NMO that has more similar clinical and demographic profiles to acute demyelinating encephalomyelitis (ADEM) [73]. Visual impairment and axonal damage in the retina after optic neuritis in MOG-IgG-positive patients are as severe as AQP4-IgG-positive patients [74]; episodes are more severe in the MOG-IgG$^+$ group, but MOG-IgG$^+$ are more likely to be monophasic, and recovery is usually better [73]. Whilst AQP4-IgG seropositivity is more common in females, males comprise the majority of the MOG-IgG$^+$ NMO group [73].

Table 1 The 2015 diagnostic criteria for NMO spectrum disorder

NMOSD with AQP4-IGG
At least 1 core clinical characteristic
Positive test for AQP4-IgG using best available detection method
Exclusion of alternative diagnoses
NMOSD without AQP4-IGG or unknown AQP4-IGG status
At least two core clinical characteristics occurring as a result of one or more clinical attacks and meeting all of the following requirements
At least one core clinical characteristic must be ON, acute TM with LETM, or area postrema syndrome
Dissemination in space (two or more core clinical characteristics)
Fulfilment of additional MRI requirements, as applicable
Negative test for AQP4-IgG using best available detection method, or testing unavailable
Exclusion of alternative diagnoses
Core clinical characteristics
ON
Acute TM
Area postrema syndrome: episode of otherwise unexplained hiccups or nausea and vomiting
Acute brainstem syndrome
Symptomatic narcolepsy or acute diencephalic clinical syndrome with NMOSD-typical diencephalic MRI lesions
Symptomatic cerebral syndrome with NMOSD-typical brain lesions
Additional MRI requirements for NMOSD without AQP4-IGG or unknown AQP4-IGG status
Acute ON requires brain MRI showing (a) normal findings or only nonspecific white matter lesions or (b) optic nerve MRI with T2-hyperintense lesion or T1-weighted gadolinium-enhancing lesion extending over 1/2 optic nerve length or involving optic chiasm
Acute TM requires associated intramedullary MRI lesion extending over three or more contiguous segments (LETM) or three or more contiguous segments of focal spinal cord atrophy in patients with history compatible with acute TM
Area postrema syndrome: requires associated dorsal medulla/area postrema lesions
Acute brainstem syndrome: requires associated periependymal brainstem lesions

LETM longitudinally extensive transverse myelitis, *NMOSD* NMO spectrum disorder, *ON* optic neuritis, *TM* transverse myelitis

Seronegative NMO About 20% of NMO patients test negative for AQP4-IgG and MOG-IgG [75]. The pathophysiology of seronegative NMO remains unclear. One possibility is that current antibody assays are not sensitive enough to detect low levels of AQP4-IgG or MOG-IgG. AQP4-IgG assay sensitivity has improved to ~90% [73], but there are still patients who are falsely classified 'seronegative' even though they have AQP4-IgG. Another possibility is that patients may have antibodies against astrocyte targets other than AQP4 or MOG [2]. There are also several diseases that clinically mimic NMO (i.e. with optic nerve and spinal cord inflammation) but are immunologically distinct [73] and, therefore, some seronegative NMO patients are probably misdiagnosed. Diseases that can mimic NMO include multiple sclerosis, acute disseminated encephalomyelitis, systemic lupus erythematosus, neurosarcoidosis, Sjögren's syndrome, varicella zoster infection and certain paraneoplastic diseases.

Diagnosis

The most recent diagnostic criteria for NMO are from 2015 (Table 1) [12]. Revision was necessary due to rapid advancements in NMO research that had rendered the 2006 diagnostic criteria inadequate. To diagnose NMO spectrum disorder, in addition to optic neuritis and acute myelitis, four other core clinical characteristics of NMO include: area postrema syndrome (unexplained hiccups or nausea and vomiting), acute brainstem syndrome, narcolepsy or acute diencephalic clinical syndrome, and cerebral syndrome [12]. Current diagnostic criteria differ depending on the presence of the AQP4-IgG antibody. In order for an NMO diagnosis to be confirmed in a seropositive patient, only one core clinical characteristic is required. Conversely, if AQP4-IgG is undetectable, the requirements for a diagnosis are more stringent: two core clinical characteristics and additional MRI features must be present. Approximately a third of MOG-IgG-positive patients fulfil the diagnostic criteria for seronegative NMO [17]. Due to the association of MOG-IgG to a wider clinical phenotype, it has been debated whether MOG-IgG$^+$ patients should be treated differently from those with NMO; the term MONEM (MOG-IgG-associated optic neuritis, encephalitis and myelitis) has been proposed [17].

NMO versus MS Whilst a number of clinical and radiological features (e.g. transverse myelitis and spinal cord lesions) are shared between NMO and MS [1], there are many differences; in general, NMO manifests later in life; the average age of onset for NMO is 39 years old compared to 29 for MS. [19] 80–90% of patients with NMO experience relapsing episodes of optic neuritis and myelitis, with little recovery from each attack. In NMO, the transverse myelitis is largely longitudinally extensive, i.e. the signal change on MRI spans at least three vertebral levels. NMO has a relapsing course with median time to first relapse at 8–12 months [76]. In contrast, most MS patients experience attacks of milder severity that usually recover well; only 15% of MS cases are primary-progressive [19]. NMO tends to cause substantial and irreversible damage to the CNS after every episode, eventually resulting in permanent visual, motor, sensory and urinary bladder defects. The degree of deterioration in NMO can be predicted by the number of relapses within the first 2 years of disease onset, the age at disease onset and the severity of the first episode. NMO fatality commonly follows neurogenic respiratory failure and mortality rates range from 25% to 50%.

Cerebrospinal fluid Cerebrospinal fluid analysis differs markedly between NMO and MS. Prominent cerebrospinal fluid pleocytosis (>50x10 [6] leucocytes/L) with a high proportion of neutrophils is characteristic of NMO-specific myelitis, whereas MS attacks usually involve milder pleocytosis with more lymphocytes and no neutrophils. Excessive IgG oligoclonal bands in the cerebrospinal fluid, which indicates intrathecal immunoglobulin synthesis, are detected in only 15–30% of NMO patients, compared to 85% of patients with MS. [19].

MRI features Clinical features and serological findings alone are adequate for a diagnosis of AQP4-IgG$^+$ NMO. Nonetheless, MRI investigations are instrumental in aiding the diagnosis of NMO regardless of AQP4-IgG status. The 2015 set of diagnostic criteria list MRI features characteristic of NMO involving the spinal cord, optic nerve, dorsal medulla, pons, dorsal midbrain, area postrema, thalamus, hypothalamus, corpus callosum, cerebrum and corticospinal tract [12]. The MRI hallmark of NMO is LETR that involves at least three vertebral levels. There are, however, additional MRI features of NMO not mentioned in the diagnostic criteria such as diencephalic lesions surrounding the third ventricles and cerebral aqueduct, as well as the anterior border of the midbrain [77]. Current research using high-field 7 T MR scanners [78] and advanced MRI techniques, such as MR diffusion and MR spectroscopy [79, 80], may shed further light on characteristic imaging features that distinguish AQP4-IgG$^+$ NMO, MOG-IgG$^+$ NMO, seronegative NMO and MS.

AQP4-IgG diagnostic assay Different assay methods are currently being used for the detection of AQP4-IgG, tissue-based indirect immunofluorescence assay (IIF), ELISA-R, GFP-AQP4 fluorescence immunoprecipitation assay (FIPA), visual fluorescence-observation cell-based assay (CBA) and quantitative flow cytometry (fluorescence-activated cell sorting assay). Of those, the fluorescence-activated cell sorting technique (FACS) has the highest detection rate [81] of up to 87% [82]. The FACS assay works when human embryonic kidney cells (HEK 293) are transfected with a plasmid encoding the M23 isoform of AQP4; then, FcR blocking reagent and patient serum are added, followed by incubation and washing. Finally, anti-human IgG tagged with fluorescent dye is added, and the cells are washed, fixed and examined by flow cytometer. Binding of a patient's IgG to the AQP4-transfected cells is then measured in terms of the intensity of the fluorescent dye [82]. Transfection of target cells using M23-AQP4 (that forms OAPs) rather than M1-AQP4 has increased the sensitivity of the AQP4-IgG assay [83].

MOG-IgG diagnostic assay MOG-IgG autoantibodies can now be identified with high precision [17]. Similar to the FACS assay for detecting AQP4-IgG, MOG-IgG cell-based assays involve the transfection of human embryonic kidney cells (HEK 293) with full-length human MOG [84]. This assay is a vast improvement from previous techniques such as western blotting (which detected false denatured MOG epitopes) and ELISA (which could not distinguish specific antibodies against conformational MOG epitopes) [17].

Treatment

Acute attacks Most acute attacks of NMO are treated with high dose methylprednisolone [2]. If there is response to methylprednisolone, plasmapheresis (plasma exchange) is used [2]. Plasmapheresis removes blood from the patient, with the plasma subsequently replaced; this technique removes circulating AQP4-IgG or

MOG-IgG antibodies, but the plasma cells that produce these antibodies remain. There is substantial evidence that plasmapheresis is both immediately beneficial and also in the long-term [85]. Other treatments for acute NMO include intravenous immunoglobulin (which has multiple mechanisms of action that suppress harmful inflammation) and cyclophosphamide (which forms DNA cross-links at guanine N-7 positions thus inhibiting mitosis and causing apoptosis in rapidly dividing cells) [44].

Maintenance treatments These treatments aim to prevent relapses and currently include general immunosuppressants such as corticosteroids, azathioprine, mycophenolate and mitoxantrone (type II topoisomerase inhibitor) [2]. Azathioprine inhibits purine synthesis; mycophenolate inhibits inosine-5′-monophosphate dehydrogenase (which is essential for the synthesis of guanosine-5′-monophosphate) and mitoxantrone, which is a type II topoisomerase inhibitor that disrupts DNA synthesis and DNA repair. Anti-B-lymphocyte treatments are also being used including rituximab, which is a genetically engineered chimeric murine/human monoclonal antibody against CD20, a differentiation antigen found on normal and mature B lymphocytes, but not plasma cells. Interleukin-6-dependent plasmablasts also seem to play a role in AQP4-IgG antibody production in NMO [86, 87]; interleukin-6 receptor blockade using the humanized monoclonal antibody tocilizumab reduces relapse rate and volume of spinal cord lesions in NMO [88]. Eculizumab, a monoclonal antibody that neutralizes complement protein C5, has been shown to significantly reduce attack frequency [89] and is an emerging treatment for NMO. Currently used NMO treatments are summarized in Table 2. Though there is now a variety of treatments for NMO, the optimum treatment is unknown and may vary between patients. Some patients respond well to some treatments, though in other patients, who are on the same treatment, NMO may progress leading to death [87].

Interferon beta and natalizumab exacerbate NMO The MS therapy, interferon beta [2], exacerbates NMO and increases relapse frequency, most likely by increasing B-cell activating factor and augmenting Th17-mediated inflammation and demyelination [2]. Another MS treatment, natalizumab that inhibits the entry of leucocytes into peripheral organs, is also known to exacerbate NMO, even after only a single dose [90]. These observations reinforce the importance of making an accurate diagnosis and the dangers of misclassifying NMO as MS.

Future treatments and challenges Currently, there is no cure for NMO and prognosis is poor; therefore, the development of novel therapeutic agents is a priority. Possible future therapeutic approaches include inhibiting the binding of AQP4-IgG to AQP4 (aquaporumab is in its developmental stage), inhibition of complement proteins (e.g. eculizumab, which works by inhibiting C5), inactivation of the AQP4-IgG antibody using bacterial enzymes and reducing permeabilization of the blood-brain barrier [44]. Aquaporumab is a non-pathogenic monoclonal antibody treatment that competitively inhibits AQP4-IgG by selectively binding to AQP4

Table 2 Currently used NMO treatments

Treatment	Typical dose
Acute attack	
Methylprednisolone	1 g daily i.v. for 3–5 days
Plasma exchange	5–7 cycles
i.v. immunoglobulin	0.7 g/kg for 3 days, treatment period 8 weeks
Cyclophosphamide	2 g daily for 4 days
Maintenance	
Prednisolone	2–20 mg/day
Mycophenylate	750–3000/day
Cyclosporine	2–5 mg/kg/day
Axathioprine	1–3 mg/kg/day
Methotrexate	7.5–25 /week
Mitoxantrone	Start 12 mg/m^2/month for 3–6 months, maintenance with 6–12 mg/m^2 every 3 months; maximum cumulative dose of 120 mg/m^2
Rituximab	For example, 1 g at day 1 and day 14, repeat every 6 months (optional: monitoring of CD19 counts)
Tocilizumab	6–8 mg/kg monthly injection
Eculizumab	600 mg i.v. each week for 4 weeks, then 900 mg i.v. at the fifth week, then 900 mg every 2 weeks
i.v. IgG	0.7 g/kg for 3 days, treatment period 8 weeks
Treatments that may exacerbate NMO	
Interferon beta	N/A
Natalizumab	N/A
Fingolimod	N/A

i.v. intravenous, N/A not applicable

without causing complement activation [44]. Given its high AQP4 selectivity and Fc mutation, which eliminates its CDC and ADCC cytotoxic effects, aquaporumab is predicted not to cause immunosuppression or toxicity, and seems to be a promising future NMO treatment [44]. Several existing treatments (developed initially for other diseases) targeting neutrophils, eosinophils and complement proteins are under evaluation to be repurposed for NMO [44]. Tolerization of the immune system to AQP4 has also been proposed as a cure, but there is no published data regarding the efficacy of such an approach in NMO [91, 92]. At present, no NMO treatment has been proven to be safe and effective in randomized controlled trials [93]. There is an obvious need for more interventional studies to be done; unfortunately, conducting a clinical trial for NMO poses major challenges. Given the small patient population, recruitment is an issue as is competition between trials for NMO patients. The severity and frequency of NMO attacks are suitable endpoints for clinical trials, thus strict definition of relapses must be followed [93]. The most challenging aspect of implementing randomized controlled trials relates to the ethics of administering placebo to patients with such a dangerous condition; innovative approaches such as shared placebo groups may facilitate the investigative pipeline [93].

Conclusions

The NMO field has progressed rapidly due to the discoveries of NMO-IgG and MOG-IgG. It is now evident that NMO pathophysiology is distinct from that of MS and, therefore, NMO is no longer considered to be a variant of MS. Despite the rapid progress, several unanswered questions remain including what causes AQP4-IgG production, what triggers an NMO attack in an asymptomatic patient who has had circulating AQP4-IgG for many years and what causes seronegative NMO.

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Atypical Inflammatory Demyelinating Syndromes of the Central Nervous System

Todd A. Hardy

Abstract The atypical demyelinating syndromes are a group of conditions, characterised pathologically by demyelination, that form part of the differential diagnosis of multiple sclerosis (MS) but differ from it due to variations in clinical presentation, MRI appearance, pathology, and response to treatment. The potential for some of these syndromes to overlap with conventional MS means that diagnostic uncertainties are common and therapeutic decision-making often focuses on whether to commence MS disease-modifying therapies (DMTs) or other immunosuppression. In this chapter, the spectrum of atypical demyelinating diseases is reviewed. I discuss the difficulties in diagnosing and distinguishing between conditions such as acute disseminated encephalomyelitis (ADEM), tumefactive demyelination, Baló's concentric sclerosis, Marburg's multiple sclerosis, and Schilder's diffuse myelinoclastic sclerosis and contrast these conditions with conventional MS. Advances in magnetic resonance imaging (MRI) and immunobiology may prove useful in our future understanding of these conditions.

Keywords Neuroinflammation · AHL · Hurst's · Myelin oligodendrocyte glycoprotein · MOG · Haemorrhagic leukoencephalitis · CNS

Introduction

The diagnosis of multiple sclerosis (MS) is rarely challenging in patients who present with a typical clinical history, magnetic resonance imaging (MRI) appearance, and cerebrospinal fluid (CSF) findings. It is more difficult when patients present with findings consistent with demyelination, but standard diagnostic criteria for a

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_17

conventional clinically isolated syndrome (CIS), or MS, are not fulfilled [1]. Classical scenarios are when there is a single, large demyelinating MRI lesion in the brain or cord, or conversely, when there are multiple, simultaneously gadolinium-enhancing demyelinating lesions in a patient who is deteriorating rapidly. Sometimes these atypical presentations correspond with a previously described atypical syndrome such as neuromyelitis optica spectrum disorder (NMOSD), acute disseminated encephalomyelitis (ADEM), tumefactive demyelination (TD), Baló's concentric sclerosis (BCS), Marburg's MS, or Schilder's diffuse myelinoclastic sclerosis (Table 1).

The rarity of these forms of demyelination means that clinical experience with them, and knowledge of their overlap with traditional MS, and with each other, remains limited. The discovery of the association between NMOSD and aquaporin-4 (AQ4) antibody, as well as a greater appreciation of imaging findings, clinical presentation and response to treatment, has meant that NMOSD, once considered an atypical form of MS, is now considered a separate disease to MS [2, 3]. While ADEM appears to be a distinct disease from MS, the difficulty in distinguishing it from a first attack of MS highlights the need for better validated diagnostic criteria [4]. It is also not clear whether other atypical demyelinating syndromes, such as tumefactive demyelination or BCS, should be thought of as MS variants, distinct diseases, or patterns of injury that can occur in different demyelinating diseases [5].

The aim of this chapter is to review the growing knowledge regarding the spectrum of acquired CNS atypical inflammatory demyelinating syndromes, with attention to nosology, clinical findings, immunopathogenesis, and treatment.

Acute Disseminated Encephalomyelitis (ADEM)

Acute disseminated encephalomyelitis (ADEM) is a rare, multifocal demyelinating disorder of the CNS, mostly seen in children from 5 to 8 years of age [6, 7], which very rarely occurs in adults. Incidence in the paediatric population is in the range 0.2–0.6 per 100,000 patient-years [7, 8]. Neurologic deficits include myelopathy, optic neuritis, seizures, and encephalopathy. Symptoms are rapidly progressive, often accompanied by a fever, and usually peak within days of onset [6]. Unlike other demyelinating diseases, ADEM occurs more commonly in males [6, 7]. ADEM often arises in the weeks following an antigenic challenge from a respiratory or gastrointestinal infection, or vaccination. A greater incidence occurs in winter and spring, in keeping with increased seasonal rates of infection [9].

Diagnosis It can be difficult to distinguish ADEM, which is generally a monophasic illness, from an atypical first presentation of relapsing-remitting MS. Encephalopathy, including behavioural change and an altered level of consciousness (LOC) or coma, is thought to be particularly discriminatory in distinguishing ADEM from MS. The 2013 International Pediatric Multiple Sclerosis Study Group (IPMSSG) diagnostic criteria require encephalopathy not due to intercurrent fever, as well as a polyfocal

Table 1 The atypical inflammatory demyelinating syndromes of the CNS

	Clinical	Radiology	Pathology
Acute disseminated encephalomyelitis (ADEM)	Monophasic Polysymptomatic Encephalopathy Often reduced level of consciousness Seizures Fever Focal neurological signs MOG-IgG	*MRI brain* T2/FLAIR signal Bilateral Asymmetric Patchy Grey matter Juxtacortical and deep white matter Thalamus Basal ganglia Corpus callosum Infratentorial Often lesions >1 cm with poorly defined margins May enhance with gadolinium T1 black holes rare Lesions forming after 3 months from onset suggest alternative diagnosis *MRI spine* T2 signal Intramedullary Confluent May enhance with gadolinium	Perivenular demyelination Relative axonal sparing Perivenular inflammation Intracortical microglial aggregates Subpial microglial activation and demyelination Diffuse meningeal inflammation
Acute haemorrhagic leukoencephalitis (AHL)	As for ADEM but rapidly progressive	Similar to ADEM but haemorrhage in some or all lesions	Similar to ADEM but with: More axonal damage, oedema, and haemorrhage Astrocytopathy may precede demyelination
Tumefactive demyelination (TD)	Headache Seizures Encephalopathy Focal neurological signs	*MRI brain* T2/FLAIR Lesions >2 cm with minimal to moderate surrounding oedema for size Rim of T2 hypointensity DWI Peripheral restriction ADC Peripheral hypointensity Gadolinium enhancement Open-ring most characteristic but any pattern of enhancement possible including venular pattern	Similar to MS: Confluent macrophages admixed with reactive astrocytes and Creutzfeldt cells May have other pathology when occurred in different contexts, e.g. NMOSD or ADEM

(continued)

Table 1 (continued)

	Clinical	Radiology	Pathology
Baló's concentric sclerosis (BCS)	Headache Seizures Encephalopathy Focal neurological signs	*MRI brain* T2/FLAIR Concentric rings of hyperintensity alternating with rings of isointensity Minimal oedema DWI Peripheral restriction Gadolinium enhancement Often at lesion edge	Alternating rings of demyelination and relatively preserved myelination
Marburg's multiple sclerosis	Monophasic Polysymptomatic Encephalopathy Often reduced level of consciousness Seizures Multifocal neurological signs	*MRI brain and spine* T2/FLAIR signal Periventricular Juxtacortical Deep white matter Rarely infratentorial Rarely cord Lesions of different ages Often TD Gadolinium enhancement	Similar to MS but: Tendency to be more destructive Axonal injury Necrosis Cavitation
Schilder's diffuse myelinoclastic sclerosis (SD)	Headache Seizures Encephalopathy Focal neurological signs	*MRI brain* T2/FLAIR signal White matter; confluent, mostly symmetrical in frontoparietal lobes Centrum semiovale Often corpus callosum DWI Restriction when acute Gadolinium enhancement Minimal	'Identical' to typical MS

Key: *ADC* apparent diffusion coefficient, *AQP4* aquaporin 4, *DWI* diffusion weighted imaging, FLAIR fluid attenuated inversion recovery *gad* gadolinium, *IgG* immunoglobulin G, *LETM* longitudinally extensive transverse myelitis, *MRI* magnetic resonance imaging, *MOG* myelin oligodendrocyte, *MS* multiple sclerosis, *ON* optic neuritis

CNS onset, for a diagnosis of ADEM [10], but the need for encephalopathy and polyfocal symptoms as a requirement for diagnosis in all patients has been questioned [7].

The IPMSSG criteria use multiphasic ADEM to describe new episodes of clinical or radiological inflammatory disease activity, in the same or different parts of the CNS, separated from the first episode by 3 months. Further attacks beyond this invalidate the diagnosis of ADEM and suggest alternative demyelinating diseases such as MS or NMOSD. Appreciation that ADEM can relapse beyond 3 months

further complicates the distinction from MS but relapsing ADEM appears to be a rare phenomenon [11–13].

MRI is helpful in diagnosing ADEM, although MRI cannot absolutely distinguish ADEM from other demyelinating syndromes. Typical brain findings in ADEM are bilateral, asymmetric patchy areas of hyperintensity on T2-weighted images within white and grey matter (Fig. 1) [14]. Lesion size is variable, but many patients have large (>1 cm) uniform lesions with poorly defined margins [11]. Gadolinium enhancement of lesions occurs in up to 30% of cases [6]. Apparent diffusion coefficient values are often increased in children with ADEM related to lesional vasogenic oedema [15]. In contrast to MS, ADEM tends to be associated with relative sparing of the periventricular white matter and affects the juxtacortical and deep white matter [16]. Involvement of the thalamus and basal ganglia particularly

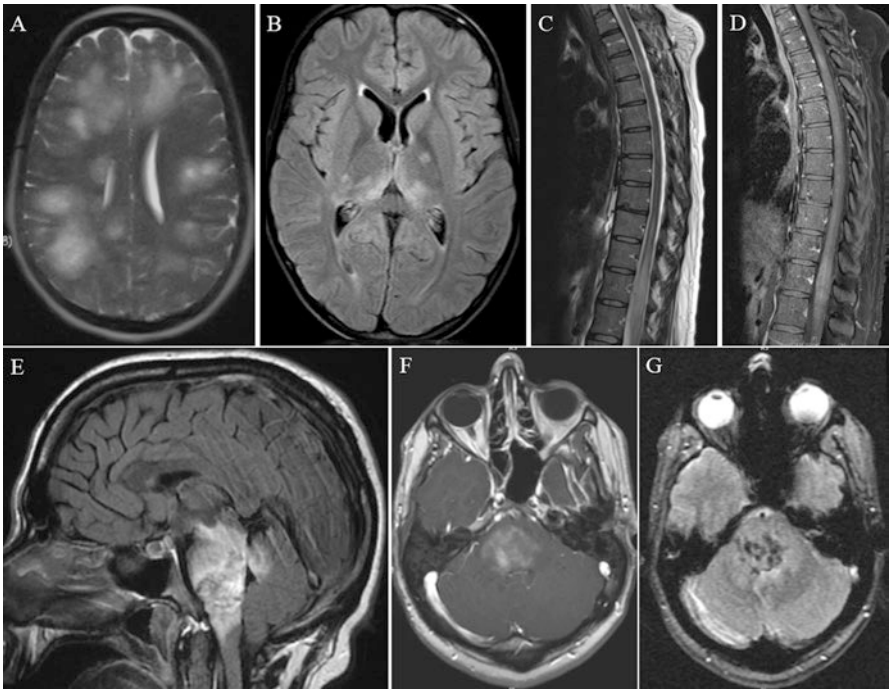

Fig. 1 Acute disseminated encephalomyelitis. (**a**) Axial T2 MRI showing patchy, ill-defined, confluent lesions throughout the deep and juxtacortical white matter with relative sparing of periventricular regions. (**b**) Axial fluid attenuated inversion recovery (FLAIR) images from another patient showing bilateral patchy, ill-defined, thalamic hyperintensities. (**c**) Sagittal T2 image showing patchy lesions throughout the thoracic cord with involvement of at least three segments of the distal cord and conus medullaris, where these is considerable oedema, in a patient who was MOG-IgG negative. (**d**) Sagittal T1 image showing the cord lesions enhance with gadolinium. (**e**). Sagittal FLAIR image showing diffuse brainstem and cerebellar hyperintensities in a patient who later died from biopsy-confirmed acute haemorrhagic encephalomyelitis. (**f**) Axial T1 image shows gadolinium enhancement of the brainstem lesion. (**g**) Axial susceptibility weighted imaging (SWI) sequence shows hypointense areas of haemorrhage within the brainstem lesion

favours ADEM. T1 hypointense 'black holes' seen commonly in MS are rare in ADEM. The optic nerves, corpus callosum, brainstem, cerebellum and spinal cord can all be affected. Spinal cord lesions in ADEM are intramedullary and tend to be confluent and longitudinally extensive.

In most cases, follow-up imaging reveals partial or complete resolution of lesions without development of new lesions [11, 17–19]. This is unlike MS where lesions tend to have more clearly defined margins, complete resolution of lesions is rare, and the development of new lesions is common [11, 18]. Features that may indicate a monophasic course include the absence of a clinical flare following the initial symptoms, the absence of new MRI lesions, and marked resolution of lesions on MRI in the early phase [19]. Performing a re-baselining MRI scan at 3 months after ADEM for comparison with future MRIs might help to distinguish ADEM from MS [20].

Serum myelin oligodendrocyte glycoprotein (MOG) IgG has been measured in children and adults with ADEM. Persistent seropositivity following a clinical episode appears to indicate a worse prognosis than if MOG antibodies are only transitorily positive [21]. It is not clear if MOG antibodies in these patients have a pathogenic role, or are an epiphenomenon reflecting tissue damage. MOG antibodies are associated with recurrent ADEM and ADEM followed by optic neuritis [13, 20, 22]. Tumefactive demyelinating lesions can occur in ADEM, and simultaneous large, enhancing lesions, makes multifocal primary CNS lymphoma an important differential diagnosis.

CSF examination in ADEM commonly shows a mild mononuclear pleocytosis of 50/μL (range 0–270/μL). CSF protein may be normal or elevated. CSF-restricted oligoclonal bands may be transitorily present but are absent in most cases [6, 11].

Pathology The pathology of ADEM is very different to that of typical MS. Lesion borders in ADEM are less distinct, with the hallmark being numerous perivenular 'sleeves' of demyelination accompanied by macrophage-predominant inflammatory infiltrates with comparatively fewer T and B lymphocytes [23]. Perivenular lesions may coalesce leading to large areas of pathology, but the broad zones of demyelination with macrophage infiltrates seen in MS are not present, although overlap cases have been described [24]. Lesions are of a similar age to each other and, as in other demyelinating diseases, there is relative axonal sparing. Unique to ADEM over MS are distinct, multifocal, intracortical microglial aggregates occurring patchily throughout the cortex but particularly associated with cortical layer three [24]. Subpial microglial activation, diffuse meningeal inflammation, and demyelination are also recognised [23, 24].

Treatment Treatment of ADEM is with intravenous corticosteroids initially, with plasma exchange (PLEX), or less often, intravenous immunoglobulin (IVIg), used in patients who fail to respond [25]. Oral corticosteroid taper of less than 3 weeks is associated with an increased risk of relapse [11]. Early PLEX may be beneficial in patients at risk of complications from cerebral oedema and raised intracranial pressure (ICP), but neurosurgical decompression is required in some. Although mortal-

ity from ADEM in adults approximates 12%, those patients who survive often recover well over several months, and mortality is much lower (1%) in children [26]. About 33% of children will have ongoing minimal or residual disability [7].

Acute Haemorrhagic Leukoencephalitis (AHL)

Acute haemorrhagic leukoencephalitis (AHL), also known as Hurst's disease, or Weston Hurst's disease, is a rare form of demyelinating disease usually regarded as a fulminant form of ADEM [27], comprising 2% of paediatric ADEM cases [6]. Patients present with rapidly progressive severe encephalopathy and polyfocal symptoms in the CNS usually leading to death within 1 week of onset. The MRI appearance of lesions in AHL is reminiscent of those in ADEM, except that haemorrhage, best seen on susceptibility weighted imaging and T2∗ sequences, can be seen in some or all lesions [28, 29]. Cases with isolated brainstem involvement have been reported [30] (Fig. 1).

The pathology of AHL has similarities to ADEM. There is perivenous demyelination in conjunction with inflammatory changes including in the meninges but also fibrinoid vascular necrosis, granulocyte infiltration, and perivenous microhaemorrhages [31]. Evidence that an astrocytopathy may precede demyelination comes from the observation that, in areas free of demyelination or substantial oligodendrocyte damage, there can be end-feet swelling and degeneration of astrocytic processes and cell bodies [31]. Perivascular inflammation comprises both mononuclear cells and neutrophils, often with a neutrophil predominance in the early days, and CSF examination often reveals neutrophils. Axonal damage, haemorrhage, and oedema reflect that AHL is a more significant pathological insult than occurs in typical ADEM.

Acute necrotising encephalopathy (ANE) is a genetic condition, most commonly seen in children, which can mimic AHL and which is associated with missense mutations in the nuclear pore gene Ran-binding protein 2 (RANBP). The condition has a predilection to affect the thalamus bilaterally. A family history of encephalopathy with fevers, and a lack of inflammatory activity in CSF or brain tissue favours ANE, as neutrophilic infiltrates are also frequently seen in the CSF and brains of patients with AHL [32].

Treatment of AHL does not differ from treatment for 'standard' ADEM, with the mainstay of therapy being early use of corticosteroids and PLEX.

Tumefactive Demyelination

Tumefactive demyelination (TD) is usually defined as any large inflammatory demyelinating lesion of the brain of a size greater than 2 cm. Patients present with symptoms and signs related to the size and location of the lesion including seizures,

impaired consciousness, cognitive deficits or focal neurological signs [33]. When these lesions occur singly, they can be difficult to diagnose with certainty and initially may be mistaken on MRI for a neoplasm. Other differentials include cerebral abscess, ischaemia, or other infections, and a careful workup to exclude these possibilities is often required.

TD can be diagnosed most confidently when it occurs in a patient with an established diagnosis of MS, but dual pathology is possible, and patients should be followed closely once a lesion is identified. One study of 31 patients found that as many as 6.5% of patients with biopsy-proven TD were later re-diagnosed as having a cerebral neoplasm [34]. TD lesions are not only associated with MS, occurring in as many as 1.4–8.6% of MS patients [35, 36], but may also be seen in ADEM and in aquaporin-4 antibody and MOG antibody seropositive NMOSD [11, 37, 38]. Hence, TD may not strictly be a separate demyelinating disease, but rather a final common lesion type that reflects different but related disease processes. The international magnetic resonance imaging in MS (MAGNIMS) collaboration has identified four different subtypes of TD lesion depending on their MRI appearance: infiltrative, ring-enhancing, Baló-like, and megacystic lesions [39, 40]. TD lesions can occur multiply at onset, and very rarely, patients may develop relapsing tumefactive disease [41–43].

Tumefactive demyelination is the preferred term to tumefactive MS as it does not imply an underlying disease process. Also, tumefactive MS is imprecise and could mean variously that a lesion has occurred in the context of established MS, that a patient with a solitary lesion will later develop MS, or that a patient has relapsing TD.

Diagnosis MRI clues that support TD include open-ring enhancement, minimal surrounding oedema, a rim of T2 hypointensity, peripheral restriction on diffusion-weighted imaging (DWI), and venular enhancement [44–46] (Fig. 2). Rapid ADC changes at the edge of TD lesions evolve dynamically over time which is unlike the more static DWI findings of abscesses or tumours. Also, in ring-enhancing TD lesions, peripheral DWI restriction favours TD over tumours or abscesses, whereas central DWI restriction favours an abscess [47]. CT findings of hypodensity corresponding to MRI areas of enhancement in these lesions may also predict TD over neoplasm [48].

Magnetic resonance spectroscopy (MRS) for diagnosing TD lesions is promising, but its role is yet to be fully defined [49]. Changes in MRS metabolites can occur according to lesion age, whether short or intermediate echo time proton MRS (TE 1H-MRS) is used, and due to a lack of standardised studies comparing between relevant differential diagnoses on a single type of scanner. Increased glutamate/glutamine peaks on short TE 1H-MRS may favour TD [49]. An increase in choline to N-acetyl-aspartate (NAA) ratio on either short or intermediate TE 1H-MRS is commonly seen in TD lesions but does not reliably distinguish TD from tumour [50, 51]. CT-PET may also be helpful in distinguishing TD from neoplasms, which have greater metabolic activity than TD [52]. However, some inflammatory disorders such as neurosarcoidosis may also be hypermetabolic on CT-PET [53]. In future, the combined use of MRS and CT-PET may be sufficiently sensitive to distinguish TD from neoplasm [52].

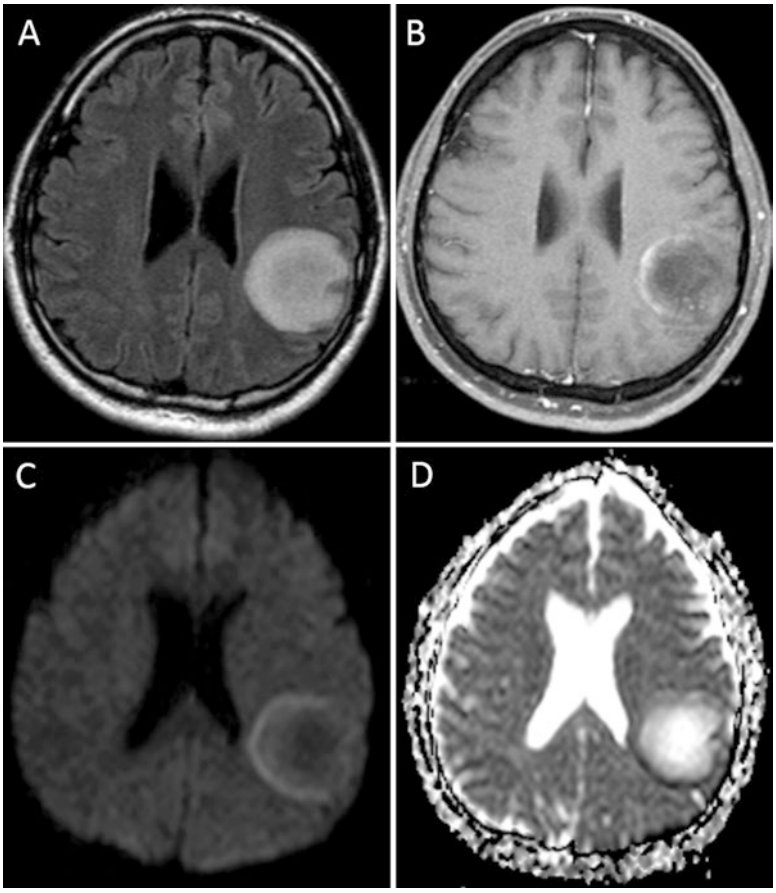

Fig. 2 Tumefactive demyelination. (**a**) Axial FLAIR image showing a tumefactive demyelinating lesion in the left parietal peripheral white matter with minimal surrounding oedema. (**b**) The axial post-gadolinium T1 image demonstrates 'open-ring' enhancement of the lesion. (**c**) Diffusion-weighted image (DWI) and (**d**) apparent diffusion coefficient (ADC) sequences show restricted diffusion at the periphery of the lesion

CSF examination in patients with TD lesions is usually normal or yields a slightly elevated protein and/or cell count [46]. The presence of CSF-restricted OCBs can be an important clue that favours MS-related TD over alternative pathology, such as a neoplasm, but OCBs are only positive 50–70% of the time in patients with TD lesions, so negative OCBs do not exclude demyelination [43, 54]. Conversely, positive oligoclonal bands have been detected in the CSF of patients with lymphoma [55].

Pathology Biopsy of TD lesions is not usually necessary, unless atypical features cast doubt on the diagnosis [33]. The pathology of TD lesions is reminiscent of typical MS lesions and consists of confluent areas of demyelination with relative axonal sparing (although widespread axonal damage can occur), reactive astrocytes and lesional and perivascular inflammatory infiltrates – particularly foamy macrophages [45].

Creutzfeldt cells, classically associated with demyelinating disease, should not be mistaken for mitotic glia more indicative of malignancy, but glioblastomas, in which Creutzfeldt cells and mitotic glia co-existed, have been reported [56].

Treatment The prognosis of patients with TD lesions is variable and reflects the underlying disease process when this is known, e.g. MS, NMOSD, or ADEM. Some patients may acutely experience a fulminant course unresponsive to immunotherapies. In one large series, more than 50% of patients made a full recovery from a TD lesion [57]. Indeed, there is some evidence that patients presenting with isolated, diagnostically undifferentiated TD lesions may have an overall better prognosis than patients with conventional MS [58, 59], but data regarding long-term follow-up of TD patients are limited.

Treatment of patients with an acute presentation of TD is based on limited reports and series and is usually with corticosteroids, with second-line treatment being PLEX [33], although in patients with fulminant or rapidly evolving TD the two can be used simultaneously at the outset. Decompressive craniectomy is considered a last resort when brainstem herniation either due to direct mass effect or raised ICP appears imminent [60].

When lesions are evolving more slowly, cyclophosphamide and rituximab may be reasonable options and have the benefit of medium-to-longer term immunosuppression. In patients with TD lesions in the context of established demyelinating diseases such as MS or NMOSD, then standard immunotherapies should be considered [33]. Case report-level evidence supports natalizumab as an effective therapy in relapsing TD [61]. Reports of TD lesions occurring in the context of fingolimod therapy mean that some caution should be applied before using fingolimod in MS patients with TD [62–65]. TD lesions can also occur following withdrawal of fingolimod therapy as a 'rebound' phenomenon suggesting that the mechanism by which fingolimod affects compartmentalisation of lymphocyte subsets in the CNS and the periphery may be relevant to TD lesion formation [65, 66]. Further support for lymphocyte involvement in TD lesion formation comes from the observation of a TD lesion in a patient following their first course of the anti-CD52 monoclonal antibody MS therapy, alemtuzumab, an agent which depletes peripheral B and T lymphocytes but following which there is a more rapid and differential reconstitution of B lymphocytes over T lymphocytes [67].

Baló's Concentric Sclerosis

Baló's concentric sclerosis (BCS) refers to a lesion or lesions in the CNS composed of alternating rings of demyelination and relatively preserved myelin [68]. Patients may present with focal neurological signs and symptoms of MS accompanied by features reflecting a cerebral mass lesion such as headache, reduced LOC, cognitive dysfunction or seizures. A prodromal illness of fevers and headache occurs in some

patients [69]. Females are affected more often than males [68, 70]. BCS is rare but may be more common in patients of Han Chinese and Filipino descent in whom more traditional forms of MS are less common than in Caucasians [71].

BCS lesions can be large and, as with TD, patients may undergo biopsy in the mistaken belief that they are primary brain tumours [70]. Although simultaneous BCS lesions can occur at different sites in the brain [5], relapsing BCS is rarely reported [69, 72]. In those patients who present with an initial isolated BCS lesion, approximately 40% go on to develop more typical MS [73], but the precise relationship of BCS to MS is not well studied, as the rarity of the condition means that data are from small case series. BCS lesions may also occur in aquaporin-4 seropositive and seronegative NMOSD [71, 74], and have been described in children, occurring multiply, as part of an ADEM-like presentation [75].

Diagnosis The typical 'onion ring' or 'target' appearance of BCS on MRI occurs when concentric rings of T2 and/or FLAIR hyperintensity and T1 hypointensity alternate with rings of isointensity. Often there is minimal surrounding oedema for lesion size [68] (Fig. 3). DWI changes and gadolinium enhancement are often noticeable at the lesion edge but may also occur in concentric layers. In as many as 55% of patients, there may be other typical MS lesions on MRI at the time of presentation which, when present, are a helpful clue to diagnosis [73].

CSF examination in patients with BCS is usually bland with as many as 82% of the patients negative for OCBs, which some authors have argued indicates that BCS is an immunologically distinct disease from conventional MS, in which the frequency of CSF-restricted OCB approaches greater than 95% [76].

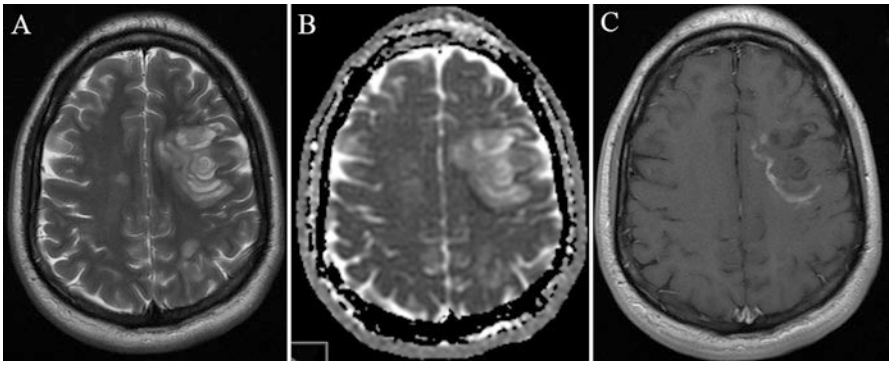

Fig. 3 Baló's concentric sclerosis. (**a**) Axial T2 image showing a large, demyelinating lesion with concentric rings in the white matter of the left frontoparietal lobe. There are additional, smaller and more typical demyelinating lesions in the white matter of both cerebral hemispheres. (**b**) The rings of the Baló lesion are observable on the ADC sequence where there is true restricted diffusion at the lesion edge. (**c**) The Baló lesion demonstrates open gadolinium enhancement of its outermost ring

Pathology The pathology of BCS is described as a variant of MS immunopathological pattern III, characterised by cerebral white matter oligodendrocyte apoptosis and loss of myelin-associated glycoprotein within demyelinated areas. The demyelinated rings of BCS occur around a central perivenular demyelinated zone and contain foamy macrophages, activated microglia, reactive astrocytes and areas of axonal loss, as in typical MS [23].

The reason for the concentricity of BCS lesions has long been debated. One hypothesis is that lesions arise from this central venule, where the BBB is 'leaky', allowing certain chemical mediators to spread radially in successive waves from that point inducing macrophage-mediated demyelination [77]. According to the ischaemic preconditioning hypothesis (IPH), at the leading edge of each successive wave, certain hypoxia-inducible factors are expressed which confer neuroprotection such that there is partial preservation of myelination in these areas leading to the overall concentric appearance of the lesions. IPH is supported by pathological evidence of upregulated iNOS in macrophages and microglia of active lesions, and increased expression of hypoxia-inducible proteins such as hsp70, HIF-1α, and D-110 at lesion borders [77].

It is not clear whether a chemical mediator might induce demyelination directly or via its action on microglia and macrophages causing them to produce nitric oxide intermediates and oxygen radicals locally and inducing hypoxia through impairment of mitochondrial function [77]. Modified biophysical modelling of Liesegang ring formation suggests concentric rings could also be explained by non-linear chemotaxis of macrophages and microglia toward a chemoattractant which is inhomogeneously and radially distributed [78].

Oxidative stress in BCS lesion formation has plausibility, as HIF-1 has been shown to be upregulated in glial cells beyond the edge of demyelinating layers, but is also found in increased amounts in hypertrophic astrocytes on the inner aspect of lesions, and in oligodendrocytes in non-demyelinating layers [72]. As these astrocytes express CC motif chemokine 2 and/or interleukin-1b, which are inducible by hypoxia-inducible factor-1a, they have the potential to be the neuronal cell type responsible for inducing demyelination [72] – a hypothesis supported by the observation by some, but not all authors, of extensive loss of aquaporin-4 in glial fibrillary acidic protein-positive hypertrophic astrocytes in BCS lesions in AQP4 antibody-negative patients (Fig. 4) [71, 79]. Indeed, disruption of astrocyte and oligodendrocyte interactions contributing to BCS lesion formation is also suggested by the lack of staining for the gap junction protein, connexin 43, which connects astrocytes to oligodendrocytes [71, 80].

The centrifugal enlargement of BCS lesions is supported by the temporal evolution of lesions seen on standard serial MRI sequences [72, 81–83] and by evolving changes in different lesion layers seen on DWI, ADC, and MRS [72, 84, 85]. DWI restriction initially forms at lesion edges to be followed by layers of gadolinium enhancement at their inner aspect. As gadolinium enhancement is lost, lesions become T2 hyperintense as demyelination occurs [72]. New diffusion-restricted layers then emerge in a new ring around the initially affected area and the lesion continues to expand radially in the same manner [72].

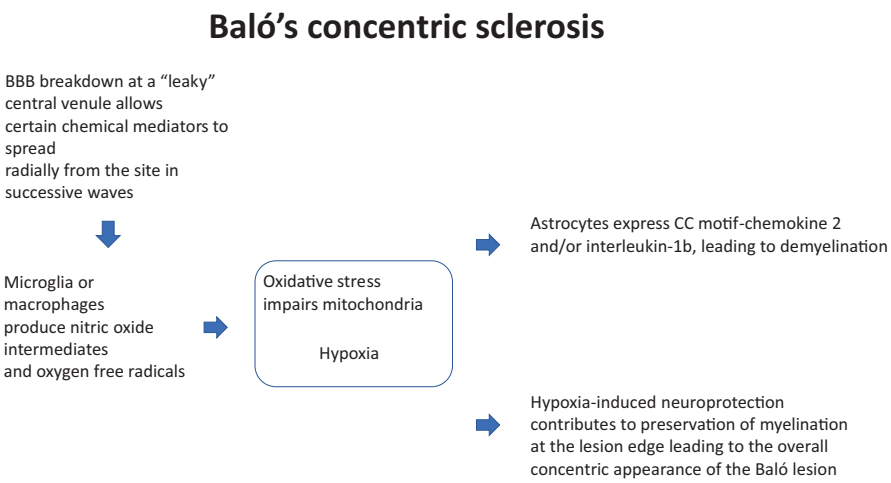

Fig. 4 A proposed scheme for the immunopathogenesis of Baló's concentric sclerosis

The existence of Baló-like lesions which have some features consistent with BCS but manifest in a more limited way, as well as recent cases of BCS and TD occurring in the same patient, and BCS evolving from an apparent TD lesion, all suggest possible overlaps in the mechanisms underlying the development of these BCS and more typical MS and TD lesions, and in this regard, BCS lesions can be considered a subtype of TD [86–88].

Treatment The prognosis of BCS was originally based on post-mortem diagnosis in patients who deteriorated and died rapidly over days to weeks from onset [89]. Fortunately, the use of MRI to investigate neurological symptoms in the modern era means that a fuller picture of the clinical spectrum of BCS can be appreciated, and it is now understood that many patients make a full recovery – presumably due to earlier detection and treatment [68].

Anecdotal evidence suggests that treatment of acute BCS lesions should be with corticosteroids, with the use of immunosuppression reserved for more extreme cases, with no evidence beyond occasional case reports to guide immunotherapy [68]. PLEX is often used as an adjunct to corticosteroids but a recent retrospective review of PLEX in the treatment of demyelinating disease has indicated that pattern III MS lesions, such as BCS, may not respond as well to PLEX as other subtypes of demyelination [90].

Evidence is limited regarding longer term treatment with DMTs in patients whose BCS lesion occurs as part of established MS, or when the BCS lesion occurs as part of the fulfilment of dissemination in space and time leading to an MS diagnosis [68]. Injectable MS disease-modifying therapies (DMTs) such as interferon-beta and glatiramer acetate have a good safety profile, although their efficacy is uncertain in patients with Baló lesions as part of their MS.

Marburg's Variant of Multiple Sclerosis

Marburg's MS is the name applied to a fulminant form of demyelinating disease with high morbidity and mortality that was first described post-mortem by Marburg in 1906 [91]. Patients can present polysymptomatically with seizures, headache, bilateral ON, and gait disturbance with hemi- or quadriparesis with symptoms progressing rapidly either in a stepwise manner, as successive relapses occur, or as a relentless monophasic decline. Encephalopathy raises the possibility of ADEM but does not exclude Marburg's MS and, indeed, it may be difficult to distinguish the two conditions at onset. A standard definition of Marburg's MS is that patients die within 1 year of onset, although cases living beyond 1 year are reported [92], and it may be that advances in the management of acute demyelination and in intensive care mean that the mortality for this condition is improving. When patients survive, they are usually left with significant neurological morbidity. Death is usually due to lesional brainstem involvement or brainstem herniation related to raised ICP [93].

Diagnosis In Marburg's MS, the typical MRI appearance is of multifocal demyelinating lesions in the periventricular, juxtacortical, and deep white matter, brainstem, cerebellum, and spinal cord. Perilesional oedema and gadolinium enhancement are often present (Fig. 5). Lesions of different ages favour Marburg's MS over ADEM. In many cases, lesions will be confluent or frankly tumefactive and so Marburg's MS is often also synonymous with fulminant multifocal TD.

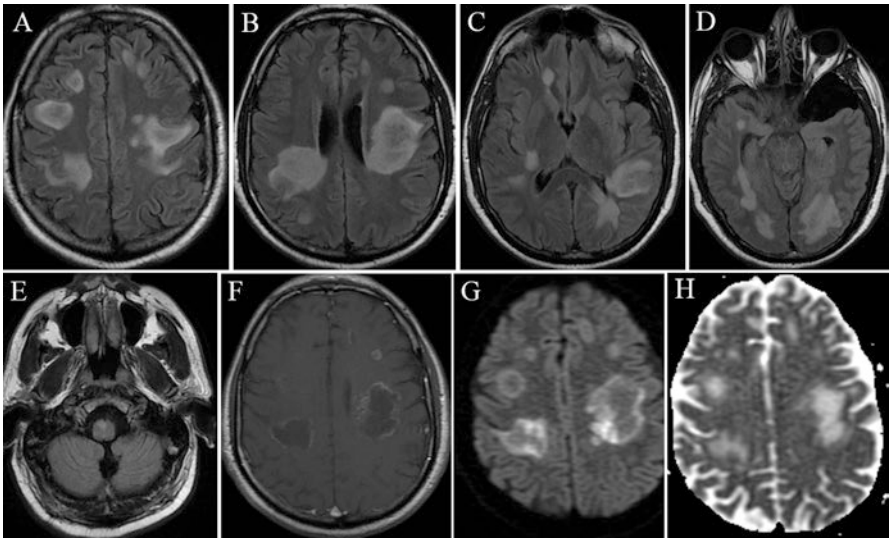

Fig. 5 Marburg's MS. (**a**–**d**) Axial FLAIR images showing large demyelinating lesions throughout the cerebral white matter involving the corpus callosum and (**e**) brainstem. (**f**) The lesions ring-enhance with gadolinium. (**g**, **h**) DWI and ADC sequences are consistent with facilitated diffusion, mainly at the lesion edges

In Marburg's MS, CSF is usually unremarkable without pleocytosis or OCBs [94]. In one case of autopsy-proven Marburg's MS, pretreatment CSF showed elevated Th2 cytokines/chemokines such as eotaxin, interleukin-5, and interleukin-10, distinct from the Th-1-dominant cytokine profile of prototypic MS [95].

Pathology Lesional pathology resembles typical but more destructive MS, with marked macrophage infiltration, axonal injury, necrosis, and areas of focal or confluent hypercellular demyelination [23]. Hypertrophic and giant astrocytes may also feature. Cavitating lesions, often infiltrated by neutrophils and eosinophils, can occur, bearing in mind that historical descriptions of pathological findings do not always adequately exclude conditions such as NMOSD. Cases in which demyelination is accompanied by fibrinoid necrosis and a perivascular mixed cell infiltrate have raised the possibility that at least some cases may overlap with cerebral vasculitis [96, 97]. Meningeal inflammation and grey matter lesions and predominantly B-cell-rich inflammatory infiltrates and perivascular inflammation have also been described [95].

The reason for the destructive course in Marburg's MS compared to typical MS is not understood. A less cationic, and therefore probably less compact, isoform of myelin basic protein (MBP) has been found in Marburg's MS patients which indicates that some individuals may be intrinsically predisposed to more aggressive MS [98, 99]. It is also possible that the fulminant disease course could reflect an inhibition of normal neurogenesis at lesion sites as, in one autopsy patient, lesional expression of neural stem cell markers GFAPδ, SOX2, and PAX6 were low, as were markers of proliferation such as Ki-67, and intermediate precursors such as NG2 [100].

Treatment Treatment of Marburg's MS is challenging. As with other fulminant forms of atypical demyelination, intravenous corticosteroids, often followed by PLEX, are the first-line treatment. Older literature suggests treatment with mitoxantrone as a viable next step [101], and high-dose cyclophosphamide has been used with success in a single patient [102]. There is little published experience of the use of higher efficacy MS DMTs, but alemtuzumab has been shown to arrest the decline of a patient with Marburg's MS [103].

Schilder's Diffuse Myelinoclastic Sclerosis (Schilder's Disease)

Schilder's disease (SD) is a controversial diagnosis, as there is very limited evidence to suggest that demyelinating lesions occurs as the result of a separate disease process from what might be better described as bilateral, simultaneous TD or, in some cases, ADEM. The disease is described in children and young adults and may be defined as 'one, or more commonly, two roughly symmetrical bilateral plaques measuring at least 3 × 2 cm in two of the three dimensions, involving the centrum semiovale of the cerebral hemispheres' [104]. It is said to occur slightly more commonly in males [105]. The clinical phenotype is of a monophasic course

characterised by focal neurological signs and symptoms consistent with typical MS or, depending on the size and location of the lesions, with symptoms of a mass lesion or lesions, including encephalopathy and seizures [106].

Attempts to review the literature on SD are also complicated by the fact that the term has been applied historically to what we now recognise as distinct disease processes. The confusion stems from the fact that Schilder first described an inflammatory demyelinating condition in 1912 but then subsequently described cases in 1913 and 1924 which are now recognised to be due to adrenoleukodystrophy and subacute sclerosing panencephalitis, respectively [104]. Hence, the published literature on SD includes cases of both demyelinating and non-demyelinating diseases, and leukodystrophies are sometimes referred to eponymously as SD.

Diagnosis Criteria were developed by Poser and colleagues to make the diagnosis more restrictive and hence to try to identify typical cases [104]. These Poser criteria specify that lesions must be absent from elsewhere in the CNS, that the peripheral nervous system is not involved, and that patients have normal adrenal function and fatty acid carbon-chain length. Updated versions of the criteria attempt to exclude ADEM-like features such as fever or prodromal infection, and emphasise the *bilateral* large lesions atypical from MS and negative OCBs [107]. However, even updated criteria do not easily distinguish multifocal presentations of TD or ADEM, complicating attempts to recognise SD as a separate form of atypical demyelination. Diagnostic criteria for SD were also devised prior to the availability of testing for AQP4-IgG and MOG-IgG, and it may be that some historic cases would now be reclassified as NMOSD or MOG antibody-associated demyelination if found to be antibody positive.

MRI descriptions are plagued by nosological difficulties, but favour features that might suggest typical or tumefactive demyelination over neoplasm. In some MRI cases, there is confluent, mostly symmetrical T2 and FLAIR hyperintensity in the white matter of the frontoparietal lobes involving the centrum semiovale and often the CC [108] (Fig. 6). These lesions are minimally enhancing, demonstrate restricted

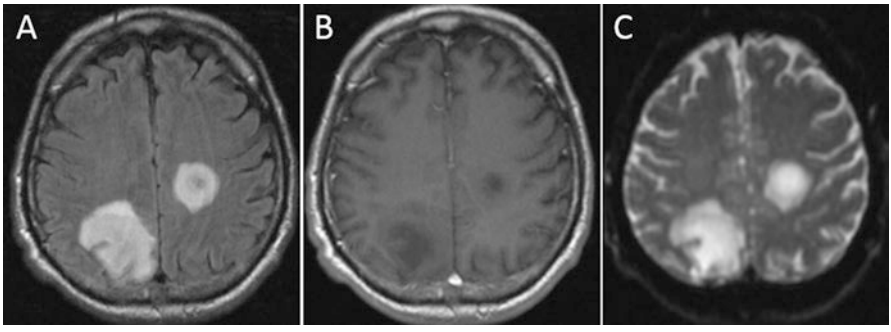

Fig. 6 Schilder's variant of MS. (**a**) Axial FLAIR images showing discrete ovoid area of increased signal involving the right parietal white matter with an asymmetric, smaller area of high signal in a similar region in the left hemisphere. (**b**) Axial T1 image showing the lesions do not enhance with gadolinium (**c**). DWI sequence shows that the lesion is associated with facilitated diffusion

diffusion when acute, and when chronic can resemble the confluent demyelination seen in leukodystrophies. Other published cases resemble the more discrete, ovoid, ring-enhancing lesions of TD or ADEM, or are bilateral but asymmetrical [106]. The radiological phenotype also extends to incorporate symmetrical 'butterfly' lesions in the parieto-occipital lobes, with or without posterior CC involvement, but this pattern can be seen in other demyelinating conditions such as TD, ADEM, and MS. There may be a role for MRS and PET imaging in SD but this has not been well defined [109].

CSF examination is usually negative for pleocytosis and oligoclonal bands [106].

Pathology The pathology of SD, when obtained, is said to be 'identical' to MS [104], including well-defined lesion regions of demyelination with reactive gliosis, relative axonal sparing, perivascular lymphocytic infiltration, foamy macrophages, and GFAP-positive astrocytes [108]. It is difficult to comment more on specific distinguishing features, because pathological descriptions in the literature incorporate a diverse group of demyelinating lesions.

Treatment Early treatment with corticosteroids may be beneficial and the addition of IVIg has been beneficial in some patients [110, 111]. Prognosis is variable and the disease can be fatal or disabling in a subset of patients, but many exhibit complete recovery.

Discussion

The atypical CNS inflammatory demyelinating diseases are represented by a spectrum of clinical presentations, MRI and pathological lesion types which overlap with MS, and with each other, but which are distinct enough that they may be separate, but related, demyelinating diseases. They occur infrequently enough that it is difficult for clinicians to gain experience with the diseases, and very difficult to accumulate enough cases for research or for clinical trials. When they do occur, however, they can have a devastating impact on patients, and they can provoke anxiety in the treating clinician who may harbour concern about whether they have made the correct diagnosis, and whether a particular treatment pathway is appropriate. Diagnostic uncertainty means that patients with atypical demyelination often undergo more investigations than patients with typical MS, resulting in greater costs to the healthcare system, and a greater risk of iatrogenic complications.

The nosological classification of the atypical demyelinating conditions of the CNS is complicated by the fact that the diseases are often ill-defined and many cases, overlap with each other and with MS. Many of these conditions were first described as pathological entities but the capacity to see these types of demyelinating lesions and syndromes using MRI has changed our appreciation of the full spectrum of these lesions and their clinical consequences. Patients are now less likely to have biopsies and instead to have their conditions followed with serial MRI to assess treatment response, and so clinicians reach diagnoses based strongly on clinical

features and MRI appearance, but it is not always clear how accurately MRI appearance reflects underlying pathology.

When an atypical demyelinating event occurs in a patient, questions arise such as is it merely an unusual lesion occurring in the context of otherwise typical MS, is it how MS presents in susceptible individuals, or is it a manifestation of a unique disease process? In the case of TD and BCS, for example, it is not clear whether the radiological and pathological differences between them mean they should be very clearly defined as separate diseases or merely different manifestations of demyelination of any cause. Even their very distinction from typical MS or ADEM is not defined, and both lesion types can occur in NMOSD. BCS and TD lesions, therefore, are not exclusive to MS and could represent a pattern of injury common amongst demyelinating lesions. The very existence of SD as a separate form of atypical demyelination is questionable, and whether Marburg's MS is merely the name given to the most aggressive cases on the normal spectrum of MS, or a separate but similar disease is not known.

The discovery of aquaporin-4 antibodies as a diagnostic marker in most cases of NMOSD serves as an exemplar as to why research into different atypical forms of demyelination is needed as the capacity to confidently distinguish new diseases from each other can lead to changes in prognosis and treatment. The emergence of MOG antibodies as a potential discriminator of yet further subtypes of NMOSD, and ADEM, has been a promising recent development, and highlights the importance of identifying new biomarkers which can help to further refine atypical demyelination disease definitions, and provide important information regarding prognosis.

While short-term therapies for acute demyelinating attacks tend to favour corticosteroids and/or PLEX, the more difficult question is judging when a condition is likely to be monophasic and when it is likely to relapse. When a tendency to relapse is suspected, the next questions are when to use standard MS DMTs and when to use other forms of immunosuppression, and whether treatment duration needs to be lifelong or can be withdrawn after a period of disease remission.

To clarify these important clinical questions, ongoing advances in MR imaging, serological biomarkers, immunogenetics and pathology will be important, as will the scrupulous collection of clinical data and outcomes after longer term follow-up. Rare disease registries are a particularly promising strategy to advance knowledge in the field. Only through a full understanding of the immunopathogenesis of these different atypical forms of demyelination will it be possible to help distinguish between them, or in other cases unify them, and to refine understanding of their clinical course, prognosis, and treatment.

Acknowledgements, Competing Interests, and Funding TAH: Has received honoraria or travel sponsorship from Bayer-Schering, Novartis, Biogen Idec, Merck-Serono, Teva, Roche, Alexion and Genzyme.

This work received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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Autoimmune Limbic Encephalitis

Shahar Shelly, Ram Narayan, and Divyanshu Dubey

Abstract Autoimmune encephalitis is now being widely recognized as a common and potentially treatable cause of encephalitis. The majority of the autoimmune encephalitis cases clinically present with limbic system dysfunction. Many neural autoantibody biomarkers of autoimmune limbic encephalitis have been described, and novel antibodies are being recognized every year. These antibodies are either directed against cell surface epitopes or intracellular antigens. Learning about the specific clinical presentations of autoimmune encephalitides, their pathophysiology, and cancer association is crucial for patient care. We highlight the typical clinical and radiological features of autoimmune limbic encephalitides. We also describe the treatment strategies and immunotherapy agents utilized.

