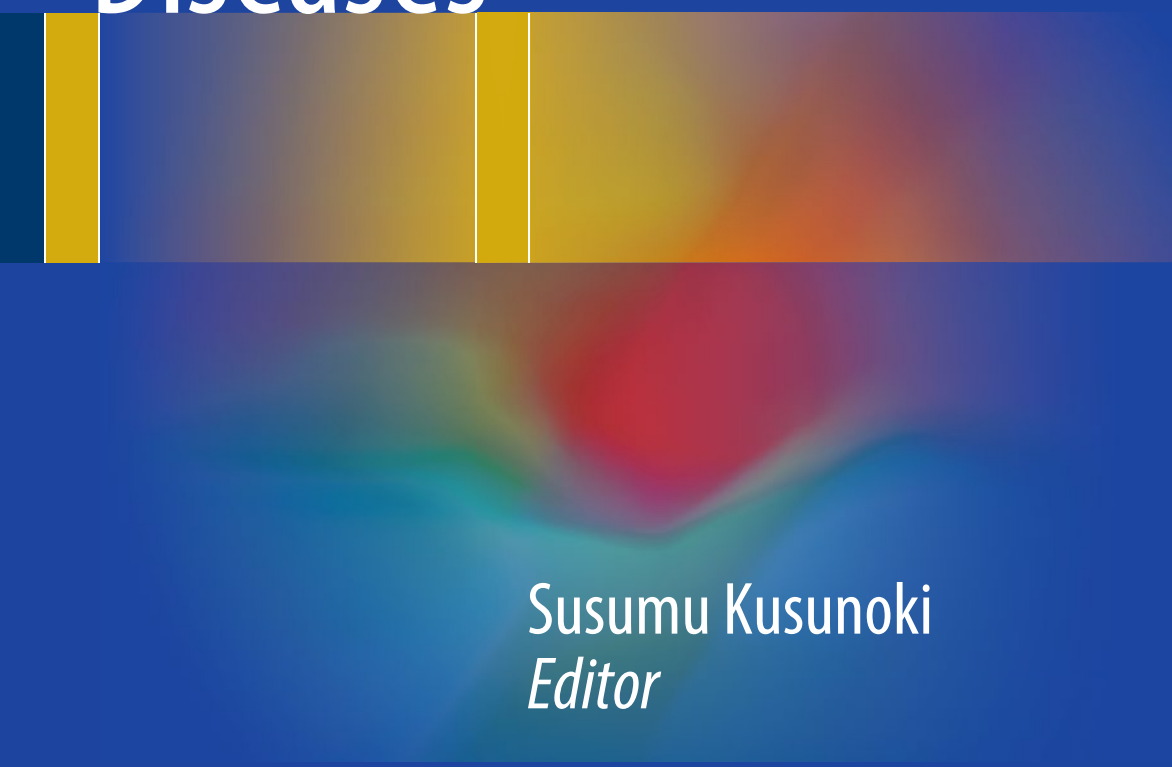
Neuroimmunological Diseases



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Preface

Neuroimmunological diseases comprise neurological disorders caused by immunological mechanisms. Affected areas are varied: the central nervous system in multiple sclerosis and neuromyelitis optica (NMO), the peripheral nerve in Guillain-Barré syndrome (GBS), and the neuromuscular junction in myasthenia gravis. There are many players involved in the pathogenetic mechanisms of neuroimmunological diseases, including autoantibodies, activated lymphocytes, macrophages, cytokines, chemokines, and others.

I served as chairman of the Research Committee on Neuroimmunological Diseases sponsored by the Ministry of Health, Labour and Welfare of Japan from 2008 to 2014. During that period, many novel research findings were reported in the annual meetings of the committee. This book is intended to provide readers with cutting-edge research on neuroimmunological diseases by collecting the papers mainly from the members of the research committee.

The book is divided into 18 chapters. Basic immunology associated with neuroimmunological diseases are described in the early chapters. In the remaining chapters, individual diseases are described with regard to epidemiology, pathogenesis, diagnosis, treatment, and so on. Recently, several autoantibodies have been reported in neuroimmunological diseases. For example, aquaporin-4 antibodies are specifically detected in NMO. Antibodies to glycolipids such as gangliosides are frequently detected in GBS. In particular, GQ1b antibodies are specifically detected in most patients with Fisher syndrome, a subtype of GBS. In myasthenia gravis (MG), acetylcholine receptor (AChR) antibodies are well known. In addition, in AChR antibody-negative cases, other antibodies including muscle-specific kinase antibodies have been reported. Several autoantibodies, including N-methyl-D-aspartate receptor (NMDAR) antibodies, have been reported in autoimmune encephalitis. Those autoantibodies are not only useful diagnostic markers but also causative factors directly involved in the pathogenetic mechanisms. In multiple sclerosis, significant progress has been made in the research of cell-mediated immunities and in genetic analyses. Therapeutic strategies have also been improved recently in neuroimmunological diseases. This book covers those recent advances.

I am convinced that the book is useful for clinical neurologists as well as researchers in neuroimmunology.

I would like to thank the authors for their superb contributions. We would also like to express our appreciation to Springer for giving us an opportunity to gather our works for publication.

Osaka-Sayama, Japan Susumu Kusunoki

Contents

Part I
Basic Immunology

Chapter 1
Cellular Immunity and Multiple Sclerosis: Current Understanding

Wakiro Sato and Takashi Yamamura

Abstract We here focus on a single representative neuroimmunological disease, multiple sclerosis (MS), and illustrate how cell-mediated immunity, in which immune reactions occur independently of antibodies but are dependent on the recognition of antigens by T cells, contributes to the disease mechanism, especially with respect to the dynamics of relapse and remission. The findings from the most useful animal model of MS, the experimental autoimmune encephalomyelitis (EAE), and recent genome-wide association studies clearly demonstrated the causative role of cell-mediated immune response in MS. However, we are witnessing a rapid increase of prevalence of MS as evident in Japan, which hardly explained by genetic factors or established environmental factors, such as vitamin D or smoking habit. We will introduce new players of immunology, such as innate lymphoid cells or gut microbiome, which may provide a clue to the mystery.

Keywords Multiple sclerosis • Th17 cells • Innate lymphoid cells • Cytokine • Chemokine

1.1 Introduction

Multiple sclerosis (MS) is an autoimmune disease affecting the central nervous system (CNS), of which pathogenesis has been intensively investigated for many years. Although basic research aiming at the cure of MS would cover broad ranges of subjects and use various methodologies, immunology-driven research could be regarded as being the most successful in the past, because of its contribution to the development of new drugs. Before T cell antigen receptor gene was cloned and sequenced, it was already known that adoptive transfer of lymphoid cells but not immune sera into syngeneic rodent could recapitulate the neuroinflammation associated with experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Afterward, T cell lines and clones reactive to myelin basic protein (MBP)

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have been established, which are able to induce EAE upon adoptive transfer [1–3]. Similar MBP-specific T cell clones were isolated also from the peripheral blood of patients with MS [4, 5], allowing us to believe that pathogenesis of MS is mediated by autoreactive T cells. Because of ethical reasons, encephalitogenic potentials of human MBP-reactive T cells have been explored only ex vivo. However, in 2000, Roland Martin and his colleagues have reported that an MBP peptide analogue designed to inactivate MBP-specific T cells would activate those T cells and induce relapses of MS [6]. They failed to develop a new drug on the concept of altered peptide ligand (APL) but ironically revealed that MBP could be a target autoantigen in MS. Peripheral blood T cells from patients with MS are also sensitized against other autoantigens, including proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG). Because of the presence of numerous antigenic epitopes recognized by autoimmune T cells in MS, antigen-specific therapy for MS is thought to be a challenge.

1.1.1 MS Disease Course

MS is described as a chronic inflammatory demyelinating disease of the central nervous system (CNS). The term "chronic" does not always mean "progressive"; rather, the disease is mostly characterized by repetitive attacks of neurological deficits with various degrees of recovery. Attacks, also called relapses, typically occur at different ("multiple") CNS locations and at different ("multiple") times [7]. Clinically, each attack is usually visualized as lesion(s) or plaque(s) by magnetic resonance imaging of the brain or the spinal cord. MS is not a so-called common disease, like diabetes or cancer; however, it is the most common neurologic disease in young adults in Western countries. Moreover, epidemiological studies suggest that it is increasing worldwide. Its prevalence among the Japanese population has increased by more than tenfold within a few decades [8, 9]. The reason for this increase is unclear, but environmental factors may be involved (discussed later). Our knowledge of MS has progressed according to our general knowledge of medicine and biology [10]. The etiology of MS has been a mystery for a long time, but the critical role of the immune system is now well accepted.

Although the disease course for most patients usually starts with relapsing-remitting type (RRMS), it typically transitions into secondary progressive type (SPMS). SPMS is characterized, clinically, by slowly progressive neurological deficits without recovery and is usually accompanied by a decreased rate of relapses. Some patients initially experience a progressive course without any episodes of relapse (i.e., primary-progressive MS or PPMS). Recently, a simple, up-to-date version of the clinical course classification has been proposed as follows [11]: (1) RRMS and (2) progressive course. These two courses can co-occur with different contributions in each patient and clinical stage.

Based on numerous studies, the following basic theory explaining these two conditions has been proposed: (1) relapse and remission are controlled mainly by

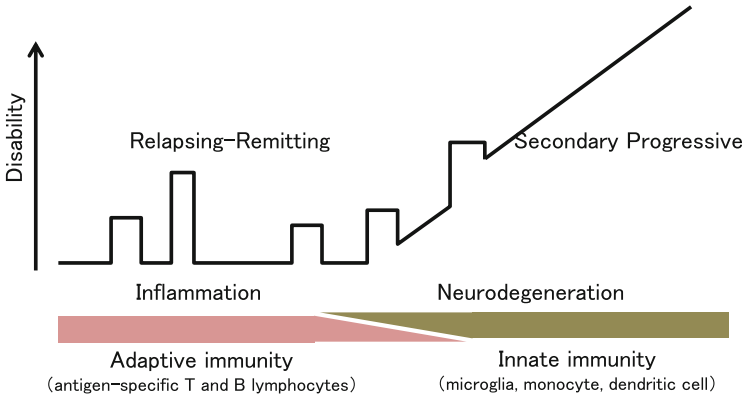

Fig. 1.1 Clinical course of MS and a proposed pathomechanisms. At the stage of Relapsing-Remitting course is driven by the adoptive immunity with the central role of CD4+ helper T cells, whereas later stage of secondary progressive disease is driven mainly by innate immunity including brain-resident microglia, monocytes (Modified from Ref. [12])

the adaptive immune system, in which $CD4^+$ T cells play a pivotal role, and (2) progression is less dependent on adaptive immunity, and more influenced by the innate immune system, in which microglia, monocytes, and dendritic cells serve decisive roles (Fig. 1.1) [12, 13]. Although this is still a working hypothesis, accumulating evidence supports the basic concept.

The mechanism of relapse has been extensively studied, resulting in the development of several effective drugs to reduce MS relapse rates, providing proof of concept regarding the understanding of relapse. However, the mechanism of progression has not been sufficiently elucidated, making it an important issue in the field of neuroimmunology. It should be mentioned that relapse and progression are not completely independent phenomena. Rather, it is suggested that they are fundamentally associated with each other [14]. Very recently, Oki, Raveney, and Yamamura have revealed that $CD4^+$ T cells expressing transcription factor Eomes are involved in the pathogenesis of chronic EAE model and SPMS [15]. As such, chronic neuroinflammation associated with microglial activation is under control of inflammatory T cells which appear to be distinct from Th1 or Th17 cells.

1.2 The Immunology of Relapse

As mentioned above, the disease course for the majority of MS patients starts with a clinical pattern of RRMS [16]. Let us start by describing the importance of helper T (Th) cells in the pathogenesis of MS, especially in relapses.

1.2.1 EAE: A Model of MS

For decades, the mechanisms of MS relapse have been studied extensively using the experimental autoimmune encephalomyelitis (EAE) animal model [17]. Around 40 years ago, it was shown that the injection of a defined protein of the myelin sheath (e.g., myelin basic protein (MBP)) combined with an adjuvant causes either acute or chronic EAE (active EAE) in recipient animals [18]. Encephalitogenic potentials of proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG) were demonstrated much later until synthetic peptides were available for EAE studies. EAE can be induced by adoptive transfer of in vitro activated CD4+ Th cells that are specifically reactive to myelin antigens (passive EAE) [19]. The disease course of EAE, such as monophasic or chronic progressive, depends on the genetic background or antigen type, reminiscent of the variety of clinical courses of MS. In 2009, Pollinger et al. reported that a new transgenic mouse model showed a relapsing-remitting course of EAE [20]. Because Th cells play decisive roles in the pathogenesis of EAE, extensive studies have examined human Th cells in MS.

Some evidence shows the role of Th cells in the pathogenesis of MS. For example, Th cells that are reactive to myelin antigens can be detected in patient blood samples. Histopathological studies of lesions have revealed the accumulation of oligoclonal Th cells [17], suggesting that T cells reactive to specific antigens are recruited to and expand in the lesions (antigen specificity). These and other findings support the autoimmune nature of the disease, with a special role of Th cells in the pathogenesis of MS. However, the antigen(s) recognized by such oligoclonal T cells are not yet identified and are a topic of further investigation.

1.2.2 Genetic Factor

According to epidemiological data, the concordance rate in MS of monozygotic twins is about 30 %, showing a contribution of genetic factors. The most significant genetic risk factor for MS is major histocompatibility complex (MHC) class II (HLA-DR and HLA-DQ), and MHC class I is additionally involved. In 2011, a genome-wide association study of 9772 Caucasian individuals was reported [21]. Association of MHC classes I and II, IL-2 receptor, and IL-7 receptor was confirmed. Moreover, 29 newly identified genes demonstrating associations were involved in CD4+ Th cell function or differentiation, which are related to adaptive immunity. In addition, about a half of the associated genes were the same as those associated with other autoimmune diseases such as type 1 diabetes, Crohn's disease, and rheumatoid arthritis. These results indicate that MS is primarily an autoimmune disease, in which the role of adaptive immunity is related to genetic factors, suggesting the adaptive immune component is causal but not a result of the disease. However, the contribution of each allele was small, suggesting substantial contributions of environmental factors.

There are many potentially useful directions for further investigation. Genome-wide association studies evaluate only common variants. There is a possibility that minor variants also play roles in MS. Additionally, the genetic backgrounds that influence the disease phenotype of MS (MS heterogeneity) should be examined. Other important questions are related to the interaction between environmental factors and genetic factors and the role of gene-gene interactions (i.e., epistasis). Moreover, it is not yet clear whether different ethnic groups, such as the Japanese population, share the same genetic risks with Caucasians.

1.2.3 The Mechanism of Relapse

The initiation mechanisms of EAE (and presumably MS relapse as well) are usually divided into the following three stages: (1) activation of autoreactive CD4+ T cells in the peripheral lymphoid organs, (2) migration of activated T cells through the blood-brain barrier (BBB) to the CNS, and (3) events involved in CNS tissue damage [7]. The last process involves the infiltration of various cell types, such as monocytes or CD8+ T cells.

Such autoreactive, invading T cells are termed pathogenic or encephalitogenic T cells. In the first stage, T cells react to various CNS components, presumably including myelin antigens such as MBP and may become activated. The mechanisms of activation are not completely understood, but it is thought to occur after microbial infection or exposure to other inflammatory stimuli, which activate the innate immune system. Two potential underlying mechanisms have been proposed. The first, called molecular mimicry, considers that autoreactive T cells cross-react with an infectious agent. The second, referred to as bystander activation, assumes that autoreactive T cells are activated as a result of nonspecific inflammatory events that occur during infection [7].

Activated T cells may then migrate to the CNS by crossing the BBB, which is a functional unit that sequesters the CNS from the systemic environment for CNS homeostasis. The topic of BBB will be discussed in Chap. 4. The effectiveness of drugs that suppress MS relapse provides proof of concept supporting the importance of the second step. For example, natalizumab, a humanized anti-VLA4 integrin antibody, blocks migration of T cells into the CNS at the BBB level, whereas fingolimod, an S1P1 receptor antagonist, traps the T cells in the lymph nodes. Both drugs reduce relapse rates, emphasizing the importance of the T cell migration step in relapse [22, 23].

1.3 What Are Pathogenic T Cells?

1.3.1 From Th1/Th2 Dogma to the Discovery of Th17 Cells

Since the 1990s, there has been a widely accepted dogma in immunology in which Th cells are categorized into two functionally antagonistic subsets: Th1 and Th2 [24]. Th1/Th2 balance theory was proposed to describe the immune mechanism of autoimmune diseases and allergies. According to the established dogma, EAE/MS is a representative Th1 disease, meaning that Th1 cells predominate over Th2 cells. Th1 (which produces interferon-γ: IFN-γ) but not Th2 (which produces interleukin (IL)-4) cells are regarded as pathogenic in EAE/MS. In fact, treating MS patients with IFN-γ worsens the disease, providing direct evidence of the pathogenic role of IFN-γ in MS [25]. Moreover, an altered peptide ligand of MBP administered to MS patients in a phase 2 clinical trial revealed a potential encephalitogenic effect via the activation of Th1 cells [6].

In 2003, a breakthrough was achieved as described in a report by Cua et al. [26]. -IL-12 is a critical cytokine to induce Th1 cells. However, IL-12-deficient mice, which lack Th1 cells, developed EAE, suggesting that Th1 cells are unrelated to EAE. Instead, IL-23-deficient mice were completely protected from EAE. IL-23 is similar to IL-12, but important to induce IL-17 production, instead of IFN-γ. To sum up the results of several studies, the IL-23-IL-17 pathway, which induces pro-inflammatory cytokines, such as IL-6, tumor necrosis factor-α (TNF-α), and granulocyte-macrophage colony-stimulating factor (GM-CSF), is critically involved in the pathogenesis of EAE [27]. A few years later, IL-17-producing Th cells were shown to be induced in vitro by IL-6 and TGF-β in mice and to express the characteristic transcription factor RORγt; they were classified as a new Th subset that is fundamentally different from Th1 and Th2 cells [28]. The number of cells needed to induce EAE in an adoptive transfer model was much smaller in Th17 cells than in Th1 cells, implying strong pathogenicity of Th17 cells [29]. Interestingly, however, IL-17-deficient mice are only partially protected from EAE [30]. Moreover, the therapeutic effect of IL-17 blockage was moderate [31], suggesting an insufficient pathogenic role of IL-17.

1.3.2 Diverse and Flexible Th17 Cells

Recent studies have revealed that Th17 cells are more diverse in function (i.e., they exhibit heterogeneity) and more flexible during development (i.e., they are plastic) than originally thought. The possibility of the in vivo transition between regulatory T cells (Treg) and Th17 cells has been reported. Zhou et al. reported that Tregs could be converted into Th17 cells in vivo [32]. Conversely, another report has shown that IL-17-producing Th cells could exert regulatory function [33]. Similarly, phenotypic changes for Th17 cells and Th1 cells have been reported. IL-17A

reporter mice were generated to track IL-17A expression in vivo [34]. EAE of the reporter mice depended on the ex-TH17 cells; IFN-γ and other pro-inflammatory cytokines were produced exclusively by IL-17-producing cells before conversion.

The epigenetic mechanism underlying such Th cell plasticity was examined by generating genome-wide histone H3 lysine 4 (H3K4) and lysine 27 (H3K27) trimethylation maps for various Th cell subsets [35]. Genes encoding transcription factors for Th cell differentiation, such as T-bet, Foxp3, or Rorc, exhibited a broad spectrum of epigenetic states, resulting in high plasticity of differentiated effector T cells.

1.3.3 Pathogenic and Nonpathogenic Th17 Cells

How do such heterogeneity and plasticity of Th17 cells connect to pathogenic and nonpathogenic phenotypes? In one study, Th17 cells generated in response to IL-23 signaling, independently of TGF-β signaling, efficiently induced EAE [36]. These pathogenic Th17 cells co-expressed RORγt and T-bet. Another group has reported that TGF-β3 together with IL-6 induces pathogenic Th17 cells [37]. TGF-β3 was produced by developing Th17 cells in an IL-23-dependent manner, again implying a critical role of IL-23. Th1-related T-bet may be a key factor in inducing the pathogenic functions of Th17 cells, although another study did not reproduce these results [38]. Several factors probably cooperate to determine the fates of various Th17 cell types. Supporting this hypothesis, a study by Kuchroo et al. demonstrated a dynamic regulatory network with as many as 39 regulators controlling Th17 cell differentiation [39]. A model has been proposed in which mouse Th17 cells are related to a wide spectrum of effector phenotypes (Fig. 1.2) [40]. In this model, TGF-β and IL-23 play major antagonistic roles in shifting the Th17 phenotype toward regulatory (nonpathogenic) and alternative (pathogenic) phenotypes, respectively, although other cytokines also contribute to the fine-tuning of the spectrum. The nonpathogenic Th17 subtype is characterized by the production of IL-9 and IL-10 and the expression of c-Maf and AhR, whereas the pathogenic Th17 subtype is distinguished by IFN-γ, GM-CSF, and IL-22 signatures with T-bet expression. Two simultaneously published papers suggested that GM-CSF is required for the acquisition of pathogenicity in Th17 cells [41, 42], supporting this model.

1.3.4 Pathogenic Th17 Cells in MS

Using MS patient samples, a potentially pathogenic role of human Th17 cells has been reported. The frequency of Th1 and Th17 cells in the cerebrospinal fluid (CSF) of patients with RRMS during relapse has been investigated [43]. Both Th1 and Th17 cells are significantly more abundant in the CSF than in the peripheral blood.