Keywords Limbic Encephalitis · Paraneoplastic Syndromes · Anti-N-Methyl-D-Aspartate Receptor Encephalitis · Antibodies · Immunotherapy

Introduction

Autoimmune encephalitis is now being widely recognized as a common and potentially treatable cause of encephalitis [1]. A considerable proportion of the paraneoplastic or autoimmune encephalitis cases clinically present with limbic system dysfunction. The concept of limbic system was first introduced by Pierre Paul Broca (1878), and further characterized via functional studies by Klüver and Bucy (1937) [2, 3]. It is comprised of hippocampus, amygdala, hypothalamus, cingulate gyrus, and the limbic cortex. MacLean named these cortical and subcortical connections as

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_18

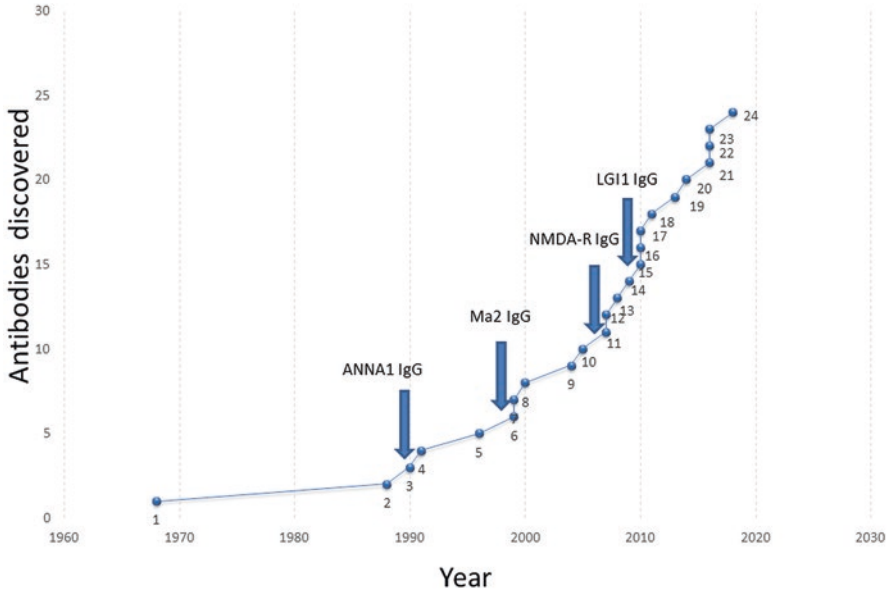

Fig. 1 Autoantibodies associated with autoimmune encephalitis. Key: 1, detailed description of autoimmune limbic encephalitis; 2, glutamic acid decarboxylase 65 Kd IgG; 3, ANNA-1, anti-neuronal nuclear antibody type-1 (ANNA-1, a.k.a. anti-Hu) IgG; 4, myelin oligodendrocyte glycoprotein (MOG) IgG; 5, amphiphysin; 6, Ma-1 and Ma-2 IgGs; 7, collapsin response-mediator protein 5 (CRMP5) IgG; 8, Purkinje cell cytoplasmic type 2 (PCA-2) IgG; 9, voltage-gated potassium channel-complex (VGKCc) IgG; 10, aquaporin 4; 11, anti-neuronal nuclear antibody type-2 (ANNA-2) IgG; 12, N-methyl-D-aspartate receptor (NMDA-R) IgG; 13, adenylate kinase 5 (AK5) IgG; 14, glycine receptor IgG; 15, amino-3-hydroxy-5-methyl-4-isoxazolepropionic receptor; 16, leucine-rich, glioma-inactivated-1; 17, contactin-associated protein-like 2; 18, gamma-aminobutyric acid type B (GABA-B) receptor IgG; 19, metabotropic glutamate receptor 5 (mGlur5) IgG; 20, dipeptidyl-peptidase-like protein 6 (DPPX) IgG; 21, gamma-aminobutyric acid type A (GABA-A) IgG; 22, glial fibrillary acidic-α (GFAPα) IgG; 23, neurexin 3-α; 24, neurofilament light chain IgG

the "limbic system" [4]. Furthermore, Papez elucidated the association of complex emotions and motivational processes with the limbic lobe [2, 3].

Many neural autoantibody biomarkers of autoimmune encephalitis have been described, and new antibodies are being recognized every year (Fig. 1). Immunofluorescence assays continue to play a critical role in clinical testing as well as discovery of neural-specific autoantibodies (Fig. 2). Few neural-specific antibodies directed against cell surface epitopes are pathogenic, whereas those targeting intracellular antigens are biomarkers of autoimmunity and/or underlying cancer (Fig. 3) [5]. Learning about the specific clinical presentations of autoimmune encephalitides, their pathophysiology and cancer association is crucial for patient care. In this review, we highlight the typical clinical and radiological features of autoimmune limbic encephalitides. We also describe the treatment strategies and immunotherapy agents utilized in the management.

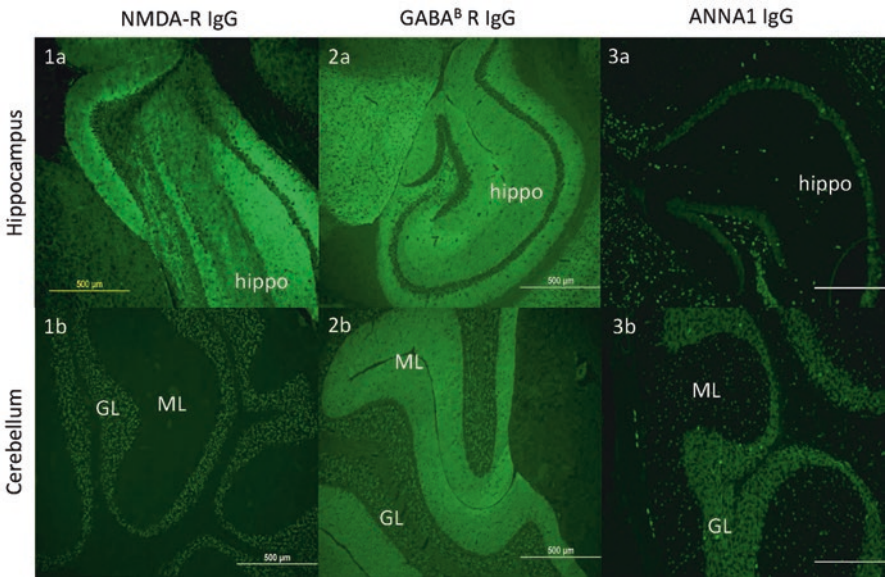

Fig. 2 Unique indirect immunofluorescence assay on mouse brain with antihuman IgG staining. Key: NMDA-R, N-methyl-D-aspartate receptor (1a,b); GABA-BR, γ-aminobutyric acid-B receptor (2a,b); ANNA-1, anti-neuronal nuclear antibody type-1 (a.k.a. anti-Hu, 3a,b); GL, granular layer; hippo, hippocampus; ML, molecular layer

Epidemiology

Although it was initially thought to be relatively rare, there is growing consensus that autoimmune encephalitis is responsible for considerable proportion of encephalitis syndrome previously considered idiopathic [1]. A recent population-based epidemiology study of autoimmune encephalitis in Olmsted county, Minnesota [1], showed that autoimmune encephalitis had prevalence of 13.7/100,000. Antibody-positive definite autoimmune encephalitis as per the proposed autoimmune encephalitis diagnostic criteria was the most prevalent category; the next was acute disseminated encephalomyelitis (ADEM) [6]. The most frequently identified neural autoantibody specificities were myelin oligodendrocyte glycoprotein (MOG) and glutamic acid decarboxylase (GAD65) [1]. Furthermore the incidence of autoimmune encephalitis is increasing over time from 0.4/100,000 person-years in 1995–2005 to 1.2/100,000 person-years in 2006–2015. This is mostly attributable to increased detection of autoantibody-positive cases (Fig. 1, Table 1). A prospective study based in the United Kingdom that enrolled encephalitis patients from 24 hospitals over a period of 2 years found 21% had autoimmune encephalitis. In their study ADEM and NMDA-R encephalitis were the most common subcategories [7]. Moreover, relapsing nature of some of these syndromes imposes a significant disease burden and morbidity [1].

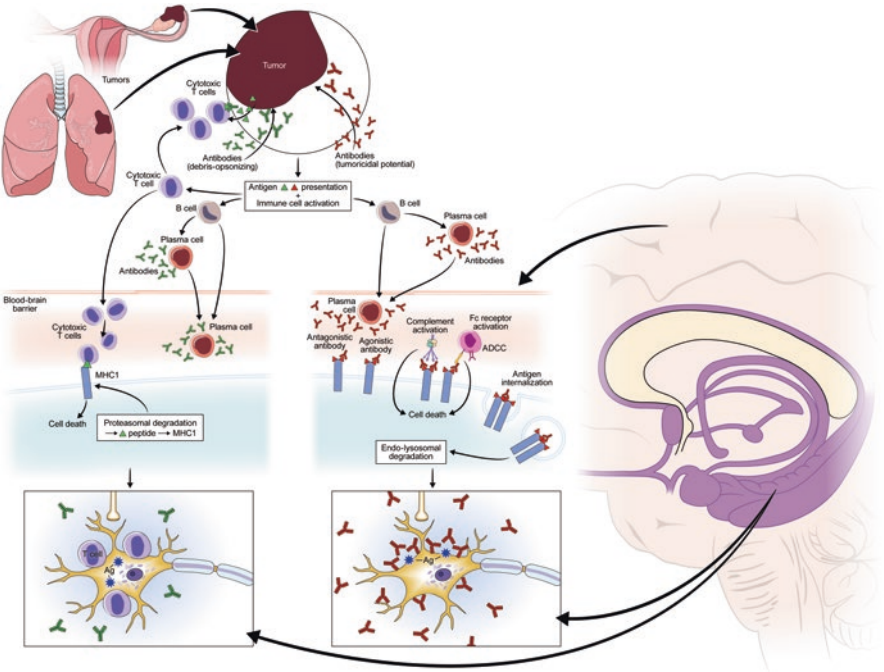

Fig. 3 Pathophysiological mechanisms for paraneoplastic neurological disorders. Tumor-targeted immune responses are initiated by onconeural proteins expressed in the plasma membrane, nucleus, cytoplasm, or nucleolus of certain cancers. These antigens are also expressed in neurons or glia and thus are coincidental targets. Antibodies directed at neural cell surface antigens (e.g., N-methyl-D-aspartate [NMDA] receptors) are effectors through multiple mechanisms. In contrast, intracellular antigens are not accessible to immune attack in situ, but peptides derived from intracellular proteins are displayed on upregulated major histocompatibility complex (MHC) class I molecules after breakdown in the proteasome and in turn are targeted by peptide-specific cytotoxic T cells. Antibodies (e.g., anti-Hu) targeting these intracellular antigens are not pathogenic but serve as diagnostic markers in clinical practice of a T-cell-predominant immune response Key: green triangle, intracellular antigen; red triangle, cell surface antigen; Ag, Antigen; ADCC, antibody dependent cellularcytotoxicity; MHC, major histocompatibility complex

Clinical Manifestation

Even though autoimmune encephalitis subtypes might have some difference in clinical presentations, behavioral changes, seizure, and/or focal neurologic deficits are the predominant manifestations among the majority of these cases (Table 1) [8]. Autonomic dysfunction (e.g., orthostatic intolerance, cardiac rhythm dysfunction, hyper- or hypothermia) can be a part of the neurological syndrome. CSF analysis may mimic viral etiologies, with lymphocytic pleocytosis and elevated CSF protein being common abnormalities [9]. However, nearly half of the cases may have non-inflammatory CSF studies [10]. Diagnostic criteria, as a part of expert consensus for autoimmune encephalitis, have been proposed [6]. These criteria may help the

Table 1 Clinical features of specific neural autoantibody-associated syndromes

Antibody	Neurological presentations	Brain MRI	Cancer association (3+, 2+, 1+)	Specific cancer type
NMDA-R	Oral dyskinesia, catatonia, neuropsychiatric dysfunction, autonomic dysfunction, refractory epilepsy (EEG: extreme delta brush)	Normal or non-specific cortical and/or subcortical changes	2+	Ovarian teratoma
AMPA-R	Limbic encephalitis	Cortical atrophy, deep gray nuclei FLAIR hyperintensity	2+	Thymoma, small cell lung cancer, breast adenocarcinoma
LGI1	FBDS, piloerection seizures, limbic encephalitis, paroxysmal dizzy spells	Medial temporal FLAIR hyperintensity, T1 basal ganglia hyperintensity (FBDS cases)	1+, 2+[a]	Thymoma
CASPR2	Neuromyotonia, Morvan's syndrome, limbic encephalitis, refractory epilepsy, sleep disorder	Normal or medial temporal FLAIR hyperintensity	1, 2+	Thymoma
Glycine	SPS, PERM	Normal or non-specific cortical and/or subcortical changes	Rare (<5%)	Thymoma
DPPX	Diarrhea, hyperekplexia, ambiguous sleep, parasomnias, PERM,	Normal or non-specific cortical and/or subcortical changes	Rare (<10%)	Lymphoma
GABA-A	SE, autoimmune encephalitis	Multifocal cortical and subcortical hyperintensity	1+	Thymoma
GABA-B	SE, limbic encephalitis	Medial temporal FLAIR hyperintensity	2+	Small cell lung cancer
GAD65	SPS, hyperekplexia, brain-stem encephalitis (especially African Americans)	Multifocal cortical and subcortical hyperintensity or brainstem hyperintensity	1+	Thymoma

(continued)

Table 1 (continued)

Antibody	Neurological presentations	Brain MRI	Cancer association (3+, 2+, 1+)	Specific cancer type
mGluR5	Encephalopathy, mood changes, movement disorder, and seizures, SE in children.	Normal in 50%, limbic/cortical FLAIR changes	2–3+	Hodgkin lymphoma
MOG	ADEM, ON, TM	Multifocal demyelination, involvement of corpus callosum, deep gray nuclei	–	–
ANNA-1/Hu	Limbic encephalitis, sensory neuronopathy, autonomic dysfunction, rarely SE	Normal or medial temporal FLAIR hyperintensity	3+	Associated with history of 80–90% SCLC and neuroendocrine tumors
ANNA-2/Ri	Stridor, laryngospasm, jaw dystonia, opsoclonus myoclonus	Brainstem FLAIR hyperintensity and/or atrophy	3+	Small cell lung cancer, breast cancer
Ma-1/Ma-2	Limbic encephalitis, brainstem encephalitis	Brainstem FLAIR hyperintensity or medial temporal FLAIR hyperintensity	3+	Testicular germ cell tumor[b], small cell lung cancer[c]
Amphiphysin	SPS, PERM, transverse myelitis, limbic encephalitis can occur in up to 30% of patients	Normal or non-specific cortical and/or subcortical changes	2+	Small cell lung cancer, breast cancer
GFAPα	Meningo-encephalomyelitis, tremor, ataxia, autonomic dysfunction	Peri-radial/ patchy enhancement or diffuse subcortical hyperintensity	1+	Ovarian teratoma
CRMP5	Choreo-athetosis, optic neuritis, retinitis, limbic encephalits, ataxia, transverse myelitis, polyradiculoneuropathy	Normal or medial temporal FLAIR hyperintensity	3+	Small cell lung cancer, thymoma

(continued)

Table 1 (continued)

Antibody	Neurological presentations	Brain MRI	Cancer association (3+, 2+, 1+)	Specific cancer type
IgLON5	Parasomnias, REM and NREM dysfunction, brainstem dysfunction, hyperexcitability disorder, rarely associated with nocturnal frontal lobe epilepsy	Normal or non-specific cortical and/or subcortical changes	Unknown	–
Neurexin-3a	Prodrome: fever, headache GI symptoms followed by encephalopathy and seizures	Normal	–	–
Adenylate kinase 5	Limbic encephalitis	Bilateral medial temporal FLAIR hyperintensity	–	–

Key: 1+, 10–30%; 2+, 30–60%; 3+, >60%; *ADEM* acute disseminated encephalomyelitis, *AMPA-R* amino-3-hydroxy-5-methyl-4-isoxazolepropionic, *ANNA-1* anti-neuronal nuclear antibody-1, *ANNA-2* anti-neuronal nuclear antibody-2, *CBA* cell-based assay, *CASPR2* contactin-associated protein-like 2, *CRMP5* collapsin response-mediator protein 5, *DPPX* dipeptidyl-peptidase-like protein-6, *EMG* electromyography, *FLAIR* fluid-attenuated inversion recovery, *GABA-A* gamma-aminobutyric acid type A, *GABA-B* gamma-aminobutyric acid type B, *GAD65* glutamic acid decarboxylase 65, *GFAP* glial fibrillary acidic protein, *LGI1* leucine-rich, glioma-inactivated-1, *MOG* myelin oligodendrocyte glycoprotein, *NMDA-R* N-methyl-D-aspartate receptor, *ON* optic neuritis, *PERM* progressive encephalomyelitis with rigidity and myoclonus, *REM* rapid eye movement, *SPS* stiff person syndrome, *TM* transverse myelitis

[a]Coexisting LGI1 and CASPR2 antibodies

[b]Ma2 antibodies

[c]Ma1 antibodies with or without Ma2 antibodies

clinicians to diagnose autoimmune encephalitis at the time of initial presentation. Predictive model based on clinical features and initial neurological assessment (Antibody Prevalence in Epilepsy and Encephalopathy [APE2] score) may aid in the identification of these patients as well. Furthermore, scoring systems for response to immunotherapy (Response to Immunotherapy in Epilepsy and Encephalopathy [RITE2] score) may also be utilized for immunotherapy trials (Table 2). APE2 score ≥ 4 was found to be a sensitive and specific predictor of neural-specific antibody positivity, whereas RITE2 score ≥ 7 was a predictor for initial immunotherapy response. Familiarity with various types of antibodies can help with the diagnosis. In the following section, we will elaborate in detail various neural-specific antibody subtypes.

Table 2 Components of the APE2 score (1A) and RITE2 score (1B). The assigned APE[2] and RITE[2] scores are the sum of values for all components

1A: Antibody Prevalence in Epilepsy and Encephalopathy (APE2 score)	Value	1B: Response to Immunotherapy in Epilepsy and Encephalopathy score (RITE2 score)	Value
New onset, rapidly progressive mental status changes that developed over 1–6 weeks or new onset seizure activity (within 1 year of evaluation)	(+1)	New onset, rapidly progressive mental status changes that developed over 1–6 weeks or new onset seizure activity (within 1 year of evaluation)	(+1)
Neuropsychiatric changes; agitation, aggressiveness, emotional lability	(+1)	Neuropsychiatric changes; agitation, aggressiveness, emotional labiality	(+1)
Autonomic dysfunction [sustained atrial tachycardia or bradycardia, orthostatic hypotension (≥20 mmHg fall in systolic pressure or ≥10 mmHg fall in diastolic pressure within 3 minutes of quiet standing), hyperhidrosis, persistently labile blood pressure, ventricular tachycardia, cardiac asystole or gastrointestinal dysmotility][a]	(+1)	Autonomic dysfunction [sustained atrial tachycardia or bradycardia, orthostatic hypotension (≥20 mmHg fall in systolic pressure or ≥10 mmHg fall in diastolic pressure within 3 minutes of quiet standing), hyperhidrosis, persistently labile blood pressure, ventricular tachycardia, cardiac asystole or gastrointestinal dysmotility][a]	(+1)
Viral prodrome (rhinorrhea, sore throat, low-grade fever) to be scored in the absence of underlying systemic malignancy within 5 years of neurological symptom onset	(+2)	Viral prodrome (rhinorrhea, sore throat, low-grade fever) only to be scored in the absence of underlying malignancy within 5 years of neurological symptom onset	(+2)
Faciobrachial dystonic seizures	(+3)	Faciobrachial dystonic movements	(+3)
Facial dyskinesias, to be scored in the absence of faciobrachial dystonic seizures	(+2)	Facial dyskinesias, to be scored in the absence of faciobrachial dystonic seizures	(+2)
Seizure refractory to at least to two antiseizure medications	(+2)	Seizure refractory to at least to two antiseizure medications	(+2)
CSF findings consistent with inflammation[b] (elevated CSF protein >50 mg/dL and/or lymphocytic pleocytosis >5 cells/mcL, if the total number of CSF RBC is <1000 cells/mcL)	(+2)	CSF findings consistent with inflammation[b] (elevated CSF protein >50 mg/dL and/or lymphocytic pleocytosis >5 cells/mcL, if the total number of CSF RBC is <1000 cells/mcL)	(+2)
Brain MRI suggesting encephalitis[b] (T2/FLAIR hyperintensity restricted to one or both medial temporal lobes or multifocal in gray matter, white matter, or both compatible with demyelination or inflammation)	(+2)	Brain MRI suggesting encephalitis[b] (T2/FLAIR hyperintensity restricted to one or both medial temporal lobes or multifocal in gray matter, white matter, or both compatible with demyelination or inflammation)	(+2)
Systemic cancer diagnosed within 5 years of neurological symptom onset (excluding cutaneous squamous cell carcinoma, basal cell carcinoma, brain tumor, cancer with brain metastasis)	(+2)	Systemic cancer diagnosed within 5 years of neurological symptom onset (excluding cutaneous squamous cell carcinoma, basal cell carcinoma, brain tumor, cancer with brain metastasis)	(+2)

(continued)

Table 2 (continued)

1A: Antibody Prevalence in Epilepsy and Encephalopathy (APE2 score)	Value	1B: Response to Immunotherapy in Epilepsy and Encephalopathy score (RITE2 score)	Value
	Total (max: 18)	Immunotherapy initiated within 6 months of symptom onset	(+2)
		Neural plasma membrane autoantibody detected (NMDA-R, GABA-AR, GABA-BR, AMPA-R, DPPX, mGluR1, mGluR2, mGluR5, LGI1, IgLON5, CASPR2, MOG)	(+2)
			Total (max, 22)

Key: *AMPA-R* amino-3-hydroxy-5-methyl-4-isoxazolepropionic, *ANNA-1* anti-neuronal nuclear antibody-1, *ANNA-2* anti-neuronal nuclear antibody-2, *ANNA-3* anti-neuronal nuclear antibody-3, *CASPR2* contactin-associated protein 2, *CRMP5* collapsin response-mediator protein-5, *DPPX* dipeptidyl-peptidase-like protein 6, *FLAIR* fluid-attenuated inversion recovery, *GAD65* glutamic acid decarboxylase-65, *GABA-BR* γ-aminobutyric acid-B receptor, *GFAPα* glial fibrillary acidic protein, *LGI1* leucine-rich glioma-inactivated-1protein, *MOG* myelin oligodendrocyte glycoprotein, *NMDA-R* N-methyl-D-aspartate receptor, *PCA-1* Purkinje cell cytoplasmic antibody type 1, *PCA-2* Purkinje cell cytoplasmic antibody type 2

[a]Scored only if no history of autonomic dysfunction prior to onset of suspected autoimmune syndrome and the autonomic dysfunction not attributable to medications, hypovolemia, plasmapheresis, or infection

[b]Patients scored zero if MRI of the brain or CSF analysis not performed

Cell Surface, Ion Channels, and Other Surface Epitopes

There are four IgG isotypes (IgG1–4) which have different ability to activate the complement system. Antibodies of the IgG1–3 subtypes are able to cross-link the antigens because of their bivalent nature, whereas IgG4 subtypes are hetero-bivalent but behave as monovalent antibodies in most situations [11]. They lose their cross-linking ability after the Fab-arm links with other unrelated IgG4 molecules. Cross-linking autoantibodies are believed to bring the antigens close together on the cell membrane and promote the degradation of the ligand–receptor complex [11]. IgG1–3 can activate the complement system by forming the membrane attack complex (MAC) and leading to membrane damage of targeted cells [6].

N-Methyl-D-Aspartate Receptor (NMDA-R) IgG

NMDA-R encephalitis is an important diagnosis to consider especially in young patients (<40 years) with autoimmune encephalitis. The California Encephalitis Project found that the number of young patients in the study with NMDA-R encephalitis was greater than those with any single viral etiology (51). NMDA-R IgGs are

predominantly IgG1–3 subtype and target the NR1 subunit in the receptor complex. It leads to reduced number of the synaptic and extra synaptic receptors, causing decreased synaptic plasticity and transmission (Fig. 4) [12]. Clinical presentation usually begins with a prodrome of a headache or fever, followed by psychiatric manifestations including delusions, hallucinations, mania-like episodes, and alternating episodes of extreme agitation and catatonia. Patients may then progress to develop seizures, encephalopathy, stereotyped movements (orofacial dyskinesias, chorea, ballismus, or myoclonus), rigidity, or opisthotonos and autonomic dysfunction. Seizures in NMDA-R encephalitis are usually focal non-motor seizures that might progress to refractory status epilepticus [13, 14]. Nearly 12–20% of cases have clinical relapses [15].

Majority of NMDA-R encephalitis patients have normal brain MRI (Magnetic resonance imaging) on initial presentation [16, 17]. Among the subset of patients with MRI abnormalities, the changes are usually non-specific [18, 19]. "Extreme delta brush" (EDB) was initially considered specific electroencephalography (EEG) findings for anti-NMDA-R encephalitis [20]. However, recent studies have described EDB with other metabolic and structural encephalopathies [21].

In about half of the patients with NMDA-R encephalitis, an immunogenic "trigger" can be identified. The two main triggers are the presence of ovarian teratoma [22] and a history of herpes simplex virus (HSV) encephalitis [23, 24]. Approximately two-third of adult women between ages 18 and 45 years with NMDA-R encephalitis have been reported to have ovarian teratoma [25]. In the case of ovarian teratoma, the tumor itself contains mature or immature neural tissue [26]. Ovarian teratomas in association with NMDA-R encephalitis are extremely rare in children younger than 12 years or older adults (≥45 years) [18].

Leucine-Rich, Glioma-Inactivated-1 (LGI1) Immunoglobulin G (IgG)

Voltage-gated potassium channels (VGKCs), typically formed by four different α subunits, each associated with a β subunit [12, 27]. Typically, tetramers of four α subunits arranged as a ring formation, creating the transmembrane K+ pore. Additionally, there are other associated proteins like LGI1, contactin-associated protein-like 2 (Caspr2), Contactin 2, a disintegrin and metalloproteinase protein 22 (ADAM22), and a disintegrin and metalloproteinase protein 23 (ADAM23), all of which can affect the function of VGKC (Fig. 4) [28]. In 2010 the discovery of autoantibodies against the extracellular proteins LGI1 and Caspr2 facilitated a change of view regarding the clinical importance of VGKC complex antibodies [29].

LGI1 encephalitis seems to be more prevalent in older individuals especially men [30]. However, few pediatric cases have also been described as well [31]. Typical clinical presentations include seizures and memory deficits [31]. About 60% of the patients will have hyponatremia [32], and some patients have preceding or concomitant myoclonic-like jerks involving the face, arm, or leg—described as faciobrachial dystonic or tonic seizures (FBDS). These are focal dystonic motor seizures and are usually brief, and they occur multiple times a day [33]. They have

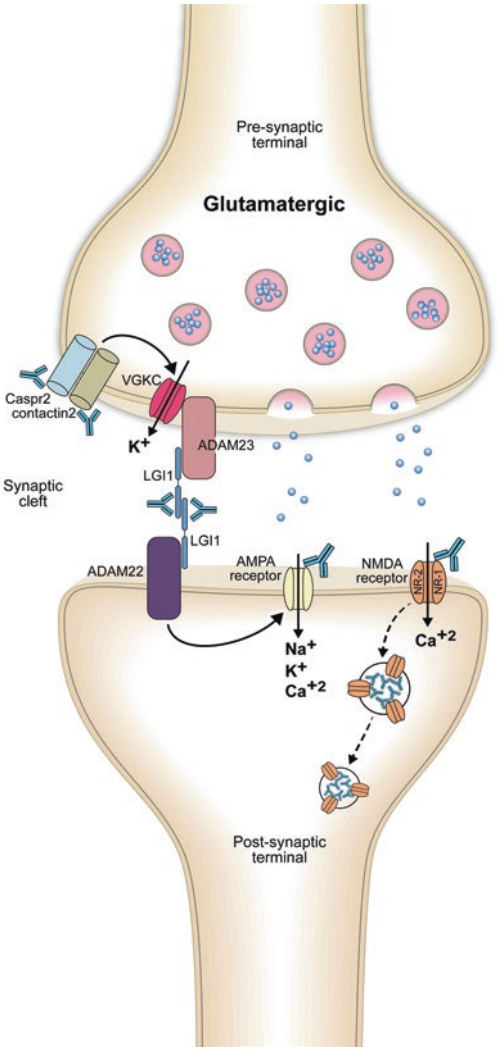

Fig. 4 Pathophysiological mechanism for glutamatergic synapses that represent major primary excitatory neurotransmitter at almost all synapses in the central nervous system. Leucine-rich glioma-inactivated-1 (LGI1) IgG and/or contactin-associated protein-like 2 (Caspr2) IgG are present in a subset of patients with voltage-gated potassium channel-complex (VGKCc) antibodies. LGI1 interacts with presynaptic ADAM23 and postsynaptic ADAM22 forming a complex that includes presynaptic Kv1.1 potassium channel and postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor. LGI1 IgG effects this complex, potentially altering postsynaptic AMPA receptors and presynaptic Kv1 channels, leading to increased neuronal excitability. N-methyl-D-aspartate [NMDA] receptor IgG in the autoimmune encephalitis cases predominantly bind to an epitope on NR1 subunit. NMDA receptor IgG binding disrupts the interaction between NMDA receptor and EphB2, and causes internalization of the NMDA receptor

a characteristic stereotypic contraction of the face, arm, and leg [33]. FBDS usually has no ictal EEG correlate, but preceding contralateral frontotemporal electrodecrement or sharply contoured rhythmic delta activity has been reported in few cases [34].

Another, characteristic seizure semiology is unilateral piloerections episodes [35]. More recently, paroxysmal dizzy spells have also been described in a subset of patients [30]. These "dizzy spells" or "out of body experiences" may precede encephalopathy by several months. Recognition and treatment of these seizures with immunotherapy may lead to better clinical and cognitive outcome [33, 36, 37].

CASPR2 IgG is found in a minority of VGKCc IgG-positive cases. These patients seem to have predominant peripheral nervous system involvement. Two clinical syndromes which have been described in association with antibodies are Isaacs' syndrome (neuromyotonia) and Morvan's syndrome (neuromyotonia, myokymia, or dysautonomia). In a considerable proportion of these cases, CSF analysis and brain MRI are usually normal or with non-specific findings. A subset of cases (10–20%) may have thymoma, and the risk is significantly higher among patients with coexisting LGI1 IgG (20–40%) [30].

α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid Receptor (AMPA-R) IgG

AMPA-R encephalitis was initially described in 2009 [38]. Median age of onset is around 60 years (range, 23–81 years), and it occurs more frequently in females (64%) [39]. Typical presentation includes anterograde and/or retrograde memory deficits, mood changes, and temporal lobe seizures. Recent studies have supported direct antibody-mediated pathogenicity [40, 41]. The majority (60–70%) of the patients have underlying malignancy, mainly small cell lung cancer or thymoma [38, 42]. In a series of ten patients (nine women), seven had thymoma or cancer of the lung or breast. A considerable proportion of patients have a refractory course and go on to develop diffuse cortical atrophy [40, 41, 43]. It is thought that the antibodies are directed against GluR1 and GluR2 subunits (Fig. 5) causing downregulation of the receptor and decrease of its synaptic clustering [40, 44].

γ-Aminobutyric Acid Type A (GABA-A) Receptor IgG

GABA receptors are ionotropic receptors (Fig. 5) [45]. There are several subunit isoforms (α, β, and γ) for the GABA-A receptor, which determine the receptor's agonist affinity, chance of opening, conductance, and other properties [46]. For GABA-A autoimmunity varied clinical features have been described (Table 1) [47]. One study reported six patients with encephalitis and refractory status epilepticus [48]. Brain MRI abnormalities are common and have been reported in up to 88% of the patients, showing multifocal, asynchronous, cortical–subcortical T2/FLAIR abnormalities predominantly involving temporal (95%) and frontal (65%) lobes

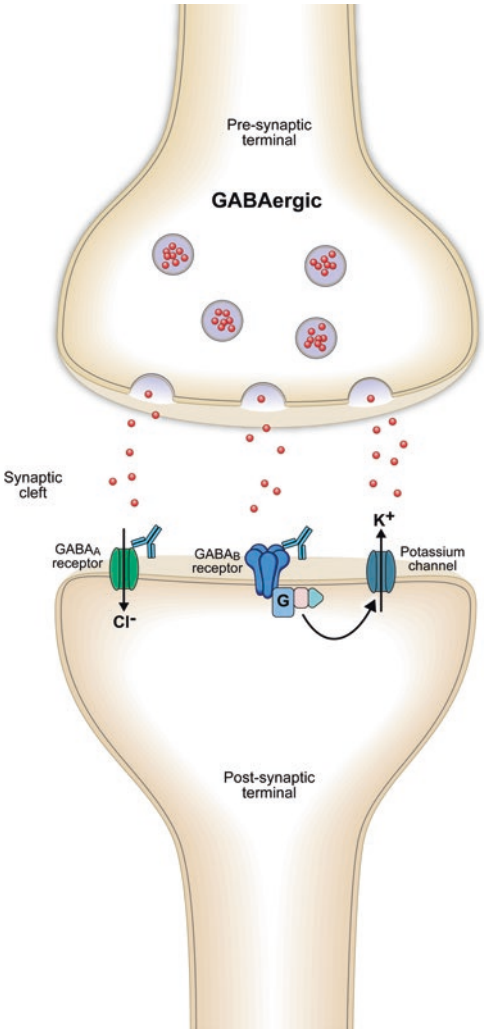

Fig. 5 Pathophysiological mechanism for γ-aminobutyric acid (GABA) synapses and represents the major inhibitory neurotransmitter in the CNS. Mediates its action via two classes of receptors, ionotropic GABA-A and metabotropic GABA-B receptors seen here. GABA-A receptors are ligand-gated ion channels, whereas GABA-B receptors are guanine nucleotide-binding (G) protein-coupled receptors that modulate calcium (Ca2+) and potassium (K+) channels and elicit both presynaptic and slow postsynaptic inhibition

[49, 50]. Age of symptom (median age, 40 years) onset tends to be younger than cases with GABA-B encephalitis [51]. Even though the disorder is severe, most patients respond to treatment [50].

γ-Aminobutyric Acid Type B (GABA-B) Receptor IgG

GABA-B receptors are metabotropic transmembrane receptors that are linked to G-protein-gated potassium channels (Fig. 5) [52]. There are two GABA-BR subtypes, GABA-B1R and GABA-B2R, assembling into functional heterogenic complexes [53]. A significant proportion of GABA-B receptor IgG-positive cases have

detectable or occult small cell lung cancer (SCLC) [54]. The clinical features of GABA-BR IgG-positive patients are variable, but the usual presenting symptoms are seizure, confusion, disorientation, memory loss, or behavioral changes consistent with limbic encephalitis [55]. Few cases of refractory status epilepticus have also been described [39, 56]. The median age of symptom onset is 61 years old (16–77 years) and tends to occur more common in men [51]. MRI of the brain usually demonstrates unilateral or bilateral medial temporal FLAIR hyperintensity. However, the initial MRI of the brain may be normal in some cases [17].

Dipeptidyl-Peptidase-Like Protein 6 (DPPX) IgG

DPPX is a part of the VGKC complex. It is a cell surface auxiliary subunit of the Kv4.2 potassium channel family [57]. Most patients have both IgG4 and IgG1 DPPX antibody subtypes [39, 57]. Reported median age of symptom onset is 57 years [58]. The clinical syndrome usually includes prodromal symptoms, weight loss, and diarrhea followed by cognitive dysfunction, memory deficits, CNS hyperexcitability (hyperekplexia, myoclonus, and tremor), seizures, and brainstem or cerebellar dysfunction [58]. Tumor screening is usually negative; there is one report of mantle cell lymphoma associated with DPPX autoimmunity [58, 59].

Metabotropic Glutamate Receptor 5 (mGluR5) IgG

Anti-mGluR5 limbic encephalitis has also been referred to as the "Ophelia syndrome" [60, 61]. The syndrome is named after a character from Shakespeare's play *Hamlet*. Patients usually present with subacute onset of encephalopathy, mood changes, movement disorder, and seizures [62]. Status epilepticus has been commonly reported in pediatric cases [60]. The clinical syndrome is associated with Hodgkin's lymphoma.

Intracellular Epitopes

These clinical syndromes are mediated by a cellular immune response. Many of these biomarkers have strong paraneoplastic associations [63]. Additionally, these patients tend to have a refractory clinical course in comparison to patients with antibodies against extracellular epitopes [64].

Glutamic Acid Decarboxylase (65 kd, GAD65) IgG

GAD65 antibodies target the synaptic isoform of the enzyme necessary to synthesize GABA. It is known to be associated with diverse clinical conditions including type 1 diabetes, stiff person syndrome (SPS), progressive encephalomyelitis with rigidity and myoclonus (PERM), autoimmune epilepsy, cerebellar ataxia, and limbic encephalitis [65, 66]. Higher titers (≥ 20 nmol/L) have a more specific association with neurological autoimmunity. The median age of symptom onset is 30 years (range, 5–80 years). Patients with SPS present with muscle rigidity and spasms that may occur spontaneously or triggered tactile stimuli, auditory stimuli, or emotional disturbances. The disorder predominantly affects axial and proximal muscles of the extremities. Electromyography (EMG) of the involved muscles shows continuous motor unit activity as a result of dysfunction of the inhibitory GABAergic system. Few patients with limbic encephalitis associated with GAD65 IgG have also been reported. T-cell-mediated cytotoxicity has been hypothesized to contribute to the refractory nature of the disease.

Anti-Neuronal Nuclear Antibody Type-1 (ANNA-1, a.k.a. Hu) IgG

ANNA-1 or "anti-Hu" has a strong association with small cell carcinoma (pulmonary or extrapulmonary) and childhood neuroblastoma (Figs. 2 and 3) [67–69]. Additionally, small cell lung cancer patients (~15–20%) without paraneoplastic neurological syndrome also have antibodies to HuD antigen [70]. Some studies have identified HuD-specific T cells among these patients with paraneoplastic autoimmunity. However, two different kinds of HuD-specific T-cell responses have been reported, either a classical IFN-γ-producing cytotoxic T-cell response or T cells producing type 2 cytokines such as IL-13 and IL-5 that lacked cytolytic activity [71]. The clinical presentation is with various central and peripheral nervous system manifestations such as sensory neuronopathy and autonomic dysfunction, especially gastroparesis [5]. However, a considerable proportion (10–17%) of cases present with limbic encephalitis or refractory seizures. Both temporal and extratemporal localization of the seizures have been reported [72].

Ma1 or Ma2 IgG Antibodies

Antibodies to Ma1 and Ma2 proteins are associated with paraneoplastic disorder that affects the limbic system, brainstem, and cerebellum [73, 74]. Anti-Ma2 encephalitis (with or without anti-Ma1 antibodies) should be suspected in patients with limbic diencephalic or brainstem dysfunction common symptoms are visual or eye movement deficits: diplopia, opsoclonus, difficulty opening the eyes, memory deficits; confusional state and decline of cognitive function [75]. Up to 70% of patients will have MRI abnormalities in these regions, and inflammatory changes in the CSF. Anti-Ma2 antibodies are strongly associated with testicular tumors in

young men and small cell lung cancer or breast cancer in older patients [75]. Clinical presentation mimicking motor neuron disease has also been described in associated with Ma2 IgG seropositivity [76]. Male gender, younger age (<45 years), presence of testicular tumor with complete response to treatment, and absence of anti-Ma1 antibodies have been associated with better clinical outcomes [75].

Collapsin Response-Mediator Protein-5 (CRMP5) IgG

CRMP5 is a paraneoplastic biomarker of small cell lung cancer or thymoma [77]. Patients with CRMP5 IgG usually manifest with various neurologic signs including chorea, cranial neuropathy, dementia, cerebellar ataxia, myelopathy, and peripheral neuropathy [49, 78, 79]. Among patients with choreiform movement disorder prominent T2/FLAIR hyperintense lesions in the striatum have been described. Management of underlying malignancy and early initiation of immunotherapy may be associated with favorable outcome [80].

Adenylate Kinase 5 (AK5) IgG

AK5 IgG is a rare autoimmune limbic encephalitis biomarker. So far just 12 patients have been described in the literature, with detailed clinical data on 10 of them [81]. The median age of symptom onset was 64 years (range 57–80 year), and majority of these (7/10) were men. Subacute anterograde amnesia was common among all the ten cases. A prodromal state was present in five patients, which consisted of depression (5/10), asthenia (4/10), anorexia (3/10), or headache (1/10). None of the patients had a detectable malignancy [81, 82].

Hashimoto's Encephalopathy (HE) or Steroid-Responsive Encephalopathy with Autoimmune Thyroiditis (SREAT)

Clinical characteristics of HE include encephalopathy, seizures, stroke-like episodes, and myoclonus [5]. These patients typically have thyroid peroxidase (TPO) antibodies but may or may not have history of thyroiditis. Seizure presentations are variable including new onset refractory status epilepticus or progressive myoclonic epilepsy [83, 84]. Triad of encephalopathy, evidence of thyroid autoimmunity (clinically or serologically), and a favorable response to steroids have been traditionally utilized for identification of these cases [6].

Pathogenesis

Pathophysiology and Triggers of Autoimmunity

Tumors: Paraneoplastic neurological disorders are a rare and heterogeneous group of disorders caused by immune response against cancer, rather than an effect of tumor itself, nutritional or metabolic deficits. This immune response is misdirected toward shared neural target antigens causing an immune-mediated neurological syndrome [85]. Lately with the use of immune checkpoint inhibitors, the frequency of these syndromes has relatively increased. Due to the difference in immunogenic autoantigens expressed by various tumors, antibody response seems tumor-specific. Malignancies which are commonly associated with these autoantibodies are small cell lung cancer, thymomas, ovarian teratomas or adenocarcinomas, breast adenocarcinomas, and testicular germ cell tumors. Paraneoplastic neurological symptoms often occur prior to tumor detection; the diagnosis may aid in the identification of the underlying cancer (Table 1) [86].

Infections: Infection may also serve as an initial trigger of autoimmune neurological syndrome. Several mechanisms have been suggested including molecular mimicry, epitope spreading and bystander activation. Some proteins expressed by the pathogens share structural or amino-acid sequence homology with a self-antigen. This may elicit cross-reactive immune response implicated in neurological autoimmunity. This phenomenon is referred to as molecular mimicry. In other instances host cells may be damaged by the infection, leading to the release of self-antigen and triggering an autoimmune response. In bystander activation, infection leads to activation of antigen-presenting cells and/or pre-primed autoreactive lymphocytes, which can then evoke an autoimmune disorder [86].

Mechanisms of Antibodies Targeting Neuronal Surface Antigens

Internalization of receptors: Antibodies can cross-link to two adjacent receptors via their Fab fragments. These antibody-linked receptors can be endocytosed, internalized, and degraded. A good example is NMDA-R encephalitis. NMDA-R IgGs recognize an extracellular, conformation-dependent epitope region on the GluN1 subunit of the NMDA-R. Binding of the autoantibody does not interfere with glutamate binding, but cross-links NMDA-R, thereby initiating the internalization of the receptor. The reduced NMDA-R density on the neuronal surface results in neuronal dysfunction. It is important to note that this process is reversible and, after removal of NMDA-R IgGs, we see a good recovery of the synaptic function [87, 88].

Agonistic effects: Direct agonistic effect is another mechanism of antibody-mediated neuronal dysfunction. GluR1 and GluR2 have been reported to be the common antigenic target among patients with AMPA encephalitis [40, 44]. However,

in a subgroup of patients, the antigenic target has been reported to be the GluR3 subunit [89]. Binding of antibodies to GluR3 leads to opening of the ion channel receptors. This allows excessive Ca2+ influx through the receptor-operated cation channels causing exocytotic neural death [90, 91].

Ion channel deficiency: Antibodies such as LGI1 IgG might cause their pathogenic effect by ion channel dysfunction. Disruption of LGI1-ADAM22 interaction may lead to reduce synaptic AMPA receptor function in rat hippocampal neurons. Additionally in epileptic LGI1 knockout mouse model, levels of AMPA receptor are greatly reduced. By affecting AMPA receptor function (Fig. 4), LGI1 IgG might lead to disrupted calcium influx [92, 93]. This antibody-mediated ion channel dysfunction results in increased excitability, which results in seizures and some of the other neurological manifestations [94].

Diagnosis

The differentials for autoimmune limbic encephalitis are varied. The most important groups are summarized in Table 3. Autoimmune and viral encephalitides can resemble one another, and sometimes autoimmune encephalitis may have para-infectious associations such as post-HSV infection NMDA encephalitis. Among immunocompromised individuals, infectious etiologies should be higher as a differential. The presence of meningeal signs is usually more suggestive of infectious etiologies, only exception being GFAPα IgG which is frequently associated with meningoencephalitis. Human herpes virus-6 (HHV-6) encephalitis that is usually seen in immunocompromised patients may be difficult to differentiate from autoimmune limbic on brain MRI. Temporal lobe gliomas may also have a radiological appearance similar to limbic encephalitis [95].

Treatment

General Principles

The current evidence for the treatment of autoimmune encephalitis is primarily based on experience from retrospective case series, case reports, and expert opinions. Efforts are underway for designing randomized control trials for this condition although there are many challenges in this regard as discussed later in this section. Treatment of a patient with autoimmune encephalitis can be broadly conceptualized as immunotherapy (main stay of treatment), removal of an immunologic trigger, such as a tumor, when applicable and symptomatic therapy to manage comorbidities associated with this condition (Table 4).

Table 3 Differential diagnosis of autoimmune limbic encephalitis

Disorder	CSF pleocytosis	Distinctive features	Diagnostic tests
Herpes simplex virus encephalitis (HSE)	Yes	Fever (>38 °C) MRI showing hemorrhagic lesions, medial temporal lobes involvement	HSV DNA in CSF Can be negative if done too early (≤24 hours) or too late (after 10–14 days) Consider determination of intrathecal HSV antibody synthesis for atypical or protracted cases
HHV-6 encephalitis	Occasional	Common in immunocompromised patients	HHV-6 DNA PCR in CSF
Neurosyphilis	Yes	Common in immunocompromised patients Meningeal signs and cranial nerve involvement common Sequela of other organ involvements due of syphilis	CSF treponemal antibody tests
Whipple disease	Yes	Systemic symptoms (polyarthralgia and intermittent diarrhea) Oculomasticatory myorhythmia	*T whipplei* DNA PCR in CSF
HIV	Yes	Low CD4 cell count	Positive HIV serology.
Status epilepticus	Occasional	History of seizure, alternative etiology for epilepsy, antiseizure medication non-compliance	Reversible diffusion weighted images restriction Mesial temporal sclerosis in some cases
Gliomas	No	Contrast enhancement and mass effect on MRI brain, usually unilateral lesion	Brain lesion biopsy

Key: *CSF* cerebrospinal fluid, *LE* limbic encephalitis, *MRI* magnetic resonance imaging, *FLAIR* fluid-attenuated inversion recovery, *CT* computed tomography, *DNA* deoxyribonucleic acid, *HSV* herpes simplex virus, *HHV-6* herpes simplex virus-6, *PCR* polymerase chain reaction

The principles in the management of autoimmune encephalitis in the acute, maintenance, and chronic phases:

1. Achieving maximal clinical recovery at the lowest risk of exposure of such therapy. An aggressive approach during the initial phase followed by titration it to the least effective dose to maintain remission is recommended.
2. Early commencement of immunotherapy is commonly associated with a better outcome [6]. Treatment should start as soon as alternative etiologies are excluded. This said, initiating treatment on the grounds of convincing clinical, radiological, and serological findings after a preliminary exclusion of common mimics is very reasonable. In this regard, since infectious encephalitis like herpes simplex virus (HSV) encephalitis is a very close mimic of autoimmune encephalitis, it is not unreasonable to consider an empiric course of antiviral agents like acyclovir (or antibiotics in suspected cases of bacterial meningoencephalitis) until preliminary testing comes back negative.

Table 4 First-line and second-line immunotherapy agents for management of autoimmune encephalitis

Medication	Dose	Route	Frequency	Mechanism	Adverse effects	Safety monitoring
Methylprednisolone	1000 mg (30 mg/kg)	IV	3–5 days for acute phase treatment	Acts on nuclear glucocorticoids receptors to reduce cytokine and chemokine production, Reduces migration of leukocytes to the target tissue	Insomnia, increased appetite, psychiatric disturbance (psychosis), diabetes, cataracts, osteoporosis, hip avascular necrosis, delayed wound healing	CBC, electrolytes, blood pressure. Glucose. PJP prophylaxis, proton pump inhibitors. Osteoporosis prevention
Intravenous immunoglobulin (IVIG)	2 g/Kg over 3-5 days	IV	3–5 days for acute phase treatment, maintenance, 1–2 g/kg every 3–4 weeks	Interaction with antigen binding fragment on the antibodies and/or crystallizable fragment on the antibodies or the antigen-presenting cells	Headache, aseptic meningitis, deep venous thrombosis, renal failure, pulmonary edema	IgA levels prior to administration. Electrolytes and renal function
Plasma exchange (PLEX)	5–7 exchanges	IV	10–14 days for acute phase treatment (one exchange every other day)	Extracorporeal blood filtration designed to remove large molecular weight molecules, including immunoglobulins, immune complex, and complements	Hypotension, electrolyte imbalance, perioral paresthesia (hypocalcemia), coagulopathy, central IV catheter-related infection, hemorrhage, thrombosis, and pneumothorax	PT, INR, PTT, Fibrinogen
Rituximab	1000 mg followed by same dose at 2 weeks or 375 mg/m^2 weekly ×4 doses	IV	Every 6 months (Approximately) or based on CD 19 counts; goal <0.1	Anti-CD20 monoclonal antibody. B-cell depletion by antibody- and complement-dependent mechanisms	Infusion reactions, rash, pruritus, edema, hypertension, fever, fatigue, chills, headache, diarrhea, cytopenias, neutropenic fever, liver toxicity, hepatitis B reactivation	Hepatitis B and tuberculosis screening. Periodic CBC

Mycophenolate mofetil	500–3000 mg/day (renal adjustment necessary)	PO	Twice a day	Inhibition of inosine monophosphate dehydrogenase-mediated guanosine nucleotide synthesis	Vomiting, diarrhea, hypertension, creatinine elevation, edema, lymphoma, myelotoxicity, teratogenicity	CBC, renal function, pregnancy test prior. CBC weekly first month, increase interval thereafter. Limit sun and ultraviolet light exposure, dermatology evaluation at least yearly
Cyclophosphamide	500–1000 mg/m^2/mo (IV) 1–2 mg/kg/day (PO) (renal adjustment necessary)	IV/PO	Monthly (IV), daily (PO)	Alkylating agent with interferes with DNA synthesis	Nausea, vomiting, alopecia, infertility, mucositis, hemorrhagic cystitis, myelotoxicity	CBC, liver and kidney function test, urinalysis at weekly intervals during first month, increase interval if stable. Patient should not receive live vaccines while on this treatment. PJP prophylaxis recommended
Azathioprine	1–3 mg/kg/day	PO	BID	Converted to cytotoxic 6- thioguanine nucleotides, which leads to incorporation as a false base into DNA inducing lymphocyte apoptosis	Fever, malaise, myalgias, nausea, vomiting, diarrhea Leukopenia, anemia, thrombocytopenia, myelotoxicity, liver toxicity, hypersensitivity reaction, rash, lymphoma	TMPT/pregnancy testing prior to first dose. CBC, LFTs weekly for the first month, increase interval thereafter. Limit sun and ultraviolet light exposure. Therapeutic target of five-unit increase in mean corpuscular volume from baseline. Discontinue for leukopenia/neutropenia

(continued)

Table 4 (continued)

Medication	Dose	Route	Frequency	Mechanism	Adverse effects	Safety monitoring
Methotrexate	7.5–20 mg (gradually titrated)	PO/ SC/ IM	Once a week	Irreversibly binds to and inhibits dihydrofolate reductase resulting in inhibition of purine synthesis and interfering with DNA synthesis, repair, and cellular replication	GI intolerance, stomatitis, oral ulcers, abnormal liver enzymes, rash, alopecia, headache, fever, hematological abnormalities	CBC, liver enzymes, renal function, Hep B and C screening, periodic screening for neuropathy. Leucovorin/ folic acid rescue

Key: *BID* twice a day, *CBC* complete blood count, *DNA* deoxyribonucleic acid, *GI* gastrointestinal, *IM* intramuscular, *IV* intravenous, *PO* per oral, *PT* prothrombin time, *PTT* partial thromboplastin time, *INR* international normalized ratio, *SC* subcutaneous

3. Immunotherapy should not be delayed while waiting confirmatory testing for antibodies in the serum or CSF [18].
4. The main goals of the acute phase treatment are to reduce acute inflammation to prevent or minimize irreversible neurological sequela and to restore normal neuronal function and enable maximum clinical recovery as early as possible [96].
5. The main goals of the maintenance phase treatment are to prevent immune-mediated disease progression and to prevent relapses (as applicable in select antibody-mediated disorders) and adverse effects of steroids (steroid spearing).
6. Treatment should be guided by clinical improvement. Currently there are no reliable biomarkers to measure treatment response in AE. Antibody titers are not reliable markers of disease severity and are not used to measure treatment response in most instances. They, however, have been shown to predict relapses in few NMDA-R IgG encephalitis cases (antibody titers in CSF) and hence potentially guide long-term immunotherapy [87].
7. If there is lack of response to an adequate immunotherapy regimen, it should prompt a detailed evaluation for alternative etiologies (infectious, metabolic, genetic, neurodegenerative, etc.) before escalating immunosuppression [97].
8. Lack of response or more often, partial response to immunotherapy (especially in the setting of onconeural antibodies) should also prompt comprehensive work-up for neoplasms.
9. It is important for the treating clinician to have an understanding of the likely underlying immune mechanism in each patient. In general, T-cell-mediated mechanisms are primarily implicated in paraneoplastic encephalitides that involve intracellular antigen targets where the classic "onconeural" antibodies are not directly causative to the patient's clinical picture. These conditions are poorly responsive to immunotherapy. Broad-spectrum immunosuppressive agents like cyclophosphamide and mycophenolate are preferentially used over targeted B-cell therapies. In autoimmune encephalitis related to neuronal surface antigens, B-cell- and plasma cell-mediated mechanisms are primarily implicated, and the response to immunotherapy is generally good (and robust in specific antibody-mediated disorders), and in these conditions, targeted B-cell therapies are preferred.
10. Lastly, patients with autoimmune encephalitis also tend to have multiple comorbidities including seizures, cognitive impairment, psychiatric symptoms, speech and language impairment, spasticity, dystonia, tremors, gait and balance difficulties, ataxia, disordered sleep, fatigue, and autonomic dysfunction. Some patients also tend to have non-neurological symptoms like gastrointestinal dysfunction, sphincter disturbances, and cancer-related comorbidities. It is important to identify and address these comorbidities as they significantly affect the quality of life.

Acute Phase Treatment (Table 2)

High-dose intravenous corticosteroid therapy, plasma exchange (PLEX), and intravenous immunoglobulin (IVIG) is commonly used first-line therapies in the acute phase treatment of autoimmune encephalitis. An initial course of IV methylprednisolone (IVMP) at 30 mg/kg (~1000 mg) per day for 5 consecutive days combined with or followed by PLEX (5–7 cycles; one exchange every other day spread over 10–14 days) or IVIG (0.4 g/kg per day for 5 consecutive days) is recommended. If PLEX and IVIG are both used, it is highly recommended that PLEX should precede IVIG therapy and not be given concurrently with IVIG or shortly after a course of IVIG as it can remove the immunoglobulins from circulation and render IVIG therapy ineffective.

In a study of 472 patients with NMDA-R encephalitis, first-line treatment along with tumor removal, if applicable, resulted in improvement in 53% of the patients within the first 4 weeks of therapy, and 97% of these patients showed a good outcome at 24-month follow-up (modified Rankin Scale score 0–2) [18].

Response time varies based on the antibody type; for NMDA-R encephalitis, this is typically 2 weeks to a few months, whereas with LGI1 and GFAPα encephalitis, response to steroid therapy is robust and is seen in a few days. Early and sustained response to therapy is considered a good prognostic sign. If the response is unsatisfactory at 10–14 days, second-line immunotherapy can be considered. In the NMDA-R encephalitis study of 472 patients mentioned above, 47% of patients failed to respond to first-line immunotherapy at 4 weeks, and such patients tended to have better outcomes when treated with second-line treatments. A systematic review of treatment in autoimmune encephalitis concluded that patients who received second-line immunotherapy during the initial episode of encephalitis had fewer relapses and a better outcome [98].

Commonly used second-line immunotherapy agents in clinical studies include rituximab (1000 mg IV given as one time dose or as two doses 2 weeks apart for a total of 2000 mg) and cyclophosphamide (750 mg/m^2 IV given monthly for 3–6 months) [18]. Clinical response is assessed at 2–3 weeks after the administration of these agents, and if the response is still unsatisfactory (as noted in a small number of patients), third-line agents like bortezomib, inebilizumab, and tocilizumab are considered [99–101]. These agents have not been validated in clinical studies but have been used on an anecdotal basis only.

In the case of onconeural antibody-mediated paraneoplastic encephalitis, a major component of the acute phase treatment is the prompt removal of the potential immunogenic source, i.e., neoplasm. If this is not possible, concurrent treatment of the underlying malignancy and autoimmune encephalitis with an immunosuppressant like cyclophosphamide has been shown to be beneficial. Mycophenolate mofetil (at the dose of 1000–4000 mg per day) has also been used in such cases with reasonable success.

During the acute phase of treatment, supportive care and interdisciplinary care is crucial. A longer intensive care unit stay is considered a predictor of poor response

to therapy. Seizures should be treated aggressively with antiepileptic medication and if required even with instituting a pharmacological coma.

Maintenance Phase

Maintenance therapy in the treatment of autoimmune encephalitis is variable and is mostly based on the specific antibodies identified. The utility of instituting maintenance immunotherapy after the initial treatment phase in all patients is yet to be studied. In most instances, maintenance therapy is initiated based on clinical response to the initial therapy. For instance, maintenance therapy is initiated in a patient who tends to have worsening symptoms during a corticosteroid taper or if early relapses occur when off immunotherapy. Commonly used immunosuppressive medications in the maintenance phase include rituximab, mycophenolate, azathioprine, cyclophosphamide, methotrexate, IVIG, and even PLEX in a few patients (Table 4). One suggested approach is to begin maintenance therapy with rituximab in patients with antibodies against neural cell surface antigens and with mycophenolate or cyclophosphamide in the cases of autoimmune encephalitis associated with antibodies against intracellular neural antigen targets. In the case of starting therapy with mycophenolate or azathioprine, an overlapping prolonged steroid taper over 2–3 months is advisable until these agents take effect.

Although the clinical utility of following antibody titers after the initial phase is unclear, persistent antibody titers or rebound antibody positivity (after an initial phase of seroconversion) in an appropriate clinical setting can be useful in guiding maintenance therapy. In one study of patients with NMDA-R encephalitis, an early decrease in antibody titers from CSF correlated with improved outcome, but this was not statistically significant [87]. In general, the current recommendation is to tailor treatments according to the patient's clinical status rather than antibody levels.

There are typically no guidelines regarding the duration of treatment, and the decision on this has been made after weighing the risk of prolonged immunosuppression with the risk of a relapse or clinical worsening. Antibody titers in certain diseases can be useful to guide decision on discontinuing therapy. In a study, persistent NMDA-R antibody titers in the CSF predicted a relapse [87]. Similarly, persistent MOG antibody titers have been associated with relapses in ADEM [102]. It is reasonable to consider maintenance therapy for a period of about 3 years after clinical stability is achieved or earlier if antibody titers are undetectable and if the patient is clinically stable.

In patients with cancer risk factors and in a setting where they have paraneoplastic encephalitis from onconeural antigens, periodic cancer surveillance (at least once in 5 years or more frequently depending upon antibody) is advised during the maintenance phase. This is both in lieu of the underlying autoimmune condition and in a setting of prolonged exposure to immunosuppressive therapy. Finally the need for frequent lab monitoring of complete blood counts and liver function cannot be overemphasized.

Vaccination

Live vaccines are contraindicated during treatment with immunosuppressive medications especially rituximab, mycophenolate, and azathioprine. Inactivated influenza vaccines and pneumococcal vaccines are recommended prior to the initiation of chronic immunosuppressive therapy [31].

Implications of Pregnancy

Treatment of autoimmune encephalitis during pregnancy can be challenging. The mainstay of treatment is corticosteroids, IVIG, and PLEX. Search for teratoma and prompt removal results in significant improvement. In patients refractory to first-line therapy, rituximab is a potential option [103]. In a case series of 102 pregnancies in patients with multiple sclerosis and neuromyelitis optica where rituximab was used within 6 months of conception, no major safety signal was observed. B-cell counts were low in 39% of newborns and normalized within 6 months [104].

Clinical Trials in Autoimmune Encephalitis

Designing clinical trials in autoimmune encephalitis is an urgent need. This is however fraught with many concerns. Firstly, given the heterogeneity of various autoimmune encephalitis syndromes, it is challenging to develop unifying outcome measures. It is important to validate objective clinical tools for monitoring treatment response like brain MRI, PET, formal neuropsychological assessment, EEG, seizure diaries, etc. Secondly, given that serum and CSF antibody titers do not necessarily correlate with clinical severity, we would have to rely on clinical outcomes which are very variable across the spectrum of autoimmune encephalitis. Thirdly, given the rarity of the condition and the potential rates of drop out in the event of complete return to baseline after initial therapy, powering a study would be difficult. A multicenter trial might help overcome this problem. Fourthly, there are a sizable proportion of patients with seronegative autoimmune encephalitis and it is only a matter of time before novel antibodies are identified. This might affect the inclusion/exclusion parameters of a clinical trial. Finally, the testing of antibodies has to be standardized to minimize variability of testing across centers. At this point, there is enough evidence to render a placebo-controlled randomized trial in autoimmune encephalitis to be unethical; however a randomized controlled trial of first-line therapy versus early combination of first-line and second-line therapy would be a potential option [98].

Conclusion and Future Directions

Despite the significant growth in field of autoimmune neurology over the last two to three decades, we continue to face many challenges. Many more autoantibodies with specific clinical and/or oncological associations are likely to be discovered over the coming years. The use of metagenomics, proteomics, and phage immunoprecipitation sequencing techniques may accelerate the rate of discovery. Future research is likely to improve our understanding of the mechanisms of antibody-mediated and cytotoxic T-cell-mediated syndromes. Hopefully greater insight in the etiopathogenesis of autoimmune encephalitides will help us choose individual-specific therapeutic approaches and will allow us to more accurately predict the disease prognosis.