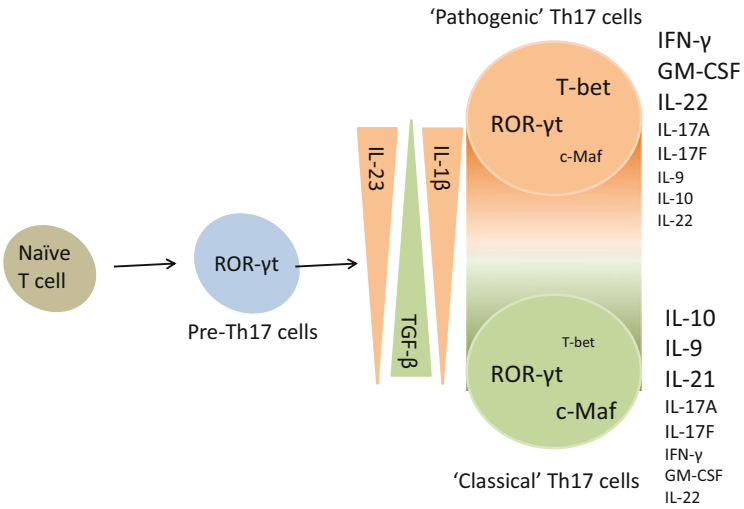

Fig. 1.2 Spectrum view of Th17 cells. pre-Th17 cells which express RORgt will be skewed into either 'classical' or 'pathogenic' depending on different cytokine milieu. Classical type of Th17 cells are induced by abundant TGF-b to produce IL-10 etc, play an important role in tissue homeostasis. On the other hand, pathogenic type of Th17 cells are induced by IL-23 or IL-1b to produce IFN-g or GM-CSF etc, exert an autoimmunity as shown in EAE model (Modified from Ref. [10])

Interestingly, adhesion molecules such as CD49d, CD6, and the melanoma cell adhesion molecule MCAM/CD146, which mediates T cell attachment to endothelial cells, were expressed more abundantly in Th17 cells than in Th1 cells. Together with other features such as the high proliferative capacity and reduced susceptibility to suppression, these Th17 cells are regarded as pathogenic. Another group has reported that lymphocytes from the peripheral blood of MS patients in relapse show an increased propensity to expand into IFN-γ-producing Th17 cells than those of controls [44]. These cells preferentially crossed an in vitro BBB model [45]. In addition, the importance of IL-1 and IL-12 in the induction of pathogenic IL-17/IFN-double-producing phenotype has been emphasized [46]. Another line of research defines the pathogenic human Th17 cell subset based on chemokine receptor expression. In one study, a fraction of CCR6+CXCR3hiCCR4loCCR10-CD161+ cells expressing a P-glycoprotein (multidrug resistance type 1 protein: MDR1) displayed a pro-inflammatory phenotype with a transcriptional signature akin to that of pathogenic mouse Th17 cells [47]. Such MDR1+Th17 cells are enriched and activated in the guts of patients with Crohn's disease and are refractory to glucocorticoids, possibly conferring a treatment-resistant phenotype. We reported that CCR2+CCR5+Th cells exhibit an IL-17/IFN-γ-double-producing phenotype and are enriched in the CSF of relapsing MS patients. Interestingly, this subset possessed a high BBB invasive capacity when activated in vitro [48].

1.3.5 Th Cell Types and Heterogeneity of MS

Following the discovery of Th17 cells, new Th subsets, such as IL-9-producing Th9 or IL-22-producing Th22 cells, were recognized. Interestingly, in EAE, different Th subsets cause different disease phenotypes. For example, in vitro induced Th17, Th1, Th2, and Th9 cells cause EAE with different pathologies, both with respect to severity and anatomy [49]. Other studies have shown that Th1 cells are more likely to induce a classical-type EAE, which mainly affects the spinal cord, whereas Th17 cells tend to induce an atypical-type EAE characterized by the presence of brain or cerebellar lesions [50–52]. Collectively, these studies suggest that various Th phenotypes mediate CNS inflammation, possibly via different immunological mechanisms. There is a good possibility that heterogeneity in the anatomical distribution of MS patients is related to variation in Th subsets.

1.4 Transmigration of Pathogenic T Cells

1.4.1 The Blood-Brain Barrier

The pathogenicity of T cells is fundamentally coupled to the invasion capacity into the CNS through the BBB. The important role of the BBB in neuroimmunology is described in Chap. 4, and we will not go into the details here.

The transmigration of T cells into the CNS is a multistep process characterized by a series of sequential steps that proceed in the following order: rolling, activation, arrest, crawling, and transmigration. The transmigration step can be further subdivided into (1) transmigration from blood vessels to the perivascular space (where CSF is filled) and (2) transmigration from perivascular space to the CNS parenchyma (through glia limitans formed by astrocytic end feet). The precise mechanism of transmigration has not yet been elucidated; however, the development of multicell culture systems recapitulating the BBB is beginning to uncover the molecular mechanism regulating this step [53, 54]. Moreover, a live cell imaging technique has been developed, enabling us to observe in vivo trafficking of lymphocytes at the BBB during the course of EAE [55]. T cell-antigen-presenting cell interactions appear to be necessary for migration into the CNS, suggesting an antigen-specific process is involved in this step (Fig. 1.3).

1.4.2 Key Molecules Involved in MS Relapse

One of the key factors in the transmigration of T cells into brain parenchyma is matrix metalloproteinases (MMPs) [56, 57]. MMPs are a family of proteolytic enzymes capable of remodeling and degrading the extracellular matrix and have

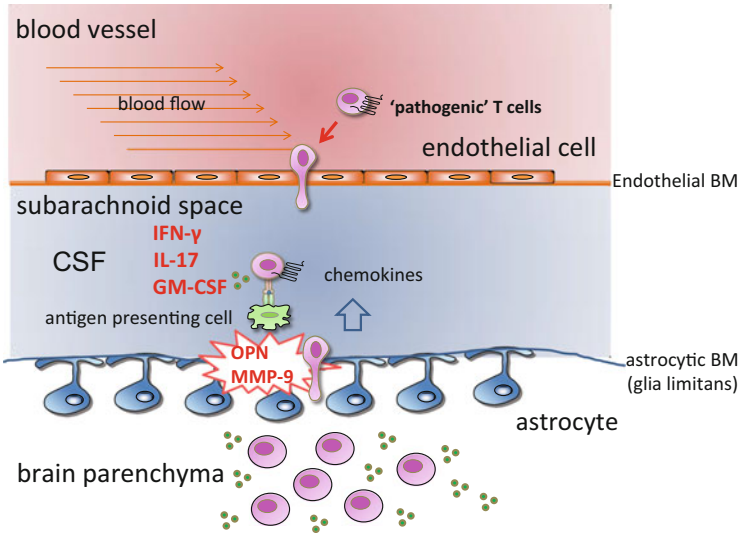

Fig. 1.3 A model in which 'pathogenic' T cells migrate from periphery to brain parenchyma, through subarachnoid space, triggering MS relapse. With activation by putative myelin autoantigen such as MBP, 'pathogenic' T cells gain the capacity to pass through the BBB. MMP-9 presumably degrades parenchymal basement membrane of the BBB, and OPN augments CNS inflammation

important roles in development and physiology. Previous studies have revealed that several MMPs, including MMP-2, MMP-7, MMP-8, MMP-9, and MMP-14, are upregulated in the serum, CSF, and brain tissues from MS patients [58]. In particular, the role of MMP-9 (gelatinase B) has been emphasized [59–61].

Another key molecule mediating CNS inflammation in MS is osteopontin (OPN). The involvement of OPN in EAE and MS was first demonstrated by Steinman and colleagues [62]. OPN is a member of a family of small integrin-binding proteins, termed SIBLING proteins, which execute various biological functions, including inflammation [63]. The injection of OPN induces relapses in the EAE model, and OPN knockout mice are protected against CNS inflammation, suggesting a critical role of this molecule in EAE. OPN transcripts are also greatly upregulated in human MS lesions [64], and increased OPN levels in plasma in RRMS patients have been reported [65–68]. Moreover, OPN binds to α4β1 integrin on T cells to stimulate expression of pro-inflammatory mediators, including Th1 and Th17 cytokines, and promotes the survival of autoreactive T cells by inhibiting apoptosis [69, 70]. As natalizumab is an inhibitor of α4 integrin, it can block OPN signaling, which may be one mechanism for preventing relapses [62]. OPN is produced by macrophages, microglia, astrocytes, and CCR2+CCR5+ T cells [48].

1.5 New Players in Cell-Mediated Immunity in MS

1.5.1 Commensal Gut Microbiota and Gut Immunity

As previously described, the number of MS patients in Japan has increased dramatically during a single generation [8, 9]. It is difficult to explain such a rapid increase by improvement of diagnostic tool or the established environmental risk factors such as vitamin D, smoking, Epstein-Barr virus, and others [71]. Recent developments in the field of gut immunity and commensal microbiota are beginning to provide important clues [72].

The mammalian immune system plays an essential role in maintaining homeostasis with gut microbial communities. At the same time, resident bacteria profoundly shape mammalian immunity and contribute to the mutualistic relationships between bacteria and hosts. It is not an exaggeration to say that gut microbiome is a kind of ecosystem.

Many studies have examined the role of gut microbiota and EAE. The development of regulatory T cells and Th17 cells depends on the gut microbiota. Our group and others have reported that changing the microbiota of an experimental mouse model actually changes the disease course of EAE [73, 74]. Even one strain of bacteria could influence the disease course. A segmented filamentous bacterium was shown to be critical to the induction of Th17 cells in mice and contributed to EAE [75, 76]. In a spontaneous CNS inflammatory mouse model, the disease did not develop under germ-free conditions but showed signs only after commensal microbiota were induced [77]. These mouse studies indicate the important role of microbiota in the pathogenesis of MS [78]. Actually, dysbiosis of disease cases (abnormal microbiome structure or balance) has been reported in various autoimmune diseases such as inflammatory bowel diseases [79]. Just recently, our group proved moderate dysbiosis of Japanese RRMS patients, with a striking depletion of species belonging to *Clostridia* XIVa and IV clusters [80]. Interestingly, the other species classified in the same clusters were reported to inducing Treg [81]. However, the functional significance of the depleted species is needed to be elucidated.

1.5.2 Innate Lymphocytes

Recently, an increasing number of studies have examined a group of immune cells that link innate and adaptive immunity. Natural killer, or NK, cells, γδ T cells, innate lymphocytes (ILCs), invariant NKT (iNKT) cells, and mucosal-associated invariant T cells (MAIT cells) are members of this group.

Unlike conventional αβT cells, which possess unique T cell receptors, they basically lack such antigen specificity. For example, iNKT cells and MAIT cells are characterized by very limited T cell receptor diversity owing to their expression of a single α-chain (Vα24-Jα18 in human iNKT cells and Vα7.2-Jα33 in human

MAIT cells). One important feature of ILCs is the ability to produce various cytokines.

iNKT cells are a unique subset of lymphocytes that recognize glycolipid antigens presented by CD1d [82]. α-Galactoceramide (α-GalCer) is an antigen of iNKT cells that activates iNKT cells to produce various cytokines, both pro-inflammatory and regulatory, including both IFN-γ and IL-4. In MS, a decreased frequency of iNKT cells [82] and a possible regulatory role of iNKT cell via IL-5 have been reported [83]. An α-GalCer analogue called OCH, which has a shorter sphingosine chain, was discovered to selectively stimulate IL-4 production from iNKT cells, ameliorating the disease course of EAE [84]. Various preclinical studies have led to drug development targeting MS. OCH is now under a clinical trial including healthy subjects and patients with RRMS.

MAIT cells are enriched in the gut (as can be inferred from their name, mucosal associated) but are represented at a substantial frequency (several %) in blood lymphocytes in humans [85]. In mice, MAIT cells exert regulatory roles in EAE, suggesting a similar role in MS [86]. MAIT cells are decreased in the peripheral blood [87] and are detected in MS lesions, although the on-site role has not been elucidated. MAIT cell antigens have long been enigmatic. However, it was recently reported that vitamin B2 derivatives function as MAIT cell antigens. Intriguingly, they are produced by the gut microbiome [88], providing an explanation for the lack of MAIT cells in germ-free mice. Regarding NK cells, it has been reported that NK cells of MS patients in remission produce IL-5 and exert a supportive effect on the maintenance of remission [89].

1.5.3 Role of B Cells

There is a growing body of evidence that suggests a prominent role of B cells in MS pathogenesis. Intrathecally synthesized oligoclonal bands in the CSF of MS patients have been an important diagnostic hallmark [90]. Histopathological examination has revealed that the accumulation of IgG or a complement is most often observed in MS lesions [91]. B cell depletion by the anti-CD20 antibody rituximab reduces the number of MS relapses [92]. Moreover, plasmapheresis and immunoabsorption therapy, which deplete humoral factors including antibodies, is beneficial in a subset of patients [93]. B cells can contribute to MS pathogenesis in multiple ways, including antigen-presenting cells, cytokine producers, and antibody producers. Here, we describe a few examples. Meningeal lymphoid follicle structures have been reported in a subset of SPMS patients [94]. They contain germinal centers, in which affinity maturation and clonal expansion of B cells occur inside of the CNS, probably affecting the progressive course of the disease. BAFF (B cell-activating factor belonging to the TNF family) is a factor that promotes the survival and development of B cells. It has been reported that the serum BAFF level of MS patients increased after the administration of IFN-β therapy [95]. There is discussion regarding why some patients who receive IFN-β exhibit increased disease

activity [96]. One explanation is that an increased BAFF level promoted B cell-mediated autoimmunity, which increased disease activity.

1.6 Concluding Remarks

In this chapter, we focused on cell-mediated immunity of MS and mainly described the role of pathogenic T cells in MS pathology. Numerous studies have been performed on this topic, not only revealing various roles of T cells in MS but also enabling the development of drugs to treat RRMS. We would like to mention here that other cell subsets, including those that we could not describe here, including CD8+ T cells and monocytes, are also involved in MS. In particular, the role of innate immune cells in the progressive phase of MS is a particularly interesting topic.

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Chapter 2
Glia in Neuroimmunology

Akio Suzumura

Abstract Accumulation of glia, especially microglia and astrocytes, is commonly observed in a variety of neurological disorders and has long been considered as static scar formation, called "gliosis." However, recent studies have revealed that these accumulated glial cells are activated and produce various factors, either toxic or protective to neural tissue. Gliosis is no more static scar, but inflammation actively participates in the development of disease processes and is called "neuroinflammation." The brain has long been considered as an immunologically privileged site. Innate immunity by microglia had been believed to be the only defense mechanism in the brain. However, in autoimmune diseases in the central nervous system, such as multiple sclerosis, glial cells are capable to function as antigen-presenting cells, effector cells to damage neural tissue, and even a negative regulator against inflammation. Activated glial cells are also involved in pathophysiology of neurodegenerative and psychiatric disorders. Thus, understanding of glial neuroinflammation is important to elucidate pathophysiology of various neuropsychological disorders and may give us clues for future therapeutic strategies against these diseases.

Keywords Microglia • Astrocyte • Neuroinflammation • Multiple sclerosis • Demyelination

2.1 Introduction

Microglia are macrophage-like cells and are considered to play the main role in innate immunity in the central nervous system (CNS). Microglia appear in the fetal and early postnatal period in the brain parenchyma, before developing other glial cells. Therefore, they are considered to play a role in the development of neural cells and/or neuronal network. After development of the CNS, microglia play roles as immune cells in the CNS. Microglia function as a first line of defense in the cases

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of infection or injury of the brain. They express a variety of surface receptors, such as CD14 and toll-like receptors (TLRs) 1, 2, 3, 4, 6, and 9, which are upregulated in inflammatory lesions in the CNS. Microglia recognize lipopolysaccharide (LPS) or peptidoglycan on the bacterial membrane and viral glycoproteins or poly(I:C) via TLRs to produce inflammatory cytokines, such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and other inflammatory mediators like nitric oxide (NO) or reactive oxygen species (ROS). Microglia are also activated through complement receptors and Fc receptors. It has been shown that microglia express the ionotropic ATP-gated receptors, such as P2X7. They also express receptor for advanced glycation end products (RAGEs) to interact with amyloid. Recent studies indicated that damage-associated molecular patterns (DAMPs), such as heat shock proteins, high-mobility group box 1 (HMGB1), ATP, and micro RNA, from injured CNS cells could bind to the receptors on microglia to activate them. Thus, microglia can be activated not only by bacterial or viral components but also via other various types of receptors (Fig. 2.1). The brain had long been considered as an immunologically privileged site where specific immune responses do not usually occur. The classical innate immunity by microglia had been considered to be the only defense mechanism in the CNS. However, studies so far have clearly revealed that this is no more the case. In response to inflammatory cytokines or some neurotropic viruses, most glial cells are induced to express major histocompatibility complex (MHC) class I antigen [1–4] that is necessary for interaction with CD8 T cells. Then, they can interact with autoreactive T cells. Microglia are also induced to express MHC class II antigen [5] that is essential for antigen presentation to T cells. Although

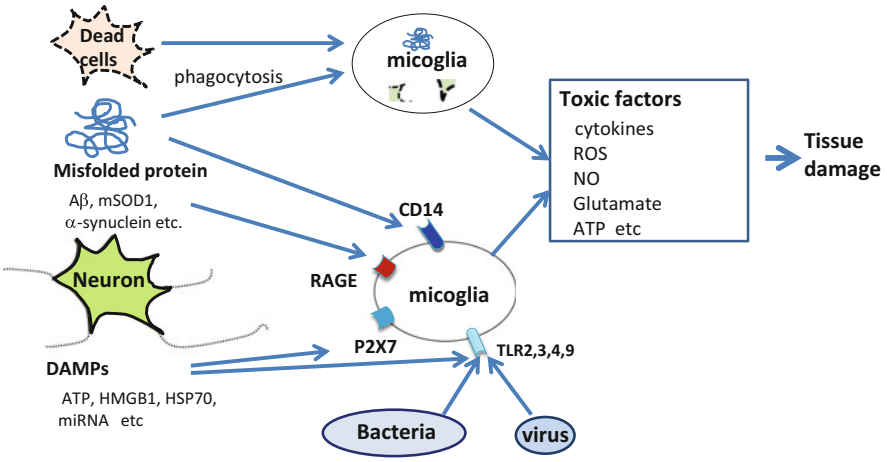

Fig. 2.1 Mechanisms to activate microglia. Bacterial lipopolysaccharide, peptidoglycan, and viral glycoproteins bind to TLRs to activate microglia. Phagocytosis of cell debris or accumulation of misfolded protein can activate microglia. Damage-associated molecular patterns (DAMPs) from damaged neural cells also are able to activate microglia via their surface receptors

astrocytes are reportedly MHC class II antigen-positive [6, 7], the functional antigen-presenting cells (APC) that express all set of co-stimulatory molecules are microglia.

Microglia and astrocytes function as effector cells in inflammation, demyelination, and neuronal degeneration via producing various inflammatory mediators. In addition, the recent studies have shown the involvement of immune and/or inflammatory mechanisms in neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and Huntington disease. Activated glial cells are accumulated in or around degenerating neurons in these diseases before the onset of clinical manifestation and have been shown to play a principal role in neuronal degeneration and regeneration. These observations suggest that glial cells play much more critical causative roles on the development of neurological diseases, than expected before. In this chapter, I focus on the role of glial cells in either immunological or other inflammatory conditions in the CNS.

2.2 Microglia in Neuroimmunological Disorders

2.2.1 Microglia as Antigen-Presenting Cells

The immune response begins when a specific antigen is presented to sensitized T cells by APC. The APC processes peptide fragments to express them on the surface as a MHC and peptide complex. T cells are activated when the MHC-peptide complex interacts with T-cell receptors (TCR). APC requires co-stimulatory molecules that bind a specific counterpart on T cells to fully activate T cells. The cells constitutively expressing MHC class II and co-stimulatory molecules are considered to be professional APC. Those include macrophages, B cells, and dendritic cells. Nonprofessional APC differs from them by expressing little or no MHC class II molecules constitutively and by not having a complete set of co-stimulatory molecules. The candidates for the nonprofessional APC in the CNS are microglia, astrocytes, pericytes, and endothelial cells. They do not usually express MHC class II antigen either in vivo or in vitro, but are induced to express MHC class II molecules by certain inflammatory cytokines, especially IFNγ [5], and some of the co-stimulatory molecules [8]. Endothelial cells, astrocytes, and pericytes reportedly process and present protein antigens to primed CD4-positive helper T cells in vitro. However, the specific role of these cells as APC in vivo is still unclear. At least, astrocytes do not usually express MHC class II antigens in vivo, even in the presence of inflammatory cells. Microglia express co-stimulatory molecules, such as B7, intercellular adhesion molecule (ICAM)-1, and lymphocyte function-associated antigen (LFA)-3, but only some in astrocytes. It has been shown that

human microglia, but not astrocytes, express both B7-1 and B7-2, suggesting that microglia is a much more suitable candidate for local APC in the CNS. In fact, microglia when stimulated with IFNγ can present antigen to antigen-specific T cells in vitro. Microglia have been shown to function as APC in pathological conditions in vivo [9]. In the inflammatory conditions, dendritic cells (DC), a professional APC, are observed and are considered to function as professional APC in the CNS. We have shown that microglia when stimulated with granulocyte macrophage colony-stimulating factor (GM-CSF) acquire DC-like phenotype and function as APC [10]. Thus, microglia-derived DC may also be a candidate for professional APC in the CNS.

Professional APC such as DC or macrophages produces IL-12 and IL-18 that regulate differentiation of T helper 1 (Th1) and natural killer (NK) cells. Microglia also produce a functional heterodimer of IL-12 upon stimulation. LPS-stimulated microglia produce IL-18, which induces interferon (IFN)γ production by T cells in synergism with IL-12. Another IL-12 family cytokine, IL-23, is a heterodimer of IL-12 p40 and p19. It has been shown that p35 knockout mice that does not produce functional IL-12 are highly susceptible to the induction of EAE, while p40 knockout mice that does not produce either IL-12 or IL-23 does not develop EAE, suggesting that IL-23, but not IL-12, is a critical cytokine for the Th-1 development [11, 12]. IL-27, an IL-12 family member, plays a role in the early stage of Th-1 development. Both IL-23 and IL-27 are produced by microglia upon activation [13]. Since all Th-1-inducing cytokines are produced by microglia, while IL-4 that induces Th-2 response is not produced in the CNS, Th-1 response occurs much more easily in the CNS than Th-2 response.

Most potent stimulus to induce MHC class II antigen on neural cells is IFNγ. IFNγ has been considered to be produced exclusively by lymphoid cells, such as T cells or NK cells. However, microglia can produce IFNγ upon stimulation with IL-12 and/or IL-18 [14]. Thus, induction of MHC antigen expression on neural cells may occur without immune cell infiltration in the CNS.