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Autoimmune Ataxias

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Abstract The dramatic progress to genetically characterise the ataxias, using next-generation sequencing (NGS), has also facilitated our appreciation that a substantial number of sporadic ataxias are not due to genetic defects but likely to be immune-mediated. At the same time, the recent identification of an increasing number of antibodies linked to sporadic ataxias has aided the diagnostic pathway for immune-mediated cerebellar ataxias (IMCAs). However, the diagnosis of IMCA remains problematic if it is solely dependent on the serological screening for such antibodies and also because there is significant phenotypic overlap with non-immune forms of ataxia. In the majority of cases, serological screening for known antibodies associated with IMCA may not be readily available. In others no specific antigenic trigger or associated antibodies have been identified as yet. Therefore, recognition of IMCA relies on clinical expertise, indirect evidence of autoimmunity (additional autoimmune diseases or family history of autoimmune disease) and appropriate investigations. It is imperative to consolidate quickly such a diagnosis as therapeutic interventions can be effective in preserving the cerebellar reserve.

Keywords Immune-mediated cerebellar ataxias (IMCAs) · Gluten ataxia · Anti-GAD ataxia · Paraneoplastic cerebellar degeneration · Opsoclonus-myoclonus ataxia syndrome · Anti-DPPX ataxia · Anti-MAG ataxia · CLIPPERS syndrome · Sjogren's ataxia · Lupus ataxia

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_19

Introduction

Immune-mediated cerebellar ataxias (IMCAs) are increasingly recognised as a group of cerebellar ataxias which can be treated successfully if therapies are administered early enough. IMCAs can be divided into two groups – first group where the antigenic stimulus is known (e.g. paraneoplastic cerebellar, gluten ataxia) and second group where the antigenic stimulus is as yet lacking. Certain antibodies may be present in the serum of patients with progressive cerebellar ataxia and may serve as a marker of IMCA (e.g. GAD antibodies). The presence of other organ-specific autoantibodies in the context of a progressive cerebellar ataxia (e.g. thyroid antibodies) may not necessarily define a disease entity or imply direct antibody-mediated pathogenicity, but simply raise the suspicion of an IMCA, given the propensity for autoimmune diseases to cluster. Similarly, a family history of autoimmune diseases in a patient with progressive sporadic ataxia may be a pointer to IMCA.

In a consecutive series of 1500 patients with progressive IMCAs accounted for about 25%, the commonest of which were gluten ataxia (20%), paraneoplastic cerebellar degeneration (2%), anti-GAD-associated ataxia (2%) and opsoclonus-myoclonus ataxia syndrome (1%) [1]. In about 20% of the 1500 cases, a precise diagnosis of the cause of ataxia was not possible despite extensive investigations (the term idiopathic adult-onset sporadic cerebellar ataxia (IAOSCA) has been used to describe such cases). Whilst the aetiology of the ataxia within this group is likely to be heterogeneous, extensive genetic testing using next-generation sequencing (NGS) panel of ataxia genes (175 genes in total) has confirmed genetic ataxia in only 15%. Patients with degenerative forms of ataxia such as cerebellar variant of multiple system atrophy (MSA type C) were excluded from this cohort. This suggests that a substantial number of this group of patients may have IMCA. The term primary autoimmune cerebellar ataxia (PACA) has been proposed to describe this entity [2]. This chapter will cover IMCAs that have a well-characterised antigenic stimulus (gluten ataxia), anti-GAD ataxia as well as primary autoimmune cerebellar ataxia (PACA) (Table 1). In addition the chapter will also cover some less common immune ataxias with and without immunological markers.

Gluten Ataxia

Epidemiology

Gluten ataxia (GA) is defined as sporadic ataxia with positive antigliadin antibodies (AGA) [3]. It accounts for 20% of all patients with ataxia and 51% of all otherwise idiopathic sporadic ataxias. This is by far the commonest immune-mediated ataxia and one of the few where the antigenic stimulus is known (gluten proteins).

Table 1 Summary of autoimmune and clinical features in representative immune-mediated ataxias

	Gluten ataxia	Anti-GAD ataxia	Primary autoimmune cerebellar ataxia	Paraneoplastic cerebellar degeneration
Prevalence amongst all progressive cerebellar ataxias	20%	2%	Unknown (amongst 20% of idiopathic sporadic ataxias)	3%
Clinical manifestations				
Time course	Insidious and chronic	Insidious and chronic, or subacute	Insidious and chronic	Subacute and acute
Age and gender	50s, female (55%)	60s, female (mostly)	50s	50–60s
Dominant symptoms of cerebellar involvement	Gait ataxia	Gait ataxia	Gait ataxia	Pancerebellar ataxia In acute onset, mimicking stroke with dizziness, associated with diplopia and gait ataxia
Associated neurological symptoms	Cortical myoclonus in some, neuropathy	Stiff person syndrome, epilepsy, impairments in ocular movements	–	–
Associated autoimmune diseases	Coeliac disease (47%),	Thyroid, type 1 diabetes, pernicious anaemia	Thyroid, Sjogren's, type 1 diabetes, primary biliary cirrhosis, pernicious anaemia, vitiligo	–
Abnormality in cerebrospinal fluid	Generally no	Sometimes; CSF oligoclonal bands	Not studied	Frequently; elevation in WCCs and protein, oligoclonal bands
Cerebellar atrophy on MRI	Present depending on duration of ataxia before diagnosis	Present depending on duration of ataxia	Present depending on duration of ataxia	Not at onset but may develop rapidly
Autoimmune backgrounds for diagnosis				
Trigger of autoimmunity	Gluten ingestion	Unknown	Unknown	Cancer (ovarian, breast, Hodgkin's lymphoma, uterus, small-cell lung carcinoma, and others)

(continued)

Table 1 (continued)

	Gluten ataxia	Anti-GAD ataxia	Primary autoimmune cerebellar ataxia	Paraneoplastic cerebellar degeneration
HLA	Type DQ2 or DQ8	–	Type DQ2	–
Well-characterised autoantibodies	Anti-gliadin (IgG/IgA), Anti-TG2, TG6	Anti-GAD65 (high titer)	No	Anti-Yo, Anti-Hu, Anti-CV2, Anti-Ri, Anti-MA2
Less well-characterised autoantibodies	–	–	Anti-cerebellum (immunohistochemistry) Anti-GAD65 (low titre), anti-VGCC, Anti-Homer3, Anti-Gluδ2, Anti-Ca/ARHGAP26, Anti-DPPX, and many more	Anti-Tr, Anti-VGCC, Anti-SOX1, Anti-ZIC4, PCA-2, Anti-Homer3, Anti-CARP VIII, Anti-PKCγ, Anti-Ca/ARHGAP26, Anti-mGluR

Prevalence is cited from a study by Hadjivassiliou et al. [1]

Clinical Manifestations

GA usually presents as pure cerebellar ataxia, sometimes associated with an axonal neuropathy or rarely as ataxia in combination with cortical myoclonus [4]. The ataxia is usually of insidious onset and of slow progression. Rarely it can be rapidly progressive (in less than 5% of cases) mimicking paraneoplastic cerebellar degeneration or even acute cerebellitis. Of 500 patients with GA assessed so far at the Sheffield Ataxia Centre, 55% were female. Mean age at presentation was 52 (range 16–95), and mean duration of ataxia was 13. Mild ataxia (walk unaided) affected 74%, moderate ataxia (walking aid) 18% and severe ataxia (wheelchair bound) 8%. Enteropathy was seen in 47% and peripheral neuropathy in 8%. There are no gastrointestinal or neurological features that distinguish those patients with gluten ataxia who have an enteropathy from those who do not. MRI and MR spectroscopy of the cerebellum showed primarily vermian involvement [5].

Pathogenesis

Gluten ataxia belongs to a spectrum of gluten-related diseases with diverse manifestations. Coeliac disease (CD), also known as gluten-sensitive enteropathy, is an autoimmune disease triggered by the ingestion of gluten. The autoantigen responsible for CD has been shown to be tissue transglutaminase 2 (TTG2) [6]. Another disease within this spectrum is Dermatitis Herpetiformis (DH). DH is characterised by an itchy vesicular rash affecting primarily the extensor surfaces of limbs. Like CD, DH responds to strict gluten-free diet (GFD). In 2002 Sardy and

colleagues demonstrated that the epidermal transglutaminase TG3 was the autoantigen in DH [7]. In 2008, patients with GA were shown to have circulating autoantibodies recognising a novel neuronal transglutaminase, TG6 [8]. TG6 is primarily expressed in the central nervous system but shares common characteristics with TG2 and TG3. All three transglutaminases share 65% homology, may deamidate gliadin and are eliminated from the serum by strict adherence to gluten-free diet (GFD). It is therefore possible that depending on the antigenic target, patients with gluten sensitivity may develop a range of manifestations that may affect the gut, the skin or the nervous system [9]. However, there is considerable overlap between such manifestations. For example, 40% of patients with CD presenting to gastroenterologists have TG6 antibodies. Such patients appear to have significantly reduced regional grey matter (cerebellum and thalamus) when compared to those CD patients who do not have TG6 antibodies. Similarly patients with DH have evidence of enteropathy on duodenal biopsy even if they do not have much in the way of gastrointestinal symptoms. Additional pathophysiological findings based on post mortem data, demonstrate an inflammatory perivascular infiltrate within the cerebellum, IgA deposition against TG2 and TG6 in brain vessels and neural tissue and cross-reactivity between antigliadin and transglutaminase antibodies and Purkinje cells [9].

Diagnosis

Endomysium and transglutaminase 2 antibodies alone are not sufficient to diagnose GA as 53% of patients without enteropathy would be negative for these antibodies [9]. Antigliadin antibodies (AGA) remain the most reliable test in the diagnosis of GA. However, the type of AGA assay used and in particular the serological cut-off for AGA positivity requires adjustment for GA patients [10]. GA patients without enteropathy have a primarily CNS-based immunological response often resulting in low serum levels of AGA. Novel biomarkers of GA are currently being developed and in particular TG6 antibodies. Transglutaminase antibody type 6 (TG6) has already been shown to be present in up to 72% of patients labelled as having GA on the basis of positivity for AGA [11]. Patients with GA primarily have gait ataxia, and MR spectroscopy shows primarily involvement of the cerebellar vermis. This is a pattern distinct to what is often observed in genetic ataxias where cerebellar involvement is much more global. MR spectroscopy of the cerebellum can therefore be a useful additional diagnostic clue [5].

Treatment

The response to treatment with gluten-free diet (GFD) depends on a number of factors: firstly the duration of the ataxia and extent of cerebellar damage prior to the diagnosis of GA. Prompt diagnosis and treatment is more likely to result in

improvement or stabilisation of the ataxia [12]. Secondly, response to treatment depends on the strictness of the gluten-free diet. A recent MR spectroscopy study showed that only those patients with strict adherence to a gluten-free diet with complete elimination of AGA antibodies improve (increase of NAA/Cr ratio) [5]. Since NAA/Cr ratio reflects metabolic activity of neurones, such an increase implies improved cell functioning and can be a useful monitoring tool. Those patients not on diet deteriorate and those on partial diet (persistently positive AGA) also deteriorate but at a slower rate [5].

Most reports on the effect of GFD concern patients with established CD who then develop neurological symptoms. These studies suggest overall favourable responsiveness to a GFD. A small, uncontrolled study used intravenous immunoglobulins (IVIgs) in the treatment of four patients with GA without enteropathy [13]. All patients improved. Another study reported three patients treated with IVIgs whose ataxia and neuropathic pain (small fiber neuropathy) were resistant to strict gluten-free diet [14]. All patients responded to IVIg. Another study reported a transient response to IVIg in two patients with GA [15]. These results suggest that continuous immunosuppression may be necessary in GA patients. However, in all of these reports, strict adherence to the GFD was assumed but no serological evidence of elimination of AGA and other serological markers of gluten sensitivity was provided. Only one systematic study of the effect of gluten-free diet on a cohort of patients presenting with ataxia and gluten sensitivity, with or without enteropathy, has been published [16]. This is the only study that also reported serological evidence of elimination of the AGA as a confirmation of strict adherence to the diet. Forty-three patients with GA were enrolled. Twenty-six adhered strictly to the gluten-free diet, had serological evidence of elimination of AGA and comprised the treatment group. Fourteen patients refused the diet and comprised the control group. Patient and control groups were matched at baseline for all variables (age, duration of ataxia). There was no significant difference in the baseline performance for each ataxia test between the two groups. There was significant improvement in performance in test scores and in the subjective global clinical impression scale in the treatment group when compared to the control group. The improvement was apparent even after excluding patients with an enteropathy. The study concluded that strict gluten-free diet is an effective treatment for GA. This study also demonstrated for the first time that patients with cerebellar ataxia with positive AGA without enteropathy also benefit from strict GFD and should be treated the same way as patients who have CD. Indeed a large study comparing patients with GA with and without enteropathy concluded that there are no clinical or radiological differences between these two groups (with or without enteropathy) [17]. More importantly both groups responded to the GFD.

The current recommendation is that patients presenting with progressive cerebellar ataxia should be screened for gluten sensitivity using validated IgG and IgA AGA assays, anti-TG2 antibodies (some centres still use endomysium antibodies which is an immunofluorescent method for detecting TG2) and if available anti-

TG6 antibodies. Patients positive for any of these antibodies with no alternative cause for their ataxia should be offered dietetic advice for a strict GFD with regular follow-up to ensure that the antibodies are eliminated (usually between 6 and 12 months). Stabilisation or improvement of the ataxia (both on clinical and/or on MR spectroscopy assessments) at 1 year would be a strong confirmation that the patient suffers from gluten ataxia. By far the commonest reason for lack of response is poor compliance with the diet. If patients on strict GFD (after repeat dietetic review) and elimination of antibodies, continue to progress, it is important in the first instance to revisit the diagnosis of gluten ataxia (e.g. may be dealing with a degenerative ataxia such as cerebellar variant of multisystem atrophy). Secondly it would be important to rule out refractory CD by repeating the gastroscopy and the duodenal biopsy [4]. The use of immunosuppressive medication such as mycophenolate should be considered for those patients who are strict with their diet but still show evidence of progression and/or ongoing inflammation on duodenal biopsy after ruling out other causes. It could be argued that these cases may in fact have primary autoimmune cerebella ataxia and that the gluten sensitivity is a coincidental additional autoimmune disease.

Anti-GAD Ataxia

Epidemiology

Anti-GAD ataxia is a rare form of IMA. Amongst 1500 patients with different types of progressive ataxia, there were 30 (2%) patients with high titres of anti-GAD antibodies [1]. This means that anti-GAD-associated ataxia accounted for 9% of all sporadic, otherwise idiopathic ataxias.

Clinical Manifestations

Anti-GAD ataxia has a slight preponderance for women and it is a late onset ataxia (over 50). The presentation is usually subacute or chronic sometimes with significant fluctuations. Patients often have other autoimmune diseases (e.g. thyroid disease, type 1 diabetes, pernicious anaemia) or are at risk of developing other autoimmune diseases. The ataxia is usually pure and may follow a variable course, sometimes with prolonged periods of stability, even without any treatment, but ultimately these patients accumulate disability over time. Clinical characteristics are of mainly gait ataxia, mild dysarthria and less commonly nystagmus and limb ataxia. Brain imaging may show mild atrophy of the cerebellum with preferential involvement of the vermis, as is often the case in immune-mediated ataxias.

Pathogenesis

Glutamic acid decarboxylase (GAD) is the rate-limiting enzyme in the synthesis of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). GAD is found in both the central and peripheral nervous systems as well as in the pancreatic beta cells [18]. There are two isoforms of GAD, GAD65 and GAD67. GAD65 is associated with the cytosolic face of GABA-containing vesicles at nerve terminals and is involved in the synthesis and the packaging of GABA. Anti-GAD65 (anti-GAD) antibodies were first identified in type 1 diabetes, and subsequently found in patients with stiff person syndrome and then in some patients with idiopathic late onset ataxia [19]. Anti-GAD antibodies are a marker of multiple autoimmunity as one or more additional autoimmune disorders are present in 60% of anti-GAD positive patients with SPS versus 6% in anti-GAD negative patients [20]. Thus the finding of high prevalence of anti-GAD antibodies in patients with idiopathic sporadic ataxia may signify that the ataxia is autoimmune in origin. In 2001 Honorat et al. collected 14 cases of patients with idiopathic ataxia and positive anti-GAD antibodies establishing this entity as a specific form of immune-mediated ataxia [21]. As a result of the diversity of the neurological phenotypes seen in the context of anti-GAD positivity (e.g. SPS, ataxia), the issue of the role of these antibodies in the pathogenesis of the ataxias has been questioned. Interestingly, most patients with SPS (the other group of patients where anti-GAD is present) have a degree of cerebellar atrophy and/or abnormal MR spectroscopy of the cerebellum on MR imaging, but in these patients it is the stiffness and spasms that tend to be the presenting features. Recent neurophysiological studies make a compelling case in favour of anti-GAD pathogenicity [22, 23]. This is based on both in vivo and in vitro studies: intracerebellar administration of CSF from anti-GAD positive patients impairs cerebellar modulation in rats and also decrease the release of GABA from GABAergic neurones. Absorption of GAD antibodies using recombinant GAD65 diminishes the above effects. Finally monoclonal human GAD65 antibodies mimic the effects of the CSF both in vivo and in vitro, and these effects disappeared in GAD65 knockout mice. These studies show that binding of GAD65 by GAD65 antibodies impairs GABA release, leading to the development of cerebellar dysfunction (Fig. 1). A possible explanation for the diversity of neurological manifestations is the epitope specificity of GAD antibodies [23].

Diagnosis

As anti-GAD antibody testing is readily available in most immunology labs, the diagnosis of anti-GAD-associated ataxia is feasible as compared to other autoimmune ataxias where associated antibodies if already identified and described are not always readily available in everyday clinical practice. Still, it is important to ensure that there is no other cause for the ataxia in patients with anti-GAD antibodies,

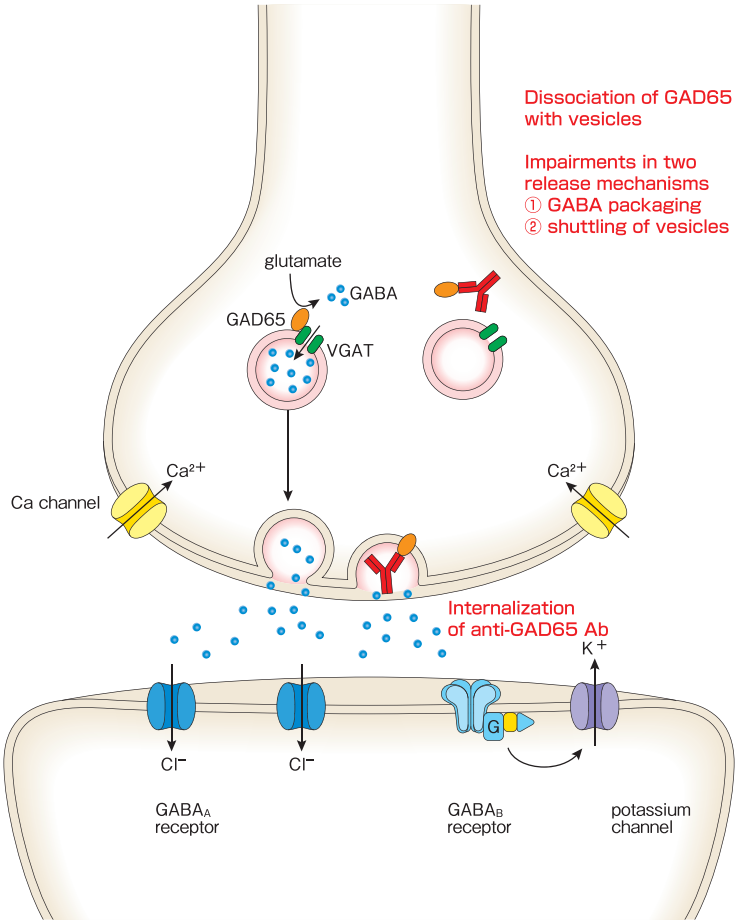

Fig. 1 Mechanisms underlying anti-GAD65 Ab-induced impairments at GABA synapses [31, 34]. Anti-GAD65 Ab is internalised into cerebellar neurons, presumably by exo- or endocytosis. Anti-GAD65 Ab disturbs the association of GAD65 with vesicles, which results in impairments in two release mechanisms, the GABA packaging and the shuttling of vesicles to the release site

particularly if intending to treat with immunosuppression. The circulating anti-GAD antibody titres usually exceed the levels seen in the context of type 1 diabetes by 100-fold.

Treatment

Treatment studies suggest that early intervention (even when the patient is well) may be associated with better long-term outcome. A retrospective cohort study reported a series of 34 patients with anti-GAD antibodies of which 9 had SPS, the

remaining 25 having primarily cerebellar ataxia [24]. Twenty patients with long-term follow-up data received immunotherapy (IVIgs, corticosteroids, azathioprine, mycophenolate). Seven patients (35%) improved. Predictors of good clinical response included subacute onset and prompt introduction of immunotherapy, at a stage where the cerebellar reserve is moderately affected. There was no specific immunotherapy recommendation, but examples include intravenous immunoglobulins, plasma exchange and rituximab and maintenance therapies using azathioprine or mycophenolate. As most patients with anti-GAD ataxia follow a subacute course, the authors favour the use of mycophenolate, a drug that is well tolerated and with minimal long-term side effects. For a comprehensive review of the treatment of anti-GAD ataxia, see "Guidelines for the treatment of immune-mediated cerebellar ataxias" by Mitoma et al. [25].

Primary Autoimmune Cerebellar Ataxia (PACA)

Introduction

PACA is the proposed term used to describe immune-mediated ataxia where no obvious external or internal antigenic trigger factor is known as yet. The evidence in support of PACA comes from a number of observations: Firstly, the Human Lymphocyte Antigen (HLA) type DQ2 is significantly overrepresented in patients with idiopathic sporadic ataxia, 74% vs 35% in the healthy population [26]. The HLA DQ2 has been shown to have a strong association with autoimmune diseases. Secondly it has been shown that there is a significantly higher prevalence of one or more autoimmune diseases in patients with idiopathic sporadic ataxia when compared to the general population and to patients with genetic ataxias, 47%, 3% and 5%, respectively [26]. Thirdly it has been shown that cerebellar antibodies can be present in at least 60% of patients with idiopathic sporadic ataxia by contrast to 5% in patients with genetic ataxias [26]. Four different staining patterns were observed in this study, three resembling those seen in patients with gluten ataxia (cytoplasmic with processes, cytoplasmic alone, nuclear) and the fourth showing staining of the granular layer of the cerebellum. Finally, studies have shown that idiopathic sporadic cerebellar ataxia can be associated with the presence of a number of different autoantibodies such as anti-GAD (see section above), anti-Voltage Gated Calcium Channel, antibodies against Homer 3 protein, glutamate receptor ∂ 2, ARHGAP26, DPPX and many more [25]. It remains debatable currently if the presence of any of the above antibodies in the context of a progressive ataxia defines a specific disease entity or if such ataxia should come under the umbrella of PACA. To some extent this will depend on the demonstration of pathogenicity of such antibodies as has been the case with anti-GAD ataxia (see section above).

Epidemiology

Epidemiological data are difficult to gather, primarily because there is no single serological or other marker that defines PACA. In a study of 1500 patients with progressive ataxia, 20% were labelled as having late onset idiopathic sporadic ataxia despite extensive investigations [1]. Even after applying extensive genetic testing using next-generation sequencing with a panel of ataxia genes (>170), the pick-up rate for a genetic cause was just 5%. This suggests that amongst the 20% of patients labelled as idiopathic sporadic there is a substantial number that may have PACA.

Clinical Manifestations

Like with other immune-mediated ataxias, PACA is a late-onset ataxia (early 50s) that primarily affects gait, less commonly causing limb ataxia, nystagmus and dysarthria. The ataxia in general tends to be slowly progressive but not as slow as in some genetic ataxias. There is gradual development of cerebellar atrophy with disproportional involvement of the vermis. In a few cases, there may be a rather more acute onset (a picture not dissimilar to that seen in paraneoplastic cerebellar degeneration). In fact some of these patients may have originally been diagnosed as having "post-infectious" cerebellitis [27]. Subsequently, however, such patients follow a progressive course by contrast to patients with true post-infectious cerebellitis who tend to make a full recovery.

Pathogenesis

The ever-increasing identification of new antibodies implicated in immune ataxias confirms the immune-mediated pathogenesis of this entity. Nevertheless, some of these antibodies may simply be markers of an immune ataxia rather than being directly pathogenic. It has been shown that cerebellar antibodies can be present in at least 60% of patients with idiopathic sporadic ataxia by contrast to 5% in patients with genetic ataxias [26]. Four different staining patterns were observed in this study, three resembling those seen in patients with gluten ataxia (cytoplasmic with processes, cytoplasmic alone, nuclear) and the fourth showing staining of the granular layer of the cerebellum. As more studies on ataxias associated with specific autoantibodies (e.g. DPPX) are ongoing, it is likely that we will gain more insight into the pathogenesis of PACA.

Diagnosis

The diagnosis of PACA requires several clues and no single test is enough to secure a diagnosis. As the HLA DQ2 is found in up to 35% of healthy individuals, this test alone cannot serve as a sole marker for patients with autoimmune ataxia. Furthermore not all PACA cases are HLA DQ2-positive. The presence of additional autoimmune diseases in either the patient or their first-degree relatives may be another clue. The presence of antibodies implicated in immune-mediated ataxias is also a helpful clue. Ultimately characterisation and easy accessibility to laboratory testing of the various cerebellar antibodies in patients with idiopathic sporadic ataxia may prove to be a useful additional biomarker for PACA. MR spectroscopy of the cerebellum may also provide some useful clues as most immune-mediated ataxias primarily involve the vermis (hence the phenotype of gait ataxia), unlike most genetic ataxias where the cerebellar involvement is more global.

Treatment

A review of small case series showed that immunotherapy (IVIgs, prednisolone, plasma exchange or rituximab) was associated with good response in 4/6 patients treated early as opposed to 9/19 in patients with chronic disease. Another small series of patients with idiopathic sporadic ataxia showed benefit with the use of intravenous immunoglobulins [28]. A retrospective study looked at 118 patients with immune-mediated ataxias, 55 of which had non-paraneoplastic ataxia. All patients had received some form of immunotherapy, and neurological improvement was reported in 54 patients. Regression analysis revealed that improvements were significantly more common amongst patients with non-paraneoplastic ataxias. Interestingly the study also showed that progression to wheelchair dependence occurred significantly faster in those patients with neuronal nuclear and/or cytoplasmic antibody than those patients with plasma membrane protein antibody positivity [29].

Paraneoplastic Cerebellar Degeneration

Introduction

Paraneoplastic neurological syndromes (PNS) are a group of immune-mediated neurological disorders triggered by cancer which is often occult. In the last 20 years or so, the discovery of specific antibodies that are present in the serum of patients with such syndromes resulted in better identification and clinical characterisation of PNS. Such syndromes are divided into classic and non-classic on the basis of how

strong is their association with cancer. Classic PNS comprise subacute or acute cerebellar ataxia, limbic encephalitis, ospsoclonus-myoclonus, encephalomyelitis, Lambert-Eaton myasthenic syndrome, sensory neuronopathy, dermatomyositis and rarely intestinal pseudo-obstruction. In this section we will concentrate on paraneoplastic cerebellar degeneration (PCD).

Epidemiology

PNS are rare neurological disorders. Based on laboratory data from serological screening of patients suspected of having PNS, only 0.9% of patients had paraneoplastic antibodies. By contrast, in a more specialised centre with a particular interest in both clinical and serological characterisation of patients suspected of having PNS, 25% were positive for such antibodies [30]. Not all patients with PNS have such antibodies. There are certain types of cancer that are more commonly associated with PNS such as small-cell lung cancer. Up to 5% of patients with this type of cancer develop PNS. In the case of PCD, the commonest types of cancer implicated are ovarian, breast and lymphomas. In a series of 1500 patients with progressive ataxias, PCD accounted for just 2%.

Clinical Manifestations

PCD usually presents in an acute/subacute manner but is characterised by a rapid progression unlike any other disorder seen in the context of progressive cerebellar diseases. Acute presentations may mimic stroke with sudden onset of "dizziness," sometimes associated with diplopia and gait ataxia. The patient quickly becomes disabled and wheelchair bound. Other prominent cerebellar signs include slurred speech, truncal and limb ataxia. Initial brain imaging tends to be normal despite the severity of the clinical signs. MR spectroscopy of the cerebellum, however, reveals severely reduced NAA/Cr ratio implying reduced cellular metabolic activity [31]. Such presentation is so typical of PCD that the management should be that of a neurological emergency in terms of investigation for cancer.

Pathogenesis

The best evidence for immune pathogenesis comes from the demonstration of antineuronal antibodies in both the serum and the CSF of patients with PNS. These antibodies react with neuronal proteins that are usually expressed by the tumour. Patients with PNS often have lymphocyte pleiocytosis in the CSF and oligoclonal bands detected by isoelectric focussing of CSF. The target antigen can be either

exposed on the cell membrane or be intracellular. Some antibodies seem to have a direct pathogenic role in causing PNS. However a pathogenic role of other paraneoplastic antibodies has not been proven as transfer of these antibodies into animal models failed to induce disease [32]. Circumstantial evidence of T-cell-mediated pathogenesis in these syndromes comes from studies on patients with anti-Hu and anti-Yo antibodies where antibody-specific T cells have been identified in both serum and CSF [33]. The same researchers reported a role for cytotoxic T cells in the autoimmune destruction of Purkinje cells in paraneoplastic cerebellar degeneration. T cells in the CSF were predominantly Th1 cells. Further support for T-cell-mediated mechanisms includes the fact that it is difficult to treat these disorders with immunosuppression directed against humoral immune response and that there is evidence of extensive T-cell infiltration in the CNS of patients with PNS.

Diagnosis

This should be suspected based on the rather dramatic and in some respects unique presentation outlined above. Whole-body PET scan has to be used if initial imaging does not identify any obvious malignancy. Whole-body PET scan has been shown to improve the diagnostic yield of malignancy in patients clinically suspected of having PNS [34].

Treatment

If the malignancy is treatable, treatment such as oophorectomy and mastectomy that may also include chemotherapy and radiotherapy has to be given urgently to avoid permanent and often severe neurological disability. Immunosuppression has been used in all of the PNS but with very limited benefit, particularly when the cancer cannot be fully eradicated. Because of the rapidity of progression, many patients remain disabled even after complete eradication of the cancer. Such patients eventually end up with significant cerebellar atrophy on subsequent imaging.

Other Immune-Mediated Ataxias

Introduction

As discussed in the PACA section, there are an ever-increasing number of antibodies that have been associated with suspected immune-mediated ataxias. The term PACA encompasses a heterogeneous group of immune-mediated ataxias where no specific antibody has been implicated in its pathogenesis. Here we discuss some

entities that are increasingly being recognised as immune-mediated ataxias. Whether these entities should come under the spectrum of PACA remains a subject for debate. The authors are currently of the opinion that PACA should include all entities where no pathogenic antibody has been described but also those ataxias where a link with an antibody has been made but no clear pathogenic mechanism has been elucidated.

The list is by no means exhaustive but we have selected these additional aetiologies as they highlight the diversity of the immunological insults that can result in cerebellar ataxia. The authors also propose that immune-mediated ataxias should include not only aetiologies that damage the cerebellum alone, but also aetiologies that impair the cerebellar afferents/efferents.

Opsoclonus-Myoclonus Ataxia Syndrome (OMAS)

OMAS, sometimes referred to as dancing eye syndrome, is characterised by subacute onset of opsoclonus (involuntary saccades that occur at random directions and are usually associated with oscillopsia), ataxia and myoclonus. It affects children under the age of 2 years with an incidence of 0.18 cases per million per year in the UK [35]. In 50% of children with OMAS, the aetiology is paraneoplastic usually due to a neuroblastoma. In some of the remaining cases, the aetiology is thought to be post-infectious although other autoimmune aetiologies such as gluten sensitivity should be considered [36, 37]. The aetiology of OMAS in adults is very similar and can broadly be divided into three categories: paraneoplastic, post-infectious and idiopathic. The prevalence of OMAS amongst patients with adult onset progressive ataxia was 12/1500 (0.8%) [1]. Paraneoplastic OMAS is associated with poor prognosis unless the underlying cancer is treatable. Post-infectious OMAS is usually self-limiting, and the prognosis is good.

In a retrospective series of 24 patients reported by the Spanish OMAS collaborative group, 10 patients had idiopathic OMAS [38]. The age at onset in the idiopathic group was 40, and all patients had evidence of predominantly truncal ataxia. No obvious trigger factors were identified and all patients had normal imaging at presentation. Eight of the 10 patients had what appeared to be a monophasic illness and five of them made a full recovery. The three patients who did not recover had received steroids and/or intravenous immunoglobulins. The two remaining patients followed a relapsing course and appeared to respond to immunomodulation during the relapses. The spontaneous recovery observed in some patients with idiopathic OMAS makes the condition difficult to study in terms of long term treatment.

The aetiology of idiopathic OMS remains obscure but an autoimmune aetiology seems likely. Some autoantibodies have been found in patients with OMAS but the majority of cases have no detectable serum antibodies. There are no reports of passive transfer of OMAS to animals. In view of this, a predominantly cell-mediated pathogenesis has been proposed [39]. Despite normal CSF cell counts, CSF from

patients with OMAS was shown to exhibit expansion of CD19 B-cell and gamma-delta T-cell subsets which persisted even years after disease onset. A study used a combination of rituximab, ACTH and IVIgs in 12 children with OMAS [40]. There was significant improvement of the opsoclonus and the myoclonus. The ataxia improved more slowly.

A practical approach to a patient presenting with OMAS would be to look for malignancy preferably using whole-body PET scan and check for paraneoplastic antibodies. If malignancy has been excluded, then it may be worth keeping a close observation looking for stabilisation or improvement even without any intervention (particularly in suspected post-infectious OMAS). Symptomatic treatment of the myoclonus should be used if the patient is troubled by the tremor. If the patient appears to be progressing, then immunosuppression should be considered, but the drug choice is currently unclear.

Anti-DPPX Ataxia

In 2013, dipeptidyl-peptidase-like protein-6 (DPPX), a subunit of Kv4.2 potassium channels, was identified as the target autoantigen in four patients with encephalitis of unclear aetiology [41]. Symptoms included agitation, mild confusion, myoclonus and ataxia. There was also a history of profound weight loss associated with diarrhoea often preceding the onset of the neurological symptoms. A further series of three patients published in 2014 highlighted a distinct syndrome involving hyperekplexia, prominent cerebellar ataxia and trunk stiffness [42]. The authors suggested that this was a variant of DPPX associated with progressive encephalomyelitis with rigidity and myoclonus (PERM) thus expanding the aetiology of PERM. DPPX-associated encephalopathy therefore belongs to the CNS hyperexcitability spectrum. A series of 20 patients with DPPX antibodies was published in 2014 [43].

The majority of patients had some cognitive/psychiatric complaints including memory deficits, delirium, psychosis and depression. Ataxia was a prominent feature in almost half of these cases. The weight loss can be striking and a useful diagnostic clue. One of the authors has seen DPPX patients referred to the Sheffield Ataxia Centre with progressive ataxia but minimal additional (cognitive) features other than the myoclonus. Startle is also a very prominent feature. As this entity may present with ataxia, DPPX antibody testing, if available, should be part of routine testing for patients with suspected autoimmune progressive ataxia, particularly when myoclonus and weight loss are present.

Anti-MAG Ataxia

Myelin-associated glycoprotein (MAG) is a glycoprotein specific to Schwann cells in the peripheral nervous system and also plays a role in maintenance of myelin integrity and inhibition of axonal regeneration in cerebellar neurons in the

CNS. Anti-MAG antibodies are commonly associated with distal acquired demyelinating sensory and motor (DADS) neuropathy in patients with IgM monoclonal gammopathy of unknown significance (MGUS). These patients exhibit sensory disturbances resulting in sensory ataxia. Recently, a series of five patients (four males and one female, 60–80 years of age) with MGUS and anti-MAG-associated cerebellar ataxias were reported [44]. These patients presented with severe gait ataxia, mild limb ataxia, and gaze-evoked nystagmus. Four of these five patients also had peripheral neuropathy (DADS). The cerebellar origin of their ataxia was identified using MR spectroscopy that showed reduction in vermian N-acetylaspartate (NAA) to creatine (Cr) ratio in all patients, which correlated with treatment-induced (retiximab) improvement of the ataxia. It should be acknowledged that patients with sensory ganglionopathy show no abnormalities on cerebellar MR spectroscopy. MRI showed no cerebellar atrophy. Three of these patients showed improvement following treatment with rituximab, although in the other two patients, rituximab was discontinued due to drug-induced vasculitis (in one) and refusal of treatment in another patient. The therapeutic response to rituximab supports an immune-mediated mechanism.

CLIPPERS Syndrome

Chronic lymphocytic inflammation with pontine perivascular enhancement responsive to steroids (CLIPPERS) is characterised by marked perivascular T-cell inflammation mainly in the pons with compatible perivascular gadolinium enhancement on MRI, which is responsive to corticosteroids [45, 46]. The lymphocytic infiltration, mainly CD4-dominant T cells, is mostly seen in perivascular lesions, but also shows more diffuse parenchymal distribution in the white matter. The perivascular infiltration is mainly in the pons and adjacent rhombencephalic structures, such as the cerebellar peduncles, cerebellum, medulla, and the midbrain.

According to a review of 56 reported cases, there are considerable differences regarding age at presentation, ranging from 13 to 86 years (mean age at onset 52.4 years), with male comprising 67% of the patients [45]. Patients show subacute onset of varying neurological symptoms related to the brainstem pathology, frequently including pancerebellar ataxias, dysarthria, dysphagia, dysgeusia, oculomotor abnormalities, altered facial sensation, facial nerve palsy, and vertigo. Pyramidal signs and sensory disorders are also observed. MRI shows a characteristic change, which reflects perivascular lymphocyte infiltration, in the pons and peripontine lesions. The hallmark feature is multiple “punctate” and/or “curvilinear” gadolinium-enhancing lesions resulting in “peppering” of the pons with or without peripontine lesions. CSF examination is either normal or shows mild to moderate rise in protein levels with mild elevation of white cells. Early intervention with corticosteroids results in clinical improvement and often requires long-term maintenance with reducing doses [46]. It is recommended that the initial treatment with intravenous methyl prednisolone is followed by maintenance immunotherapy using the combination of oral prednisolone and corticosteroid-

sparing immunosuppressants (azathioprine, methotrexate and rituximab). Withdrawal of corticosteroids results in disease exacerbation thus, long-term maintenance therapy is required.

Sjogren's Ataxia

Primary Sjogren's syndrome (PSS) is one of the commonest autoimmune diseases affecting up to 4% of the population. It has a female to male ratio of 9:1. The onset of the disease is usually in the fourth or fifth decades, but it can affect younger individuals. It is characterised by lymphocytic infiltration of the exocrine glands leading to distraction of the glands and clinically manifesting with dry mouth and dry eyes. PSS can be associated with other organ involvement including lungs (pneumonitis), kidneys, pancreatitis, myositis and occasionally lymphoma. The most recent diagnostic criteria have been published in 2012 [47]. The diagnosis relies on the presence of 2 out of 3 of the following criteria: (a) positive serum antibodies known to be associated with PSS (anti-Ro and anti-La) (b) demonstration of xerophthalmia using a special ocular staining score and (c) labial salivary gland biopsy showing focal lymphocytic sialadenitis.

The interest in the neurological manifestations of PSS started in the 80s following the publication of a series of papers by a group of researchers based at the John Hopkins Hospital, Baltimore USA. The same group came up with a figure of prevalence for neurological involvement of 20%. As PSS is a common disease often associated with other autoimmune diseases, the coexistence of PSS with common autoimmune neurological diseases such as multiple sclerosis (MS) has to also be considered. The concept that PSS may mimick MS was first put forward by the same Baltimore group in 1986 when the authors described a range of neurological signs in patients with PSS including optic neuritis, intranuclear ophthalmoplegia, cerebellar ataxia and pyramidal weakness [48, 49].

By far the most common and better characterised form of peripheral nerve involvement in PSS is that of sensory ganglionopathy. This is a form of asymmetrical purely sensory peripheral nerve involvement that affects the dorsal root ganglia. It is often associated with sensory ataxia and can often be the presenting feature of PSS. In a series of 92 patients with PSS-associated neuropathy, 93% were diagnosed with PSS after neuropathic symptoms appeared [50]. The commonest form of peripheral neuropathy was sensory neuronopathy (59%). Sensory ganglionopathy in PSS is slowly progressive but ultimately disabling because of the severe sensory ataxia. In addition to sensory ataxia, Sjogren's syndrome can be associated with cerebellar ataxia with the first such report published in 1961 [51]. The largest series of patients with PSS and cerebellar ataxia was published in 2018 by Yang and colleagues [52]. They retrospectively identified 13 patients with PSS and ataxia. 9/11 had abnormal CSF, and 11 had cerebellar atrophy on MR imaging. Twelve patients received steroids, three cyclophosphamide and one mycophenolate. During a median follow-up time of 9 months, only two patients progressed.

Our own experience is with a group of 27 patients with PSS who underwent MR imaging because of complaining of loss of balance. Of these 18 (67%) had abnormal MR spectroscopy of the cerebellum with 41% showing evidence of cerebellar atrophy. These patients were recruited from a dedicated rheumatology/neurology clinic run by one of the authors, based at the Royal Hallamshire Hospital, Sheffield, UK.

It is currently unclear if such cerebellar involvement is along the lines of PACA with Sjogren's syndrome representing an additional autoimmune disease or if Sjogren's ataxia is a distinct entity. Immunotherapies have been used in small uncontrolled and retrospective cases using intravenous immunoglobulins, steroids and cyclophosphamide.

A potential role for anti-Ro antibodies in the pathogenesis of neurological involvement comes from some in vitro studies where serum from patients with PSS containing anti-Ro antibodies was shown to stain the cytoplasm and cell membranes of endothelial cells derived from umbilical vein and from brain tissue [53].

Lupus Ataxia

Neuropsychiatric manifestations are common in systemic lupus erythematosus (SLE). Cerebellar ataxia has been reported in SLE, presenting especially with a subacute cerebellar syndrome and thus raising first the possibility of a PCD [54–55]. Ataxia of acute onset is a differential diagnosis of cerebellitis [56]. Cerebellar ataxia may develop in adolescence or in adulthood, and is rare in kids.

Some patients harbour positive anticardiolipin antibodies and lupus anticoagulants. The mechanisms of ataxia are multiple: cerebral vascular infarction or ischaemia, vasogenic oedema, vasculitis and antibody-mediated cerebral vasculopathy. There is a debate regarding the pathogenicity of antibodies. A specific pattern of antibody production is not established. Therefore the concept of "lupus ataxia" is still ambiguous.

Cerebellar ataxia responds to high doses of steroids. Pulses of cyclophosphamide may be considered in refractory cases.

Conclusion

The cerebellum is endowed with capacities for compensation and restoration, which is defined as cerebellar reserve [22, 23, 57]. The multiple forms of synaptic plasticity and the redundant inputs of sensory information would constitute the cerebellar reserve. Thus, immunotherapies should be introduced during a period when the cerebella reserve is preserved. In this regard, we argue that every effort should be made to reduce the diagnostic delay and the loss of therapeutic opportunities [57].

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Stiff-Person Syndrome Spectrum Disorders

José Fidel Baizabal-Carvallo and Marlene Alonso-Juarez

Abstract Stiff-person syndrome was first described in 1956; its further characterization as an autoimmune neurological disorder occurred more than 30 years later with the discovery of glutamic acid decarboxylase (GAD) antibodies (Abs), frequently coexisting in these patients. In the following years, clinical variants of SPS have been characterized, and a paraneoplastic presentation was also recognized, the latter mainly associated with amphiphysin antibodies. Although the presence of GAD-Abs has led to theorize that these antibodies cause disinhibition of the central nervous system through decreased production of the inhibitory neurotransmitter (GABA), the pathogenic role of GAD-Abs has not been demonstrated, although the evidence suggests that antibodies directed against amphiphysin and glycine receptor α1 are likely pathogenic. The treatment aims to attenuate the immunological response through immunotherapy, control the symptoms, mainly with GABAergic drugs, and remove an underlying tumor, if present. The course is usually chronic and the prognosis is frequently poor.

Keywords Stiff-man syndrome · Stiff-person syndrome · γ-aminobutyric acid · Glutamic acid decarboxylase · Progressive encephalomyelitis with rigidity and myoclonus · PERM · Paraneoplastic stiff-person syndrome · Amphiphysin · Myoclonus · Glycine receptor · GAD antibodies

Introduction

Stiff-man syndrome is the original name used by Moersch and Woltman in 1956 to describe a group of 14 individuals with progressive and fluctuating rigidity [1]. The disorder was latter named stiff-person syndrome (SPS) to avoid gender bias. The disorder was associated with the presence of glutamic acid decarboxylase (GAD)

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_20

antibodies (Abs) since 1988 by Solimena and colleagues [2, 3]; however, soon after, it became clear that not all patients with SPS have positive serum GAD-Abs and some of them may show a different set of antibodies and variable clinical presentations [4]; therefore, the term SPS spectrum disorder is currently used to refer to this group of patients.

The enzyme GAD is the rate-limiting step in the synthesis of γ-aminobutyric acid (GABA), which is the main inhibitory neurotransmitter in the central nervous system (CNS) [5]. It has been theorized that GAD-Abs inhibit the activity of GAD in the central nervous system of patients with SPS spectrum disorders; however, this has not been unquestionably demonstrated in vivo, and the role of such antibodies in the pathogenesis of this group of disorders is still controversial as discussed in this chapter.

Epidemiology

SPS is an acquired disorder that usually appears between the third and sixth decades of life [6–8]. It has an estimated prevalence of 1–2 cases per million; women outnumber men (2:1); there is no ethnic predilection [9].

Children may also present with SPS, they represented 5% of patients evaluated during a 40-year period in a tertiary care center [10]. The mean age at onset was 11 years (range 1–14 years). Although classical SPS is observed in children, a literature review pointed to progressive encephalomyelitis with rigidity and myoclonus (PERM) as the most common presentation in this age group [10]. SPS is not considered a hereditary disorder, although rare family cases of SPS have been described in the literature [11].

Clinical Manifestations

Classification

There is not a universal agreement regarding the classification of SPS spectrum disorders. Classifications may use the distribution of the stiffness, the presence of associated neurological manifestations, the presence or absence of GAD-Abs and other Abs. Patients may also be classified according to the occurrence of an underlying neoplasm (Table 1). The following section describes the clinical features of different SPS presentations.

Table 1 Classification of stiff-person syndrome spectrum disorders

According to distribution of stiffness
Classical stiff-person syndrome (involves axial muscle and lower limbs)
Focal or segmental stiff-person syndrome or stiff-limb syndrome (involves limbs without axial involvement)
Axial (spinal) stiff-person syndrome (involves only axial muscles)
According to associated manifestations
Myoclonus
Jerking-stiff-person syndrome
Progressive encephalomyelitis with rigidity and myoclonus (PERM)
Epilepsy or cerebellar ataxia
Overlapping syndromes: stiff-person syndrome + cerebellar ataxia or epilepsy
Brainstem manifestations and/or encephalitis
Progressive encephalomyelitis with rigidity and myoclonus (PERM)
According to presence or absence of tumor
Paraneoplastic stiff-person syndrome (usually associated with amphiphysin Abs)
Non-paraneoplastic stiff-person syndrome (usually GAD-Abs)
According to serological response to GAD-Abs
Seropositive stiff-person syndrome (+ GAD-Abs)
Seronegative stiff-person syndrome (− GAD-Abs)

Classical Stiff-Person Syndrome

Patients with classical SPS usually have an insidious onset with aching and stiffness of axial muscles, which progress and spreads to the proximal and then to the distal muscles of the limbs [12]. Co-contraction of agonist and antagonist muscles underlies the stiffness. The axial stiffness usually may lead to a characteristic hyperlordosis affecting the lumbar spine and muscle hypertrophy (Fig. 1) [12, 13]. In some instances, patients may develop a kyphotic posture with bilateral shoulder elevation and prominent limitation for head movements [14]. When chest and abdominal muscles are prominently affected, there may be dyspnea, poor exercise tolerance, inability to swim underwater and early satiety. The distal extremities and cranial muscles may be involved particularly in untreated patients. Limb rigidity asymmetry may also be observed and should not be confused with corticobasal degeneration [15]. The stiffness is usually relieved by sleep, but such improvement may be lost in advanced stages; at that point patients require general anesthesia or neuromuscular blocking agents to relieve the muscle stiffness [16].

The stiffness is accompanied by paroxysms of transient but usually intense superimposed muscle spasms. The spasms have variable duration ranging from seconds to hours and may be quite painful. The paroxysms are usually triggered by external stimuli such as noise and manipulation as well as emotional stimuli and sudden movement [4]. Spasms occurring while walking may cause falls and in extreme cases, joint dislocations and hip fractures [17]. Apneic episodes from muscle spasms may lead to acute respiratory failure [18]. Muscle spasms affecting the laryngeal muscles may lead to a spasmodic dysphonia-like presentation [19]; cricopharyngeus muscle spasms may result in total esophageal obstruction [20], whereas

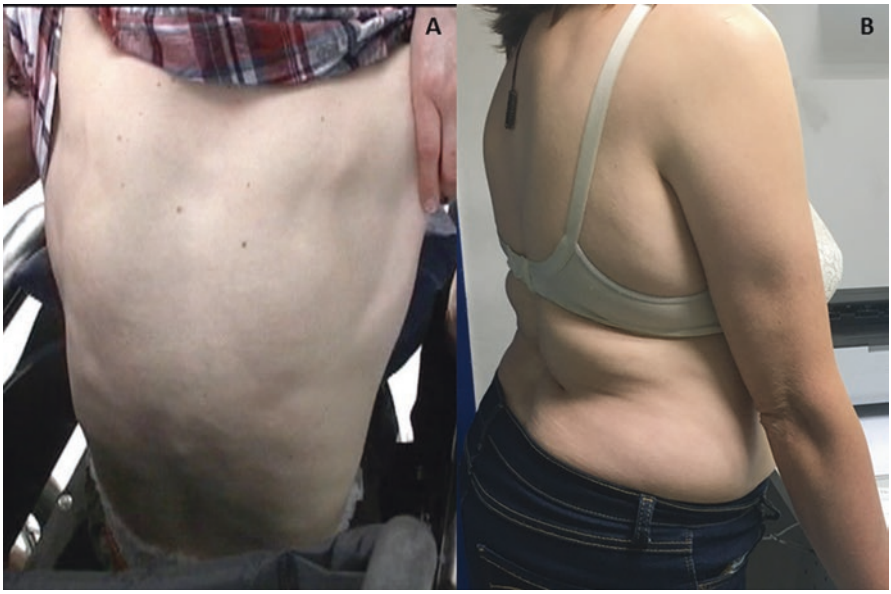

Fig. 1 (**a**) Patient with severe stiffness involving the thoracic and lumbar muscles; there is marked hypertrophy of affected muscles; (**b**) typical hyperlordosis observed in patients with classical stiff-person syndrome

dysphagia due to abnormal esophageal and gastric motility may be a more common phenomenon. Urinary retention requiring bladder catheterization, abnormal anal relaxation with anorectal spams causing constipation are recently recognized features of SPS [21].

Autonomic symptoms may accompany the paroxysms of superimposed muscle spasms with tachycardia, hypertension, hyperthermia, increased respiratory rate, pupillary dilation, profuse diaphoresis, and dysphoria [22]. Sudden death due to autonomic failure has been reported [22]. Psychiatric comorbidity is common in SPS patients and includes depression, anxiety, phobias, and chronic alcoholism [23, 24]. Phobias (particularly fear to fall) were perceived by ten patients as a realistic risk owing to motor symptoms related to SPS rather than an inherent phobic neurosis [25].

The neurological examination between spasms usually reveals “rock hard” spinal, abdominal, and proximal limb muscles [4, 12]; abnormal axial postures along with muscle hypertrophy makes possible for the examiner to bury the hand in the furrow between the paraspinal muscles in severe cases plus a paucity of movements that may resemble parkinsonism; voluntary movements are restricted in range, for example, the patient may be unable to bend at the waist to touch her or his toes or kneel. Gaze-holding nystagmus, ocular misalignment, abnormalities in ocular pursuit, and increased latency of ocular saccades may be recorded in patients with SPS [26, 27]; abnormal eye movements and vertical nystagmus may be present in some patients with positive GAD-Abs and ataxia without stiffness [28, 29]. The head

retraction reflex is hyperactive in SPS patients and may be elicited by tapping the glabella, nasal ridge, upper lip, or the chin, resulting in a backward jerk of the head sometimes with truncal retropulsion [30]; generalized hyperreflexia with plantar flexion responses is also observed. Gait may be spastic, slow, and wide-based [14]. Sensory examination is within normal limits.

Type 1 diabetes mellitus (DM1) is the most common associated autoimmune endocrine condition, present in about 35% of patients with SPS [31]; however, other autoimmune disorders may coexist with SPS, including autoimmune thyroiditis, autoimmune adrenal or ovarian failure, pernicious anemia, vitiligo, myasthenia gravis, anti-N-methyl-D-aspartate receptor (NMDA-R) encephalitis, autoimmune retinopathy, and systemic lupus erythematosus; all together these autoimmune disorders are comorbid with SPS in up to 80% of cases [31–35].

Focal or Segmental Stiff-Person Syndrome

Focal or segmental SPS (also known as stiff-leg or stiff-limb syndrome) is probably less common than classical SPS. In a series of 121 patients with SPS spectrum disorders, stiff-limb syndrome represented 20% of cases [36]. These patients usually present with stiffness and superimposed painful spasms affecting one or two legs or arms [37, 38]. Hiccup and vomiting have been reported with focal SPS, attributed to diaphragmatic spasms [39]. EMG shows the characteristic continuous motor unit activity at rest that involves agonist and antagonists muscles. Central nervous system hyperexcitability, failure of reciprocal inhibition of antagonist muscles, and altered exteroceptive reflexes can also be observed in patients with focal/segmental SPS, restricted to the affected limb [40]. The frequency of positive GAD-Abs and coexistent autoimmune syndromes seems less common than in patients with classical SPS. Amphiphysin Abs and alpha 1-glycine receptor (GlyRα1) Abs are rarely positive in individuals with focal SPS [41, 42]. Although the majority of patients with focal SPS do not have an associated cancer, an underlying malignancy is more commonly present than in patients with classical SPS. Lung cancer, breast cancer, chronic lymphocytic leukemia, and multiple myeloma are among the most common associated neoplasms [42, 43]. Unfortunately, the disorder has a protracted course with poor response to pharmacological therapy [38].

Jerking Stiff-Person Syndrome

This is a rare variant of SPS characterized by the presence of rapid and violent myoclonic jerks that may involve the axial and proximal appendicular muscles and may be nocturnal or diurnal. The myoclonus may appear years into the course of the disease and may occur either spontaneously, or it may be stimulus-sensitive, for example, following the touch of the perioral region, or stretch of head and neck

muscles [44–46]. Patients usually have symptoms that are otherwise not different to classical SPS; it is unclear whether these patients represent a continuum within the spectrum of SPS and PERM or a distinct clinical variant. Myoclonic jerks usually respond to benzodiazepines. It is uncertain why the number of reported cases of jerking SPS has dramatically decreased in the last decades, but a possible explanation is that these patients are being reclassified into another SPS spectrum disorder.

Progressive Encephalomyelitis with Rigidity and Myoclonus [PERM]

Progressive encephalomyelitis with rigidity and myoclonus (PERM) is considered within the spectrum of SPS; it is also known as stiff-person plus syndrome in some cases. However, this condition differs in several clinical and immunological aspects with respect to classical SPS. The disorder was probably first described by Campbell and Garland in 1956, under the name "subacute myoclonic spinal neuronitis" [47]. PERM distributes roughly equal between men and women [48]. DM1 is much less frequent than in classical SPS.

Muscle stiffness, myoclonus, and prominent brainstem manifestations with cranial nerve involvement are cardinal manifestations in patients with PERM; other prominent features include severe dysautonomia, corticospinal signs, gait ataxia, seizures, hypersomnia, pruritus, and behavioral changes [48–51]. The disorder is more commonly associated with GlyRα1-Abs; but about 20% of patients have positive GAD-Abs [48]. Although the term "progressive" is used in the acronym of the syndrome, a substantial proportion of patients have a relapsing-remitting course that may be fatal if left untreated. Pathological samples are dominated by inflammatory infiltrates with prominent involvement of Purkinje cells, hippocampal and pyramidal neurons, with loss of ventral horn and spinal interneurons with relative spearing of the neocortex [34, 52, 53]. Non-specific hyperintensities in the MRI are observed in about one third of cases involving the brain and spinal cord [48].

A condition resembling PERM has been associated with Abs against dipeptidyl-peptidase-like protein-6 (DPPX), which is a regulatory subunit of the neuronal potassium channel (Kv4.2) [54]. The median age at onset is 53 years, with both genders equally affected. A prominent initial manifestation is gastrointestinal tract dysfunction that manifests more commonly with diarrhea, although gastroparesis and constipation have also been described [55, 56]. This is followed by a myriad of neurological manifestations, including eye-movement disturbances, tremor, myoclonus, rigidity, exaggerated startle, hyperreflexia, hyperventilation, neuropsychiatric symptoms, and seizures [55, 56]. An underlying lymphoma or leukemia has been reported in some cases [55].

Paraneoplastic Stiff-Person Syndrome

Paraneoplastic SPS is mostly observed associated with Abs directed against the presynaptic protein amphiphysin. Patients are usually females with breast cancer; other reported malignancies are small-cell lung cancer, thymoma, and ovarian cancer [57]. These patients represent about 5% of cases with SPS [58]. A predominant upper-limb distribution of the stiffness was reported in a single study of paraneoplastic SPS [57]. However this finding has not been informed in other reports. Ophthalmoplegia and opsoclonus have also been recognized in patients with paraneoplastic SPS associated with amphiphysin antibodies [59, 60]. Comorbid DM1 is uncommon in these patients [57].

Despite the well-known association between stiffness and amphiphysin Abs, these Abs are not specific for paraneoplastic SPS, as they may be present in some patients suffering limbic encephalitis, dysautonomia, neuropathy, and cerebellar dysfunction but without stiffness; some of these patients may have underlying cancer [61]. In few cases, these disorders may coexist with stiffness; for example, a case of paraneoplastic SPS and limbic encephalitis associated with amphiphysin antibodies has been described [62]. In another study, amphiphysin Abs were positive in various paraneoplastic disorders, including neuropathy and encephalopathy; however, these patients showed positivity to other antibodies such as anti-Hu [63]. Patients that show positivity only to amphiphysin Abs, but not to other onconeural antibodies, usually presented with myelitis or SPS phenomena [63]. Recognizing paraneoplastic SPS is important, as tumor removal and chemotherapy may result in marked clinical improvement.

The paraneoplastic variant of SPS may rarely be associated with high titers of GAD-Abs; whether the association of classical SPS with underlying cancer is coincidental or not is uncertain [8, 31, 64]. However, the risk of underlying malignancy in patients with SPS is higher with older age, male gender, and positive neuronal cell-surface Abs, including GABA-B Abs and GlyRα1-Abs coexisting with GAD-Abs [65]. Anti-gephyrin Abs has been described in a single patient with paraneoplastic SPS associated with a malignant thymoma, but this finding has not been reproduced in other studies [66]. Anti-Ri (also known as anti-neuronal nuclear antibodies: ANNA-2) antibodies have been observed in patients with SPS phenomena and some malignancies [67], but such association may not be specific.

Overlapping Syndromes

Some patients with SPS may present with other neurological manifestations associated with GAD-Abs. In a retrospective study of 121 patients with SPS spectrum disorders, 8.3% were diagnosed as having an overlapping syndrome, i.e., classical or focal SP syndrome with ataxia, epilepsy, or encephalitis [36]. Among these syndromes, cerebellar ataxia is probably the most commonly associated with SPS

[68, 69]. Gait ataxia is the most common manifestation followed by limb ataxia and dysarthria [70]. The so-called brainstem attacks, characterized by transient episodes of cranial nerve, cerebellar, and long tract dysfunction preceding the onset of progressive cerebellar ataxia, are observed in about 25% of cases [71, 72]. Epilepsy may occur in few cases of SPS and it is suspected to have an autoimmune pathophysiology [2].

Pathophysiology

Increased muscle tone or hypertonia is the "*sine qua non*" condition of SPS. Muscle tone can be defined as the resistance to passive stretch of a joint. The degree of stiffness is assessed by the amount of force required to get a movement. Hypertonia can result from three different mechanisms: (1) altered mechanical properties of the muscle or joint; (2) increase in reflex response to the stretch opposing the movement, and (3) co-contraction of muscles acting on the joint [73]. The latter is the main mechanism explaining stiffness in subjects with SPS.

The increased activity of agonists and antagonists muscles in SPS is probably related to CNS disinhibition. The questions are as follows: (1) What are the molecular and neurophysiological correlates underlying such disinhibition? (2) What is the role of GAD and other Abs in the pathogenesis of SPS? (3) Where does the disinhibition originate within the nervous system? and finally (4) How can the autoimmune process be attenuated and the symptoms controlled? Currently, there are not definitive answers for these questions, but established knowledge and recent advances are provided in this chapter to better understand the pathophysiology and pathogenesis in this group of disorders.

Glutamic Acid Decarboxylase Enzymes

The enzyme GAD is specifically localized within GABAergic neurons in the central nervous system. However, an immunologically identical enzyme is present in pancreatic beta cells, the epithelium of fallopian tube, and spermatozoa [74]. The enzyme GAD is the rate-limiting step in the production of GABA. The enzyme GAD exists in 2 isoforms, one of 67 kD (GAD67) and one of 65 kD (GAD65); these enzymes are codified by two different genes [75]. GAD67 is localized in the soma of neurons and is constitutively active, providing neurons with a steady supply of GABA. On the other hand, GAD65 localizes in the cytoplasmic surface of synaptic vesicles, it provides pulses of GABA in situations requiring rapid synthesis and release of the neurotransmitter [3, 5, 74]. GAD65 is the main target of Abs in patients with SPS, but GAD67 Abs are found in the serum and CSF in a proportion of patients with SPS.

Role of GAD-Abs in the Pathogenesis of SPS Spectrum Disorders, Experimental Models

As GAD has an eminent role in the production of GABA, it can be anticipated that Abs directed against these enzymes would block the production of GABA potentially leading to disinhibition. However, GAD enzymes are intracellular which limits the interaction with pathogenic Abs. Early in vitro experiments demonstrated decreased production of GABA in crude rat cerebellar extracts, exposed to Abs obtained from the serum or CSF of patients with SPS and positive GAD-Abs [76]. Moreover, a significant increase in the frequency of post-synaptic inhibitory potentials was registered in cultured hippocampal neurons of rats after being exposed to the serum of epileptic patients with positive GAD-Abs, while no effect was observed using sera from negative controls [77]. More recently, internalization of monoclonal GAD65 Abs was shown in cultured cells, and epitope-dependent pathogenic actions of GAD65 Abs were shown in slice (normal components are preserved) and in vivo preparations [78]. Although lack of GAD-Abs internalization into cultured hippocampal rat neurons was observed in another study [79], GAD-Abs have been shown to coexist with Abs that bind to the cell surface of GABAergic neurons, but the underlying antigen was not identified [80]. Passive transfer to experimental animals of Abs directed against GAD from patients suffering SPS or cerebellar ataxia has been carried out with mixed results. Continuous motor activity with repetitive muscle discharges [81], and impaired cerebellar function due to altered motor and spatial procedural behaviors has been observed following intracerebellar and/or paraspinal administration of Abs with anti-GAD activity [82] as well as an increase in glutamate levels in cerebellar nuclei and inhibition of GAD activity [83]; stiffness-like behavior with impaired walking and decreased grip strength of the upper limbs along with postural and sensory-motor dysfunction was reported in another study following intra-lateral ventricle injection of a purified IgG fraction of an SPS patient into mice [84]. Despite this evidence, other studies have not shown the core features of SPS in mice models exposed to Abs from SPS patients but rather increased activity [80], anxious behavior or agoraphobia [85].