2.2.2 Microglia as Effector Cells in Inflammation and Neuronal Degeneration

Microglia produce a variety of cytokines, such as IL-1, IL-5, IL-6, IL-8, IL-10, IL-12 family, TNFα, and CSF-1, in response to LPS and/or IFNγ. These stimuli also induce a variety of chemokines, nitric oxide (NO), superoxide (O_2^-), and glutamate. TNFα, NO, and O_2^- have been reported to damage myelin and neuronal cells to induce demyelination and neuronal degeneration. Recently, however, it has been shown that most neurotoxic factor from activated microglia is glutamate, which disturbs mitochondrial respiratory chain to cause energy depletion in neurons [15]. TNFα was a candidate for neurotoxic factor from activated microglia. However, in our experimental conditions, TNFα did not kill neurons. Although TNFα by

itself is not a potent neurotoxic factor, it induces glutamate production in microglia via upregulation of glutaminase. Interestingly, thus produced glutamate is released through hemichannel of gap junction but not through glutamate transporters [16]. Thus, microglia can act as effector cells in either inflammatory demyelination or neuronal degeneration. In addition, since IL-1β, TNFα, and IFNγ induce proliferation of astrocytes in vitro, these microglia-derived cytokines may contribute to the formation of gliosis.

In contrast, microglia also produce anti-inflammatory cytokines, such as IL-10 and transforming growth factor β (TGFβ). Recently, we found that IL-19 produced by microglia suppress production of inflammatory factors in an autocrine manner [17]. The microarray study revealed that the most prominently upregulated mRNA in LPS-stimulated microglia was IL-19. Therefore, it is possible that microglia prepare the mechanism that self-limit their own activation immediately.

2.2.3 Microglia in MS and EAE

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the CNS that affects genetically susceptible patients under some environmental conditions. Its animal model, experimental autoimmune encephalomyelitis (EAE), is induced by immunization with myelin antigens. EAE is also produced by adoptive transfer of myelin-reactive $CD4^+$ T cells that are isolated from mice immunized with myelin peptide and restimulated in vitro with myelin peptide. EAE shows similar pathological features to MS. In both conditions, infiltrating T cells, macrophages, and B cells are found within demyelinating lesions in the white matter of the CNS. Macrophages containing phagocytosed myelin debris have been found within active lesions. Axonal degeneration is also detected in rather early active stage of MS and EAE [18]. Although the precise mechanisms of axonal degeneration remain unclear, it is mediated by infiltrating effector helper T (Th) cells, either Th1 or Th17, macrophages, and microglia. We have shown that activated microglia produce glutamate that induces axonal dystrophy [15]. Since another study has revealed that axonal dystrophy is closely correlated to microglial activation and decreased astrocytic glutamate transporter [19], it is possible that microglial glutamate plays a predominant role on axonal damage. However, it has been shown recently that oligodendrocytes maintain energy supply to axons [20, 21]. Thus, it is also possible that axonal dystrophy is a result of damage of oligodendrocytes.

In MS, Th cells require restimulation with antigen by local APC after being invaded in the CNS. The development of MS is associated with the allelic variants of MHC class II molecules, which is associated with presentation of antigen exclusively to Th cells. The mechanism of how these infiltrating cells induce demyelination is still elucidative. The factors from microglia activated by Th cells are the candidates to damage oligodendrocytes or myelin (Fig. 2.2). Following exposure to inflammatory cytokines, the blood-brain barrier (BBB) is broken down to facilitate infiltration of $CD4^+$ T cells and macrophages into the CNS parenchyma

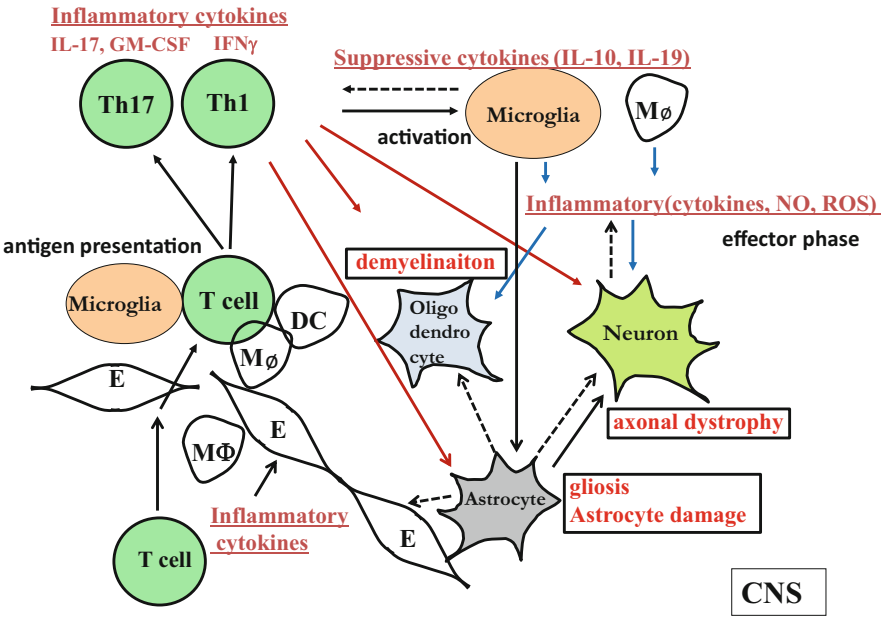

Fig. 2.2 Roles of glial cells on the development of MS. T cell-derived proinflammatory cytokines damage blood-brain barrier. Invaded T cells require reactivation in the CNS. There, microglia, dendritic cells (DCs), and microglia-derived DC function as antigen-presenting cells (APC) to induce differentiation of T cells into Th1 and Th17. These T cells activate microglia and macrophages to induce demyelination, axonal dystrophy, and gliosis. A *solid line* indicates positive or toxic function, and a *broken line* indicates protective or suppressive function. *E* endothelial cells, *Mø* macrophage

during the induction phase of MS and EAE. Disruption of BBB may be critical to induce MS. In MS, abnormalities in tight-junction-related proteins are observed in 42 % of acute lesions and 13 % of normal-appearing white matter. Inflammatory cytokines have been shown to damage BBB by downregulating tight-junction proteins [22]. Another possible mechanism to increase permeability of BBB is a damage of astrocytes, since astrocytes support maintenance of BBB integrity. In addition, we have shown that IL-25, produced by endothelial cells, protects against inflammatory cytokine-induced BBB collapse. Reduction of IL-25 observed in active MS lesion may be related to BBB damage in MS [23].

It is usually difficult to discriminate microglia from infiltrated macrophages in the CNS of MS. However, several lines of evidence have suggested that microglia may function as mediators of neuroinflammation in MS and EAE. Microglia function as immunocompetent cells in antigen presentation and production of cytokines and chemokines in the inflamed CNS (reviewed in Sobobe and Suzumura [24]). During the development of EAE and MS, microglia become activated to become CD68 positive and MHC class II antigen positive and accumulate around lesion sites. Demyelination and neuronal damage reportedly correlate with

activation of microglia in the brain of progressive MS patients [25, 26], which is characterized by an increased density of CD68-positive cells and the formation of microglia nodules. In addition, microglia are activated to become APC, which express antigens on cell surface and present them to Th, before the onset of EAE [27]. Moreover, it has been reported that pharmacogenetic ablation of microglia repressed the development of EAE [28]. These findings suggest that microglia play a key role in MS and EAE pathogenesis.

On the contrary, a recent study demonstrated that most infiltrating macrophage-like cells in early phase of EAE are blood-borne, and microglia appear in later recovery phase, suggesting that macrophages play a predominant role in acute inflammation and microglia may play a role in tissue repair [29]. Further studies are needed to conclude this.

2.3 Astrocyte in Neuroimmunological Disorders

Astrocytes have long been considered as supporting cells of neurons. Astrocytes are the most numerous among all types of glial cells. In addition to the mechanical support, they regulate glucose metabolism, glutamate concentration, synaptic, and BBB formation. They also produce a variety of neurotrophic factors, such as glia-derived neurotrophic factor (GDNF), to support neuronal differentiation and survival. The reduction of astrocytic support may result in neuronal degeneration, as shown in ALS model [30]. However, recent evidences suggest more active roles of astrocytes in the pathophysiology of the diseases. Astrocytes may be damaged from an early stage of the disease or may be actively involved to damage neuronal cells to play a causative role of the diseases. Thus, to elucidate the functions of astrocytes in various neuroimmunological disorders is important for understanding of pathophysiology of either neuroimmunological or neurodegenerative disorders.

2.3.1 Astrocytes as Antigen-Presenting Cells

The possible role of astrocytes in antigen presentation had been suggested in the mid-1980s. It has been shown that factors from immunocytes including IFNγ and a neurotropic virus can induce MHC class II antigens in astrocytes [3, 6, 7]. These stimuli also induce other co-stimulatory factors, such as B7-1 (CD80), B7-2 (CD86), and CD40. In addition, astrocytes are induced to express VCAM-1 and CS-1 that enable them to directly bind α4β1 integrin on T cells. These findings suggested that astrocytes could function as nonprofessional APC in the CNS. However, as mentioned above, evidences so far suggest that DC and microglia are much more suitable APC in various neuroimmunological conditions.

2.3.2 Astrocytes as Effector Inflammatory Cells

Astrocytes can secrete a variety of inflammatory cytokines as microglia. These include IL-1β, IL-6, TNFα, IL-12, IL-15, IL-23, IL-27, IL-33, and so on. They also produce nitric oxide (NO). Thus, astrocytes may also play a role as inflammatory cells. However, as we have shown previously, the inflammatory cytokines produced by astrocyte are much less and require more potent stimuli to produce these cytokines as compared to microglial production [31]. Astrocytes produce GM-CSF [32] and CSF-1 [33] both of which induce proliferation and activation of microglia [34]. They also produce many chemokines including CCL2 (MCP-1), CCL5 (RANTES), CXCL8 (IL-8), CXCL10 (IP10), and CXCL12 (SDF-1), to recruit inflammatory cells into the CNS lesions. We have shown that IL-9 produced by several types of T cells, including Th1, Th17, and Treg, in the presence of IL-4 acts on astrocytes to induce chemokine CCL20 [35]. CCL20, then, induce the trafficking of Th17 into the CNS through BBB. Taken together, astrocytes may also function as inflammatory cells either directly or indirectly in the CNS. It has been shown recently that gap-junction hemichannel blocker suppresses release of neurotoxic factors from astrocytes to ameliorate progression of neuronal degeneration in the animal model of neuronal ceroid lipofuscinosis (Batten disease) [36]. Astrocyte-mediated neuroinflammation may play a critical role on both inflammatory and degenerative disorders in the CNS.

2.3.3 Astrocytes as Anti-Inflammatory Cells

As microglia are called double-edged sword, astrocytes can also produce various protective factors and anti-inflammatory cytokines. Astrocytes produce anti-inflammatory cytokines, IL-10 and TGFβ, and astrocyte-derived TGFβ reportedly deactivate microglia [37]. Deletion of astrocyte results in delayed recovery of cuprizone-induced demyelination, suggesting a possible role of astrocytes in remyelination process [38]. In addition, astrocytes express death ligand CD95 that induce apoptosis of invaded T cells suggesting the defensive function of astrocytes [39].

2.3.4 Astrocytes as a Target of Neuroimmunological Diseases

Neuromyelitis optica (NMO), also known as Devic disease, is a rare inflammatory disorder of the CNS mainly affecting optic nerve and the spinal cord. NMO had been considered as a subtype of MS. However, recent evidences have clearly shown that NMO is an astorocytopathy, distinct from MS. As detailed in another chapter,

NMO is an autoimmune disease caused by autoantibodies directed against aquaporin-4 (AQP4-Ab) [40] and is now called aquaporinopathy or AQP4 channelopathy. AQP4-Ab plays an important role in NMO pathophysiology. In vitro and ex vivo experiments showed that AQP4-Ab can induce either direct astrocyte loss through complement activation or astrocyte changes via internalization of AQP4.

Recently, it has been shown that expression of astocytic connexin (Cx) is reduced in acute progressive type of NMO and MS [41], suggesting that astrocyte pathology occurs in other diseases than NMO. Since these patients did not have autoantibody against Cx, the authors have claimed that autoantibody-independent astrocytic Cx loss may relate to disease aggressiveness in both NMO and MS.

In addition to the neuroimmunological diseases, damage of astrocytes is also related to psychological disorders (reviewed in Rajkowska and Stockmeier [42]). Examination of postmortem brain from major depressive disorder and animal model or stress-induced depression has clarified the astrocyte pathology, as shown by altered expression of glial fibrillary acidic protein (GFAP), AQP-4, glutamate transporters, and so on. These findings suggested that reduction of astrocytes may cause depressive disorders without obvious neuronal loss and that astrocytes could be a therapeutic target for these disorders.

2.4 Conclusion

Glial cells play pivotal roles on the development of neuroimmunological and neurodegenerative disorders. They function as either APC, effector cells, or, in some occasion, as negative regulator for inflammation. Both astrocytes and microglia have dual functions, toxic vs trophic. Upon activation, they produce both inflammatory and anti-inflammatory cytokines and both neurotoxic factors and neurotrophins. It is possible that the dual functions may be related to the self-limiting mechanism or the chronic progression of the diseases. Thus, studies on regulatory mechanisms to exert these dual functions may give us insight for understanding of pathophysiology in various neurological and psychiatric disorders.

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Chapter 3
Animal Models for the Study of Neuroimmunological Disease

J. Ludovic Croxford and Sachiko Miyake

Abstract The development and use of numerous animal models of human autoimmune diseases have provided important advances in our understanding of pathogenic mechanisms of disease and provided robust and reliable models to test novel therapeutic strategies. However, few preclinical studies of therapeutic treatments have demonstrated efficacy in the clinic, possibly because of the biological differences between humans and other animals. Although animal models of human disease are imperfect, it is important to understand the differences between the human disease and its animal models and to design experimental studies using animal models appropriately for the questions being asked. This review provides an overview of the currently used animal models of three human neuroimmunological diseases, multiple sclerosis, Guillain-Barré syndrome, and myasthenia gravis, as well as the advantages and disadvantages of each model and how they correlate or differ from their human counterpart.

Keywords Multiple sclerosis • Neuritis • Myasthenia gravis • Animal models • Neuroimmunology • Virus models • Myelin

3.1 Introduction

Following decades of research, scientists have developed a large number of drugs and therapeutic agents that can be used to reduce the symptoms and severity of a number of neuroimmunological diseases such as multiple sclerosis (MS), neuritis, and myasthenia gravis. However, none of these treatment methodologies is curative, and many have a limited life span, such as interferon (IFN)-β that induces neutralizing antibodies in some MS patients [1], or cause serious side effects (progressive multifocal leucoencephalopathy) such as monoclonal anti-very late antigen (VLA)-4 antibodies (natalizumab, Tysabri®) [2] and thus cause patients to drop out of clinical trials or stop taking the treatment. In addition, although many

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studies have dissected the intricate pathways involved in the aetiology, development, and pathogenesis of diseases such as MS, we still do not have a definitive understanding of how these diseases manifest and, indeed in the case of MS, whether it is a single disease or rather a spectrum of disorders with similar characteristics. Therefore, researchers have used animal models to aid our understanding of disease pathways, immune cell functions, and pathology. An example of the importance of animal models was the demonstration that CD4 T cells specific for a myelin epitope injected into naïve animals were sufficient to induce a central nervous system (CNS) demyelinating disease with CNS lesions similar to those observed in MS [3, 4]. In addition, these models are a useful preclinical aid to developing novel therapeutic agents. Many different animal models of neuroimmunological disorders have been developed over the last few decades with some success. However, it is important to understand the advantages and limitations of each model to ensure that the correct model is being used for the purpose of the study.

In this review, we provide an overview of the currently available neuroimmunological disease animal models, describing their advantages and disadvantages as well as their relationship and correlation to the human disease they are attempting to model. Although no one animal model is completely identical with human disease, they are nevertheless very useful and important tools with which to increase our understanding of neuroimmunological disease when used correctly.

3.2 Animal Models of Multiple Sclerosis

3.2.1 Active Experimental Autoimmune Encephalomyelitis

MS is a human, inflammatory, demyelinating disease of the CNS, with characteristic demyelinating lesions containing immune cell infiltrate and activated CNS-resident cells located in the white matter of the brain and spinal cord; therefore, studies to investigate its pathology are difficult and often rely on autopsy tissues. In these cases, patients might have had the disease for decades and therefore the tissues are representative of disease at the end stage. Furthermore, epidemiological studies have suggested that the initiating factors for MS might be infections that occur during childhood [5]. Therefore, animal models of MS are critical for the study of susceptibility, disease initiation, and pathology during the early stages of disease. Experimental autoimmune encephalomyelitis (EAE) is an autoimmune T cell-mediated disease of the CNS mediated primarily by CD4+ T cells and a commonly used animal model for the study of MS. Disease is characterized by perivascular lesions containing inflammatory infiltrates and nerve conduction block that causes reversible hind-limb paralysis. In addition, late-stage EAE animals also develop axonal demyelination and loss, a pathological hallmark of MS, which leads

to severe permanent disability. Characterization of the immune cell infiltrate (T cells, B cells, activated macrophages, and microglia) and the pathology of perivascular demyelinating lesions have shown similarities with human MS lesions [6, 7] although in EAE the spinal cord is the target organ, whereas in MS the brain is more often targeted, especially the white matter. However, EAE is a useful model to study the inflammatory stage of MS and shares some characteristics of MS such as optic neuritis, increased susceptibility of females, perivascular lesions, axonal demyelination, and partial remyelination, as well as eventual axonal loss and flaccid-limb paralysis.

There are two commonly used models of EAE. Active EAE is induced by subcutaneously immunizing genetically susceptible animals, usually rodents, with myelin antigen in Freund's complete adjuvant-containing mineral oil and *Mycobacterium tuberculosis* strain H37RA. In some EAE low-responder mouse strains, such as C57BL/6, additional injections of pertussis toxin are required. During the induction phase, myelin-specific T cells are activated by antigen-presenting cells (APC) presenting myelin peptide fragments in the draining lymph nodes to produce T helper 1 (Th1) type cytokines, such as interferon (IFN)-γ and tumour necrosis factor (TNF)-α, which allows them to escape the lymph nodes and traffic to the CNS. The effector phase of disease involves the extravasation of activated myelin-specific T cells through the blood-brain barrier and into perivascular spaces in the spinal cord. Here, the encephalitogenic T cells encounter CNS-resident cells mediating further stimulation of pro-inflammatory cytokines and chemokines that mediate a secondary influx of other peripheral inflammatory cells including B cells and mononuclear phagocytes. A study in EAE and a viral model of MS demonstrated that APCs in the CNS restimulated myelin-specific T cells to initiate epitope spreading to other myelin antigens and perpetuate disease [8]. The consequence of this pro-inflammatory cytokine milieu is the demyelination of CNS axons, thought to be mediated in part by numerous mechanisms including phagocytosis by activated mononuclear cells, destructive effects of anti-myelin antibodies, the production of free radicals, and the direct cytotoxic effects of pro-inflammatory cytokines secreted by activated CD4+ T cells and monocytes.

Initially guinea pigs and rats were the animals of choice for EAE studies. However, with the advent of transgenic and gene knockout technology, mice have become the more commonly used animal for EAE studies. Numerous mouse models of EAE have been developed, and these are differentiated by the strain of mouse used and the immunodominant myelin peptide for that particular strain. Commonly used EAE-susceptible mouse strains include SJL, B10.PL, C57BL/6, C3H, SWR, and Biozzi ABH. Depending upon the immunizing myelin antigen and mouse strain used, different forms of EAE can be induced. The most commonly used models are SJL mice immunized with proteolipid protein (PLP) 139–151 that induces a relapsing-remitting form of disease, the most common form of MS, and C57BL/6 mice immunized with myelin oligodendrocyte protein (MOG) 35–55 that initiates a chronic-progressive type of EAE. Other strains, such as Biozzi ABH mice, can be immunized with a suspension of whole Biozzi ABH spinal cord to develop a very reproducible relapsing-remitting disease [9]. The clinical symptoms

of EAE usually develop at 7–15 days post-immunization and include weight loss, loss of tail tone and gait, and eventually paralysis in either or both hind limbs. Weight loss precedes the onset of disease symptoms and thus can be used as a marker for disease onset. Daily observation of EAE symptoms and body weight is noninvasive and is therefore a great advantage to researchers, allowing the disease process to be followed, especially when studying the effects of drug treatments.

Of note, some mouse strains are resistant to EAE (A/J, C3H/HeJ, AKR, NZW, and DBA/2). In addition to the importance of environmental factors, it is thought that multiple predisposing genetic elements might be involved in susceptibility to MS (reviewed by Ebers 1994) [10]. Therefore, the backcrossing of EAE-resistant mice with EAE-susceptible strains has been useful for genetic susceptibility studies.

3.2.2 Passive Experimental Autoimmune Encephalomyelitis

The second model of EAE is passive and involves the in vitro restimulation of encephalitogenic T cells with the myelin peptide used to immunize the original T-cell donor animals [11]. The successful culture of these cells usually requires the addition of Th1 cytokines such as IL-12 [12]. Once cells have been sufficiently activated, they are intravenously administered to naïve animals, where they traffic to the CNS and induce disease. The CNS pathology and disease course are similar to that for active EAE. The ability of MBP-specific T cells to induce EAE in rats and mice was some of the earliest evidence to suggest that MS has an autoimmune aetiology [3, 4]. Another evidence for an autoimmune inflammatory pathogenesis includes the presence of MHC class II-restricted CD4+ T cells that recognize myelin antigens such as myelin basic protein (MBP), PLP, and MOG in MS patients as well as healthy individuals and that MHC class II genes are associated with MS susceptibility [13–15]. Because T cells are already activated when administered into naïve animals, passive EAE is a suitable model for investigating the effector phase of disease. It has some advantages over passive EAE as an immunization step is not required; thus, there is no antigen reserve that continually activates naïve T cells, which likely boosts the immune system nonspecifically. Furthermore, the direct injection of effector T cells into naïve mice allows the definitive starting point of disease induction to be known, which might be useful for treatment studies, and finally encephalitogenic T cells can be directly tracked in vivo to study methods of extravasation into the CNS and for isolation of antigen-specific T cells.