In Vivo Evidence of GABAergic Dysfunction

Studies in humans have demonstrated evidence of CNS disinhibition from the cerebral cortex to the spinal cord in patients with SPS. Hyperexcitability of the motor cortex was recorded using transcranial magnetic stimulation (TMS) in patients with SPS, suggesting an imbalance between inhibitory and excitatory intracortical circuits; moreover, the degree of disinhibition seems to correlate with the titer of GAD-Abs [86, 87]. Brainstem hyperexcitability has also been documented by an abnormal recovery cycle in the R2 component of the blink reflex and abnormalities in the masseter and glabellar reflexes [88, 89]. Brain magnetic resonance

spectroscopy (MRS) has shown reduced levels of GABA in the sensorimotor and posterior occipital cortex in patients with SPS [90], and imaging of GABA-A receptor with PET-CT revealed large areas of decreased binding of 11C-flumazenil in the bilateral premotor cortex, motor cortex, and right supplementary motor cortex in few patients with SPS, suggesting downregulation of GABA-A receptors [91, 92]. These evidences suggest the possibility of supra-spinal disinhibition as the cause of muscle stiffness [93]. However, stimulation of peripheral nerves released myoclonic bursts in the trunk muscles after 60–70 ms, such phenomenon was called "spasmodic reflex myoclonus," and the recruitment order of the muscles suggested a spinal origin in the Renshaw cells or the gamma motor system [94].

Differences in Immunological Profile Between DM1 and SPS and Triggers of the Autoimmune Response

There are several quantitative and qualitative differences regarding the humoral response between patients with DM1 and SPS. GAD-Abs are observed in about 60–80% of patients with classical SPS but in a lower proportion in subjects with DM1. These Abs can also be positive in Batten disease, autoimmune polyendocrine syndrome type 1, and occasionally some neurodegenerative disorders [4]. GAD-Abs are increased 100–1000 times in patients with SPS, whereas in DM1 such increase is usually not beyond 10 times the reference range [93]. The distribution of GAD-Abs is also different; they can be found in the serum and CSF in patients with SPS, but only in the serum in patients with DM1 [4]. Epitope recognition also differs; patients with SPS have Abs recognizing linear epitopes in the N-terminal segment of GAD proteins that are not observed in patients with DM1 [95, 96]; this segment of GAD is exposed during synaptic transmission, but the pathogenic role of these GAD-Abs is unknown [97]. SPS patients also have conformational GAD-Abs that recognize discontinuous segments of the middle and C-terminal part of GAD65, some of these antibodies block the enzymatic activity of the protein [98]. Although patients with DM1 also have conformation Abs against the middle and C-terminal segments of GAD, they do not block the activity of the enzyme, and the epitope recognition is also different [76, 99]. GAD-Abs isotype is IgG1 in patients with DM1, whereas IgG1, IgG2, IgG3, IgG4, and IgE are detected in patients with SPS [5, 100].

The role of T cells in the pathogenesis of SPS has not been clarified, but activation outside the CNS followed by crossing the blood-brain barrier is possible [4]. The stimuli that trigger the T-cell response are unclear, but viral infections, including West Nile virus, coxsackievirus, and cytomegalovirus, may precede the onset of SPS [101]. Clonal CD4(+) T cells can recognize a derived epitope of the human cytomegalovirus (hCMV) processed by dendritic cells, and show cross-reactivity

with GAD65 and hCMV major DNA-binding protein [102]. This evidence indicates that T cells are involved in the loss of tolerance to GAD enzymes possibly through molecular mimicry, but this remains to be confirmed. T cells are activated in peripheral lymphoid organs, and although some of these cells cross the blood-brain barrier, it is likely that only those T cells reactivated in the CNS remain intrathecal (Fig. 2); patients with SPS and DM1 have T cells showing overlap reactivity to diverse GAD65 epitopes [103–105]; but only lymphocytes from SPS patients seem to produce a mixed Th1 and Th2 response contrasting with the Th1 response in patients with DM1 [104, 106]; Th1 response leads to cell-mediated immunity, whereas the Th2 response through interleukin-4, driven by a group of T-cell clones, facilitates switching of B-cell isotype, which seems to sustain the secretion of oligoclonal bands in the CSF of patients with SPS [104, 107]. Whether T cells mediate damage to the nervous system is unclear in classical and focal/segmental SPS, and it seems more likely to occur in patients with PERM; mice possessing monoclonal T cells against GAD65 can develop encephalomyelitis-like manifestations [108].

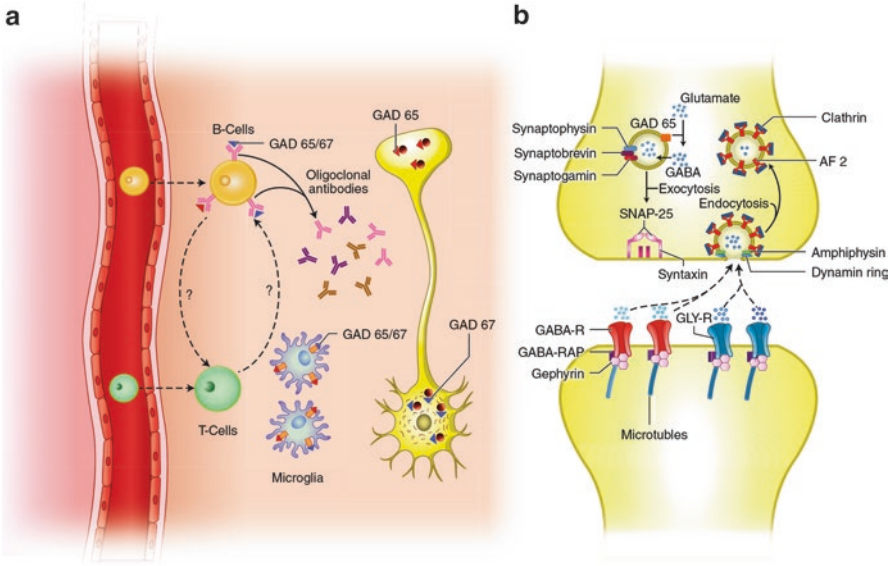

Fig. 2 (**a**) Cellular events occurring in patients with stiff-person syndrome; a set of oligoclonal GAD antibodies is produced, although how the autoimmune response is sustained within the central nervous system is unclear; (**b**) synapsis of inhibitory neurons shows the main molecular targets for antibodies found in patients with stiff-person syndrome spectrum disorders. (From Baizabal-Carvallo J.F. & Jankovic J. Reproduced with permission of BMJ Publishing Group Limited.)

Pathogenic Roles of Other Antibodies: Amphiphysin, Glycine Receptor, and DPPX

Despite the ongoing controversy about the pathogenic role of GAD-Abs, other Abs observed in SPS seem to have a more definitive role in its pathogenesis. Amphiphysin is a presynaptic protein involved in clathrin-mediated endocytosis. These Abs can be internalized into neurons by an epitope-specific mechanism and colocalize with presynaptic vesicular proteins [109]. Experimental studies have demonstrated that exposure to human anti-amphiphysin Abs provokes a depletion of the resting pool vesicles, trapping of releasable vesicular pool proteins at the presynaptic plasma membrane of GABAergic neurons with activation of alternative endocytic pathways [110]. Moreover stiffness and spasms have been induced following intraperitoneal injection of purified IgG Abs against amphiphysin from a patient with SPS and breast cancer, along with encephalitogenic T-helper lymphocytes to disrupt the blood-brain barrier, allowing Abs reaching the CNS [111]. Intrathecal passive transfer of the same type of Abs can also induce anxious behavior in rats [112]; a similar phenomenon has been documented with GAD-Abs (see above section).

By means of cell-based assays, binding of GlyRα1 IgG Abs was shown at 4 °C in controls, whereas antigen endocytosing (modulation) at 37 °C was observed in patients with SPS spectrum disorders [113]. Moreover, immunotherapy has been reported more effective in patients with positive serology to GlyRα1 IgG Abs than in seronegative patients supporting a direct pathogenic role of such Abs [114]. Abs directed against DPPX increase the excitability and action potential frequency of guinea pig and human enteric nervous system neurons; this may explain the typical diarrhea observed in patients with positive DPPX Abs; moreover decreased expression of DPPX and Kv4.2 has been observed in hippocampal neuron exposed to DPPX Abs [54–56]. Other Abs have been detected in patients with SPS spectrum disorders. Abs directed against the postsynaptic GABA-A receptor-associated protein were detected in about 70% of patients with SPS spectrum disorders in a single study [115]. However, this finding has not been yet replicated by other investigators. Abs directed to the GABA-A receptor have been associated with SPS spectrum disorders, some patients with positivity to such Abs, may present with seizures or limbic encephalitis [116]. Patients with SPS-like phenotype have low titers of GABA-A receptor Abs, whereas high titers are related to severe encephalitis [116]. Antibodies against the enzyme 17β-hydroxysteroid dehydrogenase type 4 have been identified in few patients with SPS, but their pathogenic role is unknown [117].

Pathology

There are a relative small number of pathological studies in patients with SPS spectrum disorders. It was initially suggested that neuropathological changes were scarce in patients with SPS, and the clinical features were related to functional impairment of neurons. However, more recent pathological studies have demonstrated loss of cerebellar GABAergic cells, anterior horn cells, and spinal interneurons (Renshaw cells), in some cases associated with perivascular inflammatory changes with cytotoxic T-cell infiltration, microglia infiltration, and gliosis [118, 119]. A reduction up to 70% in the spinal cord density of neurons with central chromatolytic changes has been reported in patients with SPS [120]. Vacuolation of motor neurons in the caudal segments of the spinal cord was demonstrated in a 69-year-old man with SPS and positive GAD-Abs; such vacuoles were lined by a membrane and contained invaginations with cytoplasmic matter [121]. Lipofuscin-containing lysosomes observed by electron microscopy were common in affected cells [121]. Macrophage infiltration along with neuronal cell loss in the dorsal root ganglion was also observed [121]. Affected muscles may show neurogenic atrophy [120]. Perivascular lymphocytic cuffing and parenchymal infiltrates of CD8+ lymphocytes were observed in a patient with paraneoplastic SPS and positive amphiphysin Abs [122].

Diagnosis

Diagnostic Criteria

The diagnosis of classical SPS is based on the presence of insidious onset and progressive course of muscle stiffness in the spinal and proximal lower limbs muscles secondary to co-contractions of agonist and antagonist muscles, with superimposed muscle spasms and increased exteroceptive reflexes, [123]. The diagnosis is supported by neurophysiological demonstration of continuous motor activity in the involved muscles at rest demonstrated by EMG that is usually abolished during sleep, except in advanced cases, as well as other neurophysiological features plus the absence of other neurological disorder that can explain the symptoms (Table 2). The diagnosis is also supported by the presence of high serum titers of GAD-Abs; however, negative serology does not rule out the diagnosis, particularly in patients with focal/segmental SPS; on the other hand, patients with DM1 may have low levels of GAD-Abs that should not be considered supportive of the diagnosis of SPS. In case of suspicion of paraneoplastic SPS or PERM, assessment of amphiphysin or GlyRα1 Abs should be considered, unfortunately the latter is carried out mainly in research laboratories. Secretion of GAD-Abs within the central nervous system strongly supports the diagnosis of SPS spectrum disorders.

Table 2 Criteria for the diagnosis of stiff-person syndrome

Clinical
Gradual onset and slow progression of muscle stiffness
Stiffness is related to persistent contraction of paraspinal, abdominal, and limb muscles
Abnormal postures, including hyperlordosis of the lumbar spine
Stiffness is usually abolished by sleep
Superimposed stimulus-sensitive painful spasms
Dysautonomia
Neurophysiology
Continuous motor unit activity at rest demonstrated by EMG
EMG activity abolished by sleep, peripheral nerve block, spinal or general anesthesia
Altered exteroceptive reflexes and reciprocal inhibition
Exaggerated startle reflex
Normal peripheral nerve conduction
Immunological
High serum titers of GAD65 antibodies
Intrathecal production of GAD65 antibodies

Diagnosis Workup

As previously discussed, the cornerstone of the diagnostic workup in patients with SPS spectrum disorders is the electromyographic (EMG) demonstration of continuous motor unit activity that can be abolished with the administration of benzodiazepine or anesthetics. EMG shows no signs of denervation and peripheral motor and sensory nerve conduction velocity are usually within normal limits. Ultrasonography may be used as a non-invasive method to assess for impaired relaxation of involved muscles but its specificity for the diagnosis of SPS spectrum disorders is probably low [124]. Other abnormalities that can be recorded in neurophysiologic tests include brainstem myoclonus, exaggerated startle reflex, and non-habituating exteroceptive or cutaneomuscular reflexes [125].

For detection of GAD-Abs, radioimmunoassay has 96% sensitivity and 95% specificity when compared with immunocytochemistry [126]. A newly sensitive proximity ligation assay can detect GAD levels as low as 0.65 pg/ml and GAD-anti-GAD immune complexes [127]. Detection of GAD-Abs in the CSF may be important when low levels of serum GAD-Abs are present in a patient with unclear neurological diagnosis and autoimmune endocrine disorder. CSF may show positive oligoclonal bands, but this finding is not specific. It can be assessed with the following formula, GAD-Abs CSF/GAD-Abs serum/albumin (mg/L) CSF/albumin (mg/l) serum, and a result of ≥ 1 supports intrathecal production of GAD-Abs.

Patients with SPS may show positivity for other organ- and non-organ-specific Abs, including antinuclear, anti-smooth muscle, anti-mitochondrial, antithyroid microsomal, anti-thyroglobulin, anti-parietal cell Abs, etc. [128, 129]. Systematic evaluation for underlying cancer is not indicated in patients with classical SPS; however, in male patients or those with predominant upper limb and cervical stiffness, and coexistent neuronal cell-surface Abs, a search for underlying malignancy should be considered [65]. Neuroimaging studies are usually unnecessary in patients with SPS, except in cases displaying signs of encephalomyelitis, where a proportion of cases may show non-specific MRI abnormalities.

Differential Diagnosis

The stiffness observed in patients with SPS spectrum disorders should be differentiated from other forms of hypertonic muscles, such as spasticity, parkinsonian rigidity, tetanus, or dystonia. Spasticity has a different distribution of increased muscle tone, with velocity-sensitive resistance to muscle stretch, not observed in SPS; there is absence of superimposed muscle spasms, lack of associated weakness and pathological reflexes. Moreover patients with spasticity do not show exaggerated, non-habituating exteroceptive or cutaneomuscular reflexes or increased startle reflex as patients with SPS.

Parkinson's disease and other extrapyramidal disorders present with muscle rigidity, a form of hypertonia that is not velocity-sensitive to muscle stretch, and it usually does not lead to abnormal fixed postures as observed in patients with SPS. In patients with early progressive supranuclear palsy (PSP), rigidity predominates in the axial muscles, but the characteristic hyperlordosis of SPS is not seen; other neurological manifestations such a supranuclear ophthalmoplegia and cognitive decline distinguish PSP from SPS. Members of a family affected by spinocerebellar ataxia type 3 have been described with progressive trunk and abdominal muscle stiffness, along with myokymia, painful spasms, and EMG showing continuous motor unit activity [130]. Chronic tetanus can be confused with SPS; however, trismus is more common in the former and the spasms are abrupt in onset and resolution, the syndrome lasts weeks to months, rather than years as it occurs in SPS [131]. Interestingly, a lockjaw has been reported in a patient with SPS and positive GlyRα1-Abs [132].

SPS spectrum disorders should also be differentiated with disorders associated with continuous muscle activity such as myotonia and Isaac's and Morvan's syndrome. Myotonia characterizes by delayed muscle relaxation following a voluntary contraction and it is not present at rest. Moreover, myotonia may be observed with a number of hereditary muscle disorders including dystrophies. Isaac's syndrome is characterized by the presence of spontaneous and continuous motor unit discharges with a high intraburst frequency known as neuromyotonia, often accompanied by stiffness, cramps, fasciculations, and myokymia (irregular wave-like rippling of

muscles or motor unit discharges in doublets or triplets). The latter are not observed in patients with SPS. EMG shows continuous motor activity that persists during sleep (contrasting with SPS) [73]. The distribution of muscle contraction is mostly distal, in contrast with the axial and proximal muscle involvement of SPS. Morvan's syndrome is characterized by the presence of neuromyotonia plus neuropsychiatric features, dysautonomia, and neuropathic pain; it occurs almost exclusively in males, and it is frequently associated with an underlying thymoma [133]. The disorder is caused by the presence of CASPR2 (contactin-associated protein 2) Abs and less commonly due to LGI11 (leucine-rich glioma inactivated 1) Abs, rather than Abs directed to the voltage-gated potassium channel (VGKC) [8, 134].

Treatment

Treatment of SPS spectrum disorders is divided into four main lines of action: (1) suppression of the autoimmune process with immunotherapy, (2) symptomatic control of rigidity, spasms and other neurological, psychiatric and dysautonomic manifestations, (3) tumor removal in case of underlying neoplasm, and (4) rehabilitation and support. As SPS is an uncommon disorder, there are few randomized controlled trials comparing different therapies used in SPS. Therapeutic decisions are mostly based on previous experience coming from isolated cases, small case series, and expert opinion (Fig. 3).

Immunotherapy

Immunotherapy is divided into "first line," used to achieve a relative rapid immunosuppression in order to induce remission, but it can be continued as a maintenance therapy, and "second line," which includes drugs with slower onset of action and possibly less efficacy compared to first-line therapies; however they are easier to administer providing a more sustained benefit.

First-line therapy includes intravenous immune globulin (IVIg), plasma exchanges, and steroids. In a randomized, placebo-controlled crossover study of IVIg vs. placebo in 16 patients with SPS with each therapy provided during 3 months, a significant improvement in stiffness and heightened-sensitivity scale was observed when patients were receiving IVIg, accompanied by a decrease in GAD-Abs titer [135]. This effect may result in improvement in quality of life [136, 137]. The mechanism of action of IVIg may include suppression or neutralization of Abs, inflammatory cytokines, and activated complement; blockade of leukocyte adhesion proteins; restoration of idiotypic-anti-idiotypic networks; and modulation of dendritic cell activity, among others [138]. Potential drawbacks of chronic use of IVIg are side effects (anaphylaxis) and high costs. IVIg has also provided benefit in

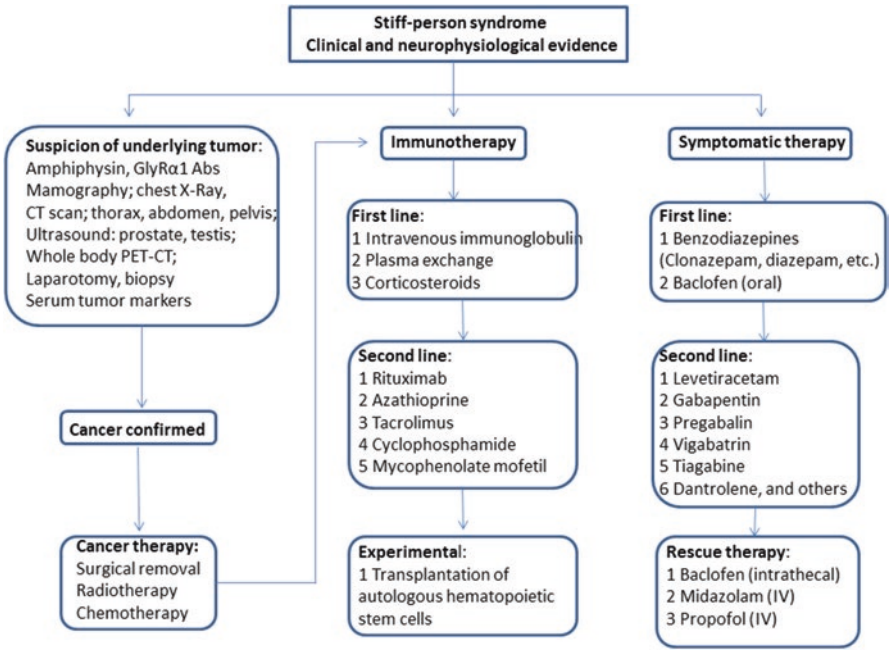

Fig. 3 Algorithm for treatment of stiff-person syndrome spectrum disorders

open trials and isolated reports [139, 140]. Plasma exchange has been used with variable success in patients with SPS spectrum disorders with transient improvements of neurological symptoms observed in 42% of cases in a meta-analysis of 26 patients treated with this therapy [141]. Monthly use of plasmapheresis has been used to maintain initial clinical benefit, but high costs and potential side effects limit this therapeutic strategy [142]. Corticosteroids (oral or pulse IV therapy) can provide variable benefit, but their use should be very cautious in patients with DM1, which is a frequent comorbid disorder in patients affected with SPS.

Second-line therapy includes rituximab, cyclophosphamide, and other immunosuppressants. Rituximab has been used as rescue therapy in patients with SPS with respiratory failure due to severe stiffness of thoracic muscles [143, 144]. However, a randomized placebo-controlled trial in 24 patients with SPS showed lack of improvement of pre-specified 50% in stiffness scores and heightened sensitivity at 6 months [145]. Quality of life improved in both groups at 3 months but not at 6 months suggesting a placebo effect [145]. Relapses are possible following initial response of rituximab [146]. Other immunosuppressants such as azathioprine, tacrolimus, cyclophosphamide, mycophenolate mofetil, and methotrexate have proved benefit in some cases [4, 147]. Transplantation of autologous hematopoietic stem cells provided sustained clinical remission on two patients with SPS, although titers of GAD-Abs remained positive and long-term benefit is unknown [148].

Patients with PERM and GlyRα1-Abs usually benefit with immunotherapy, although relapses are not uncommon [48]; if DPPX-Abs are implicated, a robust response following immunotherapy has been reported [55]. Cerebellar ataxia related to GAD-Abs may benefit from immunotherapy; in this regard corticosteroids and IVIg have shown the best results [69]. Improvement has been reported in one third of cases; subacute onset and rapid treatment initiation are the most accurate clinical predictors of response [71].

Symptomatic Control

Medications with GABAergic effect are the mainstay of symptomatic therapy aimed to decrease the rigidity and superimposed muscle spasms. Benzodiazepines are considered the first-line therapy; among these drugs, diazepam and clonazepam are among the most frequently used and possibly some of the most effective drugs to treat stiffness and spasms in patients with SPS, but clinical trials are lacking [14, 149]. The dose of benzodiazepines can be progressively escalated but are usually limited by side effects such as drowsiness or sedation. Oral baclofen is another potentially effective drug with less side effects that can be used alone or with benzodiazepines in order to achieve a combined effect on GABA receptors: type A (benzodiazepines) and type B (baclofen) [150].

Other medications with GABAergic effect or muscle relaxants have been reported useful in single case reports or small case-series of patients with SPS. Levetiracetam showed benefit in a small blinded crossover trial, but confirmation of its efficacy is needed [151, 152]. Valproic acid, clonidine, vigabatrin, tiagabine, gabapentin, and pregabalin can also be considered [153–156]. Dantrolene, tetrahydrocannabinol, cannabidiol, and other cannabis derivatives available in spray have also been reported useful [157, 158]. The role of all these drugs is not established, but they can be used as "add-on" medications in patients with incomplete response to muscle relaxants or in substitution of benzodiazepines or baclofen in case of prominent side effects from these medications, although they are probably less effective than the former. Evaluation of therapy is usually difficult in patients with SPS due to the fluctuating nature of the disorder and lack of well-validated clinical scales showing reproducibility.

In case of severe nonresponsive muscle spasms, intrathecal baclofen has been used as rescue therapy [159]. Small trials and retrospective studies demonstrated that intrathecal baclofen provides improvement in muscle stiffness evaluated by EMG or clinical assessments [160–163]. The therapy is useful for patients with SPS and PERM, although complete remissions are unlikely [161]. However, several complications may be observed with this therapy, including spasm-induced rupture of the catheter, catheter dislocation causing radicular symptoms, and inaccurate dosage administration due to pump malfunction; catheter dysfunction can be associated with severe symptomatic withdrawal and death [160, 161, 164]. Propofol and

midazolam administered intravenously are other drugs that can be used as rescue therapy in cases of frequent or prolonged muscle spasms (spasmodic storm) and dysautonomia complicated with respiratory failure, rhabdomyolysis, and myoglobinuria with acute renal lesion [165, 166]. Botulinum toxin injections may be helpful particularly in patients with focal SPS that show a lack of response to oral pharmacological therapies or to control pain in cervical muscles and reduce stiffness in facial muscles [167, 168]. Spinal cord stimulation has been reported to improve the spasms observed in cases of stiff-limb syndrome, but confirmatory studies are lacking [169].

Psychiatric manifestations, particularly anxiety, panic attacks, and phobias, can improve with the use of benzodiazepines; clinicians should be aware that medications commonly used for depression and anxiety such as serotonin-norepinephrine reuptake inhibitors and tricyclic antidepressants may potentially aggravate the motor symptoms of SPS and they should be avoided whenever possible in patients with SPS [170].

Tumor Removal

Treatment of underlying cancer is of paramount importance in cases where such condition is detected. In patients with paraneoplastic SPS, tumor removal is usually mandatory before starting immunotherapy. Dramatic improvement has been documented in patient with positive GlyRα1-Abs following removal of underlying thymoma and immunotherapy [171, 172].

Special Situations, Anesthesia, and Pregnant Patients

There is concern that patients with SPS exposed to inhalational (volatile) agents and neuromuscular blockers may suffer prolonged and severe hypotonia following anesthesia which may lead to respiratory failure with prolonged intubation. Although some patients are anesthetized with volatile agents and neuromuscular blockers develop this side effect [173], it is believed that this side effect results from the enhancement of GABA action on synapsis by medications with agonist GABAergic effect [174]. Due to this potential side effect, the TIVA technique which does not require neuromuscular blockage has been proposed for SPS patients [175]. Regional anesthetic techniques may also be used to avoid exposure to muscle relaxants [174]. Total intravenous anesthesia instead of inhalation anesthetics with close monitoring of respiratory drive and use of electrical nerve stimulator when neuromuscular blockers are used are also recommended [176].

There are few reports of patients with SPS during pregnancy, for these patients, medication adjustments to use low levels of benzodiazepines or baclofen can be tried to reduce side effects [177, 178], while immunotherapy should be withheld.

Some patients may experience transitory improvement during pregnancy [179]. Cesarean section is the preferred method of delivery but there are reports of successful vaginal delivery [178, 179]. Although newborn babies may have positive GAD-Abs until the age of 24 months, they do not seem to develop SPS phenomenology [180].

Prognosis

Patients with SPS spectrum disorders have a chronic evolution despite treatment with immunotherapy and muscle relaxants. The quality of life has been investigated in 24 patients with SPS through the Short-Form Health Survey (SF-36) showing decreased (worse) scores compared with normal controls; a strong correlation of SF-36 scores with the extent of the disease and degree of depression was observed [181].

Conclusions

SPS spectrum disorders are a group of conditions characterized by CNS disinhibition that causes muscle stiffness, spasms, and enhanced exteroceptive reflexes. Although major advances have shown that amphiphysin and GlyRα1 Abs are likely pathogenic in patients with paraneoplastic SPS and PERM, respectively, this has not been the case with GAD-Abs, and the pathogenesis of classical and focal/segmental SPS is still to be clarified; as the disorders partially respond to IVIg, plasmapheresis, and rituximab, an autoimmune humoral response is likely. Further studies should help to elucidate the pathogenesis of this group of disorders to develop better treatment strategies.

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Central Nervous System Vasculitis and Related Diseases

Hiroshi Mitoma, Mario Manto, and Jordi Gandini

Abstract Central nervous system (CNS) vasculitis can be classified into (1) primary vasculitis limited to the CNS and (2) secondary CNS vasculitis which is either a manifestation of systemic vasculitis or a complication associated with certain specific pathologies, such as infection (viral, bacterial, fungal), neoplasm, drug, connective tissue diseases (e.g., systemic lupus erythematous, rheumatoid arthritis, Sjögren syndrome), and sarcoidosis. Isolated vasculitis limited to the CNS is known as primary angiitis of the CNS (PACNS). PACNS is associated with various clinical neurological symptoms. There is no specific test for PACNS at present, rendering the diagnosis difficult. The diagnosis is currently based on the following features: (1) exclusion of other pathologies associated with CNS vasculitis, such as infection, neoplasm, drug, and systemic disease-mediated vasculitis; (2) identification of segmental arterial wall narrowing "vessel beading," followed by poststenotic dilatation; and (3) the pathological findings of granulomatosis, lymphocytic, or acute necrotizing patterns. The first line of induction therapy is the combination of corticosteroids and cyclophosphamide, followed by maintenance therapy using mycophenolate mofetil, azathioprine, and methotrexate. Involvement of larger or proximal cerebral vessels requires aggressive treatment. The diagnosis of secondary vasculitis in the CNS implies the identification of exogenous agents or conditions. Withdrawal/removal of the agents or treatment of the underlying conditions often leads to improvements in vasculitis. Due to phenotypic overlap between the various CNS vasculitis, a comprehensive work-up is often required.

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_21

Keywords Central nervous system vasculitis · Primary angiitis of the central nervous system · Primary central nervous vasculitis · Systemic vasculitis · Neuropsychiatric SLE · CNS sarcoidosis

Introduction

Vasculitis is defined as an inflammation of the blood vessel wall [1]. Various conditions can be complicated by the development of vasculitis in the central nervous system (CNS). Vasculitis of the CNS can be classified into (1) primary angiitis of the CNS (PACNS), in which the clinical manifestation is not associated with known etiologies, and (2) secondary vasculitis of the CNS associated with systemic vasculitis or certain etiologies, such as infection (viral, bacterial, fungal), neoplasm, drug, connective tissue diseases (systemic lupus erythematous, rheumatoid arthritis, Sjögren syndrome), and sarcoidosis (Table 1) [1–5]. Calabrese et al. (1997) defined secondary CNS vasculitis as "a result of an exogenous influence" [2]. In secondary vasculitis, removal of the specific inciting agent or control of the associated systemic disease can relieve CNS vasculitis-induced symptoms [2].

CNS vasculitis is characterized by infiltration of immune cells around the wall of the blood vessels, leading to its destruction [3–6]. The inflammatory reaction commonly elicits two opposite changes: thickening of the vessel wall with stenosis, resulting in ischemic lesions, and weakness of the vessel wall, causing dilatation

Table 1 Classification of CNS vasculitis

Primary angiitis of the central nervous system (PACNS) or primary central nervous vasculitis (PCNV)
Secondary angiitis of the central nervous system
CNS manifestations as part of systemic vasculitis
Takayasu vasculitis
Giant cell vasculitis
Polyarteritis nodosa
Microscopic polyangiitis
Eosinophilic granulomatosis with polyangiitis
Granulomatosis with polyangiitis
Behçet's syndrome
Vasculitis associated with connective tissue diseases
Systemic lupus erythematous (neuropsychiatric SLE, NPSLE)
Rheumatoid arthritis
Sjögren syndrome
Vasculitis associated with sarcoidosis (CNS sarcoidosis)
Infection-associated vasculitis
Cancer-associated vasculitis
Drug-associated immune complex vasculitis
Others

and rupture [6]. These inflammatory, ischemic, and hemorrhagic lesions result in focal CNS symptoms or meningeal signs, depending on the lesion site, extension, and time course of inflammation. Therefore, although various etiologies can induce CNS vasculitis, there is usually an overlap in the clinical manifestations and imaging findings. The aim of this chapter is to review specific clinical manifestations and describe the therapeutic strategies in each etiology from the overlapping features. Behçet's syndrome is discussed in Chap. 22 by Uygunoglu and Askel, and Sjögren syndrome is discussed in Chap. 23 by Annunziata.

Primary Angiitis of the Central Nervous System (PACNS)

In 1922, Harbitz described isolated vasculitis limited to the CNS as an unknown form of angiitis in the CNS [7]. The term granulomatous angiitis of the CNS was used earlier, based on the histopathological findings [2]. More recently, the terminology of PACNS [8] or primary central nervous vasculitis (PCNV) [9] has been applied. There is a consensus on the terms PACNS and PCNV, since both reflect the predominant site of pathology, and this terminology is not limited by histopathological features [2].

PACNS is a rare disease. However, since the publication of the diagnostic criteria of PACNS proposed by Calabrese and Mallek (1988) [10], several reports have been published. Especially, Salvarani et al. reported details of the clinical and pathological features based on the management of 101 patients at the Mayo Clinic [9]. Although PACNS is associated with CSF inflammatory changes, MRI abnormalities, and segmental arterial wall narrowing, these features are not specific, and there is a lack of specific autoimmune markers [9, 11–13]. Thus, to differentiate PANCS from secondary CNS angiitis, a careful work-up is necessary. The identification of multiple clinical and pathological subtypes is suggestive of a PACNS spectrum [3].

Clinical Manifestations

Typical clinical features Patients show variable and multiple clinical manifestations. PACNS equally affects males and females of all ages, with the onset of the disease typically in the late 40s [12, 13]. The excellent review by Salvarani et al. (2007) highlighted the presence of the following common clinical features: headache (63%), altered cognition (50%), hemiparesis (44%),visual symptoms (42%; visual field defect in 21%), aphasia (28%), nausea and vomiting (25%), ataxia (19%), seizures (16%), diplopia (16%), dysarthria (15%), and blurred vision or decreased visual acuity (11%) [9]. These features develop insidiously and show a slow and progressive course, although hyperacute onset also occurs in some patients [14]. Stroke and transient attacks are common, affecting 30–50% of the patients [2, 9]. Status epilepticus as the presenting manifestation has been reported [15].

Constitutional symptoms, such as weight loss and night sweat, are less commonly observed in PACNS [11]. Instead, these symptoms are suggestive of underlying systemic vasculitides [11].

Heterogeneous nature PACNS is a variable syndrome that appears to consist of several subsets of heterogeneous diseases [9]. Notably, the response to immunotherapy differs among these subtypes, confirming the heterogeneous nature of autoimmunity in PACNS. Table 2 shows the major differences in autoimmunity among the subtypes.

Epidemiology

Salvarani et al. (2007) reported that the incidence of PACNS was 2.4 cases per 1,000,000 person-years [9].

Table 2 Subtypes of PACNS

Subtype	Clinical/para-clinical features	Therapeutic response
Angiography negative, biopsy positive	Cognitive impairment Greater CSF inflammatory abnormalities Angiography: Negative Meningeal and parenchymal enhancing lesions on MRI Histopathology: Granulomatous lesions	Good
Meningeal enhancement in MRI	Predominantly males Cognitive impairment Prominent leptomeningeal enhancement Angiography: Negative Histopathology: Granulomatous lesions	
Intracranial or subarachnoid hemorrhage	Predominantly female Intracranial or subarachnoid hemorrhage on MRI Histopathology: Necrotizing features	
Amyloid-β-related cerebral angiitis	Older age, predominantly males Cognitive impairment Enhanced meningeal lesions on MRI Pathology: Granulomatous lesions with β-amyloid deposition in vessel walls	Usual
Spinal cord involvement	Spinal cord symptoms associated with cerebral manifestations Enhanced spinal cord on MRI Angiography negative Histopathology: Necrotizing features	
Rapidly progressive	Rapidly progressive clinical course Bilateral, multiple infarctions on MRI and multiple vessel lesions on angiogram	Poor, often fatal outcome

Modified from Beuker et al. (2018) [4]

Pathogenesis

Pathology The characteristic pathology of PACNS is transmural inflammation with subsequent destruction of the vessel wall [16]. The inflammatory pathological pattern is granulomatous inflammation, lymphocytic cellular infiltration, and acute necrotizing vasculitis [16, 17]. The prevalence of these pathological patterns according to one review article was 56% for granulomatous inflammation, 20% for lymphocytic cellular infiltration, and 22% for acute necrotizing vasculitis [6].

Granulomatous inflammation is characterized by a vasculocentric destructive mononuclear infiltration with well-formed granulomas and multinucleated giant cells [6]. Granulomatous inflammation is sometimes associated with β-A4 amyloid deposition in the vessel walls, which is termed amyloid-β-related angiitis [18]. Interestingly, the association of granulomatous inflammation with meningeal enhancement and cerebral amyloid angiopathy has also been described [18, 19]. In lymphocytic vasculitis, lymphocytes infiltrate with variable numbers of plasma cells, histiocytes, neutrophils, and eosinophils [6]. Necrotizing vasculitis shows acute inflammation and acute transmural fibrinoid necrosis of the vessel walls, both of which cause dilatation and rupture of the vessels [6].

Cerebral amyloid angiopathy (CAA) is sometimes associated with angiocentric inflammatory cell infiltration, which is also known as CAA-related inflammation [20]. The variability in the severity of infiltration, from little or no inflammation to marked granulomatous angiitis, is based on the extent or intensity of the inflammatory reactions to amyloid β [20]. Thus, amyloid β-related angiitis is indistinguishable from CAA-related inflammation [21].

Immunology Although an immune-mediated pathogenesis has been proposed [4], the underlying mechanism remains uncertain. The predominant infiltration of CD45R0+ T cells around the vessel wall [22] suggests the involvement of memory T cells in the process of antigen-specific autoimmunity in the wall of arteries [4]. Molecular mimicry to preceding infectious agents, such as varicella-zoster virus and mycoplasma, has also been suggested [23–25].

Diagnosis

The development of PACNS is associated with the appearance of various neurological features, but so far, there is no specific diagnostic test for this condition [5]. Other etiologies can mimic the clinical manifestations, inflammatory CSF findings, and MRI abnormalities [5]. As a result, it is sometimes difficult to establish the diagnosis and provide the appropriate therapy. Limaye et al. (2018) reported that the diagnosis in their series of patients was eventually confirmed in only 39% of the suspected cases [5]. At present, the characteristic features of PACNS on angiography are commonly used for the diagnosis. However, it should be noted that even

these PACNS-related angiographic findings may occur in patients with normal biopsy findings and also in other conditions [9]. Positive findings in brain biopsies are still considered the "gold standard" [4, 9]. Calabrese and Mallek (1988) proposed the following diagnostic criteria for cerebral vasculitis [10]: (1) history of acquired but otherwise unexplained neurological deficits, (2) presence of classic angiographic or histopathologic features of angiitis within the CNS, and (3) no evidence of systemic vasculitis or any other disorder that could cause or mimic the angiographic or pathologic findings. Almost 10 years later, Birnbaum et al. (2009) proposed modified diagnostic criteria [11]. They introduced two levels of diagnosis of PACNS: "definite" when the presence of vasculitis is confirmed by brain biopsy and "probable" in the presence of high probability of angiographic abnormalities together with CSF and MRI abnormal findings, in the absence of pathological confirmation [11].

Laboratory tests In the majority of patients, blood tests show normal leukocyte count and CRP level. Furthermore, serological tests are often negative for disease-specific antibodies [9]. Detection of antinuclear antibody, rheumatoid factor, or antineutrophil antibody suggests underlying autoimmune systemic vasculitis [4]. Tests for isolation of bacteria, fungi, and viruses are needed to rule out infectious-related vasculitis [4].

CSF examination CSF examination typically shows mild lymphomonocytic pleocytosis and high protein level, occasionally with the presence of oligoclonal bands and immunoglobulins (IgGs) in 80–90% of the patients [4, 9]. Furthermore, abundant presence of IL17-producing CD4+ T cells in the CSF was reported [26]. The absence of inflammatory changes instigates considerations for differential diagnosis [4].

MR imaging MRI is a highly sensitive imaging modality [4]. More than 90% of the PACNS patients show abnormalities on MRI [9, 11]. Infarction is the most common type (about 50%) [9]. Typical MRI findings are multifocal and bilateral T2 or FLAIR sequence abnormalities in the cortical and subcortical structures and in the deep gray-white matter [3, 9]. Stenosis and dilatation of multiple large and small vessels sometimes co-exist with ischemic lesions [4]. The ischemic lesions show variable patterns: a large-artery distribution, a branch-artery distribution, and a small-artery pattern showing multiple subcortical infarctions [9]. Hemorrhages occur in the subarachnoid space and intraparenchyma [2] in about 10% of the patients [9]. Gadolinium enhancement is observed in the parenchymal lesions and the leptomeninges in about 40% of the patients [9, 19].

Although these changes are not specific to PACNS [4], high-resolution contrast-enhanced MRI (HR-MRI) may help establish a definite diagnosis of PACNS. HR-MRI exhibits a characteristic finding around the walls of intracranial vessels, thickening, and wall enhancement [27–29].

Clues to suspect PACNS Although there are no specific neurological symptoms in PACNS, the following clinical presentations should lead to suspect PACNS: (1) cerebral ischemia affecting different vascular territories with distribution over time, in association with the appearance of inflammatory changes in the CSF; (2) subacute or chronic headache with cognitive impairment or chronic aseptic meningitis; and (3) exclusion of post-infection chronic meningitis and neoplastic disorders in such patients [3].

Digital subtraction angiography and MR angiography A typical feature of PACNS is "vessel beading," which is characterized by multiple areas of narrowing and dilatation or multilocular occlusions of intracranial vessels [30]. Other features include fusiform arterial dilatation, collateral circulation, and delayed contrast enhancement [3]. On the other hand, imaging studies have shown lack of long stenotic segments, complete occlusion, and microaneurysms in PACNS [31]. In this regard, conventional angiography is more sensitive in detecting these changes compared with MR angiography, but noninvasive MR angiography is recommended for follow-up studies during the disease course [4]. Black-blood angio-MRI (arterial wall imaging) may be helpful for the diagnosis of vasculitis. The typical pattern is characterized by thickening and enhancement of vascular wall. A typical enhancement pattern was described for several diseases of vessel wall, so this kind of imaging may become crucial for the differential diagnosis [27, 29, 32]. The forthcoming improvements of this technique should represent an opportunity to make a noninvasive diagnosis of cerebral vasculitis.

It should be noted that the "vessel beading" feature is not specific and can be observed in various noninflammatory vasculopathies, such as arteriosclerosis, following radiation, neurofibromatosis, atrial myxoma, infections, and vasospasms [17, 33, 34]. Furthermore, such inflammatory changes are invisible on standard angiography, especially in small arteries of <500 μm diameter [35]. The false-positive and false-negative nature of angiography highlights the need for a comprehensive assessment of both the CSF and MRI findings in addition to confirmation with brain biopsy [9, 12, 36].

Nuclear medicine Positron emission tomography with [11C]-PK11195 can detect vascular inflammation in patients with an important cerebral vasculitis. This technique should be utilized in patients with inconclusive imaging results [37].

Brain biopsy Brain biopsy and histopathological examination are the gold standard procedures for a definite diagnosis of PACNS. Typical findings include transmural inflammation and injury of the vessel wall [16]. The inflammatory changes encompass granulomatous inflammation, lymphocytic cellular infiltration, and acute necrotizing vasculitis [16, 17].

Due to the segmental distribution of vascular inflammation, the false-negative rate in brain biopsy is 53–74% [38, 39]. Thus, targeting the affected area based on imaging findings is recommended, in order to increase the chance of sampling from

the affected area and reduce the false-negative rate [38]. However, a definite pathological evidence cannot be made in some cases even in patients with CSF abnormalities and positive MRI and angiography findings [9]. Several groups have recommended to obtain biopsies from the nondominant frontal lobe with overlapping leptomeninges especially in cases where abnormal lesions are difficult to access surgically [21, 40].

Differential Diagnosis

RCVS The imaging findings in reversible cerebral vasoconstriction syndrome (RCVS) are similar to those of PACNS, including infarction and hemorrhage on MRI and multifocal segmental vasoconstriction on angiography [41–43]. However, RCVS is known to affect 40–50-year-old females more frequently [41–43], and the symptoms include sudden and recurrent attacks of severe headache (usually thunderclap) coupled with seizures associated with or without neurological deficits [41–43]. The attack is sometimes triggered by exposure to vasoactive medications, migraine, hypertension, and eclampsia or during the postpartum period [41, 43]. CSF examination in RCVS is mostly negative [3]. On MRI, cerebral infarctions exhibit characteristic distribution: in the superficial border zone, watershed regions, associated with cortical subarachnoid hemorrhage and lobar intracerebral hemorrhage [44]. The vasoconstriction also shows the following specific features: (1) it is rarely associated with normal parenchymal imaging in PACNS, whereas severe vasoconstriction can occur with or without parenchymal lesions in RCVS [3], (2) dissection or intact unruptured aneurysms are more frequently seen in RCVS than in PACNS [44], (3) RCVS shows short stenosis without or with only moderate wall thickening (these vessel wall changes are well observed in black-blood MRI) [45, 46], and (4) vasoconstriction often shows partial or even complete improvement after 12-week follow-up [43]. Since treatment with corticosteroids could worsen RCVS, it is important to distinguish RCVS from PACNS before treatment [4].

PRES Posterior reversible encephalopathy syndrome (PRES), first described by Hinchey et al. in 1996 [47], is a neurotoxic syndrome occurring due to the susceptibility of the posterior circulation to variations in blood pressure. It is classically characterized by a symmetric parieto-occipital white matter edema. Clinical features of PRES range from headache, altered mental status, seizures, and loss of vision to even loss of consciousness. The term describes potentially reversible imaging findings and symptomatology that is shared by a diverse group of diseases, such as hypertension, glomerulonephritis, eclampsia, preeclampsia, and drug intoxication.

The pathogenesis of PRES is unknown. Several authors postulate a vascular mechanism: a severe hypertension leads to failed autoregulation and endothelial vasogenic edema or vasoconstriction. This causes brain ischemia and subsequent

vasogenic edema. Cytotoxic agents can cause PRES in a normotensive environment. In this case, the disruption of brain-blood barrier is suspected to be a major pathogenic factor [48]. The most commonly described MRI abnormalities in PRES consist of symmetrical cortical and subcortical hyperintense signals on T2 and FLAIR-weighted MR images in the parieto-occipital lobes of both hemispheres. These areas are frequently hypointense on corresponding T1-weighted MR images and have a decreased attenuation on CT scans. Similar areas of altered signal intensity can also be seen in other locations such as the frontal lobes, cerebellum, brainstem, and basal ganglia [49]. The central variant of PRES with an isolated involvement of the basal ganglia and brainstem sparing the subcortical white matter was found in 4% of cases in the study by McKinney et al. There may be a mild mass effect with sulcal effacement and mild contrast-enhancement in some cases.

A rapid withdrawal of the triggers factors can induce a complete recovery and avoid further complications. Aggressive blood pressure management, withdrawal of immunosuppressive treatments, and delivery (in case of eclampsia) can rapidly improve the clinical status [50].

Arteriosclerosis Arteriosclerosis is associated with multiple infarctions and "beading vessel" appearance, similar to PACNS [3, 4]. However, arteriosclerosis more commonly affects older people with various risk factors, such as diabetes mellitus and hypertension, and shows no inflammatory changes on CSF examination. The infarction is specifically limited to a single vascular territory, and imaging studies show focal artery stenosis with calcification and irregularity [3, 4].

CADASIL Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an autosomal-dominant disease characterized by the development of infarction in early adulthood [51]. Thus, the clinical course of CADASIL overlaps with that of PACNS [3, 4, 51]. Affected patients also present with headache, migraine, psychiatric and cognitive disturbances, and motor disorders in early adulthood, which are induced by infarctions. However, history of strokes or dementia is specifically found in first-degree relatives of patients with CADASIL, and the infarctions are found bilaterally in the external capsule and anterior temporal lobe [3, 4, 51]. Definite diagnosis of CADASIL is established by histopathological identification of deposits of granular osmiophilic material on skin blood vessels and mutations in *notch 3* on genome examination [51].

Fabry disease Fabry disease (FD) is a multiorgan X-linked lysosomal storage disorder caused by mutation in the GLA gene, which encodes for the α-galactosidase A (α-GalA) enzyme [52–55]. Defective enzymatic activity leads to intracellular accumulation of glycosphingolipids, mainly globotriaosylceramide (Gb3) [56]. In brain tissue, Gb3 storage primary occurs in endothelium and vascular smooth muscle cells [57], but it is also responsible for glial deposition and neuronal ballooning in cortical regions and deep nuclei [58–60]. CNS symptoms can vary from very mild to severe, including manifestations related to acute cerebrovascular events and posterior circulation alterations, along with neuropathic pain, cochleovestibular

dysfunction, and a various degree of cognitive impairment and psychiatric symptoms [61]. The involvement of the peripheral nervous system (PNS) is mainly expressed by neuropathic pain [62, 63]. In addition, patients undergo a large spectrum of extraneurological signs: cardiomyopathy, progressive renal failure, and skin changes (in particular angiokeratomas) [62–64]. MRI is the reference imaging technique to evaluate possible brain damage in FD. Modern conventional MRI allows for a proper and accurate estimation of the pattern and the degree of brain alterations in patients with or without a clinical evidence of focal neurological impairment.

The radiological pattern is characterized by four pathological findings:

- Ischemic stroke [61]
- White matter hyperintensities [65]
- Dilatative arteriopathy of the vertebrobasilar system [66]
- Unilateral or bilateral hyperintensities of the thalamic pulvinar on unenhanced T1-weigthed MRI [67]

The definitive diagnosis is established through the dosage of the enzymatic activity of alpha-galactosidase [68]. Enzyme replacement therapy (ERT) is available since 2001 for FD and is the only therapeutic option till present [64, 69, 70].

MELAS MELAS syndrome is a rare inherited disorder of intracellular energy production that typically presents prior to age 40. Eighty percent of cases are associated with the mitochondrial transfer RNA A3243G point mutation [71, 72]. As with other mitochondrial disorders, MELAS syndrome displays maternal inheritance with relative penetrance based on the degree of heteroplasmy. Thus, phenotype expression and severity of disease are related to the proportion of mutant DNA and residual activity of respiratory chain complexes in mitochondria within each cell. The disease activity, penetrance, and severity can be correlated to clinical presentation and characteristic imaging findings [73, 74].

MRI is the gold standard for diagnosis and monitoring. MRI reveals global changes in gray-white differentiation, multifocal cortical and subcortical lesions that cross vascular territories, and varying degrees of generalized cerebral and cerebellar atrophy. Encephalomalacia may also be present in previously affected areas. Diffusion-weighted MRI shows a cortical ribbon-like high-intensity signal consistent with diffusion restriction. During attacks, restricted diffusion may be present in the cortex, subcortical white matter, and basal ganglia.

Advanced MRI sequences aid in the diagnosis of MELAS syndrome and provide additional insight into the mechanism of disease. Multivoxel MRS demonstrates a lactate peak in acutely abnormal brain regions associated with oxygen deficiency of cells in affected cortical areas. Several studies describe the potential value of nonanatomic MRI techniques—such as contrast-enhanced perfusion studies, blood-oxygen-level-dependent imaging, oxygen extraction fraction, arterial spin labeling, and MRS—in identifying metabolic changes within lesion foci in the acute setting [75–78].

Intravascular lymphoma (IVL) IVL is a subtype of extranodal diffuse large B-cell lymphoma, characterized by intravascular proliferation of lymphoma cells with a predilection for the CNS and skin. IVL is an important differential diagnosis of primary vasculitis of central nervous system (CNS) [79].

Treatment

Large-scale prospective and randomized clinical trials are still lacking probably due to the small number of patients diagnosed with PACNS. Thus, current treatment recommendations are based on retrospective studies, small series, or case reports. Table 3 lists the main side effects of drugs. Immunosuppressive agents increase the risk of infectious disease and malignancy.

Induction therapy There are two options with regard to the initial induction therapy. In the first option, corticosteroids are used initially. Steroids should be switched to cyclophosphamide if the patient shows resistance to glucocorticoid therapy or develops a relapse [5]. The second option is a combination therapy of corticosteroids and cyclophosphamide [4]. In a retrospective cohort study, Salvarani et al. reported the therapeutic benefits of the combination therapy with corticosteroids and cyclophosphamide compared with corticosteroids in monotherapy [13]. In addition, a multicenter cohort study showed that the combination therapy significantly improved daily life disabilities in most patients [80]. Thus, the combination therapy of corticosteroids and cyclophosphamide is recommended, especially in patients with severe and rapidly progressive symptoms [4]. The commonly used dosage regimen is either as oral prednisolone at 1 mg/kg body weight/day or as intravenous methylprednisolone at 1000 mg daily for 3–5 days [4]. Although the

Table 3 Side effects of immunosuppressive drugs

Treatment	Therapeutic effects	Side effects
Glucocorticoids	T-cell depletion, eosinophil apoptosis, macrophage dysfunction	Cushingoid syndrome, obesity, osteoporosis, dyslipidemia, hyperglycemia, cutaneous atrophy, glaucoma, cataract, gastric ulcer
Cyclophosphamide	DNA impairment and proapoptotic effect	Bladder toxicity, gonadal toxicity, malignancy, leukopenia, pulmonary toxicity
Azathioprine	Interference with de novo purine synthesis	Bone marrow suppression, gastrointestinal intolerance, hepatotoxicity, nausea, lethargy, indigestion, asthma worsening, malignancy
Mycophenolate mofetil	Inhibition of guanine synthesis	Bone marrow suppression, gastrointestinal intolerance, malignancy, teratogenicity
Methotrexate	Interference with metabolism of folic acid	Renal dysfunction, myelosuppression, mucositis, hepatotoxicity
Rituximab	B-cell depletion	Fever, pruritus, nausea, urticaria/angioedema bronchospasm, hypotension, arrhythmias, lymphopenia

latter scheme has been used in cases showing rapidly progressive clinical course, evidence is lacking for any advantage at present. The cyclophosphamide dosage used in the corticosteroid-cyclophosphamide combination therapy is either as an oral dose of 2 mg/kg body weight/day for 3–6 months or as intravenous pulse of 750 mg/m^2 body surface area/month for 6 months. However, relapse has been reported in a few patients [4]. Salvarani et al. (2007) also described relapses in 26 of their 101 patients [9].

Several other reports also highlighted the therapeutic benefits of methotrexate, azathioprine, and mycophenolate [5]. Recent studies have also shown that biological agents (rituximab and tumor necrosis factor-α blockers) have equal therapeutic benefits to induce remission when used with the corticosteroid-cyclophosphamide combination therapy [81–83]. The combination regimen of rituximab and cyclophosphamide was also recommended recently [4]. However, large-scale studies are needed to assess the therapeutic benefits of biological agents.

Maintenance therapy The aim of maintenance therapy is to maintain remission and to avoid disease worsening and relapses. Disease-modifying therapies include mycophenolate mofetil (1–2 g/day), azathioprine (1–2 mg/kg body weight/day), and methotrexate (20–25 mg/week) [5]. One cohort study of 14 children with PNSCA showed that maintenance therapy of mycophenolate mofetil was associated with less adverse events compared with azathioprine [84]. Maintenance therapy is usually administrated for 6–12 months based on the response to immunotherapies [5]. However, there are no convincing data on the effectiveness of this form of therapy at present. Careful clinical observations and periodic MR imaging and CSF examinations (every 3–4 months during maintenance therapy) are recommended for the assessment of autoimmunity [5].

Prognostic Factors

Old age, large and extensive infarctions, presence of cognitive symptoms, and involvement of large or proximal cerebral vessels are associated with a high mortality rate in PNSCA [9, 13]. Meningeal gadolinium enhancements on MRI and seizures are associated with increased risk of relapse [13]. Patients with these poor prognostic factors require a more aggressive treatment [12].

Systemic Vasculitis

Systemic vasculitis is classified based on the affected blood vessels into large-vessel vasculitis, medium-vessel vasculitis, and small-vessel vasculitis [1]. Depending on the affected vessels, each type is associated with specific CNS involvement. Constitutional symptoms and other organ involvements can provide clues for diagnosis (Table 4).

Table 4 Systemic vasculitis

Affected vessels	Disease	Neurological manifestations	Specific other organ involvement	Autoimmune markers
Large arteries	Takayasu vasculitis	Headache, confusion, cognitive impairment, stroke, meningitis, seizure	Affecting before 50 years; hypertension, cardiovascular (aneurysm)	No specific autoantibodies
	Giant cell vasculitis	Headache, blindness	Affecting older than 50 years	No specific autoantibodies
Medium-to-small arteries	Polyarteritis nodosa	Mostly subclinical	Hypertension, multisystemic, especially the skin (tender, erythematous nodules, purpura, ulcer) and mononeuritis multiplex	No specific autoantibodies
Small-to-medium vessels	ANCA-associated vasculitis			
	Microscopic polyangiitis	Ischemic infarction and intracranial hemorrhage, hypertrophy of leptomeninges, inflammation of the pituitary, spinal cord involvement	(rapidly) glomerulonephritis, pulmonary hemorrhage/ interstitial pneumonia, mononeuritis multiplex, skin; leukocytoclastic angiitis	MPO-ANCA
	Eosinophilic granulomatosis with polyangiitis		Asthma, mononeuritis multiplex, skin; leukocytoclastic angiitis	MPO-ANCA, eosinophilia and high IgE
	Granulomatosis with polyangiitis		Chronic sinusitis, otitis media and mastoiditis. Lung nodulus, (rapidly) glomerulonephritis, mononeuritis multiplex, skin; leukocytoclastic angiitis	PR3-ANCA

Takayasu Arteritis

Takayasu arteritis (TA) is characterized by granulomatous lesions in the aorta and/or its major branches [1]. Cell-mediated autoimmunity, CD8+ T cells, and autoantibodies, such as anti-endothelial antibodies, are assumed to be involved in the autoimmune inflammation.

About 20% of patients with TA show CNS involvement at disease onset [85]. The most common neurological symptoms are severe headache, confusion, cognitive impairments, stroke, meningitis, and seizure [85]. Aggressive treatment is recommended to ensure satisfactory outcome [85] and includes the combination of corticosteroids/methotrexate/cyclophosphamide followed by the combination of corticosteroids/methotrexate/tocilizumab [85].

Giant Cell Arteritis

Giant cell arteritis (GCA) affects large vessels, the aorta, and/or its major branches [1]. Histopathologically, GCA is characterized by infiltration of giant cells, lymphocytes, and macrophages, often forming granulomatous lesions. A decrease in blood flow induced by inflammation of the ophthalmic artery can induce ischemic anterior optic neuropathy leading to monocular blindness. Headache is a common symptom. Corticosteroids are used for the treatment of GCA [86].

Polyarteritis Nodosa

Polyarteritis nodosa (PAN) is a necrotizing vasculitis of medium or small arteries [1]. PAN is not associated with glomerulonephritis or antineutrophilic cytoplasmic autoantibody (ANCA) [1]. The presence of defective Treg and predominance of Th1 suggest the involvement of cell-mediated autoimmunity [87].

Although clinically evident CNS involvement is rare, the presence of small infarcts and hemorrhagic lesions has been reported in 2–28% of the cases [88]. Corticosteroids represent a first line of therapy, although the combination of corticosteroids and immunosuppressants has been used in cases with critical organ involvement [88].

ANCA-Associated Vasculitis

ANCA-associated vasculitis (AAV) is a systemic small-vessel vasculitis [1] and is divided into three subtypes: microscopic polyangiitis (MPA), eosinophilic granulomatosis with polyangiitis (EGPA), and granulomatosis with polyangiitis (GPA). Two histopathological features characterize AAV: (1) necrotizing vasculitis affecting small-to-medium vessels and (2) granulomatosis with inflammatory cell infiltration [89, 90]. ANCA plays a pathogenic role in the development of angiitis [89] (Fig. 1). ANCA, which is produced following molecular mimicry to bacteria, binds to antigens—PR3 (proteinase 3) and MPO (myeloperoxidase)—on the neutrophils. This binding results in the activation of neutrophils, leading to their transmigration to the vessel wall, degranulation, and formation of neutrophil extracellular traps (NETs) followed by their apoptosis and necrosis [89, 90]. NETs are further augmented through the complement pathways. Monocytes are also recruited at sites of acute inflammation [90].

CNS involvement is observed in less than 15% of patients with AAV. Typical CNS involvements are ischemic infarction and intracranial hemorrhage, hypertrophy of leptomeninges, inflammation of the pituitary (with hypophyseal hypofunction), and spinal cord involvement [90]. MPO-ANCA is positive in MPA and EGPA, and PR3-ANCA is positive in MPA. The combination of corticosteroids and cyclophosphamide is the first line of therapy [91].

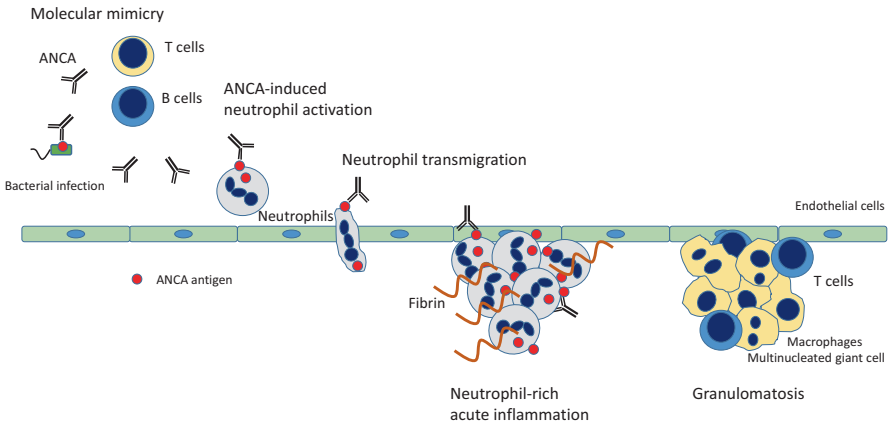

Fig. 1 Possible mechanisms underlying ANCA-associated vasculitis

Neuropsychiatric SLE (NPSLE)

Lupus erythematosus (SLE) is a chronic relapsing-remitting autoimmune disease [92]. Systemic inflammation elicits injury of various organs, including the skin, kidneys, heart, lungs, and nervous system. SLE with involvement of the latter is termed neuropsychiatric SLE (NPSLE) [92, 93]. Diverse and specific autoimmune mechanisms underlying NPSLE have been clarified recently. Antiphospholipids autoantibodies, which are generated through systematic autoimmunity, elicit a hypercoagulable state, which in turn could lead to thrombosis in the CNS. On the other hand, peripheral lymphocytes infiltrate the blood-CSF and blood-brain barriers where they secrete various proinflammatory cytokines, which trigger inflammation within the CNS. The recruitment of other immune cells and parenchymal infiltration accelerate the inflammatory process, leading to pathogenic autoantibodies- and microglia-mediated degeneration and demyelination. These inflammation-related processes are sometimes enhanced by the leakage of complements from the systemic circulation, leading to worsening of tissue damage. It is considered that vasculitis, which is characterized by focal narrowing and beading, is a rare cause for focal and diffuse symptoms in NPSLE [92, 93].

Clinical Manifestations

Various neuropsychiatric clinical features (NPs) appear during the course of this chronic disease. The first described feature was coma in 1875 [94]. In 1979, Kassan and Lokshin proposed the classification criteria for these divergent NPs [95]. In their classification, they stressed the need to include “a change from a prior state” as NP [95]. Thus, the secondary clinical features elicited by the associated infections and therapy-related events were excluded. They defined eight manifestations for NPs,

Table 5 American College of Rheumatology case classification of neuropsychiatric manifestations in the central nervous system (CNS) and peripheral nervous system (PNS)

CNS		
Focal manifestations	Diffuse manifestations	PNS
Cerebrovascular disease	Depression	Cranial neuropathy
Seizures	Cognitive dysfunction	Autonomic neuropathy
Aseptic meningitis	Mood and anxiety disorders	Mononeuropathy (single/multiplex)
Movement disorders	Psychosis	Polyneuropathy
Myelopathy	Acute confusional state	Plexopathy
Demyelinating syndrome	Headaches	Myasthenia gravis
		Acute inflammatory demyelinating polyradiculoneuropathy

which include seizure, disturbance of consciousness, disturbance of mental function, neuropathy, motor disorders, movement disorders, meningitis, and encephalitis [95].

In 1999, the American College of Rheumatology (ACR ad hoc committee) recognized 19 wide-range NPs [96] (Table 5). NPs are classified into three categories: CNS focal, CNS diffuse, and peripheral nervous system (PNS) manifestations [96]. In this classification, the CNS focal manifestations can be attributed to responsible anatomic structures, while the responsible structures are difficult to identify in the CNS diffuse manifestations [92].

The reported prevalence of NPs manifestations ranges from 12 to 95% of SLE patients [93], and the reported prevalence of each clinical feature is as follows: (1) cognitive impairment (6.6–80%), mood disorders (7.4–65%), anxiety disorders (6.4–40%), headache (12.2–28%), psychosis (0.6–11%), and acute confusion state (0.9–7%) for the CNS diffuse manifestations; (2) seizures (7–20%), cerebrovascular diseases (8–15%), demyelinating syndrome (0.9–2.7%), aseptic meningitis (0.3–2.7%), and movement disorders (0.9%) for the CNS focal manifestations; and (3) mononeuropathy (single or multiple) (0.9–6.9%) and polyneuropathy (1.5–5.4%) for the PNS manifestations [93]. Clinical evidence suggest that the severity of NPs correlates with the SLE morality rate [97]. The wide variability in the prevalence of clinical manifestations according to the 1999 ACR nomenclature probably reflects differences in the patients' selection criteria and the lack of consensus in the definition of impairment and selection of cognitive tests [92, 94].

Pathogenesis

Systemic autoimmune inflammation Antiphospholipid antibodies (aPL Abs) include lupus coagulant, anticardiolipin Abs, and anti-β2-glycoprotein I [93]. aPL Abs are associated with antiphospholipid syndrome (APS), which is characterized

by thrombosis of the venous and arterial circulation [93]. APS is associated with SLE (10–44% of SLE patients), although it can occur in isolation [98]. The risk of stroke in less than 50 years of age is eightfold higher in aPL Abs-positive individuals than in aPL Abs-negative individuals [99]. Among SLE patients, those who are positive for aPL Abs are twice more likely to develop NPSLE as aPL Abs-negative patients [100]. Thus, stroke-related NPs are attributed to aPL Abs-induced infarcts.

Leakage or peripheral lymphocytes infiltration The brain is a privileged immune site due to the presence of tightly regulated blood-brain barrier (BBB), blood-CSF barrier (choroid plexus), and meningeal barrier (Fig. 2) [92, 93]. However, the functions of these barriers are impaired in NPSLE [92, 93].

Two mechanisms have been assumed. The first encompasses leakage through the above barriers [93]. For example, the presence of serum albumin within the CNS suggests serum leakage into the brain parenchyma [101]. Leakage may be due, at least in part, to mechanical disruption caused by cerebrovascular diseases

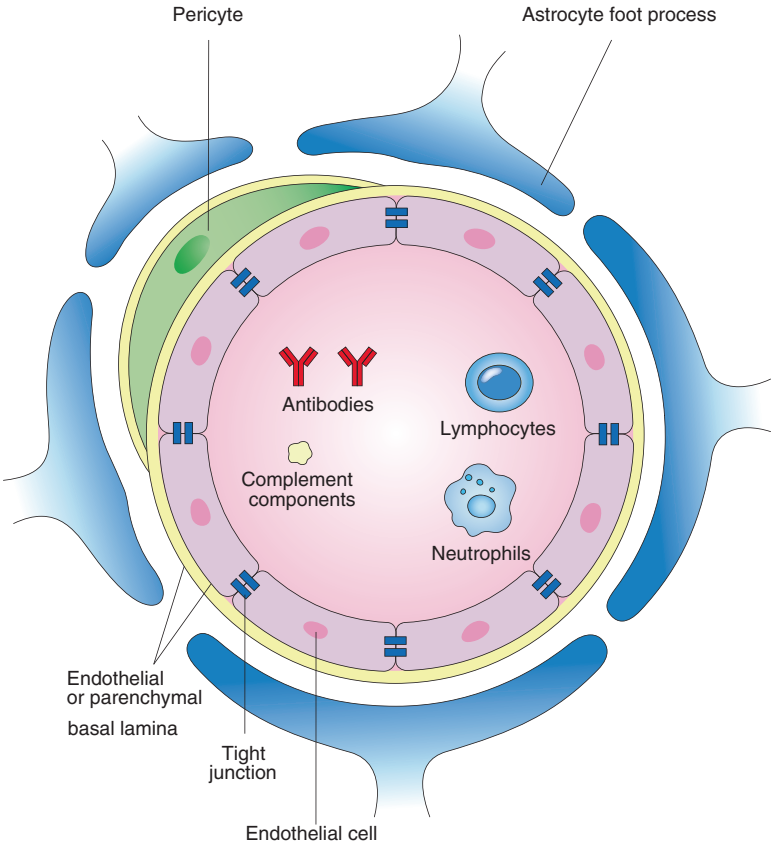

Fig. 2 Structure of blood-brain barrier

[102–106]. It has been assumed that increased permeability of these barriers allows the entry of pathogenic serum-derived autoantibodies into the parenchyma [93].

The second mechanism is inflammatory cell infiltration through these barriers [92, 93]. Direct evidence for peripheral lymphocyte infiltration through the choroid plexus into the CNS was demonstrated in MRL/lpr mice [107]. Furthermore, CD3+ T cells, including effecter CD4+ cells and inactive CD8+ cells, have been identified in many brain areas [108–110]. Differentiation of CD4+ cells into T follicular helper cells (Tfh) was also confirmed [110]. These studies suggest that CD4+ cells increase the production/release of proinflammatory cytokines upon the detection of brain-derived self-antigens, which facilitates the recruitment of other immune cells and parenchymal infiltration [111]. Consistent with this notion, deletion of CD4+ T cells attenuates CNS manifestations in mice [112].

Specific proinflammatory cytokines Clinical studies have confirmed the presence of proinflammatory cytokines secreted by infiltrating lymphocytes through the analysis of CSF samples obtained from NPSLE patients [92, 93]. For example, high intrathecal levels of IL-6, which is linked to diffuse NPSLE, have been found in NPSLE patients with acute confusion and psychosis [113–115]. IL-6 stimulates B cells to produce autoantibodies and promotes differentiation of Th17 cells [93].

On the other hand, IFN-α is implicated in NPSLE, similar to its role in SLE [116, 117]. High levels of IFN-α were found in NPSLE patients with depression, psychotic features, confusion, seizures, and neurological defects [116, 117]. In vitro studies have also shown that antibodies harvested from CSF of SLE patients induced IFN-α production [118]. Furthermore, inhibition of IFN-α resulted in improvement of psychiatric abnormalities in mice [119]. In SLE, IFN-α secreted by plasmacytoid dendritic cells stimulates B-cell proliferation and facilitates the development of memory CD8+ cells and Th17 cells, and tissue damage accelerates its production in a positive feedback fashion [120].

High levels of IL-8 and IFN-γ also correlate with the development of NPSLE [121–123]. IL-8, which is secreted by activated endothelial cells, chemoattracts neutrophils and IFN-γ, which are secreted by Th1 cells, and activates microglia [93].

Pathogenic actions of autoantibodies Various autoantibodies (Abs) have been identified in NPSLE. The pathogenic roles of anti-NMDA Abs, antiribosomal P Abs, and anti-aquaporin 4 Abs have so far been confirmed both in in vitro and in vivo preparations.

Anti-double-stranded DNA (dsDNA) Ab is characteristic for SLE. A subset of anti-dsDNA Abs cross-reacts with NR2A and NR2B subunits of NMDA receptors (NMDARs) [124, 125]. The binding of these antibodies to NMDARs induces exaggerated calcium entry, which leads to excitotoxicity and, finally, cell death [124, 125]. The CSF titer of anti-NMDAR Abs is higher in patients with active diffuse NPSLE than those with focal NPSLE or noninflammatory CNS patients [126, 127]. After experimental disruption of BBB in BALA/c mice, administration of sera containing anti-NMDAR Abs obtained from SLE patients or immunization with the NMDAR-derived DWEYS pentapeptide elicited NPSLE-like symptoms [128, 129].

Antiribosomal P protein Abs are found in up to 46% of SLE patients [130], and high titers of these Abs are associated with depression, seizure, coma, traverse myelopathy, and aseptic meningitis [131, 132]. The antigens are represented by the carboxy-terminal regions of three ribosomal P proteins (P1, P2, P0) [133]. Antiribosomal P protein Abs cross-react with the P peptide on the neuronal surface P protein (NSPA) [134]. NPSA is ubiquitin ligase that regulates NMDARs and synaptic transmission/plasticity [134]. Passive transfer of antiribosomal P protein Abs elicited depression and memory impairment in mice [135]. Consistent with these results, immunoreactivities were observed in the limbic system, hippocampus, cingulate cortex, and piriform cortex, which are brain areas involved in these affective and cognitive functions [132].

Aquaporin 4 is a water channel expressed on astrocyte foot processes that surround brain-blood vessels [136]. In neuromyelitis optica (NMO), the binding of these Abs to aquaporin 4 activates both the complement and antibody-dependent cellular cytotoxicity (ADCC), leading to inflammation and demyelination [136–138]. In this regard, there is an overlap between NMO and SLE [139]. One study reported that demyelination was observed in 27% of the patients [139]. However, it should be acknowledged that some patients with anti-aquaporin 4 Abs-positive SLE showed no neurological symptoms, including NMO, for many years [140].

Association of anti-endothelial Abs, anti-microtubule-associated protein 2, and anti-suprabasin antibodies has been identified [141–144]. However, these autoantibodies have not yet been thoroughly characterized.

Complement activities Local activation of the complement cascade within the CNS has been reported in some patients [145]. High levels of C3 of systemic origin have been found in patients with acute confusion [115, 150].The presumed mechanisms responsible for the high levels of complements include intrathecal production and leakage from systemic circulation [93].

Microglia-mediated autoimmunity In NPSLE, it is assumed that resident microglias, which are activated by type I interferons, are the predominant immune cells, acting as potent cytokine producers [93]. Consistently, suppression of microglial activation attenuated NPSLE-like symptoms in MRL/lpr mice [119, 146, 147]. Microglia are also involved in synaptic pruning [92, 93]. Thus, the binding of anti-neuronal Abs (e.g., anti-NMDAR Abs) is recognized by the complement factor C1q, leading to the production of C3b, and C3b expressed on the dendrite is recognized by IFN-α-activated microglia, which results in the elimination of the synaptic spines [92].

Diagnosis

CSF studies CSF studies often show normal cell count and protein levels in many patients [148]. However, inflammatory changes are sometimes observed in CSF. Identification of high IgG ratio and oligoclonal bands helps in the suspicion of NPSLE [148].

MRI It should be acknowledged that about half of the patients with NPSLE have normal MRI, especially those with diffuse syndromes, such as headache, mood disorders, and psychiatric disease [149, 150]. The other half of the patients show four types of MRI abnormalities, vascular abnormalities affecting large and small blood vessels, inflammatory-type lesions, and myelopathy [149].

Large-vessel disease elicits large infarct with vascular territory distribution, involving both gray and white matters [149]. Large-vessel infarcts, which elicit stroke, occur in 13–15% of patients aged 35–40 years [151]. The middle cerebral artery is the most commonly affected [149]. Infarction is not common as the initial event in SLE. However, there have been accumulating case reports of this type of manifestation, suggesting that infarction as the initial event can occur in young females (age, 31.7 ± 8.5 years), especially in the vertebrobasilar territory [152]. Since infarction in NPSLE requires the combination of immunotherapy and anticoagulation for early secondary stroke prevention, clinicians should suspect the occurrence of infarction in the vertebrobasilar territory in young females as potentially the initial manifestation of SLE [152].

Small-vessel disease encompasses lacunar stroke, recent small subcortical infarcts, microbleeds, and brain atrophy [149]. White matter hyperintensity is the most widespread type of small-vessel disease in NPSLE [149]. The two most frequent MRI abnormalities in NPSLE are multiple small-vessel lesions (30–75%) and cortical atrophy (15–20%) [151, 153, 154]. Notably, white matter hyperintensities correlate with not only cerebrovascular disease but also cognitive impairment and seizures [151].

NPSLE shows other types of stroke phenotypes on MRI, including transient cerebral ischemia, infarction, intraparenchymal hemorrhage, subarachnoid hemorrhage, and cerebral venous thrombosis [152]. These stroke types are generally attributed to thromboembolic occlusion induced by SLE-related hypercoagulable state, which correlate with high titers of autoantibodies to phospholipids [155].

Less frequently, the MRI finding in some NPSE patients is inflammatory-type lesions [92, 149]. The reported prevalence of these types of lesions is 5–10% of the patients [156]. The inflammatory-type of lesion exhibits the following specific features on MRI: hyperintensity in T2 and FLAIR, medium- or large-sized, involving the gray and white matters, with some showing contrast enhancement or diffusion restriction, without vascular territory distribution, nor clinical features of clinical and radiological features of infarction [149]. Notably, the presence of these lesions correlates with low complement levels and reversibly relieved by immunotherapy using corticosteroids [149]. These results suggest that the inflammatory-type of lesion is probably caused by inflammation-induced injury, which include disruption of the BBB, high titers of pathogenic autoantibodies, activation of the complement system, and the induction of proinflammatory cytokines [92].

Myelopathy occurs in 1–5% of the patients and is a severe condition known to elicit paralysis, sensory loss, and sphincter dysfunction [149, 157]. In half of the patients with myelopathy, myelopathy appears within 5 years of diagnosis with SLE [158]. SLE myelopathy is transverse myelopathy across one level of the spinal cord,

rather than longitudinal myelopathy involving more than four levels of the spinal cord [157]. Transverse myelitis shows swelling and focal enlargement on MRI. Enhancement is absent or poor and patchy in the most active lesion [149].

Treatment

Treatments for thrombosis Lifelong anticoagulation with warfarin is the first line of therapy for antiphospholipid-related thrombosis, using international normalized ratio (INR) of 2.5–3.0 [159]. Statins are simultaneously used to protect against endothelial cell activation secondary to antiphospholipids [160]. In patients with NPSLE and catastrophic antiphospholipid syndrome, pulse corticosteroids, intravenous immunoglobulin, and/or plasmapheresis are recommended [161].

Principles in immunotherapies for SLE Gatto et al. (2019) concluded that "corticosteroids have been the mainstay of treatment of patients with SLE for 60 years" [162]. Fava et al. (2019) added support to this: "high dose or 'pulsed' corticosteroids are important to rapidly ablate the autoimmune response in life or organ threatening manifestations in SLE" [163]. Although the duration of treatment and dosage of corticosteroids for severe manifestations have not been established [164], pulsed therapy using intravenous methylprednisolone (200–1000 mg intravenously daily for 3 days) has been recommended along with cyclophosphamide or mycophenolate mofetil for induction therapy [163]. Importantly, there is no consensus on oral corticosteroid maintenance therapy [165, 166]. Oral corticosteroid might not be necessary for the control of severe lupus manifestations [167]. On the other hand, it has been reported that a dose of 10–20 mg/day can elicit cardiovascular events and any dose above 6 mg to induce organ damage in 50% of the patients [168, 169].

Immunotherapies for NPSLE Oral prednisolone or, in severe cases, intravenous methylprednisolone has been used as the first line of induction therapy in NPSLE [170, 171]. Schwartz et al. (2019) recommended the use of high dose of corticosteroids combined with cyclophosphamide, mycophenolate mofetil, and azathioprine and stressed that "the specific choice of the steroid-sparing agent is based on the clinician's assessment of disease severity and their clinical experiences" [93].

Only one randomized controlled trial study of 32 patients with severe NPSLE compared the effects of monthly intravenous cyclophosphamide infusion versus bimonthly intravenous methylprednisolone, following induction treatment with intravenous methylprednisolone [172]. The results showed better response and outcome in patients treated with cyclophosphamide [172]. Another study of 13 patients with psychosis symptoms confirmed the effectiveness of 6-month combination induction therapy of oral prednisolone and oral cyclophosphamide followed by maintenance therapy using azathioprine [173]. As in the above studies, cyclophosphamide was widely used for induction and maintenance therapies for NPSLE [163].

However, cyclophosphamide has been lately replaced with less toxic agents, such as mycophenolate mofetil or rituximab [163]. One study examined the efficacy of rituximab during the refractory period in 10 patients [171]. After treatment with corticosteroids followed by certain immunosuppressants, these patients still showed diffuse CNS manifestations. Treatment with rituximab resulted in rapid improvement in these manifestations.

Rheumatoid Vasculitis

Rheumatoid arthritis (RA) is a chronic, progressive, systemic, inflammatory disease in which joints are the primary target. The rate of occurrence of cerebral vasculitis in RA patients is 1–8%. Cerebral vasculitis is usually associated with prominent extra-articular manifestations and minimal joint inflammation [174]. The clinical manifestations include headache, hemiplegia, partial epilepsy, cranial nerve involvement, visual impairment, cognitive impairment, confusion, and altered consciousness.

MRI shows hyperintense lesions in T2-weighted images [175]. Rheumatoid vasculitis usually responds to glucocorticoid therapy. Azathioprine [176], intravenous immunoglobulin [177], and cyclophosphamide [178, 179] are proposed in patients with corticosteroid-resistant vasculitis.

CNS Sarcoidosis

Sarcoidosis is a common granulomatous disease, affecting the lungs, heart, and skin [180]. On the other hand, the rate of CNS involvement is estimated to be 5% of cases. The basal meninges, cranial nerves, hypothalamus, and pituitary glands are the most frequently affected regions, from the early stages of the disease [180]. Granulomatous lesions sometimes elicit space-occupying focal signs [180]. Notably, the pathological findings include perivascular and vascular infiltration of meningeal and cerebral vessels by granulomatous lesions, but clinically ischemic attacks are rare and subclinical [180]. Corticosteroids form the main therapy [180], though infliximab and mycophenolate mofetil have been used in nonresponders [181].

Infection-Associated Vasculitis

Various pathogens have been reported to induce CNS vasculitis [3, 4] (Table 6). In most cases, the pathogens invade the endothelium, leading to its destruction [182]. However, infection-induced immune reactions have also been reported to elicit vessel wall damage in some cases [182].

Table 6 Main etiologies of infection-associated vasculitis

Virus: Varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, hepatitis C virus, human immunodeficiency virus
Bacteria: Syphilis, *Borrelia burgdorferi*, *Mycobacterium tuberculosis*, and various types of bacteria
Rickettsiae: Rocky mountain spotted fever, typhus
Fungi: Aspergillus, *Coccidioides*

Varicella-zoster virus (VZV)-associated vasculitis causes vascular damage, including aneurysm and dissection, cerebral/spinal cord infarction, and cerebral hemorrhage [183–185]. Angiography often shows segmental constriction, often with poststenotic dilatation [179]. The affected arteries can be both the large and small arteries (50%), large arteries only (37%), or the small arteries alone (13%) [185]. Diagnosis of VZV-vasculitis is based on the detection of anti-VZV IgG antibody and amplified VZV DNA [177–179]. Administration of intravenous acyclovir at 10–15 mg/kg body weight three times daily for a minimum of 14 days is recommended [185].

Hepatitis C virus (HCV) can elicit inflammation of vascular wall through two different mechanisms: recurrent cryoglobulin precipitation with complement activation and direct induction of innate mechanism of complement activation [186].

The clinical expression is polymorphic: fatigue, depression, and cognitive impairment are often reported. Stroke episodes, transient ischemic attacks, and lacunar infarction have also been described [187].

Several authors recommend an aggressive immunosuppressive therapy (plasmapheresis, intravenous methylprednisolone followed by oral prednisone, cyclophosphamide, and rituximab). The antiviral therapy can be delayed for 2–4 months [188].

Three scenarios have been described in human immunodeficiency virus (HIV)-related vasculitis. First, due to the existing immunodeficiency, concurrent vessel infection by other pathogens (e.g., par-VZV, CMV, and EBV) can occur in some patients [189]. The second scenario is a direct invasion by HIV. This type of HIV-associated vasculitis is rare, with an estimated incidence of 1% [189, 190]. CNS vessels are one of the targets.

Treponema pallidum invades the CNS early in the course of syphilis [191]. In most cases, *T. pallidum* is destroyed by appropriate antibiotics. However, *T. pallidum* can persist in the CNS without neurological symptomatic manifestations of neurosyphilis in some cases, which is followed by the stage of early meningeal syphilis (within 1 month), meningovascular syphilis (within 5–12 years), general paresis, and tabes dorsalis (15–25 years) [191]. Meningovascular syphilis is characterized by widespread endarteritis, causing thrombosis and infarction [191]. The most commonly affected arteries are the cerebral artery, mainly the middle cerebral artery and its branches, and their involvement elicits hemiparesis, hemianesthesia, homonymous hemianopsia, and aphasia. The recommended treatment for neurosyphilis is 18–24 MU of intravenous aqueous penicillin G daily, either as a continuous infusion or divided every 4 h, for 10–14 days [192].

Conclusion

CNS vasculitis is a group of severe but treatable conditions. The clinical manifestations can be polymorphic and aspecific. The knowledge of the pathogenesis of these disorders is crucial to make a correct diagnosis and to choose the appropriate treatment.

Although various different etiologies elicit CNS vasculitis, there is often an overlap in the clinical manifestations and MRI/angiography findings. Therefore, even in the presence of "segmental vascular constriction" on angiography, clinicians should suspect various etiologies, from primary to secondary vasculitis. The notion of "divergent etiologies and overlapping phenotypes" suggests that inflammatory destruction of the vessel wall is a final common pathway in some CNS autoimmunity disorders. With the exception of ANCA, the pathogenic process of autoimmunity remains unclear. Further studies are needed to explore the roles of innate and adaptive immune cells in invasion of the vessel wall.

Regarding the treatment of CNS vasculitis, there is no international consensus. Further trials are necessary to establish an optimal therapeutic approach.

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Behçet's Syndrome and the Nervous System Involvement

Ugur Uygunoglu and Aksel Siva

Abstract Behçet's syndrome (BS), a distinct disease with orogenital ulceration and uveitis known as the triple-symptom complex, is an idiopathic chronic relapsing multisystem vascular-inflammatory disease of unknown origin. The neurological involvement of BS is termed as neuro-Behçet's syndrome (NBS). Direct neurological involvement of BS may be classified into two forms: (1) parenchymal (p-NBS) and (2) vascular involvement. p-NBS with the rate of 75% among neurological involvements of BS usually presents with an acute-subacute brain stem syndrome. Headache, cranial neuropathy, dysarthria, ataxia, and hemiparesis are the most prominent symptoms. The most common areas affected in p-NBS are the mesodiencephalic junction (MDJ), pons, and medulla oblongata. MDJ lesions tend to extend upward to involve the diencephalic structures and downward to involve the pontobulbar region which is the most common radiological finding observed in p-NBS. The only drug that has been shown to be effective based on the Class IV evidence is infliximab for the treatment of p-NBS.

Keywords Behçet's syndrome · Parenchymal neuro-Behçet's syndrome · Cerebral venous sinus thrombosis · Bagel Sign · Infliximab

Introduction

Behçet's syndrome (BS) was originally described in 1937 by the Turkish dermatologist Hulusi Behçet as a distinct disease with orogenital ulceration and uveitis known as the triple-symptom complex. BS is an idiopathic chronic relapsing multisystem vascular-inflammatory disease of unknown origin [1]. Due to the lack of specific laboratory, radiologic, or histologic findings of BS, accurate diagnosis of BS depends on clinical features. According to International Study Group (ISG)'s classification for a diagnosis, recurrent oral ulcerations plus two of the following are

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_22

required: (a) recurrent genital ulcerations, (b) skin lesions, (c) eye lesions, and (d) positive pathergy test [2]. Given that the BS affects many organs and systems implying a "syndrome" rather than "disease," we will use the term Behçet's syndrome (BS) in this chapter [3].

Epidemiology

While BS is more prevalent in the regions along the "Silk Road," prevalence tends to increase in Western countries over the past few years due to the increased migration from some of these countries to Western countries. The estimated prevalence of BS is variable across Europe, being low in the northern part of Europe compared to the south [4]. The prevalence of BS in Turkey is between 20 and 421 in 100,000 with the highest rate across the Silk Road, giving a high rate in the Anatolia. These findings support the environmental hypothesis on BS pathogenesis [5]. Another finding supporting the environmental hypothesis is that no patient was diagnosed as BS in Hawaii where the population of Japanese (genetically susceptible) is high [6]. On the other hand, the higher prevalence of BS among Turkish immigrants living in Germany compared to native Germans, and the lower rates of this syndrome in Germany compared to Turkey, suggests that genetic influences are stronger than environmental factors in the disease etiology [7]. Furthermore, the positive family history up to 12% and a sibling risk ratio ranging from 11.4 to 52.5 support the genetic hypothesis of BS similar to complex gene disorders [8].

Around 50–80% of BS patients along the "Silk Road" carry HLA-B51, whereas the frequency of this allele is ~25% in the general population. However, in the regions where the BS is uncommon, the HLA-B51 positivity ratio is similar to the general population, and this distribution of HLA-B5 and its HLA-B∗51 subtype positivity differing among the countries may give a clue of disease origin [9]. The positivity of HLA-B51 was significantly higher in the hospital-based studies, so it can be argued that the HLA-B51 is probably related not with the disease itself but with its severity [10].

The usual onset of BS is in the third or fourth decade of life. However, onset in children has also been reported, although this is rare [11]. While BS shows equal frequency between each sex, males have a more severe disease course. Interestingly, there is no relationship between age at immigration and the risk of BS [12].

Clinical Manifestations of BS

Clinical manifestations of BS are highly variable among the patients.

Mucocutaneous Symptoms

Mucocutaneous manifestations in BS are common and include oral and genital ulcerations, papulopustular skin lesions, erythema nodosum (EN)-like skin lesions, pathergy reaction, and other rare skin lesions. Recurrent aphthous stomatitis is the hallmark of the syndrome. Aphtous lesions are usually small, round, or oval painful [13]. External genital ulcers usually occur on the scrotum in men and on the labia in women. They are deep and very painful and leave scars, producing an objective sign even in the absence of active lesions [14]. The pathergy phenomenon is a nonspecific hypersensitivity reaction of the hyperirritable skin seen in BS and is produced by inserting a 20-gauge needle into the dermis of the forearm (skin pathergy test). The reaction is considered positive if a papule or pustule is formed at the site of the puncture within 48 hours [2].

Ocular Involvement

Ocular involvement is one of the most disabling complications of BS causing progressive vision loss in half of the patients if not treated properly [15]. Anterior uveitis, posterior uveitis, cells in the vitreous on a slit-lamp examination, and/or retinal vasculitis are the primary clinical features, but optic neuritis may also be present in extremely rare instances [16].

Gastrointestinal Involvement

The most common symptoms indicating gastrointestinal involvement are right upper quadrant abdominal pain, diarrhea, and gastrointestinal bleeding, respectively. Any part of the gastrointestinal tract, especially the distal ileum and cecum, may have ulcers, and at times it may be difficult to distinguish inflammatory bowel disease from BS [17].

Arthritis

Nonerosive, nonmigrating, oligoarticular involvement of large joints, especially the knees, ankles, and wrists, is reported in 35–50% of patients and resolves spontaneously within a few weeks [18].

Neurological Involvement in BS

The neurological involvement of BS is termed as neuro-Behçet's syndrome (NBS). While there is no biomarker for NBS, the diagnosis is mainly based on the clinical and neuroimaging features. NBS is not included in the current criteria of International Study Group. Considering the rate of 5–10% neurological involvement among BS patients and taking into account the increased atypical presentation of neurological involvement especially over the last years, the importance of NBS is increasing [19, 20]. Therefore, ISG criteria need to be reevaluated in terms of neurological involvement. The criteria of NBS can be summarized as: "The occurrence of neurological symptoms and signs in a patient who meets the International Study Group Criteria for BS, when those symptoms/signs are not otherwise referable to any other known systemic or neurological disease, or treatment thereof, and in whom objective abnormalities consistent with NBS are evident either on neurological examination or MRI, or upon cerebrospinal fluid (CSF) analysis [19]."

The neurological involvement may be observed either directly or indirectly which is due to the complications of systemic involvement of BS or related with the drugs used in BS [21]. Neurological involvement developing indirectly includes increased intracranial pressure secondary to superior vena cava syndrome and cerebral emboli secondary to cardiac involvement. Central nervous system (CNS) neurotoxicity caused by cyclosporine and peripheral neuropathy secondary to thalidomide or colchicine use are neurological complications associated with BS treatment [21].

Direct neurological involvement of BS may be classified into two forms: (1) parenchymal (p-NBS) and (2) vascular involvement.

p-NBS

p-NBS with the rate of 75% among neurological involvements of BS usually presents with an acute-subacute brain stem syndrome characterized by headache, cranial neuropathy, dysarthria, ataxia, and hemiparesis as the most prominent symptoms [19]. While the headache is also the cardinal symptom of vascular involvement, differentiation of the neurologic type should be done cautiously together with the MRI features as the long-term treatment differs between these two types of neurological involvement [22]. Regarding the clinical phenotype p-NBS, it may be classified as four subtypes similar to multiple sclerosis (MS): (1) single attack, (2) relapsing form, (3) secondary progressive, and (4) primary progressive [23]. One of the main clinical differences between p-NBS and MS is that patients with the relapsing form of MS usually recover well, while those with the relapsing form of p-NBS do not [24]. The other difference between MS and NBS relies on laboratory findings: while CSF cell counts are typically within normal limits and oligoclonal bands are present in MS, pleocytosis is frequently observed, and oligoclonal bands are rarely detected in p-NBS [25].

Besides clinical features of p-NBS, MRI patterns are of utmost importance of distinguishing the p-NBS from the other disorders mimicking p-NBS (Fig. 1). The most common areas affected in p-NBS are the mesodiencephalic junction (MDJ), pons, and medulla oblongata. MDJ lesions tend to extend upward to involve the diencephalic structures and downward to involve the pontobulbar region which is the most common radiological finding observed in p-NBS (Fig. 1) [26]. Brainstem lesions extending into the diencephalic and basal ganglia during acute disease may exert mass effects caused by vasogenic edema and thus resemble tumors. Some tumefactive lesions have been confused with primary or metastatic tumors, but only a few were located in structures other than the brainstem and deep hemispheric structures such as the frontoparietal or temporal lobe or the cerebellum [27].

Out of the brainstem, spinal cord involvement is also observed in p-NBS. A long segment myelopathy occurs in most cases, which mimics neuromyelitis optica spectrum disorder (NMOSD) and myelin oligodendrocyte glycoprotein (MOG) antibody-associated disorders (MOGSD) [28–30]. However, the recently described "Bagel Sign" pattern of spinal cord involvement in BS may be helpful for

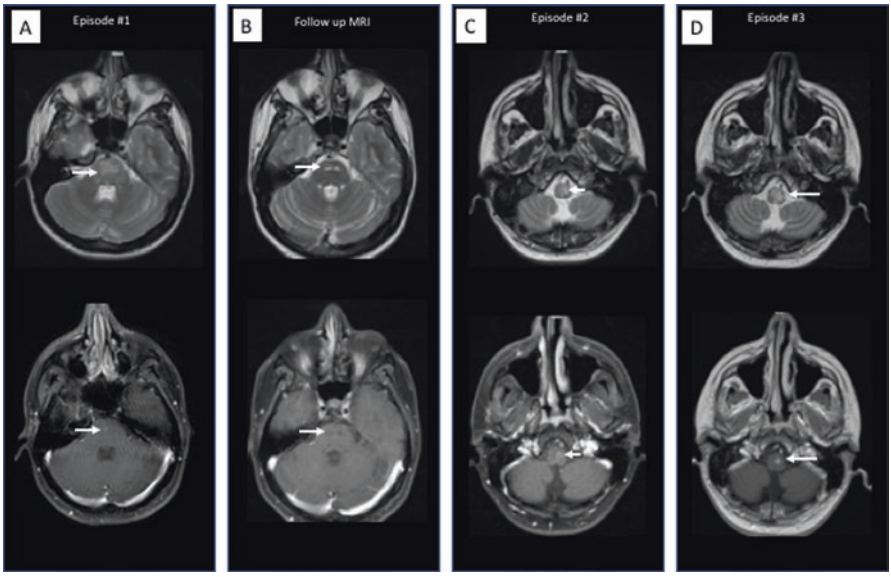


Fig. 1 Cranial magnetic resonance imaging patterns in Behçet's syndrome. (**a**) Axial T2W, axial Gd+T1W images during the episode #1. Axial T2W reveals pontine lesion and weakly gadolinium enhancement in axial Gd+T1W. (**b**) Axial T2W, axial Gd+T1W images 6 months after the episode #1. The lesion was resolved with a sequela on T2W and hypo-intensity is observed in axial Gd+T1W. (**c**) Axial T2W, axial Gd+T1W images during the episode #2, 1 year after the episode #1. Axial T2W reveals lesion in the medulla oblongata and gadolinium enhancement in the corresponding area. (**d**) Axial T2W, axial Gd+T1W images during the episode #3, 1 month after episode #2 while the patient was on oral steroid after the administration of intravenous methylprednisolone daily for 7 days. Axial T2W reveals lesion in the medulla oblongata and prominent gadolinium enhancement in the corresponding area

differentiating NMOSD and MOG antibody-associated disorders from p-NBS as this pattern has not been observed in these disorders so far [28]. “Bagel Sign” pattern is characterized by a central lesion with a hypo-intense core and a hyper-intense rim, with or without contrast enhancement.

Neuro-psycho-Behçet syndrome may be expressed with a number of symptoms such as euphoria, loss of insight, disinhibition, indifference to the disease, psychomotor agitation, or retardation with paranoid attitudes, and obsessive concerns may be seen in BS with or without cognitive impairment [31, 32].

Given that the subacute progressive presentation of the clinical findings, the distribution of the lesions prominently in the area where the venous anastomosis is few, the significant resolution of the perilesional edema with some small residue, and the pathological findings regarding p-NBS support that the venous pathogenesis plays a major role in the development of p-NBS.

Peripheral nervous system involvement is extremely rare in BS. Mononeuritis multiplex, polyradiculoneuritis, sensorimotor axonal neuropathy, or recurrent episodes of myositis had been reported in BS. However, peripheral neuropathy is much more observed in BS as a result of thalidomide or colchicine treatment rather than the direct involvement of BS [40].

Vascular Involvement

The main type of vascular involvement is cerebral venous sinus thrombosis (CVST) associated with a better prognosis than p-NBS. The clinical manifestations vary by the site and extension of venous thrombosis [33]. Major vascular involvement other than CVST includes aneurysm and/or dissection. The sites of involvement include the common carotid, internal carotid, middle cerebral, superior cerebellar, anterior cerebral, anterior communicating, and vertebral arteries [34].

CVST occurs in up to 20% of BS patients with neurological involvement. In such patients, the principal clinical features (severe headache, papilledema, and sixth-nerve palsy on neurological examination) are compatible with intracranial hypertension [19]. Most studies show that BS-associated CVST has a good prognosis in contrast to other etiologies causing CVST. The systemic features of BS in CVST patients, especially those living in endemic regions, should be looked for. CVST is usually subacute or chronic; only about 25% of cases exhibit clinical features for more than 1 month [35]. Hemiparesis, impaired consciousness, and epileptic seizures are uncommon in CVST patients with NBS. This may be explained by the extremely low probability of seeing hemorrhagic venous infarcts associated with NBS-CVST. Cranial MRI and magnetic resonance venography (MRV) will show that the most commonly involved dural venous sinuses are the superior and transverse sinuses, followed by the sigmoid and straight sinuses [33, 36]. Single-sinus occlusion is more frequent than multiple occlusions [36]. However, if treatment is delayed because of misdiagnosis, multiple sites may be affected in the later stages of BS-CVST and, in a few, may compromise the optic nerves, resulting in blindness.

Additionally, clinicians should be aware that cranial MRI and MRV scans may not show sinus thrombosis, even if the clinical findings strongly suggest its presence. In such situations, MRV of the thoracic and cervical venous structures should be evaluated. Irrespective of whether the neuroimaging data are abnormal or normal, we generally perform a spinal tap to study CSF pressure and contents in the suspected cases. Two case series found that CVST was more common in younger patients, supporting the idea that age is important in terms of NBS presentation [37, 38]. Interestingly, despite the observation of an elevated opening pressure, the CSF is free of inflammatory changes in BS-CVST patients.

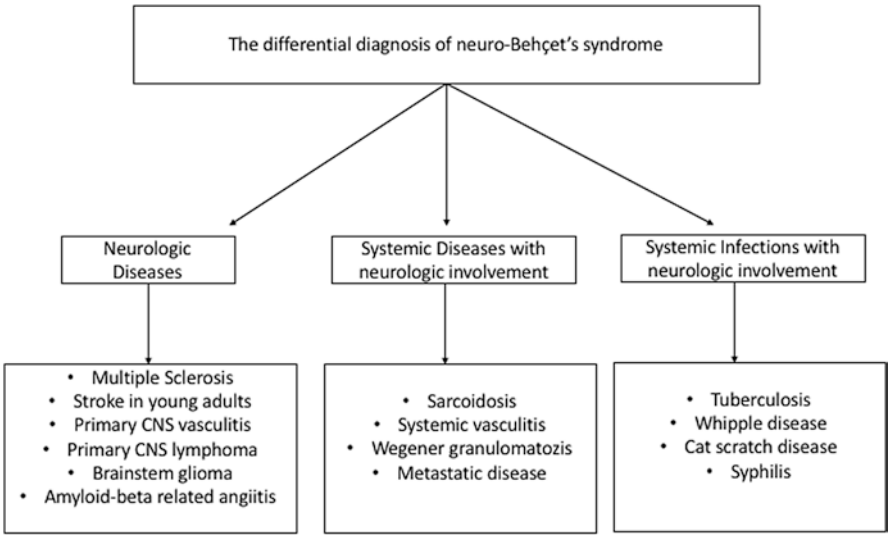
Vascular involvement other than CNS includes deep venous thrombosis, thrombophlebitis, and pulmonary artery aneurysm, and pulmonary artery aneurysm is a serious cause of morbidity and mortality in BS [39].

The differential diagnosis for NBS is summarized in Table 1 [40].

Pathogenesis

Both innate and adaptive immune systems are thought to play roles in the pathogenesis of BS [3]. Increased Th1, CD4+ and CD8+ T cell, γδ+ T cell, and neutrophil activities have been found both in the serum and in inflamed tissues of BS patients, suggesting the involvement of innate and adaptive immunity in the pathogenesis of BS [41, 42]. However, the exact pathogenesis of the disease remains unknown, but an autoimmune vasculitis predisposed by genetic determinants triggered by exogenous factors is suspected [43].

Table 1 The differential diagnosis of neuro-Behçet's syndrome



Microbial infections were thought to trigger BS either directly (streptococci and viruses) or indirectly (via heat shock proteins or molecular mimicry), since the first description of BS [44]. Although laboratory results, including the proliferation of T cells induced by heat shock proteins (HSP) associated with *Streptococcus sanguinis*, *Mycobacterium tuberculosis*, and a variety of autoantibodies support autoimmune processes as the cause of BS, it is currently thought that these antibodies are the result of tissue injury rather than the cause of BS [3].

The HLA-B51 allele located in the major histocompatibility (MHC) locus on chromosome 6p21 is strongly associated with BS and is positive in 50–80% of BS patients. However, the HLA-B51 positivity rates differ substantially among regions, and the specificity of HLA-B51 decreases in the population living along the Silk Road [45]. A recent meta-analysis revealed that HLA-B51/B5 is associated with significantly increased disability [46]. The MICA (an MHC class I related gene) and TNF genes were also found to be associated with disease susceptibility when they are present in a MHC locus other than HLA-B51. Nevertheless, it is unclear whether these genes are primarily related to the disease or the result of linkage disequilibrium with HLA-B51 [47]. Moreover, genome-wide association studies did not find an independent association between MICA and BS [48]. In addition to HLA, other candidate BS genes have also been studied. Although polymorphisms in coagulation factor V, endothelial nitric oxide synthase, and intercellular adhesion molecule-1 were found to be associated with BS, the contribution of these genetic variants to disease susceptibility remains unclear due to the limited number of cases in these studies [48]. Recently, a robust genetic association of PSORS1C1 with B and an independent genetic association of HLA-Cw∗1602 with BS, with genome-wide significance, were identified in two independent cohorts [49].

Given the presence of prolonged inflammation (i.e., pathergy), both the innate and adaptive immune systems are thought to be involved in the pathogenesis of BS [50]. Nonetheless, whether this hyperactivity results from autoimmune or autoinflammatory processes remains controversial [51]. Several autoantigens are part of the inflammatory response in BS, including anti-endothelial antigen, retinal S antigen, heat shock proteins (HSP), killer immunoglobulin-like receptors, co-stimulatory molecules, tropomyosin, and oxidized low-density lipoprotein. However, it is unclear whether these are truly pathogenic or the result of the intense inflammation of BS during disease activation [3]. Recently, *Lule* et al. identified antibodies to human and mouse neurofibrils that cross-react with bacterial HSP-65, which supports the autoimmune disease theory [52].

Several cytokines are known to be elevated isn BS, including IL-1β, tissue necrosis factor α (TNFα), IL-6, IL-10, and IL-23. IL-1β is the principal pro-inflammatory cytokine, and it leads to the expression of many chemokines and secondary mediators of inflammation and upregulates innate immunity in response to infectious agents. Gül et al. strongly implicated IL-1β in BS and observed significant improvements in patients with uveitis treated with IL-1β-regulating antibody [53]. TNFα is another pro-inflammatory cytokine that has been widely investigated in BS. The elevated TNFα levels in BS and significant association between TNFα polymorphisms and BS susceptibility suggest that the treatment of

BS with TNFα blockers is reasonable [54]. IL-6 was also found to be elevated in active BS patients compared with inactive BS and healthy controls. Although tocilizumab, an agent that blocks IL-6 signaling, has shown promising results in treating the neurological involvement of BS, the effectiveness of the drug in other forms of BS is controversial [55, 56].

In contrast to other autoimmune disorders, common autoantibodies (e.g., antinuclear antibody), female predominance, and comorbid autoimmune diseases are not observed in BS [50]. Most of the clinical features of BS satisfy some of the clinical criteria of autoinflammatory diseases, such as the self-limited nature of the disease, variable recurrence rate, and spontaneous healing of its lesions without scarring. Other factors argue against it being an autoinflammatory disease, such as the involvement of the retina, vascular structures, and central nervous system during episodes causing permanent damage, the later age of onset, the presence of vasculitis, and the ineffectiveness of IL-1β treatments in BS [3]. Nevertheless, autoinflammatory diseases should be included in the differential diagnosis of BS. Recently, McGonagle et al. proposed a new term called "MHC-I-opathy A" in a group of disease sharing immunopathogenetic basis [57]. This group of disorder mainly consists of BS and clinically distinct spondyloarthropathies whereas they are all associated with MHC Class I alleles, such as HLA-B∗51, HLA-B∗27, and HLA-C∗0602, and epistatic endoplasmic reticulum aminopeptidase 1 (ERAP-1) interactions.

As BS involves both venous and arterial vessels of all sizes, it has been classified as a "variable vessel vasculitis" in the 2012 International Chapel Hill Consensus Conference on the Nomenclature of Vasculitides [58]. The infiltrates in BS are predominantly constituted of neutrophils and lymphocytes. Elevated concentrations of pro-inflammatory cytokines, including IL-8, INF-γ, and TNFα, may be the reason for the neutrophil infiltrates [59]. Unlike the histology of other systemic vasculitides, in BS, these cells are localized around the vessels rather than inside the vessel wall. This histological "perivascular" pattern of BS, which is more similar to neutrophilic dermatosis than to classical systemic vasculitides, has been demonstrated in tissues, especially in mucosal and ocular inflammatory lesions and in pulmonary aneurysms. Similarly, skin pathergy testing reveals perivascular infiltrates of neutrophils and lymphocytes at different time points, however, without the typical features of a "true" vasculitis [60].

CSF Findings

During the acute phase of p-NBS, the CSF shows inflammatory changes in most cases of p-NBS with an increased number of cells, up to a hundred and sometimes more per ml, neutrophils being mostly the predominating cells and modestly elevated protein levels. However, an early lymphocytic pleocytosis is not an exception. When the neutrophilic pleocytosis is the case, it is later replaced by lymphocytes. The oligoclonal band positivity rate is low at a rate of 20% or less [25].

An elevated concentration of IL-6 in the CSF of patients correlating with disease activity has also been reported in p-NBS [61–63]. More recently, an increase of CSF IL-10 and CSF/serum matrix metallopeptidase-9 ratio (increased in serum and decreased in CSF compared to multiple sclerosis) was reported and suggested to be a discriminative marker between NBS and multiple sclerosis [64, 65].

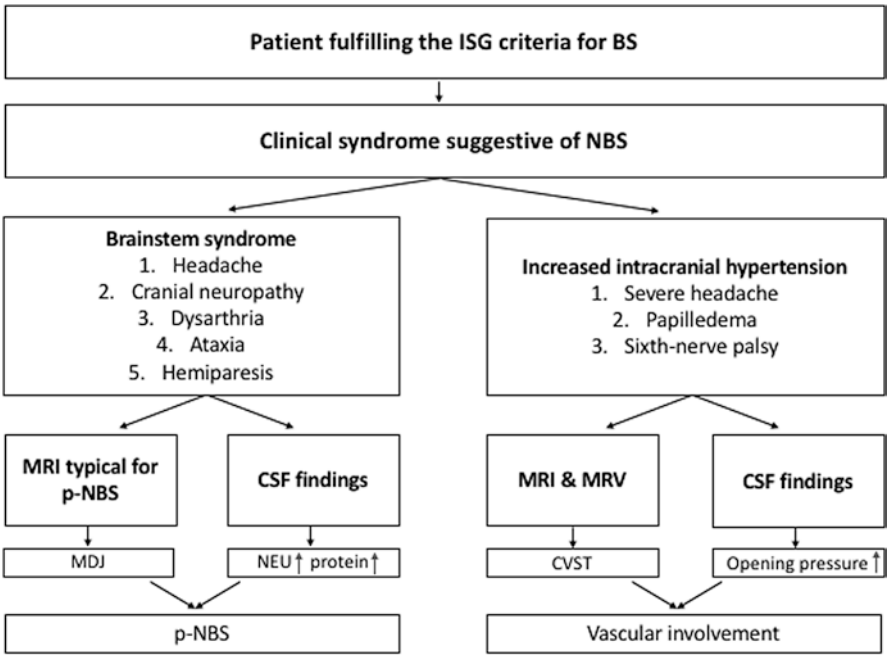
Diagnosis

The diagnostic flowchart of NBS is illustrated in Table 2.

Prognosis

Brain stem or spinal cord involvement, frequent relapses, early disease progression, and high CSF pleocytosis are the poor prognostic features for NBS, which was pointed out by International Consensus Recommendation (ICR) [66]. Initiation

Table 2 The diagnostic flow chart of NBS



BS Behçet's syndrome, *CSF* cerebrospinal fluid, *CVST* cerebral venous sinus thrombosis, *ISG* International Study Group, *MDJ* mesodiencephalic junction, *NBS* neuro-Behçet's syndrome, *MRI* magnetic resonance imaging, *MRV* magnetic resonance venography, *NEU* neutrophil, *p-NBS* parenchymal neuro-Behçet's syndrome

with severe disability, primary or secondary progressive course, fever at onset, relapse during steroid tapering, meningeal signs, and bladder involvement are possible association with poor outcome. Gender, accompanying systemic features, and age onset do not change the prognosis of NBS [67].

Treatment

Due to multisystemic involvement of BS, long-term treatments should be decided by multidisciplinary team. The first goal of the treatment in NBS is to suppress the acute episode in order to shorten the recovery time with minimal disability, and the second goal is to prevent from further attacks. However, as there are no controlled trials for the management of neurological involvement, long-term treatments depend on the clinical experience rather than trials. In this regard, prognostic factors should be taken into account for choosing the appropriate treatment together with the patients' age, gender, and patient preferences.

High-dose intravenous methylprednisolone (IVMP) pulse therapy for 5–10 days, followed by a slow oral tapering, is the first choice for treating acute episodes. The dose and duration of steroid treatment vary among centers. Colchicine, azathioprine, cyclosporine, cyclophosphamide, methotrexate, chlorambucil, thalidomide, interferon alpha, anti-TNF agents, and IL-6 blockers are among the drugs used for the preventive treatment of the systemic features of BS, which were tried for CNS involvement as well [40].

Currently, the only drug that has been shown to be effective based on the Class IV evidence is infliximab for the treatment of p-NBS [68, 69]. Although that the efficacy of azathioprine is not clear in NBS, there are a few reports suggesting that it may be effective. Due to the risk of activating latent tuberculosis among patients using infliximab, tuberculosis screening should be conducted prior to the administration of infliximab, and isoniazid prophylaxis (300 mg/day) should be prescribed for 6 or 9 months in patients with latent tuberculosis. In many centers, azathioprine is the first-line drug to be initiated once patients develop p-NBS as also suggested by the 2018 updated European League Against Rheumatism (EULAR) recommendations [69]. We tend to start infliximab in patients in whom azathioprine fails and sometimes as a first-line therapy in patients who present with a severe acute attack of p-NBS and who have poor prognostic factors [68]. Given that the cyclosporine-A is associated with the increased risk of neurological involvement, it should be avoided in patients having NBS and immediately stopped in patients developing NBS under cyclosporine-A [40].

Since the recurrence of CVST is very rare, the duration of azathioprine treatment in CVST is contradictive. In our practice, we usually use azathioprine at least 5 years, and before cessation of azathioprine, we consult the patient with rheumatologists and neuro- ophthalmologist whether AZA is required for systemic features of BS other than CVST.

Mycophenolate mofetil might be reasonable in NBS if gastrointestinal intolerance occur under AZA treatment. The addition of anticoagulant medication to steroids is controversial, as BS patients with CVST are more likely to have systemic large vessel disease, including pulmonary and peripheral aneurysms that carry a high risk of bleeding [69]. The complication rate with warfarin should be considered. Results of anticoagulation treatment in BS are controversial in CVST. Recurrence rate of deep vein thrombosis is more likely to decrease with an immunosuppressant; therefore, the use of immunosuppressants in the treatment of CVST should be the priority.

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Neuroinflammation and Sjogren's Syndrome

Pasquale Annunziata

Abstract Sjogren's syndrome (SS) is a chronic organ-specific autoimmune disease mainly involving exocrine glands such as lacrimal and salivary glands. SS may also involve central and peripheral nervous system with variable prevalence due to differences in diagnostic criteria and in time length to reach diagnosis. Clinical features of the central nervous involvement share similarities with multiple sclerosis (MS) and neuromyelitis optica spectrum disorders (NMOSD), two major neuroimmune disorders. SS may even coexist with MS or NMOSD. Sensory neuropathy, chronic polyradiculoneuropathy, cranial neuropathies as well as small fibre neuropathy are the main manifestations of the peripheral nervous system involvement. The pathogenic mechanism underlying neuro-SS is unclear even though molecular mimicry and epitope spreading have been hypothesized for central nervous involvement, whereas vasculitis with or without direct damage to nerve could account for peripheral nervous involvement. Treatment is mainly based on immunosuppressive therapies requiring a close cooperation between neurologists and rheumatologists to achieve the best management.

Keywords Sjogren's syndrome · Multiple sclerosis · NMOSD · Polyneuropathy · Autoimmunity

Introduction

Sjogren's syndrome (SS) is a chronic organ-specific autoimmune disease that is characterized by lymphocytic infiltrate and progressive degeneration of the exocrine glands such as lacrimal and salivary glands. This disorder may present with both an isolated syndrome named primary SS (PSS) and a secondary SS in association with other connective tissue diseases such as rheumatoid arthritis, systemic lupus erythematosus and systemic sclerosis (reviewed in [1]). SS may also involve central/

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_23

peripheral nervous system (CNS/PNS) and likely represents the connective disorder with most intriguing features (mainly regarding the central nervous system manifestations) that, sometimes, raise doubts in diagnosis and thus in treatment for both rheumatologists and neurologists.

Epidemiology

Data on the prevalence of PSS are heterogeneous, ranging from 0.2% in a Danish population to 3.9% in a population-based study in United States (reviewed in [2]). However, the prevalence of PSS coexisting with CNS clinical features and consistent with the diagnosis of inflammatory nervous disorders such as multiple sclerosis (MS) is questionable ranging from 0% to 16.7% [3–7]. Conversely, the prevalence of peripheral nervous system (PNS) features in SS ranges from 25% to 59% of PSS patients [8–10]. This heterogeneity is due to a number of reasons including the criteria used in performing diagnosis of SS as well as the long time needed to reach a diagnosis of SS sometimes requiring an average of 10 years [11]. Table 1 summarizes the most relevant epidemiological findings.

Clinical Manifestations

Clinical Features of Sjogren's Syndrome

The classical clinical features of PSS involve lacrimal and salivary glands and are part of the 'sicca syndrome complex' including xerophthalmia and xerostomia as well as recurrent salivary gland enlargement. However, the disease may involve other organs and systems such as the lung, liver, kidney and circulation [12]. Respiratory symptoms such as cough or rarely interstitial pneumonitis and fibrosis may be present [13]. Mild hepatitis or intestinal malabsorption occurs, while glomerulonephritis, rarely progressing towards a nephrotic syndrome, may develop [14]. SS may occur in association with other connective disorders such as

Table 1 Epidemiology of Sjogren's syndrome with nervous involvement

	Prevalence	References
CNS	5.8%–38%	[9, 20, 21]
MS	0%–16.7%	[3–7]
PNS	25%–59%	[8–10]
	1.8%[a]	[27]

CNS central nervous system, *MS* multiple sclerosis, *PNS* peripheral nervous system

[a]supported by electrophysiological findings

rheumatoid arthritis, systemic lupus erythematosus and systemic sclerosis consisting with secondary SS. Arthralgias and myalgias occur in 53% and 22% of patients, respectively [12]. The severity of clinical features is variable extending from benign course characterized by clinical remission stages up to rare severe manifestations when being associated with peripheral blood alterations such as purpura, reduced complement levels and monoclonal cryoglobulinemia that may increase mortality [15]. Other secondary symptoms are part of the various SS clinical scenario. Fatigue is present in nearly 50% of PSS patients, sometimes debilitating, and often associated with hypothyroidism [16]. Another symptom is pain that may represent one characterizing sign of fibromyalgia detected in more than 20% of PSS [17]. A mild Raynaud's phenomenon also occurs in nearly 30% of SS patients, and different skin alterations such as dryness or burning are detected. When skin ulcerations are present, vasculitis of small- or medium-sized vessels is observed [18].

Clinical Features of Central Nervous Involvement

The neurological involvement in SS consists of symptoms and signs affecting both PNS and CNS [19], but it is still matter of discussion whether central or peripheral nervous involvement may be predominant. Isolated CNS involvement was found to vary between 5.8% and 38% of PSS patients [9, 20, 21]. Brain, spinal cord and optic nerve may be affected in percentages variable and not necessarily in the same patient. The clinical features are secondary to location of inflammatory lesions in the brain and spinal cord white matter and include aphasia, hemiparesis, cerebellar symptoms, brainstem symptoms, sensory impairment, acute myelitis, chronic myelopathy, aseptic meningitis as well as optic neuritis [9, 20, 22]. The neurological manifestations in SS occur in various times developing prior to or after SS onset (reviewed in [1]) and thus leading to delay in diagnosis assessment. However, CNS involvement preceding SS diagnosis seems to be predominant ranging from 52% to 80% of subjects [9, 20, 22].

Two major inflammatory nervous disorders characterizing central nervous involvement in SS are MS and neuromyelitis optica (NMO). Clinical and laboratory features of PSS may develop during the course of MS with various diseases duration ranging from 9.3 to 13 years [5, 6] and with higher prevalence in progressive MS supporting the need to perform screening for SS in all patients with primary progressive MS [7]. An important issue is the occurrence of clinical signs or symptoms of sicca syndrome in the course of MS. This issue was addressed in a large multicentre study in Italy. Sicca syndrome occurred in 9.6% of MS patients and was detected at onset of disease in 2.3% of cases [4]. However, the presence of sicca syndrome was not related to SS development and tended to arise in progressive forms of MS patients displaying a higher disability and higher frequency of cognitive disturbances with a low inflammatory disease activity as assessed at magnetic resonance imaging (MRI). This finding suggests a different pathogenic mechanism underlying xerophthalmia and xerostomia in MS from that known in SS, linking the

Table 2 Clinical features of central nervous involvement in Sjogren's syndrome

Optic neuritis
Aphasia
Hemiparesis
Brainstem symptoms
Cerebellar symptoms (ataxia, dysmetria)
Acute transverse myelitis
Aseptic meningitis
Sensory impairment

development of sicca complex symptoms in MS to autonomic dysfunction involving both parasympathetic and sympathetic systems, previously demonstrated in this disorder [23]. Moreover, PSS may develop during MS course under immunomodulatory therapy with Interferon beta, both in non-responder and in good responder patients after a few months from MS onset [24] or after a very long time up to 29 years from MS onset [25]. Currently, a pathogenic role of immunomodulatory therapies in developing SS in course of MS has not been demonstrated. SS was found to be part of clinical onset of NMO and is currently included in NMO spectrum disorders appearing as acute transverse myelitis with MRI lesions involving one or more spinal cord segments [26]. The main clinical features are paraparesis, hypoesthesia or anaesthesia with cervical or thoracic sensory level associated or not with sphincter dysfunction. Table 2 summarizes the most frequent clinical features of central nervous involvement in SS.

Clinical Features of Peripheral Nervous Involvement

PNS involvement based on clinical assessment ranges from 25% to 59% of PSS patients [8–10]. This frequency dramatically decreased when electrophysiological assessment was used showing the prevalence of peripheral neuropathy reaching only 1.8% in a large cohort of patients with PSS [27]. The most common neuropathies reported in PSS patients include sensory neuropathy with or without ataxia, sensory-motor neuropathy, mononeuritis multiplex, chronic polyradiculoneuropathy, cranial neuropathies as well as small fibre neuropathy [8–10]. The neuropathy is mainly axonal. These patients may have extraglandular manifestations including purpura and vasculitis and laboratory features such as monoclonal cryoglobulinemia. The related clinical features include distal symmetric sensory loss, sensory ataxia due to loss of proprioceptive large fibres associated with ganglionopathy and painful dysesthesias characterizing small fibre sensory neuropathy and due to degeneration of cutaneous axons. Table 3 summarizes the main clinical features of peripheral involvement in SS.

Table 3 Clinical features of peripheral nervous involvement in Sjogren's syndrome

Sensory neuropathy with or without ataxia (dysesthesias in arms or legs, with or without pain)
Sensory-motor neuropathy (dysesthesias or hypoesthesias, limb weakness)
Mononeuritis multiplex
Cranial neuropathies (facial or oculo-motor neuropathies)
Small fibre neuropathy (painful dysesthesias)
Chronic polyradiculoneuropathy (progressive limb weakness, gait impairment with or without dysesthesias)

Pathogenesis

Immunopathogenesis of Sjogren's Syndrome

Currently, there is a wide agreement on the concept that a combination of genetic susceptibility and environmental factors may account for the pathogenesis of SS (reviewed in [1]). The role of several viral infections in the SS induction has been investigated. The list of viruses includes cytomegalovirus (CMV) and Epstein-Barr virus (EBV). An association with retroviruses such as HTLV-1 and HIV has also been reported (reviewed in [28]). Dendritic and epithelial cells of the salivary glands could be activated by viral antigens, leading to upregulation of pro-inflammatory and anti-viral genes resulting in presentation of the MHC class 2 molecules and secretion of several pro-inflammatory cytokines, mainly type-1 interferon (IFN-1) but also including B-cell-activating factor (BAFF), interleukin (IL)-6, IL-21 and IL-12 (reviewed in [1]). This strong inflammatory response results in the breakdown of innate response leading to activation of the adaptive immune response involving both T- and B-lymphocytes. These cells could migrate from peripheral blood entering the salivary gland parenchyma where they interact with antigen-presenting cells (APCs). It is likely that SSA and SSB represent the most important antigens presented by APCs. A second step of the pathogenic pathway is characterized by infiltration of lacrimal and salivary glands by $CD4^+$ but also $CD8^+$ T cells playing a role in the glandular injury. B-cell immunity also participates in the glandular injury through production of autoantibodies against M3 muscarinic receptors largely expressed on salivary gland epithelial cells (reviewed in [1]).

Immunopathogenesis of Nervous Involvement

The pathogenesis of the nervous system involvement in SS remains unclear and, to date, is mainly speculative. However, two major immunological mechanisms could be hypothesized to play a role: molecular mimicry and epitope spreading. T- and B-cell response could be triggered by a putative molecular mimicry mechanism between glandular epithelium antigens and CNS antigens that remain to be identified. This molecular mimicry could lead activated T-cell clones recognizing salivary

and lacrimal gland antigens to also recognize any putative nervous myelin antigen or, alternatively, any cerebral endothelial antigen at the blood-brain barrier (BBB) facilitating their passage into the brain parenchyma. B-lymphocytes also significantly contribute to intrathecal immune response producing antibodies reacting with specific myelin antigens. However, although IgG oligoclonal bands may be rarely detected in cerebrospinal fluid of SS patients, there is still no clear evidence for any myelin antigen recognized by these IgGs in SS. The passage of T and B cells across BBB could also be due to an epitope spreading mechanism [2] leading, in a subject with MS or SS, the immune response to extend from the peripheral blood compartment and salivary glands, respectively, to brain parenchyma. These concepts appear less appropriate for explaining the PNS involvement in SS. SS patients with clinical evidence of neuropathy do not display any circulating antibody directed against anti-myelin antigen [8]. However, vasculitis involving neural vessels is rarely present in SS patients and mainly, when peripheral blood alterations including purpura or cryoglobulinemia are found [12]. Moreover, alterations of the endoneural microvessels were observed in SS patients with neuropathy [29]. Figure 1 shows the main mechanisms underlying central nervous involvement in SS.