Early studies demonstrated that activated myelin-specific Th1 CD4+ T cells secreting IFN-γ, TNF-α, and IL-2 were encephalitogenic and sufficient to induce EAE when transferred into naïve mice of a susceptible genetic background [16–18]. However, the knockout of genes encoding IFN-γ or TNF-α [19, 20] exacerbated EAE and therefore the role of Th1-induced EAE was not straightforward. IL-17 production by CNS-infiltrating T cells is important for blood-brain barrier dysfunction and lesion formation in MS patients [21, 22], and the genetic inhibition of IL-17A in mice was sufficient to partly ameliorate EAE in mice [23]. This indicated

that Th17 cells, an alternative effector T-cell subset to Th1, might be the critical effector cells in EAE. Indeed, Th17 cells cultured in the presence of IL-23 and other cytokines become highly pathogenic and can induce EAE when transferred into naïve mice [24]. Th17 cells show an enhanced efficiency at inducing EAE compared with Th1 cells [24] and therefore might require less cell manipulation in vitro for adoptive transfer studies.

3.2.3 Other Experimental Autoimmune Encephalomyelitis Models

3.2.3.1 CD8 Experimental Autoimmune Encephalomyelitis Models

Although there is a predominance of EAE papers investigating the role of myelin-specific CD4+ T cells in EAE, MS lesions have been reported to contain greater numbers of CD8+ T cells compared with CD4+ T cells [6, 25, 26]. However, the function of CD8 T cells in MS lesions is unclear, and therefore, the study of CD8+ T cells in EAE is important. A number of EAE models induced by MHC class I-specific CD8+ T cells have been reported. MBP79-87-specific CD8+ T-cell clones isolated from MBP-immunized C3H wild-type mice induced EAE with numerous neurological deficits (ataxia, spastic reflexes, spinning) as well as hind-limb paralysis when intravenously injected into C3H wild-type mice [27]. This form of EAE was very severe and all mice were moribund by day 14. Interestingly this model showed different clinical symptoms to the CD4+ T cell-mediated type of EAE, but importantly had some similarities to MS, in that perivascular lesions were predominant in the brain, compared with CD4+ EAE where perivascular lesions are located in the spinal cord.

Another study reported that MOG35-55-specific CD8+ T cell lines could also induce a severe, chronic form of EAE when adoptively transferred to naïve C57BL/6 mice [28]. In contrast to the MBP-induced CD8 T-cell model of EAE, lesions were present in both the spinal cord and brain. Differences in genetic background, availability of myelin antigens in the CNS, or induction procedure might explain the differences observed between the two models. In contrast to these early CD8 studies, more recent investigations have indicated inhibitory roles for CD8 T cells in EAE: neuroantigen-specific autoregulatory CD8+ T cells inhibited autoimmune demyelination by modulating dendritic cell functions [29] and IL-15-dependent CD8+ CD122+ T cells ameliorated EAE by reducing IL-17 production by CD4+ T cells [30].

Humanized mouse models have also been developed to study the effect of MS-related myelin epitopes presented to CD8 T cells by human HLA molecules and showed that MOG181-189 presented by HLA-A*201 to MOG-specific CD8 T cells exacerbated CD4 T cell-induced EAE [31].

Overall, these models of CD8 T cell-induced EAE are important for determining the function of CD8 T cells during CNS autoimmune disease, i.e. pathological

versus inhibitory/regulatory effects, and have an advantage over CD4 T cell-induced EAE in that the target organ is predominantly the brain, similar to that in MS.

3.2.4 Transgenic Models of Autoimmune Encephalomyelitis Models

A number of spontaneous mouse models of EAE have been developed by the transgenic expression of T-cell receptors (TCR) specific for myelin antigens (PLP, MOG, or MOG) in T cells that overcome the negative selection of autoreactive T cells in the thymus (reviewed by Croxford 2011) [32]. Therefore, these models represent a more "natural" type of disease and are useful for examining the role of autoimmune T-cell activation by environmental stimuli. However, depending on the type of study involved, for example, drug efficacy testing, the use of spontaneous EAE models is not recommended because of the wide variance in disease onset and severity.

3.2.4.1 Advantages of Autoimmune Encephalomyelitis Models

The advantages of EAE are its robust and reproducible disease course that is useful for studying the early stages of disease initiation; the activation of immune cells, especially antigen-specific T cells; as well as mechanisms in the effector phase such as the role of resident CNS cells, regulatory T cells, and other regulatory mechanisms. In addition, pathological studies at all stages of disease can provide important information regarding sites and mechanisms of demyelination, remyelination, nerve conduction block, and axonal loss. Furthermore, depending upon the models used, the different mechanisms involved in relapsing vs chronic forms of disease can be studied. The reader is encouraged to read review articles describing the specific induction protocols for the numerous EAE models that are available [11, 12].

3.2.5 Disadvantages of Autoimmune Encephalomyelitis Models

The most significant disadvantage of EAE is the location of pathology in spinal cord, unlike MS, where many lesions occur in the brain. Furthermore, although EAE is clearly an autoimmune disease, MS does not have all the hallmarks of an autoimmune disease, and evidence for a specific immunodominant myelin antigen is lacking. Although CD4+ T cells are critical for disease induction in most EAE

models, their role in MS is less clear, where many other types of immune cells such as CD8+ T cells, B cells, and monocytes probably also have important roles. For example, B cells have little or no role in many EAE models, at least at the early time points usually studied; however, their importance in MS is suggested by the beneficial effects of anti-CD20 therapy in some MS patients [33].

EAE is a very useful tool to test potential new immunomodulatory therapies. Although four approved MS treatments were studied in EAE (natalizumab, mitoxantrone, glatiramer acetate, and fingolimod), the large majority of new treatments fail when they enter MS clinical trials. An explanation for this might be that treatment studies using the EAE model do not mimic the patient disease course in the clinic. For example, the administration of drugs before the initiation of EAE disease is only useful for indicating an effect on the activation of T cells and has no real clinical significance for MS treatment, where patients have often experienced symptoms for some time before treatments are started. In addition, treatments administered before the onset of symptoms that prevent paralysis are often said to prevent demyelination. However, if these treatments are immunomodulatory, then it is difficult to differentiate between their anti-demyelination and immunosuppressive effects. Therefore, potential treatments should be administered after the onset of EAE symptoms for clinically meaningful results. In summary, although EAE is an imperfect model of MS, its correct usage by investigators is critical when studying novel therapeutic compounds.

3.2.6 Virus Models of Multiple Sclerosis

A number of etiological studies have indicated the potential role of viruses in the susceptibility, onset, and exacerbation of MS. Therefore, in addition to immunization models that are useful to investigate the early immunological pathways involved in pathogenesis, a number of virus-induced demyelinating models have been developed that allow the study of the potential viral aetiology of MS. Using these models a number of hypotheses of the mechanism of disease onset have been developed including molecular mimicry, epitope spreading, direct bystander activation, and release of cryptic epitopes.

3.2.7 Theiler's Murine Encephalomyelitis

Theiler's murine encephalomyelitis (TMEV) is a single-strand RNA virus that belongs to the cardiovirus group of the Picornaviridae and is a natural mouse pathogen. TMEV-induced demyelinating disease (TMEV-IDD) develops in susceptible strains of mice (SJL/J) upon intracranial injection of the BeAn 8386 strain of TMEV, which persistently infect microglial cell populations in the CNS [34, 35]. The persistent infection of TMEV is thought to cause demyelination

mediated by macrophage bystander destruction activated by TMEV-specific CD4+ T-cell responses that target the persistent viral infection, leading to the release of myelin antigens that can activate autoreactive PLP156-171-specific T cells that have escaped negative selection [36]. Epitope spreading to other myelin epitopes propagates the disease, which shows similar inflammatory and demyelinating pathological hallmarks as those seen in MS patients [37, 38]. The onset of a chronic progressive demyelinating disease with no recovery or remission periods occurs around day 30–35 post-infection and continues for over 100 days. TMEV-IDD is characterized by the development of a spastic hind-limb paralysis and perivascular and parenchymal lesions in the spinal cord with demyelination of white matter tracts containing mononuclear cell infiltrates.

Although epitope spreading is thought to be involved in TMEV-IDD, molecular mimicry, the mistaken recognition of a pathogenic epitope for a "self" epitope due to shared amino acid sequences, is another potential mechanism for the induction of autoimmunity. Early studies demonstrated that the immunization of viral peptides could stimulate myelin peptide-specific T-cell responses in vivo [39, 40]. However, the need for epitope processing and the role of a "live" viral infection to stimulate the host innate immune system are not addressed by short-length peptide immunization. Therefore, recombinant TMEV strains engineered to incorporate 30-mer myelin or bacterial/viral myelin mimic epitopes were used to study the potential viral induction of autoimmunity by molecular mimicry [41, 42]. Infection of SJL mice with TMEV engineered to express a PLP mimic peptide derived from *Haemophilus influenzae*, a natural mouse pathogen, with 6/13 homologous amino acids to PLP139-151, including primary TCR and MHC class II contact residues, induced a mild but rapid onset CNS disease [42]. Interestingly, the immunization of mice with the *Haemophilus influenzae* peptide in complete Freund's adjuvant failed to induce overt disease, indicating the importance of pathogen-delivered innate immune signals for the induction of disease by molecular mimicry. Of note, viral peptide sequences in TMEV do not share any sequence homology with PLP, the immunodominant myelin peptide in TMEV-IDD in SJL mice, therefore indicating the role of the engineered *Haemophilus influenzae* peptide sequence in disease induction. These studies were expanded to include TMEV expressing 30-mer from murine hepatitis virus (MHV), which only shares 3/13 amino acids with PLP139-151, and demonstrated the importance of a proline residue at the secondary MHC class II contact point [43].

3.2.7.1 Semliki Forest Virus

Semliki Forest virus (SFV) strain A774 is a neurotropic, single-stranded RNA alphavirus of the *Togaviridae* family that induces a demyelinating disease upon intraperitoneal injection in SJL/J mice. Disease is characterized by virus-induced demyelination of the CNS. Despite the clearance of the virus by the immune system, maximal demyelinating lesions with the expression of IFN-γ and TNF-α are observed at 14 days post-infection up to 1 month in BALB/c mice, whereas both

demyelination and pro-inflammatory cytokine expression can be detected in SJL/J mice up to 1 year [44]. SFV infection induces an MBP-specific T-cell response [45] and demyelination is mediated by CD8 T cells [46, 47].

3.2.7.2 Mouse Hepatitis Virus

MHV, a group II positive-strand RNA coronavirus, is a natural pathogen of mice that upon intracranial injection causes an acute encephalomyelitis that develops into a chronic CNS immune-mediated demyelinating disease with some clinical and pathological similarities with MS [48]. MHV can induce either acute or chronic forms of disease. The acute form is characterized by the production of pro-inflammatory cytokines and chemokines that eventually reduce MHV viral load in the CNS. However, persistent infection of spinal cord white matter tracts propagates and promotes antiviral responses that cause demyelination leading to symptoms of limp tails and partial to complete hind-limb paralysis, similar to that in EAE. In contrast to EAE and other viral models of CNS demyelinating disease, myelin-specific T cells and epitope spreading are not thought to be required for demyelination; rather it is the persistent effect of antiviral immune responses including macrophages and CD4+ and CD8+ T cells that cause chronic demyelination (reviewed by Lane 2010) [49].