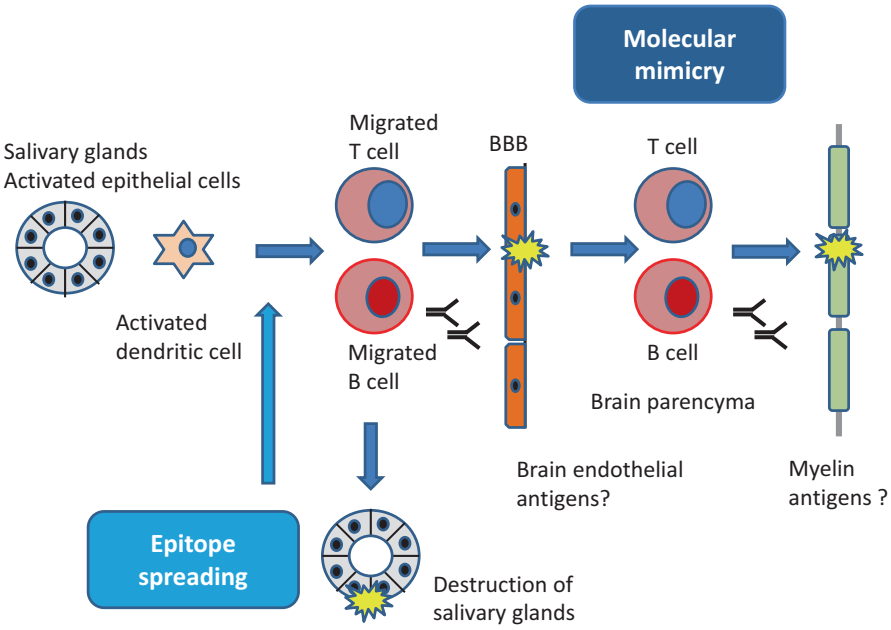

Fig. 1 Pathogenesis of the central nervous involvement in Sjogren's syndrome

Diagnosis

Currently, SS is diagnosed based on the presence of ocular and/or oral symptoms and ocular signs according to the items listed by the American-European Consensus Group (AECG) for SS as classification criteria [30]. These items also comprise a number of laboratory tests such as serum anti-Ro (SSA) and SSB antibodies as well as instrumental diagnostic tests including testing for dry eye (Schirmer's test), salivary test and minor salivary gland biopsy. The diagnosis of neurological involvement in course of SS does not appear clear at any time as due to various temporal intervals elapsing from the onset of neurological features to the SS diagnosis. This interval may take out up to 5 years [9]. Further problems come from detection of subclinical cerebral white matter lesions at MRI in PSS with no appearance of clear clinical neurological features. These lesions are not easily distinguishable from those typical of MS and thus nurture further pathogenic questions [31]. Another question is raised when, in definite MS, sicca syndrome signs and symptoms occur supporting the hypothesis of SS development. However, serum anti-Ro (SSA) and SSB antibodies are not sufficient for establishing a diagnosis even though a low frequency (7% only) of SSA antibodies has been detected in MS patients [32]. If pathogenic tests for diagnosing MS in the course of SS are lacking, more help is available for NMO diagnosis in the course of SS based on the identification of serum NMO-IgG antibody binding to aquaporin-4 (AQP4), as reliable laboratory biomarker of the NMO spectrum disorders (NMOSD) [26]. Recently, a diagnostic algorithm helping for practical managing of SS and MS has been proposed [33]. Search for sicca symptoms (xerophthalmia and xerostomia) should be performed in all patients with MS. In the presence of these symptoms, diagnostic tests (Schirmer's test) and serological screening (serum anti-SSA and anti-SSB antibodies) for SS are recommended. In case of positive findings, the diagnostic work-up should be concluded with a biopsy of minor salivary glands (Fig. 2). Conversely, in patients with PSS, a brain MRI is useful to search for any white matter lesions (mainly active as suggested by gadolinium enhancement) that should lead to perform evoked potentials and cerebrospinal fluid analysis. The positivity of these tests could support MS diagnosis according to the current revised diagnostic criteria (reviewed in [34]). Moreover, the detection of spinal cord lesions at MRI in patients with PSS requires serum anti-aquaporin-4 antibody assay to exclude a diagnosis of NMO spectrum disorder (reviewed in [35]) (Fig. 2).

On the other hand, the diagnosis of peripheral nervous involvement in course of PSS appears easier, based on clinical and neurophysiological parameters. Clinical features of PNS involvement such as sensory disturbances associated or not with pain may account for a suspected neuropathy. Electrophysiology tests such as nerve conduction studies and somatosensory evoked potentials could confirm the clinical suspicion.

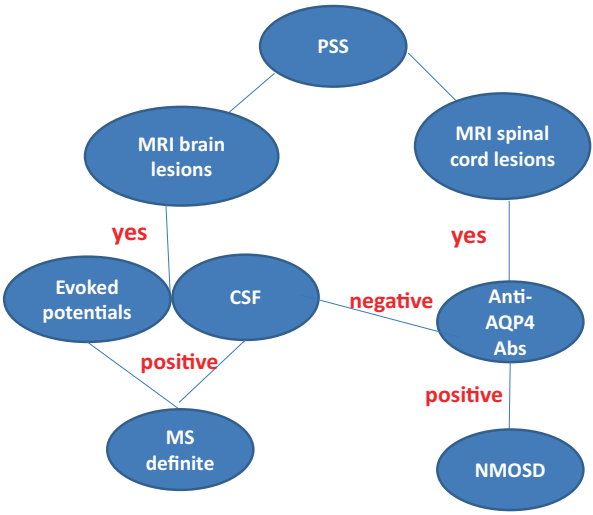

Fig. 2 Flowchart for the diagnosis of multiple sclerosis (MS) and neuromyelitis optica spectrum disorder (NMOSD) in primary Sjogren's syndrome (PSS) (Modified from Masi and Annunziata [33])

Treatment

Treatment of CNS and PNS involvement in SS aims to reduce signs and symptoms related to both SS and definite neurological disorders appearing in the course of or pre-existing to SS development. When sicca syndrome occurs in MS patients, symptomatic treatment is based on the same recommendations as PSS such as hydration, avoiding anticholinergic drugs and artificial tears. Rarely, sicca symptoms are severe in the course of MS and do not require the use of muscarinic agonists employed in PSS such as pilocarpine or cevimeline [36]. When the central nervous involvement satisfies the criteria of MS, the first-line disease-modifying therapies including intramuscular or subcutaneous interferon beta 1a or beta 1b are the established choice. Due to their well-known gastrointestinal side effects, oral immunomodulators such as dimethylfumarate appear not suitable. However, in case of MS diagnosis in the course of PSS, the treatment resembles that of definite MS and the acute clinical relapses occurring during the disease are treated with standard high-dose intravenous methylprednisolone (IVMP). In central nervous involvement not satisfying the diagnostic criteria for definite MS, clinical features may be treated with oral corticosteroids or, more frequently, with IVMP. Cyclophosphamide has been used in resistant cases. When adverse events occur or in case of low efficacy, intravenous immunoglobulins (IVIG) may be employed. In the presence of frequent relapses, other immunosuppressive drugs such as azathioprine or methotrexate have been used. In SS with severe systemic symptoms such as recurrent severe arthralgias, rituximab, an anti-CD20 monoclonal antibody, can be administered. In SS occurring in the course of NMO, IVMP could be efficacious at the first clinical manifestations. However, in the presence of relapses, azathioprine or, in case of inefficacy, rituximab may be used (reviewed in [37]). In SS patients with sensory neuropathy, dysesthesias and pain may be treated with gabapentin/pregabalin or with IVIG in absence of response. Chronic polyradiculoneuropathy is treated with

Table 4 Treatment of Sjogren's syndrome with nervous involvement

Clinical feature	Drug	Administration route	Dose
CNS	Prednisone	Os	1 mg/kg/die
	IVMP	Intravenous	1 g/die per 3–5 days
	IVIG	Intravenous	0.4 g/kg die 5 days
	Cyclophosphamide	Os	50–100 mg/die
	Rituximab	Intravenous	750 mg on day 1 and day 15
MS	Interferon beta 1a	Subcutaneous	22–44 μg (3 days a week)
	Interferon beta 1a	Intramuscular	30 μg/week
	Interferon beta 1b	Subcutaneous	250 μg each other day)
	IVMP	Intravenous	1 g/die per 3–5 days (for relapses)
NMO	IVMP	Intravenous	1 g/die per 3–5 days
	Azathioprine	Os	2 mg/kg/die
	Rituximab	Intravenous	750 mg on day 1 and day 15
PNS	Prednisone	Os	1 mg/kg/die
	Gabapentin	Os	900–1600 mg/die
	Pregabalin	Os	300–600 mg/die
	IVIG	Intravenous	0.4 g/kg die 5 days

CNS central nervous system, *MS* multiple sclerosis, *NMO* neuromyelitis optica, *PNS* peripheral nervous system, *IVMP* intravenous methylprednisolone, *IVIG* intravenous immunoglobulins

oral corticosteroids or with IVIG in severe or relapsing cases. An efficacious treatment of SS with nervous involvement requires a close cooperation between neurologists and rheumatologists to achieve the best management of all symptoms and signs appearing in the course of the related disorders. Table 4 summarizes the main treatments and related doses in SS with nervous involvement.

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Guillain-Barré Syndrome

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Abstract Guillain-Barré syndrome (GBS) is an autoimmune acute peripheral polyneuropathy, which often follows an infectious process. The most common microorganisms associated with GBS are the bacteria *Campylobacter jejuni* and *Mycoplasma pneumoniae*. Viruses such as cytomegalovirus and the Zika virus have also been associated with GBS. The incidence of GBS ranges between 0.5 and 2 cases per 100,000 population per year. The pathophysiology of GBS most likely involves molecular mimicry, in which an autoantibody against a microorganism cross-reacts with host molecules, such as GD1a, GM1, and GM1/GD1 complex located at the terminal nerves and anterior roots, and GQ1b located on oculomotor nerves and primary sensory neurons. The classical complement system has also been implicated in facilitating the development of GBS. GBS usually presents with numbness, paresthesia, and progressive weakness, but there are several clinical variants, including acute motor axonal neuropathy (AMAN), acute inflammatory demyelinating polyneuropathy (AIDP), acute motor and sensory axonal neuropathy (AMSAM), Miller-Fisher syndrome (MFS), a pharyngeal-cervical-brachial variant, a paraparetic variant, and others. Treatment of GBS mostly targets the immune response through the use of IVIg, plasma exchange, and other forms of immunomodulatory therapy.

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_24

Keywords Guillain-Barré syndrome · Acute motor axonal neuropathy · Acute inflammatory demyelinating polyneuropathy · Genetics · Major histocompatibility complex · Molecular mimicry · Complement · Immunotherapy · Intravenous immunoglobulins · Plasma exchange

Introduction

Guillain-Barré syndrome (GBS) is an autoimmune acute peripheral polyneuropathy with an incidence between 0.5 and 2 cases per 100,000 cases per year and a male predominance [1]. In many cases, an infectious episode precedes the onset of neurological symptoms, suggesting that molecular mimicry may play a role in pathogenesis [2]. Through this mechanism, an infectious agent can generate a loss of immunological tolerance against the axon, leading to the acute motor axonal neuropathy (AMAN) variant of GBS, or an immunologic reaction targeting myelin, which results in the acute inflammatory demyelinating polyradiculoneuropathy (AIDP) variant. A third variant, the acute motor and sensory axonal neuropathy (AMSAM) variant, results from a simultaneous response against motor and sensory neurons.

The clinical spectrum of the disease varies among patients but is typically characterized by a progressive bilateral motor and sensory involvement in the extremities, associated with autonomic dysfunction, cranial nerve involvement, radicular pain, and respiratory dysfunction [3]. Besides the most typical clinical variants, other variants have been described, depending on the location of the neurological deficits generated by the immune response. Among the most important are the paraparetic variant, pharyngeal-cervical-brachial weakness, and bifacial weakness with paresthesias [4]. In addition, one of the most common variants of GBS is the Miller-Fisher syndrome (MFS), which can present as acute ataxic neuropathy, acute ophthalmoparesis, acute ptosis, acute mydriasis, and Bickerstaff's brainstem encephalitis [4]. The diagnosis of the disease is generally clinical; however, diagnostic tools such as cerebrospinal fluid (CSF) analysis and nerve conduction studies are useful.

The management of patients with GBS involves immunomodulatory treatment, physical therapy, pain, and autonomic dysfunction management. Immunomodulatory management focuses on the use of intravenous immunoglobulin (IVIg), plasma exchange (PE), and other immune therapies directed against the autoimmune basis of the disease [3, 5]. The prognosis depends on the age of the patient, the clinical variant, and the response to treatment.

Epidemiology

After the eradication of poliomyelitis through vaccination, GBS became the main cause of flaccid paralysis. The prevalence increases with age reaching up to 26 per 100,000 inhabitants [1], with a male predominance [6, 7]. The incidence varies depending on the geographical location. In Europe, the incidence is between 0.84 and 1.91 per 100,000 people [8, 9], while in the United States, it is between 0.48 and

0.73 per 100,000 people [10]. In Latin America, it is between 0.46 and 1.37 per 100,000 people [11–14]. A history of recent infection is present about 40–70% of patients [15]. With regard to electromyographic variants, AIDP is the most common in America and Europe [16–18], whereas AMAN is usually the most common variant in Asia [19].

Clinical Manifestations

Characteristic symptoms of GBS include numbness and paresthesias, with the development of rapidly progressive limb weakness and decreased reflexes or areflexia [16]. Weakness usually starts in the lower extremities and in hours or days involves the upper extremities and respiratory and facial muscles [16]. Additionally, patients may experience sensory symptoms, ataxia, autonomic dysfunction, and neuropathic pain [3]. About one-third of patients can develop complications of GBS, such as respiratory failure [20], sepsis, and aspiration pneumonia [3].

Autonomic dysfunction may be observed in up to 65% of patients with GBS. Its presence usually affects patients with severe muscle weakness and respiratory failure. This condition is due to alterations in the autonomic system, generating hypertension, hypotension, bradycardia, bladder and bowel dysfunction, and abnormalities of sweating [21]. Of these, paroxysmal hypertension is usually the most frequently documented [3], generated by elevated renin levels and afferent baroreflex abnormality. Hypotension occurs in near 10% of the patients. It is observed mainly in patients with cranial nerves IX and X involvement, respiratory failure, and quadriplegia [22] and may be due to a "functional sympathectomy causing failure of reflex arteriolar and venous constriction, accentuated in some patients by inability to increase heart rate" [3]. Cardiac arrhythmias are the most ominous manifestation of autonomic dysfunction [3], due to uncontrolled vagal activity. The deficit in vagal activity generates tachyarrhythmias such as atrial fibrillation, sinus tachycardia, and ventricular tachycardia [3].

There are clinical variants of GBS, such as the pharyngeal-cervical-brachial variant which involves bulbar, cervical, and upper limb weakness [23]. Other variants include the paraparetic variant [20], those with bifacial weakness, paresthesias, and distal limb sensory disturbance [24]. Among the most important clinical variants of the GBS is MFS, which is characterized by the presence of ophthalmoplegia, cerebellar-like ataxia, and areflexia [25]. Bickerstaff's brainstem encephalitis presents with an altered state of consciousness [26].

Etiology: Genetic Factors

Genetic risk factors include certain HLA and non-HLA associations. Tables 1 and 2 [27–58] describe the genes associated with the development of GBS. Genetic risks related to HLA loci reinforce the autoimmune origin of a disease [59]. An

Table 1 Studies of HLA genes in Guillain-Barré syndrome

Genes	Population	HLA association	References
HLA-A	United States	Slight reduction in HLA-A11 frequency in GBS than in controls	[27]
	China	HLA-A33 associated with AIDP	[28]
	Egypt	HLA-A3 more frequent in GBS patients	[54]
HLA-B	Australia	HLA-Bw4/Thr80 frequently seen in subjects with GBS	[39]
	Japan	HLA-B39 associated with MFS	[50]
	Japan	HLA-B35 associated with GBS and anti-GM1 antibodies	[53]
	Egypt	HLA-B8 more frequent in GBS patients	[54]
	China	HLA-B15 and HLA-B35 associated with AMAN	[28]
HLA-C	Australia	HLA-C2 more frequent in GBS patients than in controls	[39]
HLA-DQA1	China	No association with GBS, however HLA-DQA1∗03 was associated with GM1 antibodies	[55]
HLA-DQB1	Germany	HLA-DQB1∗05:01 allele associated with severe GBS	[56]
	England	HLA-DQB1∗0301 in patients with GBS and previous *C. jejuni* infection	[57]
HLA-DQ	India	Increased HLA-DQ∗06 in GBS	[58]
	China	DQ beta epitopes were associated with AIDP	[29]
HLA-DQ5	China	Association with AIDP	[28]
HLA-DRB1	Iraq	HLA-DRB1∗03:01, HLA-DRB1∗07:01, and HLA-DRB4∗01:01 were associated with GBS risk	[30]
	Tunisia	HLA-DRB1∗14 and DRB1∗13 associated with GBS	[31]
	India	HLA-DRB1∗0701 associated with GBS with preceding infection	[58]
	Japan	HLA-DRB1∗0803 in GBS patients with previous *C. jejuni* infection and positive anti-GM1 antibodies	[32]
	China	Increased HLA-DRB1∗13 frequency in patients with AIDP	[33]
	China	DRB1 epitopes associated with AIDP	[29]
HLA-DR6	Iraq	HLADR6 could be associated with protection of GBS	[30]
HLA-DR15	China	Association with AIDP	[28]
HLA-DR3	Mexico	Increased in GBS patients	[34]

Modified from Blum et al. and *Rodríguez* et al. [17, 18]
GBS Guillain-Barré syndrome, *AIDP* acute inflammatory demyelinating polyradiculoneuropathy, *MFS* Miller-Fisher syndrome, *AMAN* acute motor axonal neuropathy, *C. jejuni Campylobacter jejuni*

association between HLA-A11 and HLA-A3 and GBS has been documented [27, 54]. Additionally, HLA-A33 was associated with the subphenotype AIDP [28]. There has also been reported an association between HLA-Bw4 and HLA-B8 with GBS [7, 9]. Interestingly, HLA-B35 is associated with the presence of anti-GM1 antibodies, while HLA-B15 and HLA-B35 are frequently associated with AMAN [28].

Table 2 Non-HLA genes in Guillain-Barré syndrome

Gene	Protein	Population	References
SERPINA1	Alpha1 antitrypsin	Australia	[35]
CD1A	CD1a	Italy	[36]
CD1E	CD1e	Italy	[36]
FAS	Fas (CD95)	Netherlands	[37]
FCGR2A	FcγRIIa	India	[58]
FCGR3A	FcγRIIIa	India, the Netherlands	[38, 58]
FCGR3B	FcγRIIIb	Norway	[40]
FCRL3	FcR-like 3	China	[41]
NRC31	Glucocorticoid Receptor	Netherlands	[42]
IGHG1	G1M marker	Australia	[43]
IGHG2	G2M marker	Australia	[43]
IGHG3	G3M marker	Australia	[43]
IL 10	Interleukin 10	Norway	[44]
IGKC	KM marker	Japan	[45]
MBL2	MBL	Netherlands	[46]
MMP9	MMP9	Netherlands	[47]
TNF	TNF alpha	Netherlands, China, India	[47–49, 51]
TLR4	Toll-like receptor 4	India	[52]

Modified from Blum et al. [17]

HLA-DQB1∗05:01 has been associated with severe GBS [57]. Concerning non-HLA genes, some polymorphisms at *FCGR3A* and *TNF* genes are the most strongly associated [38, 47–49, 51, 58]. However, others at *FCGR3B*, *NRC31*, *IL10*, and *TLR4* have been also described [40, 42, 52].

Etiology: Triggering Factors

Like other autoimmune diseases (ADs), environmental factors may play a role in the development of GBS. Several infections have been documented as triggers of GBS (Table 3) [60–88], including *Campylobacter jejuni* (*C. jejuni*), *Mycoplasma pneumoniae (M. pneumoniae)*, cytomegalovirus (CMV), and more recently the Zika virus (ZIKV). These infections generate an aberrant immune response through several mechanisms of which molecular mimicry (Fig. 1) is one of the most important. Molecular mimicry facilitates an immune response against epitopes within the axon and myelin in the peripheral nervous system. Among the main functions of these antigen-presenting cells (APCs) is the presentation of antigens obtained from external agents such as infections through the expression of MHC-II and the presentation of its own antigens through MHC-I [89].

C. jejuni or *M. pneumoniae* are the most common microorganisms found to precede GBS, with *C. jejuni* implicated in up to 40% of cases [90]. This bacterium is

Table 3 Infections associated with Guillain-Barré syndrome

Agent	Pathogen	Associated electrophysiological subphenotype(s)	Anti-ganglioside antibodies identified	Pathophysiological mechanism	References
Salmonella spp.	GBS related to enteric fever caused by *Salmonella typhi* (typhoid fever) or *S. paratyphi* (paratyphoid fever)	AIDP, MFS, and BBE	Anti-GQ1b (BBE)	It is unclear whether molecular mimicry plays a role in *Salmonella*-related GBS	[60, 61, 72, 82, 83]
Brucella	Involvement of gastrointestinal, hepatobilliary, and skeletal systems	AIDP, AMAN	Anti-GM1	Suggests that *B. melitensis* can induce autoimmunity through molecular mimicry	[84–88]
Bartonella henselae	Red bumps on the skin, fatigue, and discomfort lymph nodes near the lesion may become inflamed	AMAN	Not described	Pathogenic mechanisms have not been studied	[62, 63]
Helicobacter pylori	Various upper gastrointestinal diseases and extra digestive vascular conditions	AIDP, AMAN, MFS, and BBE	Not described	Molecular mimicry through VacA can induce myelin damage and GBS	[64, 65]
Borrelia spp.	Cutaneous involvement (e.g., "bull's eye" lesions) and systemic manifestations (i.e., neuritis, carditis, arthritis)	AIDP	Not described	Unclear whether molecular mimicry plays a role in *Borrelia*-related GBS	[66–71]
Rickettsia spp.	Exanthema, fever, headache, inoculation scar, and multisystem organ failure	AIDP	Not described	Unclear whether molecular mimicry plays a role in *Rickettsia*-related GBS	[73–76]
Ehrlichia chaffeensis	Flu-like symptoms, fever, myalgia, arthralgia, headaches, occasional rash	NA	Not described	Pathogenic mechanisms have not been studied	[77]
Coxiella burnetii	Flu-like disease, pneumonia, and hepatitis	AIDP	Not described	Pathogenic mechanisms have not been studied	[78]
Francisella tularensis	Ulceroglandular compromise, typhoidal symptoms	AIDP/AMAN?	Not described	Pathogenic mechanisms have not been studied	[79–81]

Modified from Jasti A et al. [161]

GBS Guillain-Barré syndrome, *AIDP* acute inflammatory demyelinating polyradiculoneuropathy, *MFS* Miller-Fisher syndrome, *AMAN* acute motor axonal neuropathy, *BBE* Bickerstaff's brainstem encephalitis

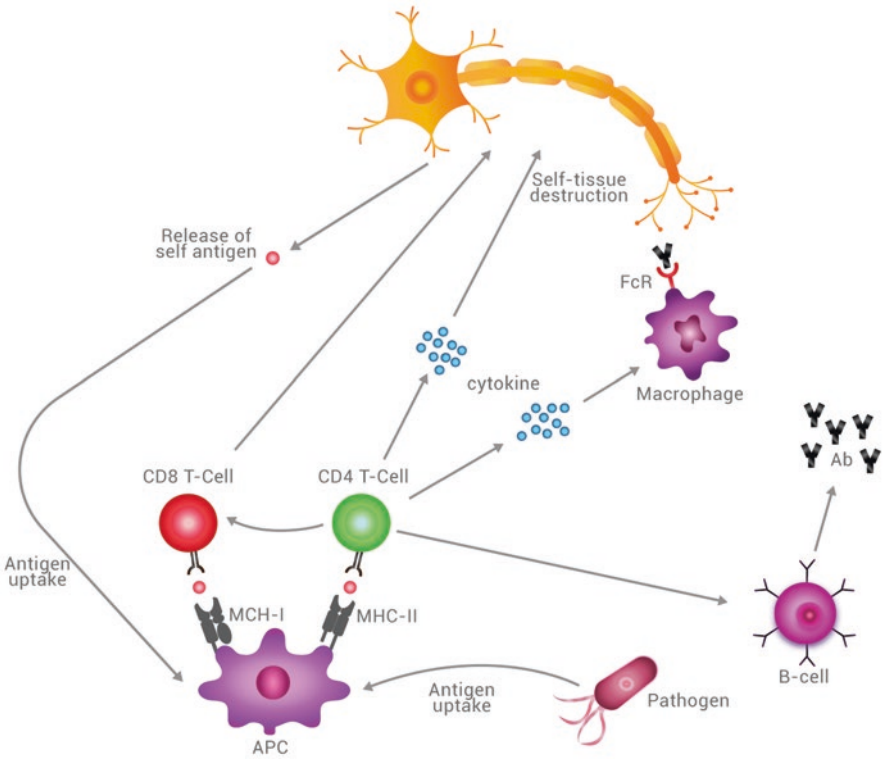

Fig. 1 Molecular mimicry**.** Immunological mechanism associated with the presence of Guillain-Barré syndrome related to previous infections. APC antigen-presenting cell, MCH I major histocompatibility complex I, MCH II major histocompatibility complex II, Ab antibody

usually associated with the presence of food-borne enteritis, which is transmitted by the ingestion of undercooked food. It has been observed that *C. jejuni* expresses epitopes capable of stimulating the immune system. Epitopes of *C. jenuni* that may stimulate the immune systems include glycoconjugates within the glycocalyx that are mainly formed by lipopolysaccharides [91, 92] The host gangliosides, composed of glycolipids which contains one or more sialic acid residues [92], are the main molecules associated with cross-reactivity between *C. jejuni* and the host [93], given its similarity with the lipopolysaccharides [94]. The main gangliosides associated with the immune response resulting from the cross-reactivity are GD1a, GM1, and GM1/GD1 complex located at the terminal nerves and anterior roots and GQ1b located on oculomotor nerves and primary sensory neurons [93].

Regarding *C. jejuni* genes associated with immune activity, the loci A, B, and C, which are related to the synthesis of lipooligosaccharides are crucial for the induction of ganglioside-like structures [94, 95]. Loci A and B are associated with *C. jejuni* sialyltransferase gene (Cst-II) [96]. This is relevant since the presence of Cst-II (Thr51) favors the synthesis of GM1-like, GM2-like, and GD1a-like lipooli-

gosaccharides, while Cst-II (Asn51) facilitates the synthesis of GT1a-like and GD1c-like lipooligosaccharides that can mimic GQ1b [97, 98].

M. pneumoniae is one of the main causes of respiratory infections. *M. pneumoniae* may also be a cause of GBS through molecular mimicry [99]. It has been proposed that the main host antigenic target is galactocerebroside (Gal-C), which is the main glycolipid associated with the formation of myelin [100]. Sensitization to Gal-C generates demyelinating neuropathy. It has been reported that up to 12% of patients with GBS and preceding infection with *M. pneumoniae* may present with anti-Gal-C antibodies [100]. In animal models, rabbit anti-Gal-C antibody bound to several glycolipids of *M. pneumoniae* and to Gal-C, suggesting a role of molecular mimicry between the glycolipids of myelin and *M. pneumoniae* [101].

Viral infections have also been associated with GBS, and again, molecular mimicry is among one of the main mechanisms. Among the main antigens associated with CMV is GM2, which is found in peripheral nerves [102]. The presence of anti-GM2 and anti-GalNAc-GD1a has been reported in patients with GBS with previous infection by CMV [103]. The envelope of CMV is formed largely by glycoproteins, some of which may cross-react with neural antigens [104]. Irie et al. demonstrated a decrease in IgM and IgG GM2 titers in the sera of patients with GBS and previous infection with CMV, supporting the interaction of anti-GM2 with cells infected by CMV. In addition, reactivity to gangliosides with a terminal GalNAc-Gal structure was described in this group of patients [102]. This was confirmed by Tsukaguchi et al., who described cross-reactivity between GalNAc-GD1a and GM2 in the host and glycoproteins of CMV as a possible mechanism of molecular mimicry occurring in CMV-associated GBS [105].

A different mechanism may be present in other viral infections such as hepatitis, where it has been suggested that the deposition of immune complexes (HBsAg-ICs) in the nerve can trigger an immune response culminating in the development of GBS [106]. In Zika virus infection, similar epitopes between the virus and neuronal antigens have been studied [107], demonstrating the presence of cross-reactivity which can lead to activation of the immune system against neuronal antigens. In fact, a number of peptides shared between the virus and proteins associated with demyelination and axonal neuropathy are present in epitopes that have been classified as immune-positive in the human host [107].

Pathogenesis

After activation of the innate immune system by an infection, the adaptive immune response is directed against the neuronal components such as the axon or myelin. In this sense, the clinical course of GBS will depend on which of these two components is mainly affected, thus generating two typical subphenotypes of the disease, AIDP and AMAN. In AIDP, the demyelination process is caused mainly by the presence of CD4+ T-cell macrophage. Unlike AIDP, AMAN is characterized by a

greater axonal involvement, the autoimmune mechanism driven by the presence of a humoral immune response against gangliosides [108].

Macrophages and Complement

The presence of endoneurial macrophages that express complement receptors, MHC-I, and MHC-II has been described [109] (Fig. 2). Additionally, these cells release proinflammatory cytokines such as TNF-α, IL-12, and molecules such as inducible nitric oxide synthase (iNOS) and matrix metalloproteinase 9 (MMP-9), that enable the expression of adhesion molecules, extending the inflammatory process, inducing damage in blood-nerve barrier, and damaging the myelin and axon [110–112]. On the one hand, it has been observed that macrophages have the ability to phagocytose myelin and axon components [113], generating segmented demyelination and axonal loss [114]. On the other hand, the activation of Th1 cells promoted by the presence of macrophages facilitates further activation of proinflammatory macrophages by the release of proinflammatory cytokines [115].

In addition, a repair process by macrophages has been described, promoting apoptosis of T cells [116] and facilitating the release of IL-10 and transforming growth factor beta (TGF-β) [108, 117]. It seems that the phenotype adopted by macrophages depends on the presence of TNF-α. In murine models, experimental autoimmune neuritis (EAN) is characterized by the presence of TNF--, which induces proinflammatory macrophages. However, the deficiency of this cytokine induces the production of anti-inflammatory macrophages with reparative functions [112].

Activation of the classical complement pathway is crucial in the development of neuronal damage in GBS (Fig. 2) [118–120]. Complement activation is generated by antiganglioside antibodies, such as anti-GQ1b antibodies, especially IgG3 or IgG1, since these antibodies have the ability to fix the complement in neuronal terminals [121]. In addition, monoclonal anti-GQ1b IgM antibody has the capacity to block nerve terminals through deposition of IgM and C3c [122]. Although the three complement pathways can all be associated with the pathophysiology of GBS, recent studies have shown that the classical pathway is the most important. This pathway allows membrane-attack complex (MAC) formation, which has been observed in experimental GBS models [123, 124]. In experimental c6 deficiency mouse models, monoclonal anti-GQ1b IgM antibody did not facilitate the formation of MAC at the nerve terminals. In mice deficient in Mac-inhibitory protein (CD59−/−), greater damage was observed in Schwann cells and neurofilament nerve terminals [123]. Inhibition of MAC formation by Ca2+-free Ringer failed to induce damage in the neurofilaments of nerve terminals. This demonstrates the importance of the classical pathway in the pathophysiology of GBS, since this pathway is dependent on Ca2+ [123].

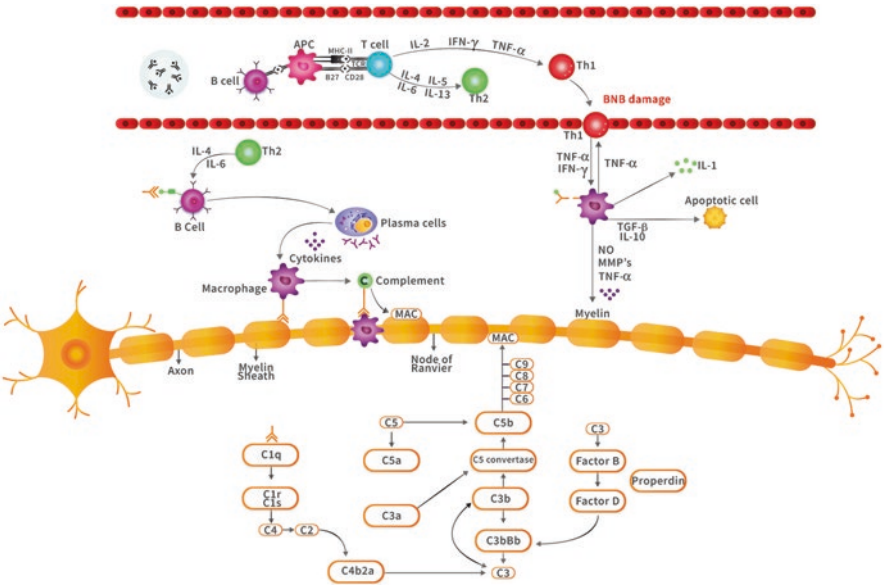

Fig. 2 Pathophysiology of Guillain-Barré syndrome. APC antigen-presenting celI, MCH II major histocompatibility complex II, Th2 T helper 2, Th1 T helper 1, TCR T-cell receptor, IFNγ interferon gamma, TNFα tumor necrosis factor alpha, TGFβ transforming growth factor beta, BNB blood-nerve barrier, NO nitric oxide, MMPs matrix metalloproteinases, MAC membrane-attack complex

Cellular Immune Response

Autopsies of patients with GBS have demonstrated the presence of inflammatory infiltrates in perivascular and endoneurial regions. These infiltrates have been observed in nerves, plexuses, and roots and are secondary to the presence of macrophages [125]. After the passage of macrophages through the blood-brain barrier, cytokines are released, thus increasing vascular permeability and facilitating the passage of activated T cells (Fig. 2) [126]. Moreover, there is an increase in the levels of IL-2 and soluble IL-2 receptor in the serum of patients during the acute phase, indicating the activation of T cells [127, 128]. Lymphocytes from patients with GBS cultured in myelinated axons generally destroy myelin directly (Fig. 1) [125]. The role of a cellular immune response mediated by T cells has been observed in murine [129, 130]. This activation of lymphocytes, such as CD8, can be generated by the entry of these cells through the permeable BNB. Upon admission, they are activated, thanks to stimulating factors released by macrophages, contributing to the autoimmune response [131].

Humoral Immune Response: AMAN

One of the main targets of the humoral immune response in the GBS is the axon gangliosides, specifically in the Ranvier node [132]. The gangliosides are anionic molecules of glycolipids that contain ceramide. This molecule is linked to residues of sialic acid and are important in the molecular structure of the peripheral nerves. GM1, GD1a, GT1a, and GQ1b differ in the number and position of sialic acids, where M, D, T, and Q represent mono-, di-, tri-, and quadrisialosil groups [132]. As previously discussed, the antibodies associated with axonal damage in AMAN facilitate complement fixation, especially the subclasses IgG1 and IgG3, which bind to the gangliosides GM1 and GD1a [133]. In animal models, it has been identified that in addition to complement fixation, the presence of these antibodies facilitates the recruitment of macrophages and the formation of MAC in the axolemma [134].

The presence of the gangliosides GM1 and GD1a is found in motor and sensory neurons [135]. These gangliosides seem to be found in a greater proportion in motor neurons, which explains their main association with the AMAN variant. On the other hand, other antibodies have been associated with the different clinical variants of GBS. This is the case of antibodies against GQ1b and GT1a, which are associated with MFS and its variant in the central nervous system, Bickerstaff's brainstem encephalitis [136, 137]. GQ1b is found in trochlear, abducens, and oculomotor nerves, as well as in muscle spindles in the limbs [137, 138]. Pharyngeal-cervical-brachial weakness is associated with the presence of anti-GT1a and GQ1b and anti-GD1a antibodies [139]. The gangliosides GT1a and GQ1b are found in glossopharyngeal and vagus nerves, which are associated with the characteristic symptoms in this variant [138]. This generates an alteration in the anatomical integrity at the level of the Ranvier node, thus blocking the nerve conduction, which is reversible in mild cases, but in aggressive cases it may lead to permanent axonal damage.

Humoral Immune Response: AIDP

In AIDP, the presence of a humoral immune response against antigens of myelin has been described [119]. Among the possible antigens are galactocerebrosides, such as LM1, and GD1b [140–144]. However, in murine EAN models, the inoculation of myelin proteins such as P0 and P2 has also been demonstrated to be associated with AIDP [18, 36]. Nevertheless, the pathogenic role of these antigens is not completely understood [145, 146]. Additionally, damage in voltage-gated sodium channels (Nav) clusters in EAN models immunized with peripheral myelin has been observed, and recently it has been shown that demyelination is associated with disruption of the Nav channel clusters in the nodes and commitment of Kv1 channels at paranodes and nodes [39, 40].

Another component of myelin that may be associated with the development of AIDP is neurofascin-186, which is found in Schwann cells. This molecule is altered after damage from Nav channel and demyelination [148, 149]. The presence of antibodies against this protein has been described in EAN models, suggesting that these antibodies may be associated with an alteration of the node in the absence of complement [149]. P2 protein may also be a target of the humoral immune response in GBS as demonstrated by the changes observed in the nodal region after immunizing rats with this peptide in an EAN model [147].

Diagnosis

Diagnostic criteria of GBS are shown in Table 4 [20] and the differential diagnosis in Table 5 [150]. The clinical course of the disease allows for early diagnosis. However, a definitive confirmation of the diagnosis requires diagnostic tools, such as CSF analysis and electrodiagnostic modalities. The presence of cyto-albuminological dissociation (normal cell count with increased protein levels) in the CSF is usually found. The first alterations in nerve conduction studies may usually be seen 2 weeks after the onset of symptoms. This tool allows differentiating between an axonal pattern and a demyelinating pattern (Fig. 3). The axonal pattern is characterized by a decrease in motor and/or sensory amplitudes, whereas the demyelinating pattern is characterized by reduced nerve conduction velocity, prolonged F-wave latency, prolonged distal motor latency, and conduction blockage (Table 6) [151].

Neuroimaging may be a useful diagnostic tool for GBS. Nerve ultrasound, 1 to 3 days after the appearance of the symptoms, may provide useful information in relation to nerve damage [152]. Morphological changes can be observed with this

Table 4 Diagnostic criteria for Guillain-Barré syndrome

	Level of diagnostic certainty			
Diagnostic criteria	1	2	3	4
Bilateral and flaccid weakness of limbs	+	+	+	+/−
Decreased or absent deep tendon reflexes in weak limbs	+	+	+	+/−
Monophasic course and time between onset-nadir 12 h to 28 days	+	+	+	+/−
CSF cell count 550/ml	+	+	−	+/−
CSF protein concentration > normal value	+	+/−[a]	−	+/−
NCS findings consistent with one of the subtypes of GBS	+	+/−[a]	−	+/−
Absence of alternative diagnosis for weakness	+	+	+	+

Taken from Fokke et al. [20]

NCS nerve conduction studies, *GBS* Guillain-Barré syndrome

[a]If CSF is not collected or results not available, nerve electrophysiology results must be consistent with the diagnosis Guillain-Barré syndrome

Table 5 Differential diagnostics of GBS

Motor neuron disease
Acute form of spinal muscular atrophy
Progressive (amyotrophic lateral sclerosis)
Bulbar form (dysarthria, dysphagia, and tongue denervation)
Acute viral poliomyelitis
Other neurotropic viruses (coxsackie, echovirus, enterovirus, West Nile virus)
Polyneuropathy
Copper deficiency
Polyneuropathy of the seriously ill in intensive care
Porphyria
Vasculitis
Neurotoxicity due to metals (arsenic, lead, thallium)
Lyme disease
Disorders of neuromuscular transmission
Myasthenia gravis
Paraneoplastic myasthenic syndrome
Botulism
Hypermagnesemia
Aminoglycosides
Neuromuscular blocking agents (pancuronium or vecuronium)
Muscle and metabolic disorders
Acute hypokalemic paralysis
Hyperkalemic periodic paralysis
Necrotizing myopathies
Acid maltase deficiency
Mitochondrial myopathy

Modified from van Doorn [150]

technique, especially in the proximal nerve segments at the level of the cervical nerve roots [152–154]. Magnetic resonance imaging (MRI) findings include post-gadolinium enhancement of peripheral nerve roots and cauda equine in up to 95% of patients. MRI may also rule out myelopathy and brain lesions [155]. Although both nerve ultrasound and MRI are promising tools for the diagnosis of GBS, further studies are needed in order to standardize protocols and to integrate them into the work-up of these patients.

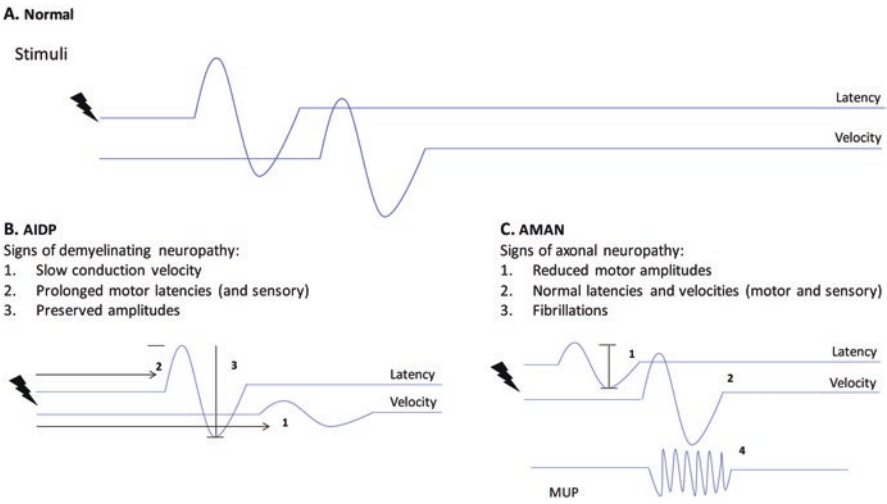

Fig. 3 Electrodiagnostic patterns in GBS. (**a**) Normal patterns of latency and velocity waves in electrodiagnostic studies (EDx). (**b**) Acute inflammatory demyelinating polyneuropathy (AIDP). Abnormal wave patterns and signs of demyelinating neuropathy. (**c**) Acute motor axonal neuropathy (AMAN). Abnormal wave patterns and signs of axonal neuropathy. MUP motor unit potential

Novel Therapeutic Strategies

The treatment of GBS is integrative, involving immunomodulatory management of GBS and treatment of complications. The most widely used immunomodulatory treatments for the management of GBS include PE and IVIg. Although effective for the treatment of most patients, there are cases in which neither of these are effective, and treatment then extends to other immunomodulatory agents.

IVIg is a pooled blood product containing mostly IgG [156]. This compound is obtained from healthy donors, so that it is enriched with antibodies directed to different pathogens, foreign antigens, and autoantigens [156]. The mechanism of action of IVIg in the treatment of GBS is unclear, but IVIg appears to neutralize or diminish immune activity against nervous system components in GBS. One proposed mechanism is the neutralization of complement pathways through the inhibition of the formation of the MAC and inhibition of C3b and C4b [156, 157]. In addition, IVIg regulates the production of proinflammatory cytokines, especially tumor necrosis factor alpha (TNF-α) and IL-1 [158]. IVIg modulates signaling through Fc receptors expressed on B cells and phagocytes, thus inhibiting demyelination induced by these cells [156, 157].

One of the most important mechanisms of IVIg is the neutralization of autoantibodies by binding to their variable regions, negatively impacting T-B cell interactions which lead to antigen presentation [159]. Several studies over the past years have been able to demonstrate the clinical efficacy of both PE and IVIg in the man-

Table 6 Criteria set employed for electrodiagnosis of GBS subtypes

AIDP	AMAN	AMSAN	Unexcitable	Equivocal
At least one of the following in at least two nerves: MCV <70% LLN DML > 130% ULN dCMAP duration >120% ULN pCMAP/dCMAP duration ratio > 130% F-response latency>120% ULN Or one of the above in one nerve, plus: Absent F waves in two nerves with dCMAP >20% LLN Abnormal ulnar SNAP amplitude and normal sural SNAP amplitude	None of the AIDP features in any nerve (demyelinating features allowed in one nerve if dCMAP <20% LLN) And at least one of the following in each of two nerves: dCMAP<80% LLN pCMAP/dCMAP amplitude ratio < 0.7 (excluding tibial nerve) Isolated F-wave absence (or < 20% persistence)	Same criteria of AMAN in motor nerves, plus SNAP amplitudes <50% LLN in at least two nerves	Distal CMAP absent in all nerves (or present in only one with distal CMAP <10% LLN)	Abnormal findings however not fitting criteria specific for other subtypes

Modified from Uncini and Kuwabara [151]
AIDP acute inflammatory demyelinating polyradiculoneuropathy, *AMAN* acute motor axonal neuropathy, *AMSAN* acute motor and sensory axonal neuropathy, *ULN* upper limit of normal, *LLN* lower limit of normal, *DML* distal motor latency, *MVC* motor conduction velocity, *CMAP* compound muscle action potential, *dCMAP* distal compound muscle action potential, *pCMAP/dCMAP* ratio between proximal and distal amplitude compound muscle action potential, *SNAP* sensory nerve action potential

agement of GBS. A 2012 Cochrane systematic review established the relative safety of IVIG and PE, with similar efficacies and a low rate of adverse effects [160]. IVIg is the preferred option in children and patients with hemodynamic issues [161].

PE is widely used in GBS due to its ability to remove immune complexes, autoantibodies, cytokines, and complement, through the use of filtration membranes [159]. The volume extracted by PE is replaced with albumin or congested fresh plasma [162]. An advantage of PE in the management of GBS is that patients can be treated with repeated exchanges to counteract the renewal of autoantibodies that can occur resulting from persistent antigenic stimulation [159]. While there is a slight but significant risk of relapse in the first year, most patients experience a complete recovery of muscle strength. The number of PE sessions usually ranges from 2 for mild illness to 4 for more severe presentations [163].

Other studies that sought to compare the efficacy and safety between PE and IVIg did not find significant differences [164, 165]. However, the ease of IVIg

administration makes it the preferred option [160]. Although the use of PE and IVIg is widely used with high rates of efficacy and safety, there are refractory patients in whom the use of other therapies such as cerebrospinal fluid filtration (CSFF), corticosteroids, biological drugs, and immunomodulatory drugs are viable options.

Given the important role of macrophages in both AIDP and AMAN [166], a mechanism of action of corticosteroids could involve inhibition of the migration and infiltration of macrophages in the peripheral nerves. However, this may also lead to an inhibition of M2 macrophages which are responsible for the repair of damaged neuronal tissue [166]. Some studies have shown no beneficial effects of corticosteroids on the clinical course of GBS [166, 167]. Moreover, two systematic reviews that compared corticosteroids with placebo found no significant differences [168, 169]. In addition, a Cochrane systematic review did not show significant differences between patients treated with corticosteroids and non-treated patients in relation to the degree of physical disability. Additionally, no difference was found between the two groups in relation to secondary endpoints such as time of ventilation recovery, unaided walking, death, and adverse events [170].

CSFF is an innovative therapy based on a high concentration of inflammatory mediators such as IL-6 and TNF-α and complement and antiganglioside autoantibodies in the CSF [171]. The mechanism involves the removal of these proinflammatory mediators that contribute to GBS and modulation of nerve demyelination [171]. This treatment has shown improvements in cell counts and protein concentrations [172]. Clinical trials have shown satisfactory clinical results with few adverse events [172, 173].

It is clear that the role of the complement in the pathophysiology of GBS is crucial, especially through the activation of the classical pathway [149]. Eculizumab, an anti-c5 monoclonal antibody used for the treatment of paroxysmal nocturnal hemoglobinuria, may be beneficial in GBS as well [174]. Eculizumab binds to C5, blocking its cleavage into proinflammatory molecules C5a and C5b and inhibiting the formation of MAC [174]. This has been shown to occur in animal models of MFS [174, 175]. In a phase II randomized clinical trial, eculizumab was shown to be efficacious and safe for the treatment of GBS [176].

rEV576 is a recombinant protein taken from the saliva of a soft tick and has the ability to inhibit complement [177]. The mechanism of action of this protein involves binding to C5 and blocking its cleavage to C5a and C5b, thereby inhibiting the classical and alternative pathway of complement [18, 23]. In vitro studies of MFS showed that rEV576 successfully inhibited MAC formation and mitigated damage to motor nerve endings [178].

Nafamostat mesilate is a synthetic serine protease inhibitor used in disseminated intravenous coagulation and acute pancreatitis [149]. Its mechanism involves inhibition of C5-convertase serine proteases, C1r, C1s, C3-convertase, and factors B and D [174, 179]. Nafamostat has been shown to prevent damage to Nav in animal models of AMAN [179].

Mirococept (APT070) is a complement regulator used in animal models of rheumatoid arthritis that can prevent complement-mediated tissue injury through the inhibition of C3/C5 convertase, thus blocking the formation of MAC [123, 149]. Human soluble complement receptor type 1 (sCR1), a cofactor of the serum

protease-I, is expressed in polymorphonuclear cells, monocytes, B cells, T cells, and erythrocytes [180] and has the capacity to indirectly degrade C3b and C4b and inhibit the activation of the classic and alternative pathways of complement [181]. In animal models, sCR1 has been shown to inhibit neuronal tissue damage by blocking demyelination [180, 182].

IFN-β is an immunomodulatory cytokine which blocks antigen presentation, regulates the activity of macrophages, and inhibits the production of TNF-α. The production of IFN- β is facilitated by the presence of Treg cells and the production of TGF-β [183]. Additionally, it has been observed that IFN- β inhibits the production of IL-12, which blocks activation of the Th1 response, facilitates the activation of Treg cells through the production of IL-10, and inhibits the cellular migration to neuronal tissue [183, 184]. In animal models, IFN- β suppresses the activity of the disease by inhibiting both the cellular and humoral immune responses [184].

The use of biologic modifiers such as rituximab can play a role in the management of GBS. Rituximab binds CD20, a molecule expressed on the cell surface of B cells, facilitating lysis of these cells [74]. Rituximab modulates the immune response mediated by complement and autoantibodies through suppression of B-cell activity [74]. Although clinical trials have not been carried out to evaluate its efficacy and safety in GBS, a case report showed the recovery of a patient with GBS after treatment with rituximab [185].

Other immunomodulatory drugs such as cyclophosphamide and mycophenolate mofetil have also been considered. Cyclophosphamide is an antineoplastic and immunomodulatory agent that causes cross-linking of tumor cell DNA, interfering with the growth of rapidly proliferating cells [186]. Although clinical trials of cyclophosphamide in GBS have not been performed, a case series showed clinical improvement in 15 patients with GBS [187]. In murine models, cyclophosphamide prevented the development of EAN, accompanied by a decrease in proinflammatory cytokines in nervous tissue and a decrease in symptoms [188]. Mycophenolate mofetil is an anti-inflammatory and immunosuppressive medication used in other ADs and in autoimmune neurological diseases such as Chronic inflammatory demyelinating polyneuropathy (CIDP).

CIDP is an autoimmune disease characterized by neurological symptoms and signs of progressive weakness, paresthesias, and sensory dysfunction. Other symptoms include reduced or absent tendon reflexes, cranial nerve involvement, autonomic symptoms, ataxia, and neuropathic pain. Unlike other autoimmune diseases, CIDP generally affects older individuals and has a male predominance. The onset is generally insidious and can take up to 8 weeks with a relapsing-recovery pattern [189, 190].

Mycophenolate inhibits guanine synthesis and the proliferation of T cells and B cells, as well as adhesion of molecules to cell surfaces [191]. One study evaluated the efficacy of methylprednisolone, IVIg plus mycophenolate mofetil compared to methylprednisolone and IVIg in patients with GBS. The results did not find differences between the two groups; however, this result could be attributed to the low doses of mycophenolate mofetil used [192].

Conclusions

Guillain-Barré syndrome is an autoimmune acute peripheral polyneuropathy with several clinical variants. The mechanism of action is not completely elucidated; however, molecular mimicry plays a significant role. Treatment of GBS mostly targets the immune response through the use of IVIg, plasma exchange, and other forms of immunomodulatory therapy. Corticosteroids are not useful for GBS. Immunosuppressives such as cyclophosphamides and mycophenolate mofetil have been used with moderate success, and the use of biological modifiers that target B-cell activity warrants a clinical trial. Eculizumab and other biological agents are promising new drugs for the treatment of GBS.

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Chronic Inflammatory Demyelinating Polyneuropathy

Miki Suzuki and Gérard Said

Abstract Chronic inflammatory demyelinating polyneuropathy (CIDP) is an autoimmune disease that targets the myelin sheaths of peripheral nerves. Lacking a specific diagnostic test, CIDP is diagnosed on the basis of the clinical presentation and demonstration of demyelination by electrodiagnostic or nerve biopsy studies. There are many phenotype variants of CIDP. Typical CIDP involves motor and sensory nerve dysfunction, with motor deficits reported in up to 94% of patients and sensory deficits in up to 89%. Half to two-thirds of patients with CIDP display the typical phenotype. The three proven treatments for CIDP are intravenous immunoglobulin (IVIG), corticosteroids, and plasma exchange. In the last years, a set of autoantibodies against proteins located at the node of Ranvier has been identified in some patients with CIDP. IVIG treatment is not satisfactory in the majority of antibody-positive CIDP patients.

Keywords Chronic inflammatory demyelinating polyneuropathy · Intravenous immunoglobulin · Steroid · Paranode · Nerve biopsy

Introduction

Chronic inflammatory demyelinating polyneuropathy (CIDP) is an acquired demyelinating neuropathy that is presumed to be of autoimmune etiology. Its clinical presentation and course are extremely variable. CIDP should be considered in any patient with progressive symmetric or asymmetric demyelinating polyneuropathy, because it often responds well to treatment. CIDP is characterized morphologically by long-standing multifocal demyelination that predominantly affects spinal roots, major plexuses, and proximal nerve trunks [1–5]. The clinical and pathological variability of CIDP accounts for the many diagnostic problems encountered in this setting.

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_25

Epidemiology

The prevalence of CIDP is estimated to be between 0.8 and 8.9 per 100,000 population [6], depending on the diagnostic criteria used [7]. In McLeod's study, its prevalence was higher in males than in females, and the age-specific prevalence reached a maximum of 6.7 per 100,000 population in the 70–79-year-old age group. The estimated crude annual incidence was 0.15 per 100,000 population. The mean age of onset was 47.6 years (median, 53.5 years) [8]. In a Japanese study, the prevalence rate per 100,000 was 1.61 in the total population, 2.01 in males, and 1.23 in females. The annual incidence rate was 0.48 per 100,000 population in the total population, 0.58 in males and 0.38 in females. The age-dependent incidence rate was 0.06 per 100,000 population in 0–15-year-olds, 0.04 in young adults (15–55-year-olds), and 0.73 in elderly persons (over 55-year-olds). These results were similar to reports in the Caucasian population [9].

In a retrospective study of disabling neuropathy in elderly persons, CIDP was the second most common cause, accounting for 14% of disabling neuropathy in this age group [10]. In another retrospective study of disabling neuropathy involving 100 patients over 80 years of age, CIDP was the most common cause, accounting for 32% of cases [11]. Most of these figures include cases of possible or probable CIDP. The stringent electrophysiological research criteria defined by the American Academy of Neurology Ad Hoc Subcommittee for the diagnosis of CIDP underestimate the actual incidence of CIDP [12].

Clinical Manifestations

The diagnosis of CIDP must be considered when a patient presents with a nonfiber-length-dependent demyelinating polyneuropathy that progresses over more than a month or has evolved chronically over many months. The subsequent course can be progressive or relapsing and remitting, often with a secondary progressive course [3, 4, 13–16]. Generally, weakness and ataxia as large-fiber abnormalities are predominant, whereas autonomic manifestations and pain as small-fiber abnormalities are less common [17]. The underlying demyelinating process is demonstrated by electrophysiological and, if needed, pathological studies.

Precipitating Factors

There is no identified genetically determined susceptibility to CIDP [18]. A history of an illness, mostly a nonspecific upper respiratory or gastrointestinal tract infection, or vaccination in the preceding 6 months was reported in 32% of cases [13].

A significantly increased risk of relapse has been documented in pregnancy. In a series of 100 patients, 16% noted an infectious event 6 weeks or less before the initial neurological manifestations; in seven patients, CIDP followed or was associated with viral hepatitis, since one patient had hepatitis A 6 weeks before the first neuropathic symptoms, and the others had chronic hepatitis B [15]. A flu-like syndrome was noted in the other patients. Three patients underwent a surgical procedure shortly before the onset of the neuropathy, and in one patient, neuropathic manifestations started during the postpartum period. Different patterns of CIDP, relapsing or progressive, have been observed at all stages of HIV infection. In most cases of HIV-CIDP, CSF pleocytosis is associated with increased protein content [19].

Age of Patients

CIDP occurs at any age [20]. In Bouchard's series of 100 adult patients with CIDP, the age of onset ranged from 10 to 82 (mean 52 ± 19 SD) years, in keeping with epidemiological studies [15]. Children can also be affected at any age from infancy [21, 22].

Neurological Manifestations at Onset

The manifestations at onset are variable. In the generalized pattern, numbness of the upper and lower extremities, spontaneous pains, and weakness progress gradually over several weeks. In some cases, a progressive sensory ataxia is the presenting manifestation, while in others, a predominantly or pure motor deficit is observed at onset. In most cases, the deficit is roughly symmetrical, both proximal and distal. In other cases, focal or multifocal involvement shows a multifocal demyelinating neuropathy with or without conduction blocks on electrophysiological testing. Some series required a motor deficit for the diagnosis [18, 23]. At onset, the incidence of motor deficit varied from 78% to 94% of cases in three large series [3, 13, 15]. In McCombe's series, a gradual onset of symptoms occurred in 84% of patients, while in 16%, the onset was acute, with the plateau of disability being reached within 4 weeks [13]. In many cases, the diagnosis of CIDP is made retrospectively because of the subsequent relapsing or progressive course or secondary involvement of other nerve territories. Pains were present at onset in 20–35% of the patients in the same series, and paresthesiae were present in 64–79% of the patients [3, 13, 15].

Neurological Manifestations at Referral or at Steady State

The clinical manifestations in the chronic phase, at steady state, or at referral reflect the symptomatic variety of CIDP. On average, a motor deficit occurs in 83–94% of patients, with sensory deficits in 72–89%. Facial palsy is observed in 4–15%, and there is loss of tendon reflexes in 86–94% of patients. Oculomotor palsy was present in 4–7% of patients [3, 13, 15]. Dysautonomia is not a feature of CIDP. In contrast to Guillain-Barré syndrome, autonomic symptoms are usually mild in CIDP [3, 13]. Autonomic dysfunction is not significant and mainly manifests as bowel and bladder complaints [24].

Increased CSF protein content is also seen in CIDP. Some authors required a CSF protein content of greater than 0.45 g/L for diagnosis, with less than 10 cells per mL [12]. In Bouchard's series, the CSF protein content was normal in 14% of patients, and cellularity was normal in all of them [15]. When the CSF is normal, it is mandatory to support the diagnosis with unequivocal demyelinative features on electrophysiological testing and/or pathological data.

Clinical Variants of CIDP

Clinical diversity in presentation and course is the most remarkable feature of CIDP. In this section, some of the most common or misleading manifestations at onset are reviewed.

Focal and Multifocal Neuropathies and CIDP

Chronic inflammatory demyelinating polyneuropathy presents in rare instances with focal or multifocal upper limb involvement. Gorson et al. reported the clinical and EMG findings in 10 such patients with upper limb CIDP, which they compared with patients with typical generalized CIDP (G-CIDP) and multifocal motor neuropathy (MMN). Symptoms began in one arm or hand in six patients and in both arms or hands in four and included numbness ($n = 10$), paresthesia ($n = 9$), weakness ($n = 8$), and pain ($n = 6$). Findings were initially restricted to the ulnar nerve distribution in three patients, and the median nerve and axillary nerve in one patient each, and they involved multiple nerves in five patients. Conduction block was detected in the forearm segment of 68% of the median and ulnar motor nerves tested; in contrast to MMN, 73% of the sensory nerves tested were abnormal, and none had anti-GM1 antibodies. Aside from the focal onset, there was no difference between the two groups. However, the magnitude of recovery following treatment was greater in patients with G-CIDP. The authors concluded that a multifocal variant of CIDP begins with upper extremity sensorimotor symptoms, simulates

isolated or multiple mononeuropathies, can be distinguished from MMN, and may have a less favorable response to treatment [25]. The multifocal variant of CIDP is also called multifocal acquired demyelinating sensory and motor (MADSAM) neuropathy, as well as Lewis-Sumner syndrome [26, 27]. The painful onset of upper limb multifocal deficits may mimic brachial neuritis [28].

Chronic Sensory Demyelinating Polyneuropathy

Some patients present with isolated sensory manifestations, including ataxia, pains, and paresthesiae of the lower extremities, which represent a subset of CIDP [29–31]. This atypical presentation, with only sensory symptoms and signs, may be seen in 6–15% of CIDP patients [32]. Two groups of patients can be identified among those presenting with isolated sensory manifestations: in the first group, sensory manifestations are followed after a variable period of time by motor deficit, which was the case in the series reported by van Dijk et al. [30]; and in the second group, the signs and symptoms remain purely sensory for years or decades.

We reviewed the data of 28 patients referred for a chronic sensory polyneuropathy, which was ascribed to a demyelinative process after electrophysiological and nerve biopsy findings and exclusion of all known causes of sensory neuropathies at referral and during follow-up: three patients developed motor deficits an average of 4.5 years after the onset of sensory symptoms [33]. They were classified as demyelinating and intermediate (some were in the demyelinating range) according to the AAN criteria. Sensory conduction velocities and motor conduction velocities in the demyelinating range were found in 3 and 2 cases, respectively. Motor and sensory conduction abnormalities in the demyelinating range were found in patients who developed weakness [34]. The AAN criteria for demyelination were a good predictor of the occurrence of secondary motor deficits in this population. Disability, mainly due to ataxia, was present at referral in 10 patients and at follow-up in 14 patients. Improvement was noted in 5/15 patients treated with oral prednisone and in 3/10 patients given intravenous immunoglobulin (IVIg) treatment [33]. In chronic sensory demyelinating polyneuropathy, demyelination may also be restricted to sensory nerves or dorsal roots [32, 35].

Katz et al. distinguished between patients with distal sensory or sensorimotor involvement, classified as distal acquired demyelinating symmetric (DADS) neuropathy, from those with proximal and distal weakness, who were classified as classic CIDP [36]. Patients with DADS present predominantly with sensory symptoms. When muscle weakness is observed, it is restricted primarily to distal muscle groups in a length-dependent fashion. Some patients with DADS neuropathy have IgM monoclonal gammopathies with anti-MAG antibodies [37] and typically do not respond to treatment with prednisone. In contrast, most patients with DADS and no IgM-protein respond readily to treatment with oral prednisone, plasma exchange, or IVIg [36].

Pure Motor Pattern

Pure motor patterns are observed in the same proportion of patients as pure sensory forms. Gorson et al. reported that about 10% of their CIDP patients showed a pure motor form [25]. A deleterious response to prednisone has been observed in pure motor CIDP [38, 39]. However, it is difficult to differentiate this worsening from lack of response to treatment with the natural progression of the neurological deficit.

CIDP in Childhood

CIDP is rarer in children than in adults, but the clinical aspects, course, and response to treatment are similar to those in adult onset CIDP [22, 40–43]. In a study comparing 12 children with idiopathic CIDP to 62 adults with idiopathic CIDP [44], the children often had more rapidly fluctuating courses than adults; a relapsing course was significantly more common in children than in adults. The recovery of children from each episode of deterioration was usually excellent and better, on average, than that of adults.

CIDP in Diabetic Patients

Patients with diabetes occasionally develop clinical and electrodiagnostic features suggestive of CIDP [45]. This diagnosis must be suspected when a predominantly motor and ataxic polyneuropathy occurs in a diabetic patient. In diabetic patients with CIDP, the nerve conduction studies showed more severe axonal loss and the degree of improvement following treatment was less in one study [46]. While some studies have suggested that diabetes mellitus occurs with increased frequency in patients with CIDP [46–48], other studies have shown that this increased prevalence of diabetes mellitus is better explained by a chance association [49].

Postural and Action Tremor in CIDP

Postural and action tremor can become very disabling in patients with CIDP. Such a tremor occurs in patients with minimal motor weakness regardless of the intensity of the sensory manifestations. It is attributable to increased physiological tremor by weakness with a possible role of decreased input from afferent large myelinated fibers [50].

Central Nervous System (CNS) Involvement in CIDP or CIDP and Multiple Sclerosis?

CIDP with CNS involvement has been reported [51–53]. CIDP was associated with symptomatic lesions of the CNS in 5% of cases in one series [15], and features of multiple sclerosis were found in the three patients who underwent MRI. All patients with CNS involvement were severely handicapped. Recently, antibodies to neurofascin 155 have been reported in some patients with combined CNS and peripheral nervous system inflammation; however, the clinical relevance of these findings is unknown [54].

Clinical Course and Prognosis

The long-term outcome of CIDP is unpredictable in the early stage of the disease. A variable proportion of cases follow a relapsing or chronic progressive course, with many patients starting with a relapsing course, followed by a secondary progressive course. In that respect, CIDP can be considered a peripheral analog of multiple sclerosis (MS). In addition, just as in MS, in which the pejorative prognostic marker of axonal degeneration has been recently stressed, loss of axons is the major pejorative prognostic marker identified in CIDP ($p < 0.0001$) [15].

In McCombe's series, two-thirds of patients followed a chronic relapsing course, and one-third had a progressive course. When reviewed after a mean interval of 10 years, six patients had died as a result of the disease, but 73% had made a good recovery; the prognosis was better in those with relapsing disease than in those with progressive disease [13].

Bouchard et al. [15] reviewed the follow-up data of 83 patients collected an average of 6 years after the first manifestations of neuropathy. Eight patients (10%) with relapsing forms were in remission, and 6 had relapsed at the time of evaluation. At evaluation, 38 patients were able to work, and 18 had retired without major disability. The outcome was good in 56% of the patients. Fourteen of the 83 patients died (17%), including nine as a result of progression of the neurological deficit to quadriplegia and respiratory and swallowing difficulty. The mean age of the patients who died was 67 years versus 49 years in Bouchard's cohort. Those who survived included 5 patients who were bedridden, 6 with severe, 11 with mild, and 23 with moderate handicaps, and 24 were fully autonomous. In Hahn's series, relapsing forms carried a better prognosis than progressive forms, in keeping with a better response of patients with acutely relapsing CIDP to IVIg treatment [55]. On the other hand, Van Doorn et al. [56] found no correlation between disease course and response to treatment. In Bouchard's series [15], 20 patients (24%), including the 9 patients who died as a consequence of their neurological deficits, were resistant to all treatment, despite a partial response for a few years.

In a study of 124 patients of different ages, Hattori et al. [57] found that half of the juvenile group had subacute progression initially, while most patients in the elderly group showed chronic insidious progression ($p < 0.0001$). Motor-dominant neuropathy was prominent in juveniles, while sensory-motor neuropathy was frequent in the elderly group ($p < 0.0001$). A relapsing and remitting course predominated in the juvenile group. Demyelinating and axonal degenerating features on sural nerve biopsy and on nerve conduction studies were common to the three age group studies. Functional recovery was common in all three age groups, but it was least apparent in the elderly group ($p = 0.00062$) [57].

A more recent study of 38 patients with CIDP found that, 5 years after the onset of treatment, 87% were able to walk and 26% experienced complete remission lasting for more than 2 years without treatment. Prognostic factors for complete remission were subacute onset, symmetrical symptoms, lack of muscle atrophy, good response to initial corticosteroid treatment, and a distal pattern on motor electrodiagnosis [58].

Pathogenesis

The pathogenesis of CIDP is incompletely understood and includes cell-mediated and several humoral mechanisms [59, 60]. The autoimmune etiology is supported by the efficacy of treatments that target the immune system, including IVIg, plasma exchange, and corticosteroids, and by evidence of an inflammatory response in the blood and peripheral nerves [60].

The presence of inflammatory infiltrations in sural nerve biopsies, changes in the frequencies/function of T-cell subsets, altered expression of cytokines, and other inflammatory mediators in the blood and cerebrospinal fluid of patients with CIDP indicate cellular immune mechanisms in the pathogenesis of CIDP [60]. T cells become activated, undergo clonal expansion, release inflammatory mediators, and cross the blood-nerve barrier (BNB). CD4+ and CD8+ T cells in variable proportions may be seen on examination of nerve biopsy specimens, but macrophages constitute the major cell component of the inflammatory infiltrate [15, 61]. During active phases of CIDP, the level of circulating tumor necrosis factor-α (TNF-α) increases [62]. It must be noted, however, that inflammatory infiltration is not very common in nerve biopsy specimens, and myelin stripping by macrophages, as observed in Guillain-Barré syndrome, is a relatively rare finding in CIDP. Expressions of TNF-α, interferon-γ, and interleukin-2 (IL-2) in the perineurium and endoneurial blood vessels may be pertinent to the breakdown of the BNB associated with CIDP [63]. Increased permeability in PNS capillaries enables access of circulating anti-myelin antibodies to the endoneurium, where they may bind to myelin sheaths and facilitate macrophage phagocytosis of myelin by Fc receptor mechanisms [64]. Activated resident and recruited macrophages, antigen presentation, and release of

proinflammatory cytokines or toxic mediators play an active role in many aspects of the immune response [60]. Macrophages also have an important role in the end stages of demyelination by stripping away and phagocytosing myelin [65]. A recent study of the T-cell repertoire in patients with CIDP found a broader activation of cytotoxic CD8+ T cells than CD4+ T cells that was reduced after treatment with IVIg [66].

The efficacy of the plasma exchange in the treatment of CIDP indicates hormonal mechanisms. Concerning the antibody-mediated mechanisms, conflicting results have recently been reported. One group found that CIDP is not associated with the antibody-mediated response to major glycolipids or myelin protein antigens [67]. On the other hand, Yuki et al. [68] reported elevated titers of immunoglobulin M (IgM) antibodies to sulfated glucuronyl paragloboside in six patients. A role for anti-PMP22 antibodies has also been suggested [69]. Yan et al. [70] successfully passively transferred disease using sera and purified IgG from 4 of 12 CIDP patients responsive to plasma exchange by bypassing the blood-nerve barrier through intra-neural injection or by opening it by activated T cells. The sera from CIDP patients or purified IgG produced marked conduction block and demyelination, which supports a role for antimyelin/Schwann cell autoantibodies in the pathogenesis of CIDP, at least in some patients. Some studies have detected autoantibody responses to P0, P2 [71, 72], and connexin [73] in CIDP serum, but these were not confirmed by others [60]. On the other hand, while antibodies to gangliosides are frequently found in GBS, such autoantibodies are typically not found in CIDP [74].

In recent years, autoantibodies against protein of the paranodes (neurofascin 155 (NF155), contactin 1 (CNTN1), or contactin-associated protein 1 (Caspr)) and nodes of Ranvier (neurofascin 186 (NF186)) occur in approximately 10% of CIDP patients [54, 74] (Table 1). These autoantibodies target nodal and paranodal structures and typically have an IgG4 isotype [75]. These autoantibodies are only found in a small subset of patients with CIDP; however, they can be used to guide therapeutic decision-making, as these patients have a poor response to IVIg [74].

In summary, both cell-mediated and humoral processes may play roles, but this still needs clarification.

Table 1 Antibodies in CIDP

Location	Antigen	Clinical phenotype
Paranode	Neurofascin 155 (NF155)	Distal motor involvement, sensory ataxia, tremor
	Contactin-associated protein (Caspr1)	Subacute onset, pain
	Contactin 1 (CNTN1)	Rapid severe onset, distal limb involvement, sensory ataxia, tremor
Node of Ranvier	Neurofascin 186 (NF186)	Subacute onset, sensory ataxia

Diagnosis

CIDP is diagnosed on the basis of the clinical presentation, evidence for demyelination on electrodiagnostic or nerve biopsy studies, and absence of other causes of demyelination [76]. In 1991, an Ad Hoc Subcommittee of the American Academy of Neurology proposed a set of diagnostic criteria for CIDP to be used for research purposes, and then several other criteria followed. The most recent widely accepted criteria were recommended by the European Federation of Neurological Societies and Peripheral Nerve Society (EFNS/PNS) in 2005 [77]. In this guideline, classical CIDP is categorized as "typical CIDP," and "atypical CIDP" includes multifocal acquired demyelinating sensory and motor neuropathy (MADSAM) or Lewis-Sumner syndrome/asymmetric CIDP, distal acquired demyelinating symmetric polyneuropathy (DADS), and pure motor or sensory CIDP. Multifocal motor neuropathy (MMN) and antimyelin-associated glycoprotein (anti-MAG) neuropathy were excluded from CIDP because of their different treatment responses.

Electrophysiological Data

Electrodiagnostic examinations are mandatory for the diagnosis of CIDP. The main purpose of electrophysiological studies in patients with suspected CIDP is to establish the presence of focal, multifocal, or diffuse demyelination, and to ascertain the anatomical extent and distribution of the abnormalities.

Furthermore, studies are needed to determine the degree of axonal loss, and EMG should therefore be included in the patient evaluation. In practice, the diagnosis of CIDP rests mainly on demonstration of an asymmetrical demyelinating process, and patients with an acquired demyelinating neuropathy often have differential slowing of conduction velocity when proximal and distal latencies of equivalent segments of two nerves in the same limb are compared. Uniform slowing of nerve conduction is suggestive of an inherited demyelinating polyneuropathy. Nonuniform slowing of nerve conduction, temporal dispersion, and conduction blocks are more common in acquired demyelinating polyneuropathies than in inherited disorders.

Due to the variability of phenotypes and the absence of specific biomarkers in CIDP [78], a large number of diagnostic criteria have been proposed to differentiate demyelinating from axonal neuropathy (for criteria review and discussion, see [79]), reflecting difficulties in the establishment of an accurate diagnosis. Most of these criteria have been developed while comparing to patients with typical axonal degeneration, such as ALS, and all revolve around the distinguishing features needed to identify demyelination as the central pathophysiological abnormality. Generalized demyelination also occurs in, for example, CMT type 1, and distinguishing features should include evidence of focal abnormalities such as conduction block or abnormal temporal dispersion [80]. Related to the multiple lesions, the

duration and dispersion of the distally evoked compound muscle action potential (CMAP) is increased in CIDP [79, 81].

Chronic inflammatory demyelinating neuropathy is typically characterized by a slowly progressive course with weakness and sensory loss in the legs and arms, and there is cranial nerve involvement in some patients. Nevertheless, the distribution of demyelinating lesions is multifocal, the disorder may have a variable clinical presentation, and the course may be relapsing [82]. Thus, the disease may have an acute initial phase and should in these cases be distinguished from Guillain-Barré syndrome, since the treatment differs in the two conditions [83]. Similarly, CIDP should be distinguished from other disorders with motor conduction block (MCB), including MMN, since steroids are effective in CIDP, but have no positive effect in MMN. Though demyelination is the main pathological lesion, axonal degeneration occurs to a variable extent and represents the main prognostic factor.

On nerve conduction study (NCS), unequivocal signs of demyelination include a reduction of motor nerve conduction velocity (MNCV) below 40% of the normal mean, along with relative preservation of CMAP amplitude [84–86], to exclude the effect of decreased conduction velocity due to loss of large myelinated fibers.

Sensory conduction studies usually do not have a prominent role in the diagnosis of CIDP, although it was demonstrated that slowing of sensory conduction is specific for demyelinating neuropathy [87]. On the other hand, the sensory conduction velocity (SNCV) was found to be less reduced than the MNCV over the same nerve segment [88], which indicates that motor and sensory myelinated fibers are affected to different degrees in CIDP. The pattern of abnormal median nerve versus normal sural sensory nerve action potential (SNAP) was found in 25–30% of cases in both AIDP and CIDP [89], and this differs from abnormalities in both hereditary demyelinating and in axonal neuropathies. Somatosensory evoked potentials can be useful to demonstrate abnormal proximal sensory conduction, particularly in sensory CIDP [35, 90].

The diagnostic criteria for CIDP have mainly been obtained by multicenter consensus discussions [91], and the sensitivity to include patients that may benefit from immunomodulating therapy has been questioned. Thus, for example, the criteria advocated by the American Academy of Neurology [12] have been found to be too restrictive [92], and more sensitive inclusion criteria have been derived by the Inflammatory Neuropathy Cause and Treatment group (INCAT) treatment studies [86]. The following criteria for CIDP have been obtained from various electrophysiological studies [12, 13, 93]:

- Motor conduction velocity less than 75% of the lower limit of normal values
- Distal motor latencies greater than 140% of normal values
- Conduction block and/or temporal dispersion of the CMAP
- Increased F-wave latency to greater than 120% of normal

Currently, the most widely accepted electrodiagnostic criteria are those recommended by EFNS/PNS, and they require demonstration of demyelinating abnormalities in at least two nerves for the diagnosis of definite or probable CIDP, or in one nerve for possible CIDP [77] (Table 2).

Table 2 Electrodiagnostic criteria of the ENFS/PNS CIDP guidelines [77]

(1) Definite: at least one of the following:
A. Motor distal latency prolongation ≥50% above the upper limit of normal values in two nerves (excluding median neuropathy at the wrist from carpal tunnel syndrome)
B. Reduction of motor conduction velocity ≥ 30% below the lower limit of normal values in two nerves
C. Prolongation of F-wave latency ≥30% above the upper limit of normal values in two nerves (≥50% if amplitude of distal negative peak compound muscle action potential (CMAP) < 80% of lower limit of normal values)
D. Absence of F-waves in two nerves if these nerves have amplitudes of distal negative peak CMAP amplitudes ≥20% of lower limit of normal values + at least one other demyelinating parameter[a] in at least one other nerve
E. Partial motor conduction block: ≥50% amplitude reduction of the proximal negative peak CMAP relative to distal, if distal negative peak CMAP ≥20% of the lower limit of normal values, in two nerves, or in one nerve + at least one other demyelinating parameter[a] in at least one other nerve
F. Abnormal temporal dispersion (>30% duration increase between the proximal and distal negative peak CMAP) in at least two nerves
G. Distal CMAP duration (interval between onset of the first negative peak and return to baseline of the last negative peak) increase in ≥2 nerve (median ≥ 6.6 ms, ulnar ≥ 6.7 ms, peroneal ≥ 7.6 ms, tibial ≥ 8.8 ms)[b] + at least one other demyelinating parameter[a] in at least one other nerve
(2) Probable
≥30% amplitude reduction of the proximal negative peak CMAP relative to distal, excluding the posterior tibial nerve, if distal negative peak CMAP≥20% of the lower limit of normal values, in two nerves, or in one nerve + at least one other demyelinating parameter[a] in at least one other nerve
(3) Possible
As in (1) but in only one nerve
To apply these criteria, the median, ulnar (stimulated below the elbow), peroneal (stimulated below the fibular head), and tibial nerves on one side are tested. If criteria are not fulfilled, the same nerves are tested at the other side, and/or the ulnar and median nerves are stimulated bilaterally at the axilla and at Erb's point. Motor conduction block is not considered in the ulnar across the elbow and at least 50% amplitude deduction between Erb's point and the wrist is required for probable conduction block. Temperatures should be maintained to at least 33 °C at the palm and 30 °C at the external malleolus (good practice points)

[a]Any nerve meeting any of the criteria (A-G)
[b]Isose S, et al. (Isose et al., 2009) [81]

In Bouchard's series of 100 patients, the criteria also included morphological features of a demyelinating process in nerve biopsy specimens. Electrophysiological tests showed a typical demyelinating pattern in 74% of patients; 4 patients had only one criterion of demyelination on nerve conduction studies. Thirteen (14%) of the 91 patients had nerve conduction blocks. No isolated or predominant axonal electrophysiological pattern was found. In some patients, undetectable sural nerve action potentials contrasted with relative preservation of nerve fiber density [15].

Several attempts have been made to improve the yield and reliability of electrophysiological tests in CIDP [80, 94], which must be tested now on a large scale and compared to pathological data.

Electrophysiological tests, although crucial for the diagnosis of demyelinating polyneuropathies, do not yield clear-cut results in many cases, due to the mixture of axonal loss with demyelinative features in patients with primarily demyelinating neuropathies. Allen and Lewis proposed 4 electrophysiological patterns that caused neurologists to misdiagnose other neuropathies as CIDP: (1) a length-dependent axonal neuropathy with an equivocal degree of slowing conduction velocities being overinterpreted as demyelination; (2) deep peroneal nerve findings from small foot muscles (extensor digitorum brevis) being interpreted as focal demyelination; (3) mild conduction velocity slowing in motor neuron disease being interpreted as demyelination; and (4) conduction velocity slowing or conduction block at sites of common nerve compression being interpreted as inflammatory demyelination [95].

Nerve biopsy should be considered when a clinical suspicion of an inflammatory demyelinating neuropathy remains in patients who do not meet the proposed electrodiagnostic criteria for demyelination [31, 96–99]. However, to avoid much of the disappointment concerning the yield of nerve biopsy, it is advisable to biopsy a nerve affected by the neuropathic process, rather than indiscriminately perform a sural or a superficial peroneal nerve biopsy [100, 101]. Nerve biopsy also has its pitfalls because the demyelinating process is not homogeneous, is basically asymmetrical, and because marked inflammatory infiltrates are seldom present at the biopsy site. Nerve biopsy may show only nonspecific lesions when demyelination and inflammation are proximal to the site of the biopsy. Thus, each method has its limitations.

Neuroimaging

An increased signal intensity on T2-weighted imaging of the brachial plexus can be seen on MR imaging of the brachial plexus in patients with CIDP and in patients with MMN, which may be useful to differentiate MMN from lower motor neuron disease [102]. Another MRI study showed hypertrophy of cervical roots and the brachial plexus in eight of 14 cases, six of whom also had hypertrophy of the lumbar plexus. Of 11 patients who received gadolinium, six showed enhancement. All patients with hypertrophy had a relapsing-remitting course and a significantly longer disease duration [103]. However, it is good to know that increased signal intensity on T2-weighted imaging of the brachial plexus and of the sciatic nerve can occur in other conditions, including infiltrating malignant lymphoma and sciatica secondary to disk herniation, as we observed. Three-dimensional nerve-sheath signal increased with inked rest-tissue rapid acquisition of relaxation imaging (3D SHINKEI) is a new technique to suppress signals of blood vessels, muscles, and fat tissue using improved motion-sensitized driven equilibrium and spectral attenuated inversion recovery. 3D SHINKEI can visualize the brachial plexus with high spatial

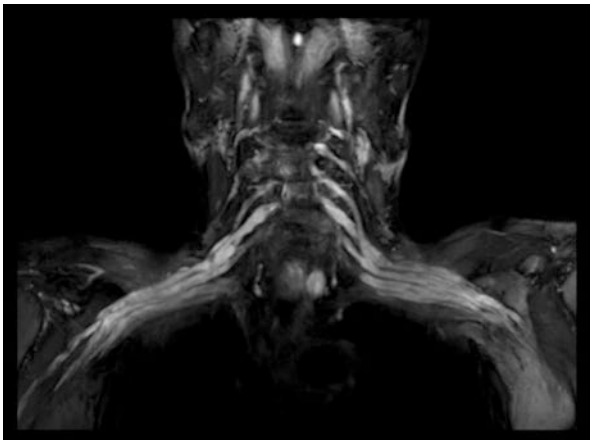

Fig. 1 3D nerve-sheath signal increased with inked rest-tissue rapid acquisition of relaxation enhancement imaging (3D SHINKEI) shows enlargement of the brachial plexus of a patient with CIDP

resolution [104] (Fig. 1). Whole-body MR neurography with diffusion-weighted whole-body imaging and background body signal suppression was also introduced as another possible method for visualizing the PNS over long trajectories in a single examination of patients with CIDP [105].

Ultrasound imaging is an emerging method for visualizing peripheral nerve pathology [106]. It can identify peripheral nerve lesions that are not apparent on electrodiagnostic testing. Nerve enlargement is common in CIDP. Nerve ultrasonography can identify the degree and pattern of nerve enlargement in multiple regions from the roots to the distal trunks [107].

In vivo corneal confocal microscopy (CCM) is a noninvasive, high-resolution imaging technique that allows the visualization and quantification of the corneal nerve network formed by small caliber Aδ and C fibers [108]. CCM has been demonstrated to quantify axonal loss in a variety of peripheral neuropathies including hereditary sensory and autonomic neuropathy, Charcot-Marie-Tooth disease type 1A, Fabry disease, and idiopathic small fiber neuropathy [109]. Loss of coronal nerve fiber has been also reported in CIDP patients [108, 109].

Morphological Findings

In patients with equivocal electrodiagnostic findings, pathological examination of nerve biopsies can help distinguish between primary demyelinating and axonal neuropathies [76].

The lesions consist of patchy lesions of demyelination and edema with variable inflammatory infiltration. Within nerve biopsy material, abnormalities can be associated with endoneurial edema, demyelinated fibers, macrophage-mediated demyelination, remyelination, Schwann cell proliferation with onion-bulb formation, inflammatory infiltration with mononuclear cells, axonal degeneration, and axon

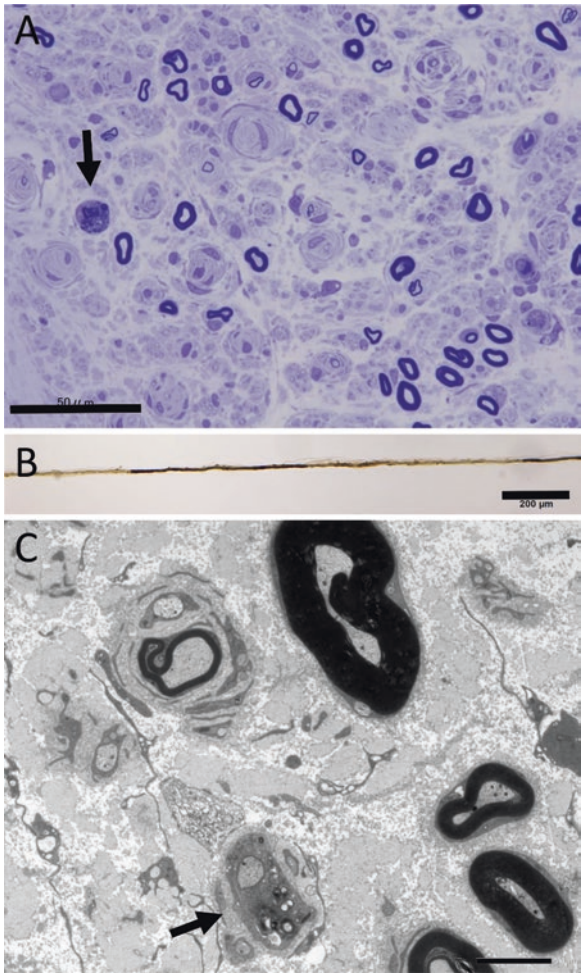

Fig. 2 Nerve biopsy of a patient with CIDP (the same patient as in Fig. 1). (**a**) 1-µm-thick cross-section of a sural nerve biopsy showing onion-bulbs and an actively demyelinating nerve fiber (arrow). (**b**) Teased fiber preparations showing segmental demyelination. (**c**) Macrophage-mediated demyelination in CIDP. Electron micrograph of the same specimen to show forming onion-bulb and demyelinated axon surrounded by macrophages filled with myelin debris (arrow)

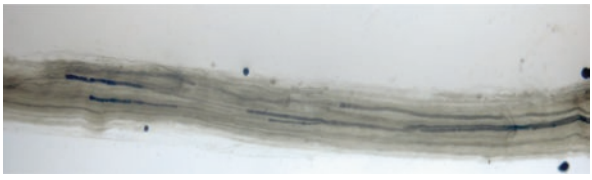

Fig. 3 Teased fiber showing segmental demyelination lesions. Normal myelin sheath is stained in black by osmium tetroxide

loss (Figs. 2, 3, 4). The presence of macrophage stripping of the myelin sheath is diagnostic of inflammatory demyelinating neuropathy [110, 111]. In a minority of cases, nerve biopsy shows variable numbers of mononuclear cells, including T cells, in the perivascular space or diffusely in the epineurium or perineurium [3, 15, 111–113]. Macrophages are the predominant inflammatory cell type. However, the demonstration

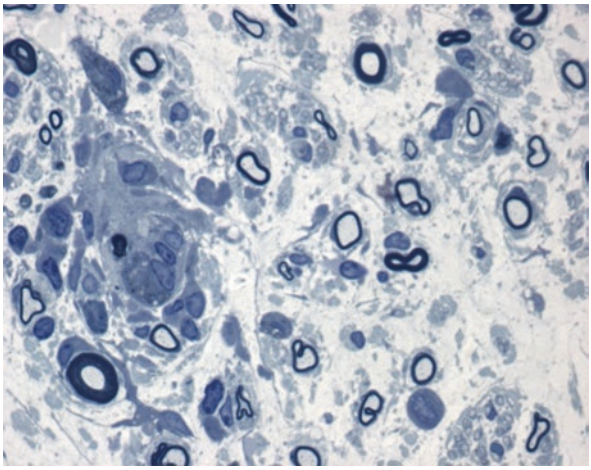

Fig. 4 Nerve specimen of a patient with CIDP showing active demyelination

of macrophage stripping of the myelin sheath and even of an inflammatory infiltrate is far from universal in nerve biopsy specimens.

In Bouchard's series, the teased fiber preparations from 95 nerve biopsy specimens showed purely demyelinative lesions in 68 patients, mixed axonal and demyelinative lesions in 20 patients, and predominantly axonal lesions in five patients. The nerve specimen was normal in two patients. Active demyelinating lesions were found in 25 nerve specimens. Endoneurial cellularity was increased in 18 samples with perivascular predominance, and only four specimens showed conspicuous inflammatory infiltration. In all cases, the inflammatory infiltration was made up of mononuclear cells. In the four specimens with marked inflammatory infiltration, immunolabeling showed a mixture of CD4 and CD8 T lymphocytes, macrophages, and a few B cells. In 17 nerve specimens, there were one or more "onion-bulb" formations per microscopic field at ×630 magnification. An important finding was that the density of myelinated fibers was below 50% of control values in 47% of patients [15]. Another study of sural nerve biopsies in 71 patients with CIDP and of motor neuron pathology following postmortem examination of the sural cords of nine patients who died of CIDP clearly confirmed the importance of axonal and neuronal involvement in this setting [114]. In this study, the overall decrease in myelinated fiber density was 65.4% of control values. Nerve biopsy may also be useful to exclude amyloidosis, vasculitis, sarcoidosis, and lymphomatous infiltration [115].

Autopsy studies in patients with CIDP show loss of spinal motor neurons, with demyelination and axonal degeneration. Focal lymphocytic infiltration, most prominently in the spinal roots and dorsal root ganglia, can be seen in approximately half of the cases [3, 114, 116].

In a morphological study recently performed by Koike et al. using electron microscopy, they observed detachment of terminal myelin loops from the axolemma at the paranodes in the neurofascin 155 antibody-positive patients that was not seen in antibody-negative CIDP patients [117].

Differential Diagnosis

Multifocal Motor Neuropathy (MMN)

MMN is a type of acquired demyelinating neuropathy different from CIDP. The main clinical features are weakness without objective sensory loss, a slowly progressive or stepwise progressive course, asymmetric involvement of two or more nerves, and absence of upper motor neuron signs [118, 119]. The presence of conduction blocks in motor nerve fibers is the hallmark of this disease. Elevated titers of IgM anti-GM1 antibodies are present in approximately 50% of patients with MMN. The distinction between LS-S and MMN could be made on the basis of IgM anti-GM1 antibody status [7]. Motor neuron disease is always considered in the differential diagnosis of this disease. The accepted treatment for MMN is IVIg [120]. In contrast to the response in CIDP, MMN does not usually respond to steroids or plasma exchange, and patients may worsen when they receive these treatments [121, 122].

Demyelinating Neuropathies Associated with Monoclonal Gammopathy

Monoclonal Gammopathy of Unknown Significance (MGUS) Polyneuropathy

The association of acquired demyelinating polyneuropathy with monoclonal gammopathy is relatively common. Monoclonal gammopathies are 10 times more frequent in patients with polyneuropathy than in age-matched controls, and 10% of adults with acquired polyneuropathy have a monoclonal gammopathy [123, 124]. The incidence of polyneuropathy among patients with IgM monoclonal gammopathy can be as high as 50%, implying that 50% of patients with IgM MGUS may have or develop polyneuropathy [123–125]. Protein electrophoresis and immunoelectrophoresis are always necessary in this setting. IgM monoclonal gammopathy of unknown significance (MGUS) polyneuropathy is usually slowly progressive, symmetrical, and predominates in the distal lower extremities. It is identified clinically and electrophysiologically by its fiber length distribution and its symmetry [126]. Morphologically, macrophage-mediated demyelination is seldom observed in MGUS-associated demyelinating neuropathy, but a widening of myelin lamellae occurs in myelin-associated glycoprotein (MAG)-positive cases. On serological testing, 50% of patients with MGUS-P have anti-MAG IgM antibodies [127, 128]. The major difference between patients with MGUS polyneuropathy and CIDP is the risk of malignancy in the following years. An increased risk of malignant transformation exists for patients with any MGUS, but it is highest for those with IgM MGUS polyneuropathy [129].

Occasionally, low levels of monoclonal IgA or IgG are found in otherwise classical CIDP patients [130, 131].

Polyneuropathy, Organomegaly, Endocrinopathy, M-Protein, and Skin Changes (POEMS) Syndrome

POEMS syndrome is a paraneoplastic syndrome due to an underlying plasma cell neoplasm [132]. It is necessary to consider the diagnosis of POEMS syndrome when a progressive sensorimotor demyelinating polyneuropathy is associated with monoclonal gammopathy and other uncommon manifestations, including skin pigmentation, hepatosplenomegaly, papilledema, enlarged lymph nodes, endocrinopathy, edema, thrombocytosis, and elevated vascular endothelial growth factor (VEGF). The most disabling feature of POEMS is the demyelinating neuropathy, frequently misdiagnosed as CIDP. Neuropathy in POEMS syndrome is typically symmetrical, sensorimotor, length-dependent, and painful [133]. Neurophysiological findings in POEMS syndrome fulfill the diagnostic criteria for CIDP in 70% of cases [134]. Conduction block and temporal dispersion are uncommon when compared to CIDP [133]. A total body scan must be performed to detect sclerotic bone lesions. This syndrome often responds well to specific treatment for plasmacytoma.

Light Chain Amyloid Neuropathy

In this setting, the occurrence of autonomic disturbances, in association with the monoclonal gammopathy, in a patient with progressive acquired demyelinating polyneuropathy suggests the development of light chain amyloid neuropathy with a very poor prognosis due to progressive axonal degeneration of the majority of the peripheral nerve fibers and multi-organ failure. Patients with late onset, sporadic cases of transthyretin-related familial amyloid polyneuropathy may be mistaken and treated for CIDP for months or years [135]. Endoneurial deposits of amyloid can induce demyelination of neighboring fibers, which can be responsible for a polyneuropathy that may mimic a case of progressive CIDP for a long period of time.

Early-Onset CIDP and the Dejerine-Sottas Phenotype

Onset of generalized CIDP during infancy or early childhood can induce a neuropathic pattern with Charcot-Marie-Tooth atrophy, which can be clinically indistinguishable from the so-called Dejerine-Sottas disease. In a series of 15 patients with the Dejerine-Sottas phenotype, the clinicopathological findings along with the absence of an identified mutation suggested the diagnosis of CIDP of infancy onset in five patients, including two who had a relapsing course. The important point to note is that such patients may respond to treatment with corticosteroids [136].

Acute or Subacute Exacerbation in Demyelinating Charcot-Marie-Tooth Disease (CMT)

CIDP can develop in some patients with CMT; this overlap should be suspected when a patient with CMT shows an unusual pace of disease progression, because in contrast to the underlying CMT, deterioration due to CIDP can improve with immunotherapy [137].

Patients with demyelinating CMT occasionally experience acute or subacute exacerbation of the neuropathy with superimposed inflammatory features, which may respond to corticosteroids [138].

Another recent study in CMT patients found an association with CIDP that was greater than would be expected by chance and stressed the importance of looking out for unexpected clinical deterioration in CMT patients, because immunotherapy may relieve these exacerbations [139].

Treatment

The first-line treatments for CIDP include corticosteroids [140], IVIg [141], and plasma exchange [55, 142]. These are all effective in about 70–80% of CIDP cases [143]. Comparative trials showed the short-term equivalent efficacy of IVIg and plasmapheresis [142] and of IVIg and prednisolone [144].

Corticosteroids are much more widely available than IVIg, they are cheaper and easier to use [145], and they may lead to long-term remission in CIDP [146]. The generally accepted prednisolone dosage is 60 mg/day or 1.5 mg/kg bodyweight on alternate days for induction, with maintenance therapy slowly tapering over months to years.

IVIg may contain anti-idiotypic antibodies and lead to inhibition of maturation and altered function of dendritic cells, as well as modulation of T- and B-cell activation, differentiation, and effector functions [147]. The efficacy of IVIg has been shown in several studies [55, 141, 148–150]. The initial dosage of IVIg is usually 0.4 g/kg bodyweight for 5 days. However, approximately two-thirds of patients with CIDP need long-term IVIg [151].

To follow the response to treatment in individual patients, several standardized outcome measures such as the Hughes functional grading scale, Medical Research Council (MRC) muscle score, hand grip strength, INCAT (inflammatory neuropathy cause and treatment) disability score, walking test, and R-ODS (Rasch-build Overall Disability Scale) have been proposed [152].

A relapse rate of approximately 45% was reported for responders who were switched from IVIg to placebo [141]. Because the benefit of IVIg is short-lived, treatment needs to be repeated at intervals and doses that need to be judged on an individual basis [153].

Subcutaneous immunoglobulin (SCIg) has been evaluated as an alternative to IVIg [154]. One recent large study supported a weekly SCIg dose range of

0.2–0.4 g/kg and showed that SCIg can be used as maintenance treatment for CIDP patients [151].

Other immunosuppressive agents, including cyclophosphamide, cyclosporine, mycophenolate mofetil, azathioprine, methotrexate, interferon-β1a, interferon-α, and rituximab, have been reported to be useful in patients refractory to conventional treatment, but none has been studied rigorously in a randomized, controlled trial [155].

CIDP patients with autoantibodies have a specific clinical phenotype that is distinct from CIDP without antibodies [54]. They typically respond poorly to IVIg, but they may benefit from plasmapheresis and rituximab [54, 156]. In patients with nodal and paranodal antibodies, corticosteroids are reported to be effective in approximately 40–60% of cases [75].

For preventing secondary axon loss and improve prognosis, early and aggressive therapy is required [15].

Conclusions

CIDP is a treatable syndrome presenting with variable manifestations. The diagnosis is based on the clinical presentation, electrodiagnostic findings, laboratory tests, and nerve biopsy in some cases. First-line treatments for CIDP include corticosteroids, IVIg, and plasmapheresis. The diagnosis should be reconsidered in patients who do not respond to one of the first-line therapies. Many questions remain unanswered regarding the pathophysiology, management, and treatment of CIDP, which is still a disabling and sometimes life-threatening disorder.

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Myasthenia Gravis and Other Immune-Mediated Disorders of the Neuromuscular Junction

Nils Erik Gilhus

Abstract Myasthenia gravis (MG), Lambert-Eaton myasthenic syndrome (LEMS) and neuromyotonia represent the three autoantibody-mediated disorders at the neuromuscular junction. They give muscle weakness and fatigability as their dominating symptoms. The weakness has usually a preferred localization to some but not all muscles. MG subgroups reflect pathogenesis and direct therapy. Patients should always be classified according to type of antibody, thymus pathology, age at symptom onset and generalized versus pure ocular symptoms. LEMS and neuromyotonia are subgrouped according to paraneoplasia or not. All conditions have well-defined autoantibodies that bind in vivo and directly induce the muscle weakness. Therapy includes symptomatic drugs influencing the acetylcholine receptor activity in the postsynaptic membrane and immunosuppressive treatment influencing the pathogenic autoantibodies. This immunoactive treatment is not yet specific for the disease-inducing antigen-antibody interaction. Treatment is usually effective, and most patients obtain mild symptoms only or a full clinical remission. Comorbidities need to be treated, especially a thymoma in paraneoplastic MG or neuromyotonia and a lung cancer in paraneoplastic LEMS. Supportive therapy is important, and a well-adapted daily training program is recommended. Severe exacerbations (myasthenic crisis) with the need for respiratory support are rare, occur mainly together with infections, and need immediate intensive care.

Keywords Myasthenia gravis · Lambert-Eaton myasthenic syndrome · Neuromyotonia · Neuromuscular junction · Autoimmunity

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_26

Introduction

The neuromuscular junction is a predilection site for disease. The site is crucial for muscle function, and disorders at this junction lead to weakness in the muscle. The disorders can be immune-mediated through the action of autoantibodies. In addition, genetic disorders and toxins can interfere with neuromuscular transmission. More than 100 mutations have been detected in either presynaptic or postsynaptic molecules, most commonly in the postsynaptic acetylcholine receptors (AChR) [1]. Such mutations usually lead to a stable generalized weakness with symptom debut during the first couple of years after birth. Rarely congenital myasthenia due to genetic disorders can be misdiagnosed as immune-mediated disease, and vice versa. The neuromuscular junction is a predilection site for animal and plant toxins. The induction of muscle paralysis is an excellent strategy both for attacking a potential prey and for defence. Botulinum toxin binds presynaptically, whereas curare and α-bungarotoxin are examples of postsynaptic toxins binding to the AChR.

Action potentials in the motor nerve lead to the release of acetylcholine from the presynaptic terminal. This release acts through the activation of voltage-gated calcium channels in the presynaptic cell membrane, allowing calcium to enter the neuron and triggering vesicles containing acetylcholine to fuse with the cell membrane. The acetylcholine traverses the synaptic cleft and binds to AChR. AChR serve as ligand-gated ion channels, so that binding of acetylcholine opens the central pore, sodium ions flow into the muscle cell, and this generates the muscle depolarization that eventually results in muscle contraction. Autoantibodies specific for immune-mediated disorders interfere with various parts of this cascade, all leading to impaired function and muscle weakness.

There are three main immune-mediated disorders of the neuromuscular junction: myasthenia gravis (MG), Lambert-Eaton myasthenic syndrome (LEMS) and neuromyotonia. These disorders should be further subgrouped according to clinical and non-clinical biomarkers (Table 1) [2–6]. The three main diseases are characterized by their clinical picture, autoantibodies and neurophysiological characteristics. The MG subgroups are less distinct. Some patients with debut after 50 years can have thymic hyperplasia, a thymoma can be detected some years after MG debut, or a patient can have purely ocular symptoms for many months before progressing into generalized muscle weakness. Such patients challenge the formal subgroup classification. The absence of antibodies in seronegative MG depends on the sensitivity of the applied tests [7]. In ocular MG, the muscle weakness is clinically restricted to the ocular muscles. This is common early in the disease, but applies to only around 10% after 2 years [8].

Both LEMS and neuromyotonia can be paraneoplastic, associated most commonly with small-cell lung carcinoma and thymoma, respectively. The disease with and without cancer is otherwise clinically indistinguishable.

This chapter will give updated information on all aspects of the immune-mediated disorders at the neuromuscular junction, but with focus on therapeutic strategies and aspects that influence therapy. MG as by far the most common of the diseases will be described most detailed.

Table 1 Immune-mediated diseases at the neuromuscular junction with the clinically important subgroups of myasthenia gravis (MG) and Lambert-Eaton myasthenic syndrome (LEMS). Circulating autoantibodies against acetylcholine receptors (AChR), muscle-specific kinase (MuSK) and lipoprotein-related peptide 4 (LRP4) are the most important biomarkers for diagnosis, pathogenesis and treatment

Disease	Antibody	Onset age	Thymus/Cancer
MG Early onset	AChR	<50 years	Thymus hyperplasia
MG Late onset	AChR	>50 years	Thymus atrophy
MG Thymoma	AChR	Any	Thymoma
MuSK MG	MuSK	Any	No
LRP4 MG	LRP4	Any	No
MG Seronegative	None detected	Any	Thymus hyperplasia or no
MG Ocular	AChR or none	Any	Thymus hyperplasia or no
LEMS non-paraneoplastic	VGCC	Any	No
LEMS paraneoplastic	VGCC	Any	Lung cancer
Neuromyotonia	VGKC	Any	Thymoma, cancer or no

Epidemiology

MG has a prevalence of approximately 150 per million in most populations, and an annual incidence of around 10 per million [4, 9, 10]. In most Western populations, there is one peak of incidence around age 30 years, and then a gradually increase from age 50 years, at least until age 80 years. In China, there is an additional incidence peak in children around age 5 years. This juvenile MG in the Far East is usually mild and often ocular and otherwise resembles early-onset MG with AChR antibodies [11]. In MG with AChR antibodies and symptom debut before age 50, there is a clear preponderance of females, a two- to three-fold increase compared to males. Late-onset MG is more common in males. This means that in the total MG population the sex ratio is near to one. In countries with a young population, MG is more common in females.

MG prevalence has increased gradually for many decades. This does not necessarily mean that the risk for getting the disease has increased [12]. Prevalence depends on disease prognosis. Today, with the improved treatment, only a slight increase in mortality will lead to a higher prevalence compared to the situation before any effective treatment with perhaps a 50% mortality after 10 years. A second reason for the reported increase in MG prevalence is an improved case-finding. Previously MG was a clinical diagnosis, and thus given only to patients with the typical clinical picture, and recognized by the responsible doctor. Today the diagnosis relies for a large part on highly specific autoantibody analyses. Such antibody tests are performed with increasing frequency, and also in individuals with atypical or mild muscle weakness and fatigue with only a minimal clinical suspicion of MG. The number of neurologists has increased and access to specialists for the whole population has improved in most countries. All this has led to a better case-finding and therefore a higher MG prevalence. Studies using well-organized national patient registries and with a detailed examination of defined cohorts are expected to

find more cases than previous and old reports from single or multiple hospital charts only. Finally, population demographics influence MG prevalence. Especially in Western countries, the ageing of the population leads to a higher prevalence of MG since MG has the highest incidence in the older age groups (Fig. 1).

MG incidence has similarly been reported higher in recent years than previously, and also when adjusted for population demographics. Improved case-finding may explain this increase. A modest and real increase in MG in elderly people has been suggested but not proven. There are no known reasons for a potential increase in MG incidence. The incidence of thymomas has not increased, but again case-finding is better, this being due to more widespread use of thoracal CT or MR. Many thymomas are detected as a coincidence at such examinations, and some few patients turn out to have a mild undiagnosed MG with AChR antibodies.

MuSK MG has a particular geographic pattern. It has a much higher prevalence in the Mediterranean area than in the Scandinavian countries, and with a clear tendency for a south-north divide [13]. However, in China the south-north divide seems to be inverse with the highest frequency in the north [14]. The geographical difference is for a large part, or entirely, explained by genetic population differences, especially HLA gene variation.

Any occurrence of MG clusters in location and time should help in finding etiologic MG factors. No such clusters have been reported in epidemiological studies. Migration studies would help in differentiating between hereditary and environmental factors causing MG. However, MG is a rare disease and good studies with sufficient statistical power are lacking. Studies support the genetic influence, whereas

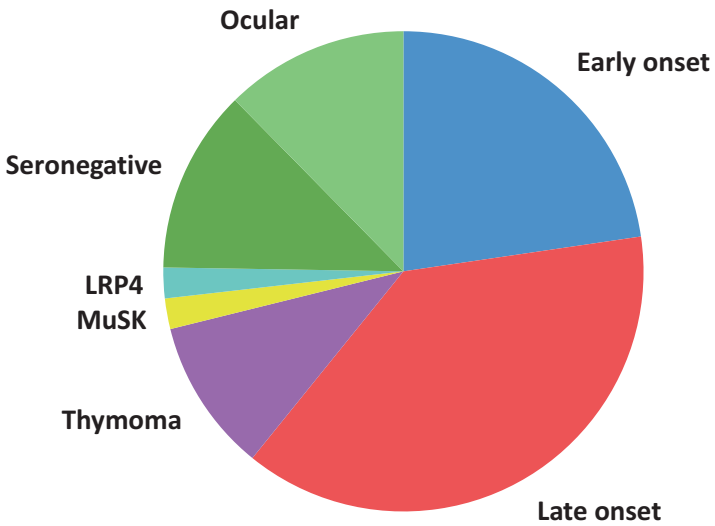

Fig. 1 Illustration of the relative prevalence of the various MG subgroups in European and North American populations. The size of the seronegative group without detectable muscle antibodies depends on the sensitivity of the assays used

no new potential environmental factors have been put forward [15]. Best estimates have hypothesized that environmental and genetic factors might be equally important in causing MG [16].

LEMS is much rarer than MG. It has been reported with a prevalence of 2–3 per million that is fifty times less common than MG [17]. The annual incidence was 0.5 per million, which was fourteen times less than MG. Approximately one half of new patients with LEMS have a small-cell lung carcinoma. These patients have a poor prognosis for survival, which explains the discrepancy between prevalence and incidence figures when LEMS and MG are compared [18]. LEMS occurs in 0.5–3% of all patients with small cell lung carcinoma and is probably not always recognized as a distinct comorbidity in these patients. The lowest incidence figures reflect what is observed in clinical practice, whereas the highest occur in prospective studies with clinical, neurophysiological and immunological follow-up of all patients. Younger patients with small cell lung carcinoma are more prone to develop LEMS than the older ones, a ten-year age difference in patients with and without LEMS [17, 19]. LEMS can occur in all age groups, but very rarely in children. Mean age at debut in a European cohort was 58 years, definitely higher than for MG. LEMS without carcinoma is equally common in males and females, whereas LEMS with small-cell lung carcinoma reflects smoking habits in the population.

Neuromyotonia is a very rare disease, much rarer than LEMS. No reliable epidemiological data exist, only small series of single patients. In up to one-third of patients, neuromyotonia co-exist with a thymoma and is paraneoplastic.

Clinical Manifestations

MG is characterized by muscle weakness. This muscle weakness is similar for all MG subgroups. Typical for MG is variation over time. The muscles are often strong in the morning and before being used. The weakness increases after repetitions and sustained use, so that fatigue is common. Patients experience this as a chronic muscle weakness, with variation over time, and with a reduced ability for physically demanding tasks. Symptoms can be matched by weakness measured by formal testing [20], but such testing is not always feasible.

The muscle weakness in MG is localized to some but not all muscles and muscle groups. It is confined to skeletal muscle. Most MG patients experience a distinct weakness in extraocular muscles. This leads to two symptoms; diplopia and ptosis. These manifestations can be observed by clinical examination. The ocular muscle weakness is often markedly asymmetrical, with ptosis on one eye only, and divergent eye movements. The asymmetry makes the clinical diagnosis easier. Eye muscle weakness is often a debut symptom of MG. In 15% of patients, the eye muscle weakness persists as the only MG symptom and sign [8]. In 90% of MG patients with eye symptoms only after 2 years, the disease will remain as a pure ocular MG.

Most MG patients have a more generalized weakness. Difficulties with swallowing and chewing and a weak voice are typical ("bulbar symptoms"). Neck and shoulder muscles are often weak, and problems with lifting the arms above the head are common. Trunk muscles are often weak, whereas muscles distally in the extremities, in hands, fingers and feet, usually have a normal strength. Apart from the eye muscles, the weakness is usually symmetrical. Variation over time is the same for all muscle groups.

Respiratory muscle weakness is the life-threatening symptom of MG. The diaphragm is usually not involved in MG. However, this can occur, especially during infections or after other triggering events such as surgery with narcosis. Aspiration due to weak swallowing, infection and respiratory muscle weakness is a feared combination. MG crisis with the need of respiratory support is rare, but a significant proportion of patients experience it, even in a well-treated cohort. Unexplained need for respiratory support, for example, during a pneumonia, can be a manifestation of an undiagnosed MG.

MG patients do not develop muscle atrophy. They do not experience muscle pain. They have no weakness in smooth muscle, and usually no cardiac muscle abnormalities.

MG muscle weakness is always reversible. Even if the paresis has lasted for a long time, one should not give up, but continue and intensify the immunosuppressive treatment to induce an improvement. This is especially important during a myasthenic crisis. Respiratory support should be maintained long term if necessary, and the weakness will improve with optimal treatment.

The clinical manifestations for early-onset MG and late-onset MG with AChR antibodies are similar. Early-onset patients tend to have a milder disease and with a better response to therapy [4, 21]. Juvenile MG with debut age below 15 years is rare in Western countries, and has the same manifestations as early onset MG in general [22]. However, the subgroup with MG onset before age 7 in China and other Far East countries usually have a mild disease, often with ocular manifestations only and with a good prognosis [11].

Thymoma MG constitutes 10% of all MG patients. They tend to have a more severe MG, and hardly ever with a spontaneous remission. Thymoma can in the same patient be associated with other rare autoimmune manifestations including neuromyotonia and the POEMS syndrome [23].

MG with MuSK antibodies has usually pronounced weakness in facial and bulbar muscles. The patients tend to have a more severe disease, with insufficient response to symptomatic treatment and with the need for long-term immunosuppression [13]. MuSK MG sometimes leads to modest muscle atrophy. This disease also tends to have less variation in muscle strength during the day. Limb weakness is uncommon, and some patients do not have any symptoms from eye muscles. Respiratory weakness can occur.

MG with LRP4 antibodies is rare, and appears even rarer because most centres do not test for this antibody. The clinical manifestations are usually mild, often with ocular symptoms being the most prominent [24].

The seronegative MG group is highly heterogeneous. We only include patients with generalized symptoms in this group, as MG patients with pure ocular symptoms and no antibodies should be categorized as ocular MG. One-third to one half of ocular MG patients do not have antibodies by standard tests. The seronegative, generalized patients include several with antibodies against AChR, MuSK or LRP4 when tested with assays that are more sensitive [7, 25]. The clinical manifestations in these patients are similar to those with detectable antibodies in routine tests, although as a group somewhat milder. Atypical clinical manifestations and no detection of muscle antibodies should always lead to a critical re-examination of the MG diagnosis.

Ocular MG is characterized by ptosis and diplopia, often intermittently and with asymmetry. These symptoms occur early, and shortly after debut, ocular MG is common. During the next weeks and months, most patients develop distinct non-ocular manifestations as well. However, if the disease is purely ocular 2 years after onset, it will remain as an ocular MG in 90% of the patients [8].

LEMS has muscle weakness as a hallmark. This weakness is usually most pronounced in the legs, leading to difficulties in walking. The weakness is usually mostly proximal, and up to 80% of the patients experience proximal weakness in both legs and arms [18]. Facial and bulbar muscle weakness is common, as well as eye muscle complaints. Some patients have also distal muscle weakness. There is little variation during the day and no fatigue as in MG. On the contrary, some patients experience an improvement of muscle strength during repetitive activity after an initial weakness. LEMS patients with small cell lung carcinoma tend to have more severe muscle weakness, and often with a gradual progression. LEMS also affects respiratory muscles. Both symptomatic and immunosuppressive treatment has a more variable effect in LEMS compared to MG, and especially so in paraneoplastic LEMS [5, 26]. Absence of tendon reflexes is typical in LEMS. LEMS patients have also autonomic dysfunction. Dry mouth, dry eyes, erectile dysfunction, constipation and reduced sweating represent common symptoms in LEMS. The autonomic symptoms are mild to moderate, and they have less significance for the patients than the muscle weakness.

Neuromyotonia has less distinct muscle weakness, but rather a feeling of fatigue and stiffness in affected muscles. This combines with muscle cramps and muscle twitching, often resembling gross fasciculations. Neuromyotonia implies a reduced capacity for using the muscles, most common being walking difficulties. The symptoms occur most commonly in the legs but can also affect the trunk, arms, face, and neck muscles. A minority of the patients experience mild sensory symptoms [27, 28].

Pathogenesis

MG, LEMS and neuromyotonia are all caused by antibodies against proteins at the neuromuscular junction (Fig. 2). These antibodies bind in vivo and thereby induce the clinical manifestations of the disorders.

AChR antibodies bind to many epitopes on the extracellular part of the receptor, and to all AChR subunits [3, 16, 29]. There is a major immunogenic region, a predilection site for antibody binding. The IgG antibodies inhibit receptor function by destruction or by blocking for acetylcholine binding. Destruction is more important than blockade and is induced either by cross-linking of AChR or by complement activation. Blockade occurs either directly or through conformational AChR changes. New synthesis of AChR is not inhibited by AChR antibodies and takes place with increased speed in MG. AChR half-life is markedly reduced in MG patients, usually to less than half the normal. This explains the great restorative potential in MG.

MuSK and LRP4 are proteins that functionally and anatomically link to AChR in the postsynaptic membrane. Binding of IgG antibodies to these membrane proteins inhibits their function, and thereby the function of AChR [4, 30, 31]. MuSK antibodies are monovalent so they do not cross-link MuSK molecules, nor do they activate complement. LRP4 antibodies are believed to interfere with the AChR-mediated neuromuscular transmission via an interaction with agrin.

MG patients can have circulating antibodies against other muscle proteins. Whereas AChR, MuSK and LRP4 antibodies very rarely occur together in the same patient, these additional antibodies are present together with AChR antibodies. They are specific or semi-specific for MG. Antibodies against titin are detected in 20–30% of MG patients [16, 32]. In thymoma MG, they appear in nearly 100% of patients; in late-onset MG, they are frequent, whereas they are seen only rarely in other MG sub-

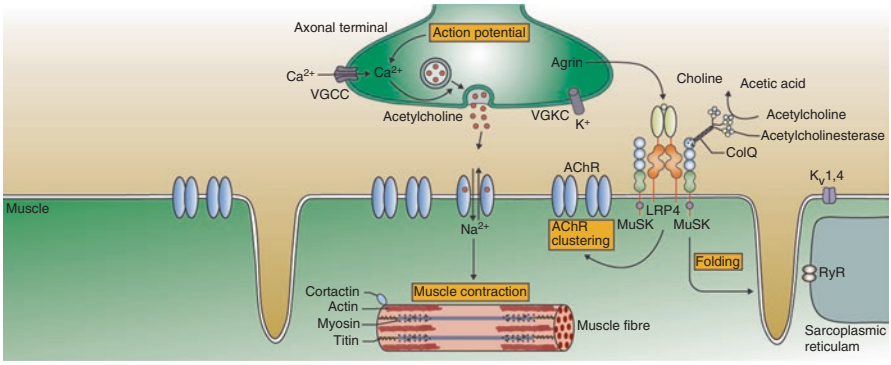

Fig. 2 The neuromuscular junction with the key molecules instrumental in the autoimmune disorders MG, LEMS and neuromyotonia. Antibodies against AChR, MuSK and LRP4 postsynaptically and against voltage-gated calcium channels (VGCC) and voltage-gated potassium channels (VGKC) presynaptically cause the muscle weakness and dysfunction

groups. Ryanodin receptor antibodies are frequent in thymoma MG, rare in late-onset MG, and very rare in the other MG subgroups [33]. Antibodies against titin and ryanodine receptor indicate a more severe disease, with a higher need for long-term immunosuppressive therapy, and in sufficient doses [32]. These antibodies are directed against intracellular antigens, and it is not known if they bind in vivo or if they are merely biomarkers. Antibodies against the membrane molecule agrin have been detected in some MG patients, and in patients both without and with other antibodies [34]. No pathogenetic role has yet been defined. Antibodies against the voltage-gated K^+-channel $K_v1.4$ in skeletal muscle are seen in many AChR MG patients. In Japanese patients, they reflect a more severe disease and often with cardiac complications [35]. This was not found in a North-European cohort [36]. Any pathogenic effect of these antibodies remains to be proven.

Thymus plays a pathogenic role in some but not all MG patients (Fig. 3). This is most obvious in those 10% of MG patients with a thymoma. One-third of all patients with a thymoma develop MG, and even more have AChR antibodies. Thymoma cells express muscle-like antigens, and they are able to present these antigens for developing thymocytes [37]. T lymphocytes that are capable of inducing antibody production against AChR and other muscle antigens are exported from the thymus with a thymoma [38]. The antibodies are produced in plasma cells/B lymphocytes in activated lymphoid tissue throughout the body. MG with a thymoma is therefore a true paraneoplastic disease. Early-onset MG patients have typically thymus hyperplasia. Thymus is enlarged, and it has a high number of lymphoid follicles. This thymus exports AChR-antibody-inducing T lymphocytes. T cells with this reactivity

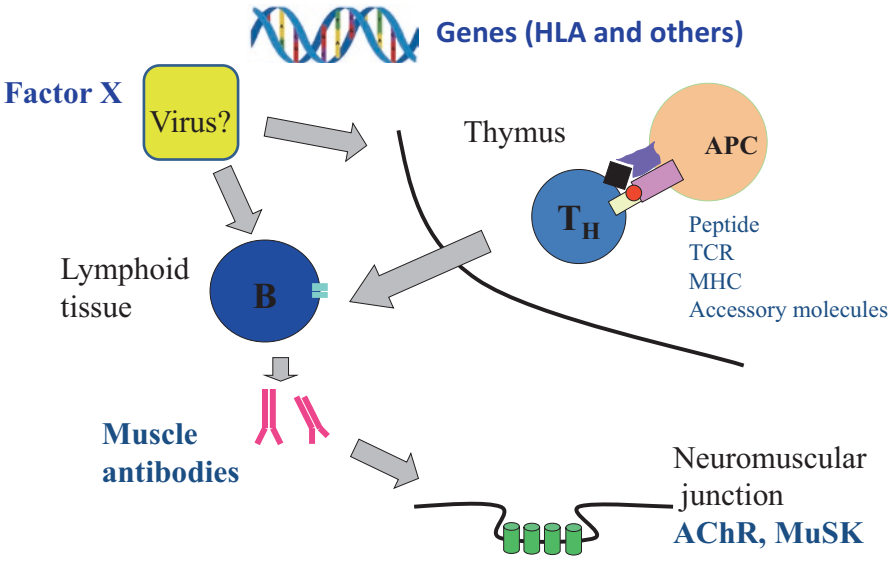

Fig. 3 MG with AChR antibodies has a pathogenesis that involves the neuromuscular junction, thymus, genetic predisposing factors and unknown triggering or causative factors. In thymoma MG, the thymic tumour represents this causative event

have been stimulated inside thymus, and they have escaped the normal intrathymic mechanisms to control autoimmunity. Myoid muscle-like cells and epithelial antigen-presenting cells probably both play a role in this AChR sensitization [16, 38]. Most late-onset MG patients with AChR antibodies and some of those with early onset have what appears as a normal thymus. In some of these, pathological biomarkers similar to those of the hyperplastic thymus can be found, and the pathogenesis is probably the same. However, in most such patients, no pathology has been identified [39]. Thus, it is questionable if thymus represents a pathogenic element in all MG patients with AChR antibodies. Ocular MG can have thymus hyperplasia, and this means an increased risk for generalization of symptoms. It is not known what triggers the immunization against AChR in the hyperplastic thymus. Virus infection has been proposed as a potential factor. Although Epstein-Barr virus was claimed to appear in MG thymus some years ago [40], no signs of infection causing MG have been convincingly shown [41]. It is therefore completely unknown why some individuals start to develop thymic hyperplasia, with MG as the consequence. MuSK MG and LRP4 MG do not have any thymus pathology.

Genetic factors are important in the development of MG. First-degree relatives have a 10–100 times increased risk [42]. Three to seven per cent of MG patients have a first- or second-degree relative with MG [43, 44]. Specific HLA alleles correlate to early-onset MG, late-onset MG, thymoma MG and MuSK MG [45]. Additional genes regulating immune processes increase or decrease the risk for MG [46]. In nearly all such studies, the MG patients have not been defined by subgroup. Some of the risk genes are common for several autoimmune disorders and not specific for MG, particularly in the early-onset MG subgroup [47]. The genetics seem to account for less than fifty per cent of the MG risk.

Neither epidemiological, clinical nor experimental studies have succeeded to identify the external factors that lead to MG. The geographical variation can be explained by genetic influence, and good migration studies are lacking. Those undertaken have failed to come up with potential external factors.

MuSK MG represents a separate disease, with separate genetic and non-genetic causative factors [25]. Thymus is not involved. HLA and non-HLA gene susceptibility is specific for MuSK MG, but explains only a modest part of the total disease risk, similar to the other MG subgroups.

LEMS is caused by antibodies to voltage-gated calcium channels in the presynaptic nerve terminal. These channels are located in the cell membrane, where their calcium transport is necessary for the release of acetylcholine after receiving the triggering nerve signal. The antibodies reduce the number of active channels, they block channel activity, and the calcium influx into the cell is reduced. The consequence is that the quantal release of acetylcholine becomes lower than it should have been [48, 49]. Most antibodies bind to the alpha-1 channel subunit, but the exact pattern of epitope reactivity varies between patients. Voltage-gated calcium channel antibodies are found in at least 85% of all LEMS patients [18]. Whether all the remaining patients have undetectable antibodies against the same channel or there are alternative disease mechanisms is not known.

Small-cell lung carcinoma represents a trigger for the autoantibody production in LEMS through molecular mimicry. Structures antigenically very similar to normal voltage-gated calcium channels appear as tumour-related neoantigens in small-cell lung carcinoma. LEMS usually starts early in tumour development. Most patients with small-cell lung carcinoma and the relevant neoantigens do not develop LEMS. However, some of them have the antibodies without any symptoms. LEMS can rarely be a paraneoplastic manifestation of other cancers [18]. No triggers have been identified for LEMS patients without a cancer. These patients do not have an increased risk for malignancies. LEMS patients without cancer have a proven genetic disposition shown by a linkage to specific HLA-patterns [50]. This is similar to other autoimmune disorders. LEMS patients with small-cell lung carcinoma do not have this pattern, reflecting the difference in etiology. The reason why some but not all patients with small-cell lung carcinoma develop LEMS is unknown. Some differences in the tumours with and without LEMS have been found, but non-tumour aspects are probably more important [19].

Neuromyotonia is caused by antibodies to voltage-gated potassium channels in the presynaptic nerve terminal at the neuromuscular junction, or by antibodies to the channel complex proteins. These antibodies bind to extracellular parts of the channels in vivo and reduce the ionic transport through the channels [51]. There seems to be a correlation between antibody concentration, channel function, and symptom severity [52]. The reduced potassium transport across the neuronal membrane leads to a hyperexcitability. Thymoma is found in 20% of patients with neuromyotonia, and also other neoplasms are associated with neuromyotonia. Antibodies generated against tumour antigens cross-react with the neuronal voltage-gated potassium channels. The same antibodies can bind in the central nervous system and give an autoimmune encephalitis. Some patients may have concurrent autoimmune paraneoplastic manifestations due to a spectrum of autoantibodies [6, 53]. The majority of neuromytonia patients do not have a paraneoplastic condition. The cause of the disease in these patients is not known.

Diagnosis

MG can in most patients be diagnosed clinically. The clue is to consider the disease when relevant. This means to evaluate the possibility in all patients with diplopia, with ptosis, and with otherwise unexplained muscle weakness. In elderly patients, stroke is a common diagnosis at referral, whereas young patients are sometimes believed to have unspecific fatigue conditions. Clinical testing can be normal. One should examine strength in the symptomatic muscles after exercise, for example, as a ptosis test or after continued arm elevation.

AChR antibody testing has a diagnostic sensitivity of 75% and a specificity of near 100% for the best commercial tests [2, 3]. Thus, it is well suited as a screening test, recommended in all patients with a suspicion of MG. The lack of false-positive results is a huge advantage. MuSK antibodies should be tested in samples without

AChR antibodies and where MG is still suspected. Sensitivity and specificity for the best commercial MuSK antibody tests are similar to those for AChR antibodies [25]. There are not yet any commercial assays for LRP4 antibodies, so such testing is done mostly for research [24]. More sensitive assays have been developed both for AChR and MuSK antibodies [7]. This shows that a proportion of patients regarded as seronegative indeed belong to one of the other MG subgroups. It is not yet sufficiently clear if these sensitive assays have the same disease specificity. They are not yet commercially available. With a strong suspicion of MG and negative tests, retesting should be done after 6–12 months.

AChR and MuSK antibody concentrations do not reflect MG severity. Some patients with mild disease and a good prognosis have high titres, and patients with low antibody concentrations can have severe MG. There is a tendency for antibody concentrations to fluctuate in parallel with disease development in the same patient [54]. Therefore, repeated AChR and MuSK antibody measurements can be helpful when considering adjustments in ongoing immunosuppressive therapy, and also when considering if a deterioration in function is due to MG or comorbidity.

Titin antibodies are a sensitive marker for thymoma, but with low specificity [32, 55]. Combined with imaging of the mediastinum, it gives an optimal test result. Presence of titin antibodies makes early-onset MG with thymic hyperplasia unlikely. Titin antibodies also indicate a more severe MG, with a long-term need for immunosuppressive treatment. Ryanodine receptor antibodies are in a similar way associated with thymoma, and with a higher specificity, but is not available as a commercial kit.

Imaging of the mediastinum should be performed in all MG patients. It is important to identify the thymoma that is present in 10% of the patients. Both sensitivity and specificity are far from 100%. CT and MR seem to be similar. However, new MR protocols are in development [56, 57]. This should improve thymoma diagnostics, and also lead to a more reliable diagnosis of thymic hyperplasia by imaging. Standard imaging often reveals only an enlarged thymus that could be due to hyperplasia, neoplasia or represent a normal variant. Specialized pathological examination of the removed thymus is important, and for both microtumours, lymphoid follicles and other hyperplasia markers [38]. In most patients with late-onset MG, histological examination of the thymus does not reveal any pathology [39].

Neurophysiological tests can be used to diagnose MG. Repetitive nerve stimulation has a suboptimal sensitivity but a good specificity. Single-fibre EMG has a higher sensitivity but lower specificity. These tests are important diagnostic tools in patients where antibodies cannot be detected or where such tests are unavailable [4]. In patients who already have a clinical and antibody diagnosis, neurophysiological tests are usually unnecessary to perform. However, in MG with purely ocular symptoms, it may be of interest to examine if there are electrophysiological signs of generalization. The selection of muscles for testing is always crucial.

Comorbidity risks should be evaluated both at time of diagnosis and during follow-up. Relevant tests should be performed [58, 59]. The same is true for potential side effects of MG treatment.

LEMS and neuromyotonia are diagnosed based on clinical suspicion, positive tests for the relevant antibody, and typical results at specific neurophysiological tests. Both voltage-gated calcium channel and voltage-gated potassium channel antibody test assays have very high specificity and high sensitivity. Repetitive nerve stimulation at the optimal frequency shows a diagnostic increment in LEMS, reflecting improved channel function and increased release of acetylcholine after multiple stimulations.

Once LEMS or neuromyotonia have been diagnosed, one should search for a small-cell lung carcinoma (LEMS), a thymoma (neuromyotonia) or another cancer (both disorders). Smokers and non-smokers should follow the same examination program, although the risk for lung cancer differs markedly. PET examination should be included if a tumour has not already been detected. In LEMS without a detected small-cell carcinoma at diagnosis, one should follow-up with PET or other sensitive techniques every 6 months for the next 2 years [19].

Treatment

MG responds to symptomatic therapy and to immunosuppression [2, 4, 60, 61] (Table 2). Acetylcholine esterase inhibition leads to symptom relief as long as the drug is active. Pyridostigmine is the favoured drug. Ambenonium chloride and

Table 2 Most frequently used drugs for MG treatment

Drug	Action	Dose
Pyridostigmine	Acetylcholine esterase inhibition	Single dose 10–120 mg Daily dose 40–600 mg
Prednisolone	Complex immunomodulation	Induction 40–80 mg daily Stable 5–20 mg daily, alternate days an alternative
Azathioprine	Suppression B and T cells	50–250 mg
Mycophenolate mofetil	Suppression B and T cells	1.5–2 g
Rituximab	Suppression B cells	0.5–1 g Repeat after 2 weeks Can be repeated at 6 months
Methotrexate	Folate metabolism inhibition	20 mg per week
Cyclosporine	Suppression T and natural killer cells	100–500 mg
Tacrolimus	Suppression T and natural killer cells	3 mg
Cyclophosphamide	Suppression B and T cells	50–500 mg every 4 weeks
Intravenous immunoglobulin	Complex immunomodulation, neutralization of autoantibodies	2 g per kg, over 2–5 days

neostigmine are usually less effective but represent alternatives. 3,4 diaminopyridine increases the amount of acetylcholine in the synapse by increasing its presynaptic release. This drug has little or no effect in most MG patients. Pyridostigmine should be given as first-choice drug to all MG subgroups. However, patients with MuSK MG have usually a limited effect of cholinergic treatment [13]. The optimal dose is decided from effect and cholinergic side effects. These are most commonly gastrointestinal, but also from other parts of the autonomic nerve system. Dose can vary from day to day, reflecting variation in patient needs and tasks. Patients can self-administer their optimal dose, regarding both single dose and dose frequency. Some patients become symptom free on pyridostigmine and do not require further drug therapy.

Most MG patients should be treated with immunosuppressive drugs. The combination of prednisolone and azathioprine is regarded as first-choice immunosuppressive treatment. Prednisolone dose should be increased gradually over a few weeks. After obtaining a remission, prednisolone dose should be gradually reduced. It is usually wise to keep a small dose long term, even if a remission seems stable. Prednisolone as MG treatment is given by many centres every second day. This gives a satisfactory effect and may reduce the side effects. Azathioprine takes some months before a clinical effect appears. This drug represents long-term treatment. Most patients tolerate azathioprine without any side effects. Patients can be tested for thiopurine methyl transferase activity before treatment. This is low in 10% of the population, which increases the risk for intolerance to azathioprine. The main reason for immunosuppressive treatment is to control present symptoms. An additional indication should be to prevent deterioration and the development of a more severe MG. This has especially been discussed for ocular MG, if early treatment with prednisolone and azathioprine can prevent generalization [8]. Data indicate that this can be true for some patients.

If the first-choice immunosuppressive drugs fail, there are several options. Failure can be due to lack of effect or side effects. One should be ambitious in the immunosuppressive MG treatment, not accepting symptoms of functional significance or side effects influencing quality of life. Often, second-line immunosuppressive drugs are combined with prednisolone or azathioprine.

Rituximab is recommended as an effective drug in MG. It binds selectively to the CD20 antigen on B lymphocytes and should therefore be well suited for antibody-mediated diseases such as MG. No controlled trials have so far been published, but widespread experience from series of patients with moderate and severe MG shows a therapeutic effect [62]. The optimal treatment schedule has not been defined, but most centres use the same induction schedule as for rheumatic disease and multiple sclerosis. Follow-up treatment depends on the clinical MG development. JC virus-related progressive multifocal leukoencephalopathy is a very rare side effect with rituximab, occurring in perhaps 1 in 30,000 patients [63]. There is no need to check for JC virus before starting with rituximab.

Mycophenolate mofetil is often used for mild to moderate MG. Clinical experience favours the use of this drug, together with uncontrolled study reports. However, two prospective and controlled studies failed to reach the primary end

points [64, 65]. This could be due to weakness of the studies, but indicates that this drug is not very potent in MG. Alternative second-line immunosuppressive drugs for MG include methotrexate, cyclosporine, tacrolimus and cyclophosphamide. Neither MG subgroup nor any other MG biomarkers favour one of these immunosuppressive drugs more specifically. However, rituximab seems to be particularly well suited for MuSK MG treatment [66].

Thymectomy should be undertaken early in the course of MG. Patients with early-onset MG have a well-proven effect on MG disease development that comes early and increases during several months after surgery. Thymoma patients should have their thymus removed together with the tumour. It is crucial that the surgeon removes all thymus tissue. This can be done by thoracoscopic, minimally invasive techniques or by traditional sternotomy. The key factor is access and visibility to the mediastinum so that all thymus tissue can be identified and removed. It is not always easy to decide whether a patient should be thymectomized. Patients with generalized MG debut before age 50 and AChR antibodies should definitely have surgery [67]. The same is true for older patients with an enlarged thymus at imaging, being suspected of thymic hyperplasia or even a thymoma. Patients up to the age 60–65 with a normal imaging result are also sometimes thymectomized, but probably not if they have titin antibodies as an indicator of late-onset MG. Patients without any detectable antibodies represent a challenge as we know that some of them in fact have AChR antibodies and thymic hyperplasia. For this group, we recommend specialized imaging of the mediastinum and sensitive antibody tests. For ocular MG, a benefit of thymectomy has not been proven [8]. However, in the presence of AChR antibodies, an enlarged thymus on imaging and neurophysiological signs of generalization, we recommend thymectomy. Even with negative imaging and a pure ocular disease after extensive tests, there are data showing a reduced risk of MG generalization after thymectomy [8]. Thymectomy should not be done in patients with MuSK or LRP4 antibodies, and not in the oldest patients.

Many therapeutic monoclonal antibodies have immunosuppressive actions. Several of them influence autoantibodies: their production, transport and binding, as well as consequences of their binding to the antigen. They might well have a benefit in MG, but the great majority have not been tested properly. Ocrelizumab is a humanized anti-CD20 monoclonal antibody and ofatumumab is a fully humanized antibody against the same antigen. These drugs should be at least as good as rituximab for MG, are very much more expensive, and have not yet been tested. Eculizumab is a humanized monoclonal antibody against the terminal complement protein C5. This drug has a proven but moderate effect in MG [68, 69]. Cost-benefit considerations make it prohibitive for MG patients now as it is extremely expensive, but in the future complement will probably be a target for immunotherapy in MG.

Intravenous immunoglobulin (IvIg) is a well-proven treatment for MG. The effect appears after a few days and is often remarkable. It lasts for approximately 3 months. IvIg is the treatment of choice for MG exacerbations, for severe MG periods, and before surgery or other challenges that could deteriorate their MG. IvIg (or alternatively plasma exchange) should always be given in myasthenic crisis when the patients have a need for respiratory support. The response rate is around 80%

[70]. Long-term treatment with IvIg is unusual, but remains an alternative in patients responding well to the other immunosuppressive treatments. IvIg treatment should be combined with immunosuppressive drugs, often in a higher dose than before, or in a combination with new and more potent drugs. IgG can be given subcutaneously. This treatment has not been tested systematically in MG, but it may be an alternative for medium- to long-term treatment [71]. There are ongoing trials using modified IgG molecules or IgG-modifying agents as long-term MG treatment [72].

Plasma exchange has the same indications as IvIg in MG treatment. The therapeutic effect is similar, and is well proven. The frequency of side effects is also similar, but the risk for severe side effects may be higher for plasma exchange. The choice between plasma exchange and IvIg usually depends on local availability, experience and organization. In some patients, one of the treatments is clearly superior. This means that both IvIg and plasma exchange should be available at centres treating patients with severe MG.

For myasthenic crisis, respiratory support and intensive care are crucial. Any infections precipitating or complicating the crisis should be treated vigorously. The patients should be mobilized as soon as possible. A myasthenic crisis is always reversible.

Patients with MG should have a daily physical exercise program. Exercise improves muscle strength also in MG patients. The program should be adapted to their disease, regarding intensity, duration and variation in strength between muscle groups [73]. The exercise program should be combined with sufficient rest. Overweight should be avoided.

MG patients with persisting diplopia and ptosis may benefit from assistive devices, or even local surgery [8]. Most patients should continue to work full time, although physically demanding occupations should be avoided. MG patients tolerate most drugs. However, both patient and doctor should be aware of the possibility of a drug-induced MG exacerbation when initiating a new drug treatment. Muscle relaxants, penicillamine, fluoroquinolones, macrolides and aminoglycosides should be avoided in MG. Statins should be initiated at the same indications with and without MG, but if MG aggravates or is unmasked, the statins should be withdrawn.

LEMS treatment includes symptomatic and immunosuppressive drugs [5, 26]. 3,4 diaminopyridine is the drug preferred to facilitate the cholinergic transmission. Most patients experience a marked and long-lasting effect of such treatment. The effect is better in patients without a small-cell lung carcinoma. Pyridostigmine usually has less effect, adds nothing but side effects in combination with 3,4 diaminopyridine, but can be tried as an alternative in LEMS patients with an inadequate response to 3,4 diaminopyridine. Most LEMS patients need also immunosuppressive drugs. There are no controlled studies, so treatment guidelines rely mostly on clinical practice. The drugs used are the same as for MG: with prednisolone and azathioprine as the first choice, rituximab and mycophenolate mofetil as second choices and several other drugs with an expected effect. IvIg and plasma exchange can be used as in MG but have a usually only a moderate effect. Treatment of the cancer is essential in those with small-cell lung carcinoma. Effective cancer treatment will sometimes improve also the LEMS.

Neuromyotonia treatment includes symptomatic and immunosuppressive measures [28, 52]. Antiepileptic drugs and botulinum toxin can improve muscle stiffness, spasms and pain. The immunosuppressive drugs to be tried are the same as for MG, but experience is limited due to the rarity of this condition. Potential treatment includes IvIg or plasma exchange for exacerbations, severe disease and critical situations.

MG, LEMS and neuromyotonia patients all need optimal treatment of any comorbid conditions. It is important to identify such conditions and to separate them from the neuromuscular disease [58, 59]. Especially in elderly patients, this can be difficult. Specialists tend to care and take responsibility only for one condition. That is a challenge for the patient and even pose a threat for the total care. The neurologist should take responsibility as others usually do not dare to interfere with the treatment for these rare neuromuscular conditions. Cardiovascular disease and respiratory disease are highly relevant, and many patients have additional autoimmune disorders. Insomnia and mild anxiety are common, as in the general population [74].

MG females in reproductive age should get specific information about pregnancy and giving birth [75, 76]. Pyridostigmine, prednisolone and azathioprine are regarded as safe during pregnancy and should be continued if they are needed for MG. IvIg and plasma exchange are also safe and represent effective treatment for exacerbations during pregnancy. Methotrexate, mycophenolate mofetil and cyclophosphamide are teratogenic, whereas rituximab should not be given during the last 6 months before conception because risk of B-cell depletion in the baby. Most patients with MG give birth in an ordinary way, but the percentage with caesarean section is somewhat higher than in women without MG [77]. Neonatal myasthenia due to transfer across placenta of mother's IgG antibodies occurs in 10–15% of the newborn babies. This can occur for both AChR and MuSK MG, and for LEMS. Neonatal myasthenia lasts for days or a few weeks, until mother's antibodies disappear. The baby does not produce any muscle antibodies. The risk for neonatal myasthenia means that all females with MG shall give birth at institutions with experience in intensive neonatal care including respiratory support. AChR antibodies induce in rare cases permanent changes in the developing child in utero [78]. Such persistent myopathy can be mild but also severe and with arthrogryposis. This is so uncommon that MG women should be supported in their wish to have children. Breastfeeding is recommended, except in the rare cases where the mother is treated with methotrexate, mycophenolate mofetil or cyclophosphamide.

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Myositis

Liliana R. Santos and David Isenberg

Abstract Idiopathic inflammatory myopathies are a heterogeneous group of chronic autoimmune disorders that mainly affect the proximal muscles. The most common types include dermatomyositis (DM), polymyositis (PM), necrotizing autoimmune myopathy (NAM), and sporadic inclusion body myositis (sIBM). Patients typically present with progressive, proximal weakness and functional impairment. Extramuscular manifestations may also be present. Laboratory investigations with raised serum creatine kinase (CK) and myositis-specific antibodies (MSA) may help in diagnosis, differentiating the clinical phenotype and confirming the myositis subtype. The major goals of treatment are to eliminate the inflammation, restore muscle performance, reduce mortality, and improve quality of life.

Keywords Myositis · Polymyositis · Idiopathic inflammatory myopathies · Necrotizing myopathy · Inclusion body myositis · Myositis-specific antibodies

Introduction

Idiopathic inflammatory myopathies (IMMs) are a heterogeneous group of diseases collectively named "myositis." IMMs share symmetrical proximal skeletal muscle weakness and raised serum levels of muscle enzymes (e.g., creatinine kinase). They may or may not also have skin and extramuscular organ involvement [1]. The IMM are most often subclassified based on patterns of presentation, age of onset, immunohistopathologic features, and response to treatment.

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1_27

The major types of IMM are dermatomyositis (DM), polymyositis (PM), necrotizing autoimmune myopathy (NAM), and sporadic inclusion body myositis (sIBM). Table 1 summarizes the main features of each type of myositis.

The diagnosis may not be straightforward. In 1975, Bohan and Peter published the first widely accepted criteria that some still use today [2]. They divided the IMMs into five groups: primary idiopathic polymyositis (PM), primary idiopathic dermatomyositis (DM), DM/PM associated with neoplasia, childhood DM/PM associated with vasculitis, and DM/PM with associated collagen vascular disease [2, 3]. Bohan and Peter criteria are very simple and very sensitive (Fig. 1); however, several limitations including the lack of IBM as a subgroup have been identified.

In 1991, Dalakas proposed a new set of IMM criteria and revised it in 2003. Three groups were defined: PM, DM, and amyopathic based on the presence of myopathic muscle weakness, electromyographic findings, muscle enzymes, muscle biopsy findings, and rash or calcinosis [4, 5]. The diagnostic criteria of Targoff and collaborators published in 1997 included muscle-specific antibodies and maintained sensitivity of diagnosis but improved specificity from 23 to 62% [6]. In 2005, Troyanov et al. took into account the discovery of myositis-specific and myositis-associated autoantibodies and proposed another set of clinico-serological criteria [7]. This classification had a broader inclusion than previous ones. However, it requires signs of an inflammatory myopathy for a patient to be classified as having IMM (Fig. 1).

Recently, the European League Against Rheumatism and American College of Rheumatology (EULAR/ACR) proposed a new scheme for subgrouping IMM [8]. Two models, with or without muscle biopsy results, were developed to reflect better clinical settings such as pediatrics, where performing muscle biopsy is not standard of care. Based on a complex but robust method, 16 variables deemed to provide better discrimination for IMM cases were weighted and included in a final criteria set presented in Fig. 1. These criteria allow the classification in IMMs in definite (probability cutoff >90%), probable (probability >55%), possible (probability >50% but<55%), and improbable (when probability is <50%). There, following categories were included: dermatomyositis, polymyositis, amyopathic dermatomyositis, juvenile dermatomyositis, and sporadic inclusion body myositis [8].

These criteria have several novelties compared with previous sets. The practical implication of the probability model and the different weight each variable contributes means that one needs to test only enough variables to reach a predefined probability providing flexibility for the classification criteria. The presence of anti-Jo1 autoantibodies gives the highest score among the variables in the new criteria, supporting a high level of specificity for this single criterion. With the new EULAR-ACR classification criteria, patients with dermatomyositis without clinical muscle weakness can also be classified as having IMM [8]. Criticisms of these criteria have been published because no account is taken of the antibodies to other tRNA synthetase enzymes [9]. The identification of the IMM subtype and the distinction of these conditions from mimics are fundamental because each subtype has a different prognosis and expected therapy response [10, 11].

Table 1 Summary of the different features of Idiopathic inflammatory myopathies (IMM) subtypes

	Pattern of muscle weakness	CK	Biopsy	Antibodies profile	Prognostic
DM	Proximal symmetric weakness + Skin rash	CK may be up to 50> ULN	Perivascular, perimysial, and perifascicular inflammation Necrotic fibers in wedge-like infarcts Perifascicular atrophy Reduced capillaries *No vacuole formation*	Anti-MDA 5 Anti- Mi-2 Anti-TIF-1 Anti-NXP2	Good 5-year survival rate around 70%
PM	Proximal symmetric weakness	CK may be up to 50> ULN	CD8+ cells invading healthy fibers Wide spread expression of MHC class I antigen. *No vacuole formation*	Anti-synthetase antibodies	Good 5-year survival 70%
NAM	Proximal and severe weakness	Very high; May be more than 50 times ULN in early active disease	Scattered necrotic fibers with macrophages No CD8+ cells Deposits of complement on capillaries *No vacuole formation*	Anti-SRP Anti-HMGCR	Good response to treatment
IBM	Proximal and distal weakness Atrophy of quadriceps and forearms; mild facial muscle weakness	Up to 10 times the ULN; can be normal or slightly elevated	CD8+ cells invading healthy fibers Cytochrome – oxidase negative Widespread expression of MHC class I antigen Ragged-red or ragged blue fibers Congophilic amyloid deposits *Vacuole formation (*Autophagic vacuoles)	Anti-cN1A	Poor even with treatment Increased functional disability

MHC major histocompatibility complex, *ULN* upper limit of Normal, *DM* dermatomyositis, *PM* polymyositis, *NAM* necrotizing autoimmune myopathy, *IBM* Inclusion body myositis, *CK* creatinine Kinase

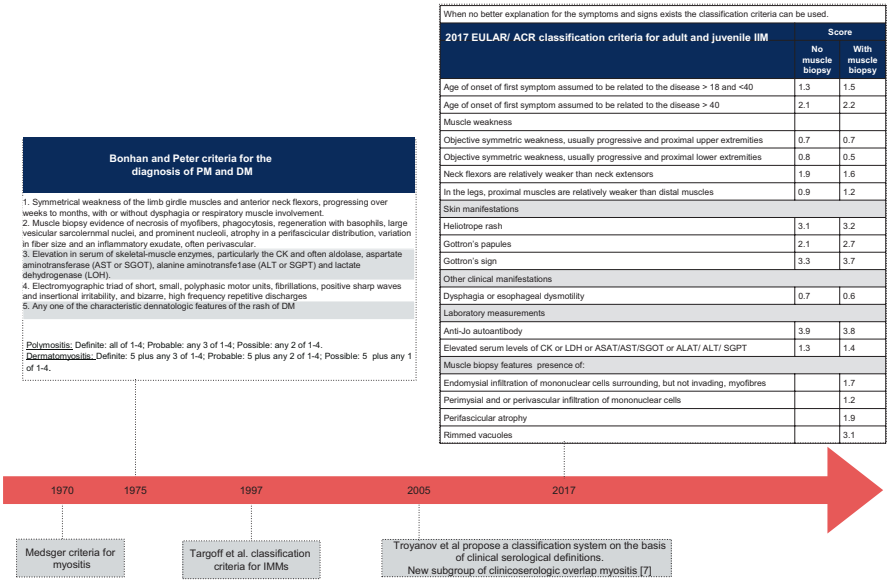

Fig. 1 Sequence of classifications and diagnostic criteria for idiopathic inflammatory myopathies proposed over time

In this chapter, we have tried to reflect the current knowledge of these conditions, updating not only the clinicopathological features of IMM but also the fundamental aspects on disease mechanisms and therapy.

Epidemiology

IMM are rare and good epidemiological studies are scarce. Between 1947 and 1990, the annual incidence of IMM ranged from 0.4–1.0 cases per 100,000 cases using older diagnostic criteria [12]. The overall annual incidence of IMM appeared to be rising [13].

Nowadays, the incidence of DM and PM combined is around 6–10 per million, affecting more women (ratio 2:1) with a peak incidence of 60–69 years in DM and 50–59 years in PM [14, 15]. A Mayo Clinic Study showed PM to be the most common clinical phenotype [16]. Others consider that sIBM is the most frequent acquired myopathy after 50 years of age [13].

Overall, myositis prevalence was estimated at 14/100,000 inhabitants (95% CI 12.84, 15.46) between 1982 and 2010 [17, 18]. The estimated total number of patients with PM/DM and the prevalence rate in Japan in 2010 were 17,000 and 13.2 per 100,000 population, respectively [18]. In South Australia, between 1980 and 2009, three hundred fifty-two biopsy-proven cases of IMM were identified [13].

Fig. 2 World incidence of myositis (per million per year); IMM inflammatory myopathies; sIBM sporadic inclusion body myositis; JM juvenile myositis

Different studies reported the prevalence of myositis in five continents and verified that there were disparities, but no clear geographical differences were found when taking into account methodological variations (Fig.2) [17]. Regarding ethnicity, according to the Sultan SM and collaborators, in a UK study 69.6% of patients were Caucasian, 13% Afro-Caribbean, 13% Asian, and 4.3% others [19].

Clinical Manifestations

In virtually all forms of IMM, the common feature is symmetrical, bilateral muscle weakness, presenting in 84% of the patients while myalgia is present in up to 75% at the initial presentation [14]. The patients have difficulty in performing tasks like getting up from a chair, lifting objects, and climbing steps [1, 4, 5, 20]. In sIBM, patients experience more distal muscle weakness and early have difficulties in tasks like holding objects, shaking hands, or buttoning up a shirt. Falling is also common due to the precoce involvement of the quadriceps muscle and weakness of foot extensors. Facial muscles are affected in the sIBM form and less common in DM or PM [20]. In all subtypes, neck extensor and pharyngeal muscle can be involved, resulting in head drop and/or dysphagia. Extramuscular symptoms notably fever, arthralgia, Raynaud's phenomenon, and cardiac arrhythmias may also occur.

Dermatomyositis Typically, DM presents as an acute or progressive proximal weakness. The skin manifestations may be simultaneous or precede muscle weakness. The typical skin rashes (Fig. 3) include red or heliotrope rash, most prominent on the upper eyelids, face, upper trunk, knees, elbows, anterior chest (often in a V pattern), or shoulders (shawl sign), and violaceous eruption on the knuckles known as Gottron's rash [21, 22]. The lesions are photosensitive and may be aggravated by

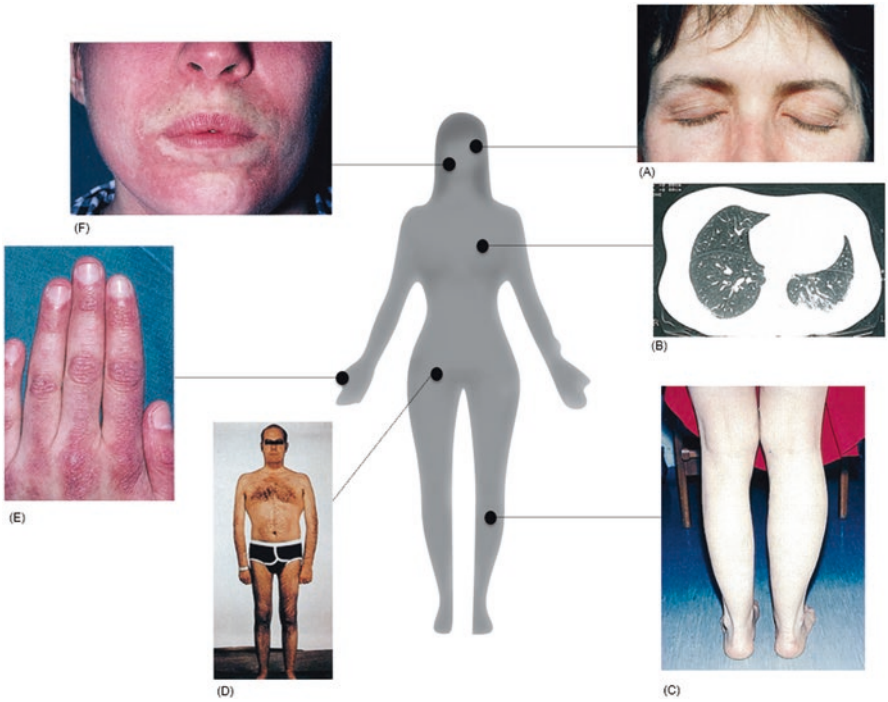

Fig. 3 Clinical features of myositis: (**a**) Heliotropic rash. (**b**) Fibrosing alvolitis shown on a CT scan of a patient with DM. (**c**) Distal unilateral gastrocnemius muscle wasting. (**d**) Striking proximal weakness of arms and legs. (**e**) Gottron's papules. (**f**) Perioral sparing of the facial rash in a patient with DM [22]. (Reproduced with the kind permission of Oxford University Press)

ultraviolet radiation. Some patients develop dyspnea related to interstitial lung disease (ILD) or ventilatory muscle weakness. Dysphagia due to esophageal or pharyngeal involvement, congestive heart failure or arrhythmia from myocarditis, and gastrointestinal bleeding due to vasculopathy of the gut may also be present [23]. Also, other characteristic features of DM are the "mechanic's hands," dilated capillary loops at the base of the fingernails, and irregular and thickened cuticles. Clinical features of DM may overlap with other autoimmune diseases such as systemic sclerosis or undifferentiated autoimmune rheumatic diseases [24].

Several variants of DM are known. Amyopathic DM is characterized by the presence of cutaneous manifestations without the muscle involvement [25]. Juvenile dermatomyositis (JDM) affects children. Multisystem involvement is common in JDM and is associated with calcinosis cutis and vasculopathy affecting the gastrointestinal tract [26]. Dermatopathic DM is characterized by weakness and histological signs which are similar to DM but without the inflammatory lesions of the skin [27].

Polymyositis PM is rare with an estimated prevalence of 5% of all cases, often misdiagnosed, and remains a diagnosis of exclusion [28]. It is best defined as a subacute proximal myopathy in adults who do not have rash, a family history of neuromuscular disease, and exposure to myotonic drugs (like statins, penicillamine, and zidovudine). It presents typically with progressive neck flexor and symmetric proximal limb muscle weakness which develops over weeks to months. Myalgias and tenderness are common complaints. Dysphagia occurs in one-third of patients. The most common extramuscular involvement is ILD and myocarditis [28, 29].

Inclusion body myositis The disease starts insidiously and develops over a period of years, sometimes asymmetrically. Inclusion body myositis may be suspected when a patient's presumed polymyositis does not respond to therapy. However, there are several features that can lead to an early clinical diagnosis including the early involvement of distal muscles notably the foot extensors and finger flexors; atrophy of the forearms and quadriceps muscles; frequent falls due to weakness in quadriceps muscles; and mild facial muscle weakness. Dysphagia occurs in more than 50% of the patients and may present as the initial symptoms [30].

Necrotizing autoimmune myositis Necrotizing autoimmune myositis (NAM) is a clinicopathologically distinct form. It accounts for up to 19% of all inflammatory myopathies [31]. A few years ago, NAM was thought to be a rare IMM, but is now being recognized more frequently. After the identification of two antibodies seen in around 60% of patients, one against the signal recognition particle (SRP) and other against 3-hydroxy-3-methylglutaryl-coA-reductase (HMGCR), the incidence rate seems to be increasing [32]. NAM can occur at any age but it is more commonly seen in adults. NAM presents with a subacute progressive proximal muscle weakness without a rash. The weakness in NAM generally develops more rapidly than in patients with pure PM [31]. Swallowing difficulties may occur. NAM may occur alone or may be associated with viral infections, with cancer, or other autoimmune diseases namely autoimmune rheumatic disorders or even in patients taking statins [33].

Antisynthetase syndrome Patients with ASS often have very specific features linked to the antibody present in their serum, anti-Jo1 being the most widely associated. This syndrome is characterized by myositis with prominent pathologic changes at the periphery of the fascicles and the perimysial connective tissue, ILD, arthritis, Raynaud's phenomenon, fever, and mechanic's hands [26].

Etiology and Pathologic Mechanisms

The etiopathogenesis of IMMs remains unknown, although much work has been done to identify the cause; a complete understanding remains elusive. It seems to be a multifactorial disorder where genetic and hormonal risk factors linked to immune responses against undefined environmental agents have been proposed (Fig. 4) [26].

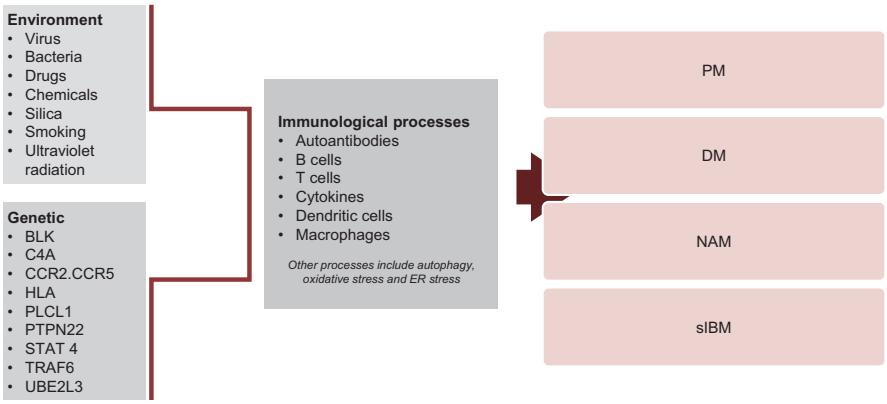

Fig. 4 Generic pathways in IMM phenotypes including polymyositis (PM), dermatomyositis (DM), necrotizing myopathy (NAM), and inclusion body myositis (sIBM) resulting from the interaction between genetic risk factors and environmental ones. ER endoplasmic reticulum, C4A complement 4A, ROS reactive oxygen species

Environmental factors As with other autoimmune diseases, the study of environmental risk factors involved in IMM has been based on animal models, case reports, and/or cases series suggesting variable roles in different IMM phenotype. Increased incidence of DM in low latitude areas (close to the equator) probably because of increased ultraviolet light exposure was reported. Viral, bacterial, and parasitic infections, foods and dietary supplements, and chemicals and drugs were commonly associated with IMM phenotypes. Specific infectious agents have been implicated, namely, infection with hepatitis B virus in PM and DM, hepatitis C virus in sIBM, HIV in PM, DM, and sIBM; *Toxoplasma* spp. and *Borrelia* spp. in PM and DM; and influenza, picornavirus, and echovirus in PM, DM, and JDM [34].

Severe case reports have documented associations after exposures to drugs or medical devices: D penicillamine in PM and DM and anti-TNF agents in DM are known examples. An increased risk of developing anti-HMGCR antibody-positive immune-mediated necrotizing myopathy (IMNM in patients with HLA:DRB1:11∗01 who were taking statins) has also been reported. Vaccines have been implicated in IMM pathogenesis, notably diphtheria typhoid pertussis; measles, mumps and rubella; Bacillus Calmette-Guerin; influenza; and hepatitis A/B [36]. Recently, an increased frequency of anti-Jo-1 antibodies in patients who are current or previous smokers was reported, highlighting the importance of environmental risk factors in the development of IMM [34].

Genetic factors Although no one gene has been identified as an underlying cause of IMM, genetic risk factors are likely to be associated with IMM. However, there are few reports of familial occurrence where the precise heritability of IMM is unknown. A nationwide study in Taiwan that investigated co-aggregation of autoimmune disease in the families of individuals with systemic lupus erythematous and

systemic sclerosis identified a higher relative risk of IMM in these families than in the general population [35]. A national study in China suggested that relatives of patients with systemic sclerosis have an increased risk of IMM. This aggregation pattern strongly suggests a shared genetic background. Genome-wide studies either in adults or juvenile individuals with DM or PM identified the strongest disease associations with the MHC region on chromosome 6. The Myositis Genetics Consortium conducted the largest genetic study which included 2566 patients with IMM. The study demonstrated the clear association with alleles of the HLA 8.1 ancestral haplotype – HLA DRB1∗03:01 and HLA B∗08:01 in PM and DM, respectively. Polymorphisms in the tumor necrosis factor-a (TNF-a) gene coding region are correlated with a longer disease course and increased disease severity [36]. Several non-HLA loci have been associated with IMM. PTPN22 was associated with PM but not with DM. Other genes including STATA4, TRAF6, and UBE2L3 were associated with different subtypes of IMM. One study linked the complement 4A deficiency to an increased risk of juvenile dermatomyositis, although the strongest risk factor identified in the study was the presence of HLA-DRB1∗03:01 along with C4A deficiency [37].

IMM pathogenesis seems to be mediated by both adaptive and innate immune pathways. In DM, the main feature is a complement-mediated vasculopathy of small vessels. The complement C5b-9 membrane attack complex is activated before the destruction of muscle fibers and deposits on the endothelial cells leading to necrosis, reduction of the density of endomysial capillaries, ischemia, and muscle fiber destruction. The action of membrane attack complex may trigger the release of pro-inflammatory cytokines. This release may also up regulate adhesion molecules on endothelial cells and helps in migration of activated lymphocytes including B cells and plasmocytoid dendritic cells to the perimysial and endomysial spaces. Histologically, there are mononuclear inflammatory cell infiltrates mainly B cells and $CD4^+$ T cells in the muscle fibers in the perivascular and perifascicular areas (Fig. 5). A specific subtype is juvenile dermatomyositis, where maternal chimeric cells may contribute to the pathogenesis of the disease [38]. The typical histological findings on skin biopsy are vacuolar interface dermatitis with vacuolar changes of the epidermal basal layer, apoptosis, necrotic keratinocytes and perivascular lymphocytic infiltrate, and mucin deposition in the dermis [39].

In PM and inclusion body myositis (Fig. 6), $CD8^+$ cytotoxic T cells surround and invade healthy non-necrotic muscle fibers that aberrantly express MHC class I [40]. This expression is absent in normal muscle fibers and is probably induced by cytokines secreted by activated T cells. The CD8-MHC class I complex is characteristic of PM and inclusion body myositis, and its detection is helpful in confirming the histological diagnosis. In fact, CD8+ T cells contain perforin granules directed toward the surface of the muscle fibers, which cause necrosis of the muscle and release [41]. B-cell activation also occurs most prominently in inclusion body myositis [42]. The main muscle biopsy features are fiber size variability, cellular invasion of non-necrotic muscle fibers expressing MHC-1 antigens, and scattered necrotic and regenerating fibers.

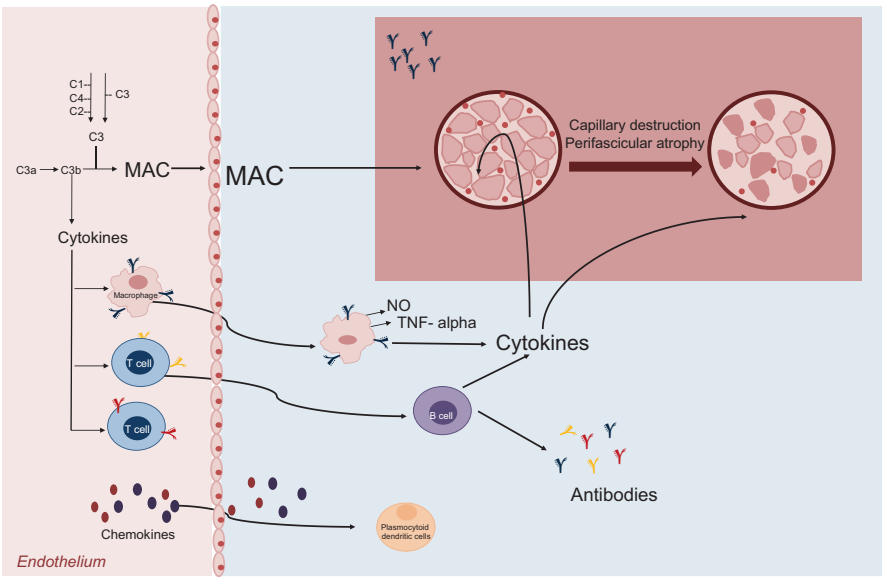

Fig. 5 Pathogenesis in dermatomyositis. Activation of the complement, component 3 (C3) that leads to the C3b formation and MAC (membrane attack complex) activation which are deposited on the endothelial cell wall of the endomysial capillaries. This leads to the destruction of capillaries mostly in the periphery of the fascicles. Cytokines released by activated complement lead to the activation of CD4+ T cells, macrophages, B cells, and plasmocytoid dendritic cells. They enhance the expression of vascular and intercellular cell adhesion molecules on the endothelial cell wall and lead to migration of lymphoid cells into endomysial tissue

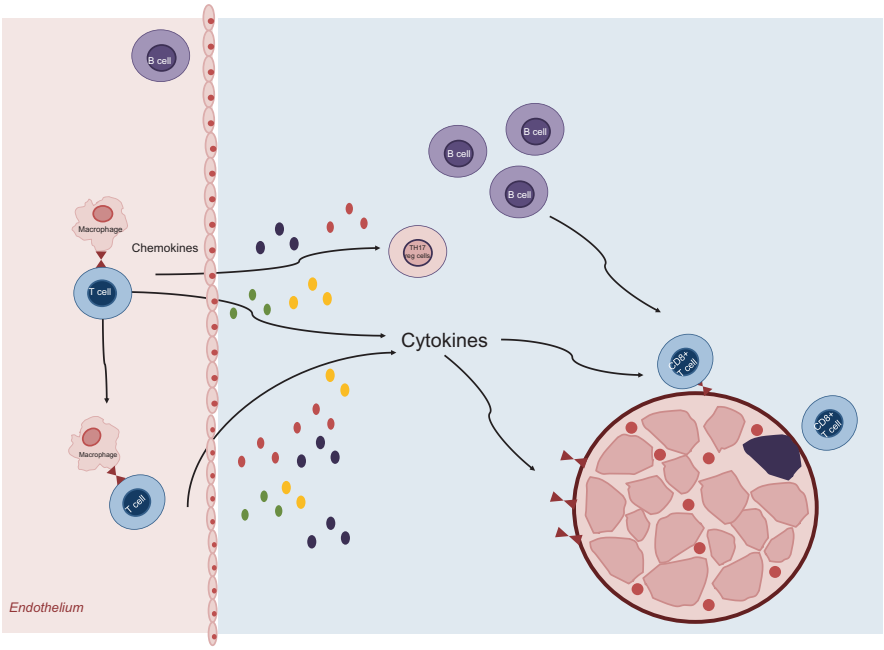

Fig. 6 Pathogenesis mechanisms underlying polymyositis and inclusion body myositis

Diagnosis

The diagnosis of IMM subtypes is based on the clinical history, time of disease progression, pattern of evolution, muscle involvement, muscle enzyme levels, electromyographic findings, muscle biopsy analysis, and presence of autoantibodies.

Complete blood tests including full blood count, urea and electrolytes, liver and thyroid function tests, and 25-OH-vitamin D should also be performed. Serum creatine kinase (CK) is elevated in all subtypes, and it is the most sensitive indicator of inflammatory myopathy, but does not necessarily correlate with the severity of the symptoms. It often improves with treatment. In necrotizing autoimmune myositis, it may be as high as 50 times the upper normal level, although in inclusion body myositis, it rarely exceeds 10 times the upper limit of the normal. Along with the creatine kinase, aspartate aminotransferase and alanine aminotransferase levels, although less sensitive, may also be elevated. Aldolase levels in serum may also be elevated specially in fascia involvement myositis. Neither the erythrocyte sedimentation rate nor C-reactive protein levels are reliable indicators, as they are usually normal or only mildly elevated [23].

Electrophysiology studies: An electromyogram (EMF) should be done to rule out a neuropathic process and confirm the presence of a typical myopathic process, namely, myopic motor unit potentials (short-duration, low-amplitude polyphasic units on voluntary activation) and positive sharp waves which are important to determine whether myopathy is active or chronic. The EMG must be done on one side of the body and the muscle biopsy on the other side.

Muscle biopsy is the most important tool for the diagnosis of polymyositis, overlap myositis, necrotizing autoimmune myositis, and inclusion body myositis. It is most useful when the biopsy site is carefully chosen. Although generally safe and well tolerated, open muscle biopsy is relatively costly and requires the assistance of a surgeon, an operating theater, and local or regional anesthetics. In contrast, various techniques of "percutaneous" needle muscle biopsy (NMB) offer a more convenient and cost-effective means of obtaining adequate muscle specimens. However, most NMB techniques require a small skin incision that leaves a scar (though much smaller than that left by an open biopsy) and an adequately sized tube that may utilize suction to deliver the muscle into the tube before it is guillotined. A technique using a spring-activated 14-gauge needle is minimally traumatic and may be capable of obtaining diagnostic results historically comparable with more invasive techniques [16, 43].

In terms of histological evaluation, DM the inflammation is perivascular and is most prominently located in the interfascicular septa or at the periphery of fascicles. In PM and inclusion body myositis, the inflammation is perivascular and is most typically concentrated in multiple foci within the endomysium. In necrotizing autoimmune myositis there are abundant necrotic fibers invaded and surrounded by macrophages [23].

Our understanding of IMM has changed considerably over the last decades. One of the most exciting recent developments has been the identification of autoantibodies in IMM patients, many of which can be linked to different phenotypes and outcomes.

Autoantibodies are currently detected in up to 60% of the patients with myositis. It is especially important in necrotizing autoimmune myositis diagnosis and also for the classification of distinct subtypes and systemic organ involvement. These antibodies include those against aminoacyl tRNA syntheses which are detected in 20–30% of patients. They have been classified into two main categories: myositis-specific autoantibodies (MSAs) which can be found in IMMs exclusively and myositis-associated autoantibodies (MAAs) which can also be found in other conditions. The latter are present in around 20% of the patients and have a lower positive predictive value or indicate another related comorbid (or overlap) autoimmune rheumatic condition [44].

Both MSAs and MAAs are summarized in Tables 2, 3, and 4. There are several methods to test for MSAs and MAAs (indirect immunofluorescence on HEp-2 cells, counterimmunoelectrophoresis, immunodiffusion, and immunoenzymatic assays such as ELISA) with variable sensitivity, specificity, costs, complexity, and feasibility in clinical and research settings [44].

Differential Diagnosis

Given the myriad of possible clinical features and investigation findings, there are many potential diagnoses to consider actively while assessing a patient with myositis hypothesis. Systemic causes such as thyroid dysfunction, electrolyte disturbance,

Table 2 Myositis-associated autoantibodies (MAAS)

Antibody	Antigen	Clinical association
MAAs		
Anti-PM-Scl	Exosome protein complex (PM/Scl75/100)	Overlap PM/SSc
Anti-C1D	Exosome-associated protein	Overlap PM/SSc
Anti-U1-RNP	U1 small nuclear RNP	MTCD
Anti-fibrillarin (anti-U3-snRNP)	Fibrillarin	SSc
Anti-Ku	DNA-PK regulatory subunit	PM/SSc. Potentially severe ILD
Anti-Ro52	Ro-52/TRIM21	ILD. Frequently coupled with other MSA
Anti-Ro60/SSA	Ro60/SS-A	SjS; SLE
Anti-La/ SSB	SS-B	SjS; SLE
Anti-cN-1A (anti-Mup44)	Cytosolic 5'nucleotidase 1A	sIBM

Table 3 Myositis-specific antibodies (MSAS)

Antibody	Antigen	Clinical association
MSAs		
Anti- Jo-1	Histidyl-tRNA synthetase	Classic anti-synthetase syndrome with more frequent muscle involvement
Anti- PL-7	Threonyl-tRNA synthetase	Anti-synthetase syndrome with prevalent ILD
Anti-PL-12	Alanyl-tRNA synthetase	Anti-synthetase syndrome with prevalent ILD
Anti-EJ	Glycy-tRNA synthetase	Anti-synthetase syndrome
Anti-OJ	Isoleucyl-tRNA synthetase	ILD or Anti-synthetase syndrome
Anti-KS	Asparaginyl-tRNA synthetase	ILD or Anti-synthetase syndrome
Anti-Zo	Phenylalanyl-tRNA synthetase	Myositis
Anti-YRS7HA	Tyrosyl-tRNA synthetase	Myositis
Anti-Mi2	Nucleosome remodeling deacetlyase	Classical DM
Anti-SAE	Small ubiquitin like modifier activating enzyme	Severe cutaneous disease that classically precede DM with severe dysphagia and systemic symptoms.
Anti-MDAS (anti-CADM140)	Melanoma differentiation-associated gene 5 (MDA5)	Hypo-amyopathic, ILD with possible RP-ILD, and severe and peculiar skin involvement
Anti-TIF1 y/a (anti p155/p140)	Transcription intermediary factor 1	Juvenile DM. Cancer-associated hypo-myopathic DM
Anti- TIF 1beta	Transcription intermediary factor 1Beta	DM
Anti-NXP2 (anti-MJ)	Nuclear matrix protein (NXP-2)	Juvenile DM, diffused calcinosis. Cancer-associated DM
Anti-SRP	Signal recognition particle	IMNM with frequent esophageal involvement. Possible ILD
Anti-HMGCR	HMG-CoA reductase	IMNM with or without history of statin exposure

and drug-related myotoxicity should be ruled out in each patient. Noninflammatory myopathies must be excluded including late onset muscular dystrophy, limb girdle dystrophy, and myotonic dystrophy type 2. Other conditions like mitochondrial myopathies which also can present with proximal muscle weakness and a raised CK, muscle biopsy should be done in order to do the differential diagnosis. Metabolic myopathies are associated with carbohydrate and lipid metabolism. Examples are the known carnitine deficiency and myoadenylate deaminase deficiency (Table 5).

Table 4 Antibodies in a miscellaneous

Antibody	Antigen	Clinical association
Miscellaneous		
Anti-RuvBL1/2	RuvBL1/2 complex	SSc, PM, Morphea
Anti-Su/Ago2	Argonaute 2	ILD in the absence of cancer. frequently coupled with MSA, Ro-52, and other antibodies
Anti-SMN	Survival of motor neuron	PM/SSc
Anti-NUP	Nup358/RanBP2, gp210, Nup90, p200/p130, Nup62	Subgroup of PM/SSc patients (so-called NUP-syndrome). PBC
Anti-mitochondrial (AMA-M2)	Branched chain alpha ketoacid dehydrogenase complex	Long-lasting myositis with muscle atrophy and cardiac involvement. PBC
Anti-KJ	Translocation factor	Anti-synthetase-like syndrome
Anti-Fer (anti-eEF1)	Eukaryotic elongation factor	Anti-synthetase-like syndrome
Anti-Wa		Anti-synthetase-like syndrome
Anti-Mas	Selenocysteine seryl-tRNA-protein complex	Non-immune-mediated rhabdomyolysis. Autoimmune hepatitis
Anti-PMS	DNA repair mismatch enzyme (PMS1, PMS2, MLH1)	Mild myositis
Anti-cortactin	Cortactin	*PM*. myasthenia graves
Anti-FHL1	Four and a half LIM domain 1	Myositis and muscular atrophy with severe systemic

Table 5 Differential diagnosis of IMM

	Differential diagnosis
Inherited	Muscular dystrophies; myotonic dystrophies; channelopathies
Metabolic	Mitochondrial; glycogen storage disorders; fatty acid oxidation defects
Endocrinologic	Hyper/hypothyroidism; Cushing's syndrome; Addison's syndrome; acromegaly
Toxic	Corticosteroids; statins; antimalarials; colchicine; penicillamine; antiretrovirals; alcohol
Infective	HIV, acute viral or bacterial infections; trichinellosis
Neuromuscular junction	Myasthenia graves; Lambert-Eaton syndrome
Miscellaneous	Malignant hyperthermia, motor neurone disease, neuropathies, diabetic amyotrophy, sarcoidosis, amyloidosis, neuroleptic malignant syndrome, chronic graft versus host disease

Cancer Screening

The association between DM and cancer is well established. All forms of myositis except IBM have been associated with 2–7-fold increased risk of cancer. Leatham and collaborators demonstrated that undiagnosed malignancy is present in <10% of

USA patients at the time of dermatomyositis onset. Around 25% of DM patients develop a cancer after 0–5 years of disease onset. In PM, the association rounds 10–15% [47]. Most of the diagnoses are made within 1 year but can take up to 3 years. The cancer is often not associated with suspicious signs or symptoms. The risk factors include male gender, older age at disease onset, extensive skin or muscle involvement, elevated inflammatory markers, and negative ANA and MSAs. The most frequent IMM-associated malignancies are breast and ovary in women, lung and prostate in men, as well as pancreatic, gastric, colorectal, bladder cancer, and non-Hodgkin lymphoma [45, 46].

However, there is little consensus about the methods of cancer screening modalities in patients with IMM. Effective malignancy screening of dermatomyositis patients often requires evaluation beyond a history, physical examination, and "age-appropriate" cancer screening – these data may help to inform future guidelines for malignancy screening in this population. The European Federation of Neurological Societies recommended that DM patients have computed tomography of chest/abdomen, pelvic ultrasound and mammography in women, ultrasound of testes in men, and colonoscopy in men and women over 50. If primary screening is negative, repeat screening is recommended after 3–4 months; thereafter screening is recommended every 6 months for years [23].

18F-Fluorodeoxyglucose positron emission tomography (FDG PET) is a standard tool for detecting malignancies. PET has the advantage that only a single test is needed, making it a more conventional approach for the patients.

Treatment

As with many autoimmune diseases, managing IMM continues to be challenging for the physician. The main aims of treatment are to suppress inflammation and to improve muscle power preventing chronic complications and/or extramuscular involvement. As IMM are rare conditions, very few large treatment trials are available to guide clinicians (Fig. 7) [23].

Conventional therapies include glucocorticoids, and it is generally agreed that it should remain the anchor drug in IMM although there is a lack of controlled clinical trials. The starting dose is approximately 0.5 mg/kg of prednisolone, but the many side effects of steroids encourage a reducing regime over the first 2 months. Oddis and Aggarwal (2018) reported on their experience using a dose initially of about 1 mg per kg per daily with an average dose of 60 mg daily. If a patient has a severe myositis or extramuscular involvement, intravenous methylprednisolone at a dose of 500 mg to 1 g daily for 3 days, prior to switching to an oral dose of prednisolone can often be administered. These patients may require a slower steroid reduction, dropping by 20–25% of dose monthly with the goal of achieving a low daily dose of prednisone of 5–10 mg daily in 6 months. Glucocorticoids have a high relapse and commonly cause adverse effects (infection, osteoporosis, diabetes, hypertension, among others) and because of this, they are rarely used alone [23].

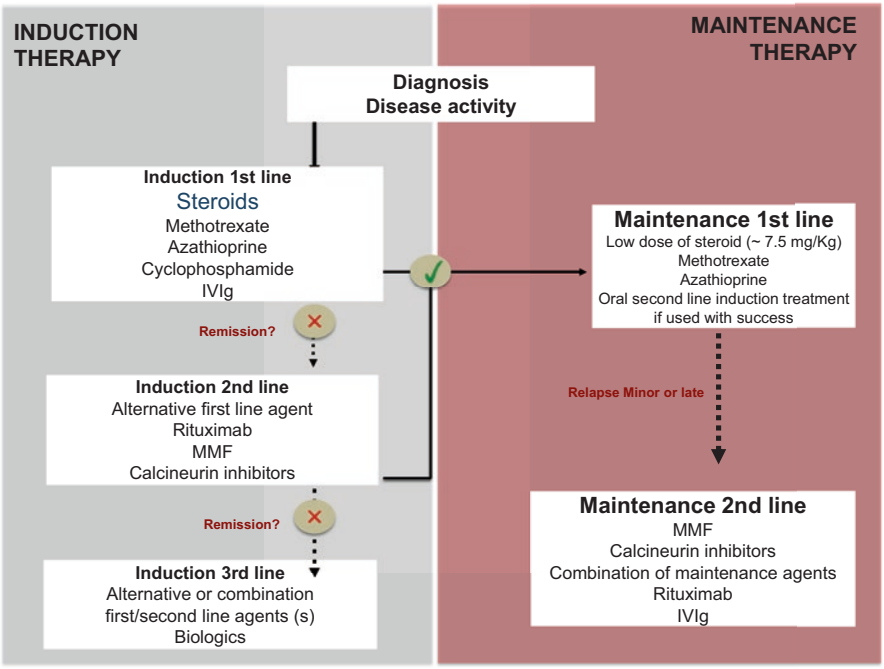

Fig. 7 Step wise approach to remission induction and maintenance therapy in myositis - update. IVIg intravenous immunoglobulins

A Cochrane analysis compared all case studies available with immunosuppressants including methotrexate (MTX), azathioprine (AZA), or mycophenolate mofetil (MMF) and other agents in myositis, and no significant efficacy was identified [47]. Methotrexate and azathioprine are often used as first-line disease-modifying anti-rheumatic drugs (DMARDS). Retrospective studies support the use of methotrexate either orally or subcutaneously with a dose of up to 25 mg weekly [48, 49], followed by 5–10 mg folic acid, 5 days of week. In 2016, a placebo-controlled, multicenter trial of juvenile dermatomyositis demonstrated that treatment with MTX in combination with prednisone resulted in a better response than prednisone alone [50]. Comparative studies have suggested that MTX and AZA have similar efficacies [48]. As azathioprine is a prodrug that is converted into the active metabolite, 6-mercaptopurine, the thiopurine methyltransferase levels should be checked prior to screening for enzyme deficiency. Those that are deficient have an increased chance of myelosuppression. If the enzyme activity is on normal range, AZA can be started at orally 50 mg/day for the first week and then increased every week up to 2–2.5 mg per kg daily, given orally once a day or divided into three doses [51]. In patients with reduced TPMT activity, an initial dose of 25 mg should be used. AZA and MTX can also be used in combination where either agent alone has not proved effective [52].

Mycophenolate mofetil (MMF) is also a prodrug of mycophenolic acid that inhibits purine synthesis impairing B- and T-cell proliferation and consequently leading to immunosuppression. The potential use of MMF is limited to case series generally involving doses of 2000–3000 mg daily, orally. It is known that improvements in skin disease and muscle strength are seen in patients who have not responded to conventional treatment [53, 54]. In an open label study involving seven patients with refractory PM and DM, all the patients achieved complete remission with MMF combined with intravenous immunoglobulin therapy [55]. Small studies have suggested that MMF also benefits pulmonary function tests in patients with DM and ILD [56]. Later, a large cohort of 125 patients with ILD were treated for a period of 897 days, and MMF was associated with improvement in the forced vital capacity and diffusing capacity of the lungs for carbon monoxide [57].

Cyclophosphamide, an alkylating agent, is reserved for the treatment of patients with severe myositis and patients with rapid progressive ILD or overlapping systemic vasculitis or patients refractory to several other therapeutic options [58]. Cyclophosphamide can be administered orally or intravenously; typically 500–750 mg is given in monthly doses up to 6 months. Its use is limited due to its toxicity and increased risk of malignancy [59].

Cyclosporine is a calcineurin inhibitor that blocks the production and release of IL2 [60]. *Tacrolimus* is a second-generation calcineurin inhibitor that binds to an intracellular protein (peptidyl-prolyl cis-trans isomerase FKBP112) leading to inhibitor of T-cell activation [61]. In a Japanese study of 16 patients with PM and 15 patients with DM, treatment with tacrolimus led to an improvement in muscle scores and a substantial decrease in serum creatine kinase levels [62]. Cyclosporine and tacrolimus have a role in the treatment of IMM with ILD [63].

A range of biologic therapies have been investigated for the treatment of myositis.

Rituximab depletes CD20+ B cells, and has been shown to be effective in patients with refractory disease. RTX is reported to be well tolerated. The most common side effects were infections mainly involving respiratory tract [64]. A randomized double-blind (RIM trial) and placebo phase study included 195 individuals, 75 with PM, 72 with DM, and 48 with juvenile dermatomyositis that were refractory to glucocorticoids and at least one immunosuppressive agent. These patients received infusion of RTX (1 g) either at baseline or 8 weeks later, and 83% of the patients met DOI which incorporates the International Myositis Assessment and Clinical Studies (IMACS's) six core set measures of disease activity [65]. Recently, a rate of response to RTX of 78.3% was found in a review of 48 studies which included 458 patients with myositis treated with RTX. The authors concluded that these results support the idea that off-label use of RTX in patients with refractory myositis [64].

Anti-TNF therapies. Although there is expected beneficial role of this potential drug in autoimmune diseases, the results in myositis seem to show little benefit. A number of case reports and case series have reported good responses to infliximab in patients with myositis. However, in a double-blind placebo-controlled clinical trial using infliximab in 12 patients (PM and DM), the response rate was below 33% after 14 weeks [66]. Nowadays, the use of anti-TNF therapy cannot be fully

approved due to the fact that these agents might cause myositis. But in patients with arthropathy, anti-TNF therapy might be considered [67].

Abatacept is a full human fusion protein of cytotoxic T-lymphocyte protein 4 (CTLA 4) and the Fc portion of human IgG1 that inhibits T-cell co-stimulation. A randomized open label trial including 20 patients with refractory dermatomyositis ($n = 11$) and polymyositis ($n = 9$) was conducted, and nearly the half (42%) of the patients achieved the primary end point. The therapy was well tolerated, and these encouraging results led to an ongoing phase III clinical trial (US National library of Medicine Clinical Trials gov).

The future of myositis treatment is likely to include biologic new therapies. However, to assess the therapeutic effect, outcome measures to gauge treatment responses effectively are necessary. The International Myositis Assessment and Clinical Studies Group have suggested core measures to monitor inflammatory myositis. These include global activity, muscle strength, physical function, laboratory assessment, and extramuscular disease. The American College of Rheumatology and European League Against Rheumatism has developed a set of criteria to monitor response based on six core set measures (physician, patient, extramuscular global activity, muscle strength, health assessment questionnaire, and muscle enzyme levels), with a total improvement score classifying patients into minimal, moderate, and major improvement groups. Although mainly designed for trials, they can also help guide therapeutic response and the need for intervention [68].

Physiotherapy is mainly advised in the acute phase to maintain a full range of joint movement. It is encouraged as patients start to recover, and full remission is not required for active therapy [67].Intensive exercise could even be considered in patients with PM and DM as an anti-inflammatory treatment. Strength training or aerobic and resistance training may reduce inflammation by decreasing fat mass and improving cardiovascular fitness and reducing levels of pro-inflammatory cytokines [69]. Recently, new methods for strength assessment have been reviewed [70].

Outcome and Conclusions

Most patients respond well to immunotherapy, but do not usually return to full strength with monotherapy and may require long-term immune-targeted treatment. So far, mortality in IMM in general has improved over the last decades, and this may be due to a better understanding of the disease and effective treatment modalities. In fact, before corticosteroids and immunosuppressives were introduced, the mortality rate was as high as 50–70%. Older series reported 5-year survival rates as low as 52% or 65% with survival rates at 7 and 8 years of 53% and 72.8%. In a retrospective study, Isenberg D and collaborators (2016) reported an estimated cumulative proportion survival at 5,10, 15, and 20 years of 94%, 82,2%, 72,1%, and 66.1, respectively [71]. The cumulative 2-year rate in a Norwegian study was 87% compared with 96% in age- and gender-matched controls and survival rates continues to be significantly below matched controls at 5- and 10-year intervals [72].

However, despite intensive treatment regimes, irreversible muscle damage with fibrosis or fat replacement still occurs, contributing for an elevated morbidity in all myositis subtypes.

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H. Mitoma, M. Manto (eds.), *Neuroimmune Diseases*, Contemporary Clinical Neuroscience, https://doi.org/10.1007/978-3-030-19515-1