3.2.7.3 Uses of Viral Models of Multiple Sclerosis

In summary, TMEV-IDD is a demyelinating model that is associated with persistent infection of the CNS, whereas SFV infection of SJL mice might represent a model of MS where immune-mediated demyelination is triggered by a virus infection of the CNS that is cleared efficiently by the host immune system. Although some studies have indicated MS might have a viral aetiology, the mechanisms involved are unknown but might involve an infection in early life that primes autoreactive T cells by molecular mimicry or a persistent infection of the CNS that causes demyelination via epitope spreading and/or molecular mimicry [50]. Thus, both mouse models allow the study of how viruses might induce CNS demeylinating autoimmune disease. In contrast, MHV provides a different scope for study, the underlying mechanisms that mediate host defence against an acute viral infection that later becomes chronic and which is associated with CNS demyelination and neurological symptoms in the absence of myelin-specific T-cell responses.

3.3 Animal Models of Guillain-Barré Syndrome

Guillain-Barré syndrome (GBS) is a rapid autoimmune-mediated acute disease of the peripheral nervous system, which is often caused by infection with *Campylobacter jejuni*. Clinical disease characteristics usually occur rapidly following onset, usually within 4 weeks, and include neuromuscular paralysis, progressive limb weakness, and deficits of the sensory and autonomic systems as well as cranial nerve involvement. The generation of antibodies to cell-surface gangliosides highly expressed on peripheral nerves by cross-reactivity to *Campylobacter jejuni* outer membrane components (lipo-oligosaccharides) mediates disease [51] and is thought to be caused by postinfectious molecular mimicry (reviewed by Shahrizaila 2011) [52].

Two forms of GBS have been characterized: (i) a primary demyelinating form (acute inflammatory demyelinating polyradiculoneuropathy [AIDP]) and (ii) those with axonal involvement (acute motor axonal neuropathy [AMAN], acute motor-sensory axonal neuropathy [AMSAN]), with either the presence or absence of anti-ganglioside antibodies [53, 54]. The pathogenic mechanisms of the AIDP form of GBS include the demyelination of the peripheral nerve myelin sheath by inflammation including upregulated adhesion molecules, pro-inflammatory cytokines, and the infiltration of CD4+ T cells and plasma cells secreting anti-ganglioside antibodies and macrophages [53–56], whereas the axonal forms of GBS (AMAN, AMSAN) usually do not include lymphocyte or monocyte involvement, rather Wallerian degeneration as well as complement and anti-ganglioside antibodies that directly mediate myelin destruction [57].

Disease is often acute and the recovery of motor function is common in approximately 60 % of GBS patients. However, in other cases, residual sensory deficits remain, and in extreme cases, severe alterations to the autonomic nervous system can cause death by respiratory failure, embolism, or cardiac arrest. Current treatment of GBS is generally nonspecific and typically includes the administration of intravenous immunoglobulins or plasmapheresis, which reduce the duration to recovery. However, neither of these treatments is curative, and therefore, the use of animal models of GBS to identify pathogenic mechanisms and to test novel treatments is important.

3.3.1 Experimental Autoimmune Neuritis

Experimental autoimmune neuritis (EAN) is a commonly used, robust, and highly predictable animal model of GBS. It was originally developed in rabbits [58], but has since been induced in a wide variety of animals including rats, mice, rabbits, guinea pigs, and monkeys. Disease is induced by the active immunization of susceptible animals with purified whole peripheral nerve myelin or specific myelin protein components (myelin protein 2 [P2], myelin protein zero [p0]) in complete

Freund's adjuvant [58–60]. EAN in rats and mice is a monophasic acute demyelinating inflammatory disease of the peripheral nervous system [58] with many similarities to GBS including clinical, immunological, and morphological characteristics. In rats, onset of disease is observed at 12–13 post-immunization with a peak of disease severity at day 16. Clinical disease is characterized by tail and limb weakness, and histopathological analyses have shown the presence of nerve oedema and demyelinated peripheral nerves accompanied by the infiltration of inflammatory cells, features which are present in GBS. Symptoms are likely to be caused by a combination of nerve conduction block and the effects of demyelination. During the effector phase of EAN disease, typical pro-inflammatory components including chemokines (MIP-1α and MIP-1β, MCP-1, RANTES, and IP-10) (reviewed by Fujioka 1999) [61, 62], cytokines (reviewed by Zhu 1998) [63], and adhesion molecules (VCAM-1) [64] have been shown to be upregulated. Th1-type cytokines might mediate the demyelination of peripheral nerves as evidence shows that the addition of IFN-γ can enhance EAN, whereas blockade of IFN-γ with neutralizing antibodies can ameliorate EAN symptoms and disease course [65].

EAN mouse models with severe clinical symptoms and pathological features similar to rat EAN have also been reported. An early study reported the induction of EAN SJL/J mice, which showed subclinical damage to peripheral nerve myelin but without clinical symptoms, in contrast to EAN Lewis rats, that developed typical hind-limb weakness and histopathology of the peripheral nervous system [66]. A subsequent study demonstrated that the addition of pertussis toxin to SJL/J mice immunized with bovine peripheral nerve myelin in complete Freund's adjuvant enhanced the mild disease seen in the absence of pertussis toxin [67]. When immunized mice with pertussis toxin were treated with recombinant mouse IL-12, the disease course duration was prolonged and recovery was delayed. Histological analysis demonstrated severe demyelination of the caudae equinae and sciatic nerves during the recovery stage as well as mononuclear cell infiltration. Of note, although C57BL/6 mice were initially thought to be resistant to EAN induction, immunization of male C57BL/6 mice with a synthetic $P0_{180–199}$ peptide induced the clinical and pathological characteristics of acute monophasic EAN [68]. As previously shown, the addition of intravenously administrated pertussis toxin increased the incidence of EAN and enhanced inflammation and demyelination of peripheral nerves.

3.3.2 Adoptive Transfer Models of Experimental Autoimmune Neuritis

Similar to that seen for EAE, in addition to immunization models of EAN, adoptive transfer models of EAN have been reported. P2- and P0-specific CD4+ T cell lines have been shown to transfer histopathologically similar EAN to naïve syngeneic Lewis rat recipients when injected intravenously [69–71]. However, the onset of

disease was earlier (day 7) in a P2-adoptive transfer EAN model compared with active immunization EAN models (day 12–13).

3.3.3 *Correlation of Experimental Autoimmune Neuritis with Guillain-Barré Syndrome*

Although EAN is a robust, reproducible model of neuritis, sharing many pathological and clinical features with GBS, one critical disadvantage is that *Campylobacter jejuni* infection, which is thought to be involved in a high percentage of human cases of GBS, does not induce disease in rats. Furthermore, immunization of rats with various gangliosides, the main immunological target of the immune system in *Campylobacter jejuni*-induced GBS, also does not induce disease [72]. The addition of gangliosides to immunization protocols failed to have an enhancing effect of EAN severity or disease onset. Although other species of animals have shown some promise in terms of developing conduction block (rabbits immunized with GM1 demonstrated sciatic nerve conduction block [73], 33/100 chickens administered *Campylobacter jejuni* isolated from a Chinese patient showed sciatic nerve Wallerian degeneration with minor demyelination) [74], these models have not been studied extensively. Therefore, the existing EAN model is useful for studies related to the effector phase immune-mediated mechanisms of peripheral nerve demyelination; it is less useful for studies to determine the pathogenic mechanisms involved following *Campylobacter jejuni* infection.

3.4 Animal Models of Myasthenia Gravis

Myasthenia gravis (MG) is a rare neuromuscular disease that causes excessive fatigue and generalized muscle weakness that can fluctuate and is characterized by clinical symptoms including ptosis and diplopia. Muscle weakness often worsens upon use but usually improves with rest. As the disease course of MG progresses, bulbar and respiratory muscle weakness worsens, which can become life-threatening, often requiring the use of mechanical ventilation by intubation.

MG is thought to be a T cell-dependent antibody-mediated autoimmune disease because approximately 80 % of MG patients have autoantibodies against acetylcholine receptors (AChR) [75, 76], possibly as a consequence of the loss of “self”-tolerance in the thymus [77]. AChR antibodies bind to AChR expressed at neuromuscular junctions (NMJ) and block neuromuscular transmission. Interestingly, the first experimental evidence to suggest AChR antibodies might be pathogenic was demonstrated using an experimental rabbit model where immunization with purified acetylcholine receptor in complete Freund’s adjuvant induced the

production of antibodies to acetylcholine receptor, which mediated neuromuscular blockade that caused flaccid paralysis and MG-like symptoms [78].

Despite the early identification of AChR antibodies as potential mediators of disease, the mechanisms involved that precede the production of AChR antibodies are less clear, although involvement of the thymus has been indicated. The symptomatic treatment of MG using acetylcholinesterase inhibitors and/or immunosuppressive drugs can improve muscle function although these treatments are limited by either a reduction in efficacy over time or severe side effects. Furthermore, plasma exchange and surgery in patients that develop thymoma are invasive procedures. Currently, no treatments directly target the autoimmune component of disease, and therefore, there is a still a need for the further elucidation of MG pathogenesis and the development of novel drugs, which highlights the importance of using animal models of MG.

3.4.1 Active Experimental Autoimmune Myasthenia Gravis

Experimental autoimmune myasthenia gravis (EAMG) was originally induced in rabbits by immunization of highly purified AChR isolated from the electric organ of *Electrophorus electricus* emulsified in complete Freund's adjuvant [78]. This induced the production of antibodies that specifically recognize AChR and bind to these receptors at NMJs, subsequently blocking neurotransmission, causing muscle fatigue and weakness, which mimic the symptoms observed in human MG. Since the first description of EAMG, it has been induced in a wide variety of animals including rabbits [78, 79] rats [80], mice [81, 82], guinea pigs [80], goats [83], monkeys [84], and frogs [85].

Currently, rat and mouse models are the animal models of choice, as gene knockout and transgenic technology has allowed a greater in-depth investigation into the specific molecules involved in disease pathogenesis. Susceptible rat strains include Lewis, Fischer, and Wistar-Munich rats, and nonresponder strains include Wistar Furth and Copenhagen rats. It is important to note that some mouse strains have different susceptibilities to EAMG; H-$2^{b,\ s}$ haplotype strains (C57BL/6 and SJL/J) are high responders, whereas H-$2^{k,\ p}$ haplotypes are nonresponders; therefore, it is important to determine the strain used before initiating studies [81, 86]. Interestingly, rats appear to be more susceptible to EAMG than mice, as usually only one immunization with AChR in complete Freund's adjuvant is required to induce autoantibodies to AChR. In contrast, susceptible mouse strains usually require two or three immunizations with AChR in complete Freund's adjuvant. Furthermore, disease severity in mouse EAMG is reduced compared with the rat model, and therefore, this is an important consideration if the efficacy of novel therapeutic agents is to be tested. Of note, susceptibility to both MG and EAMG is linked to the HLA/MHC region [87]. Thus, EAMG has many clinical and pathological similarities to human MG, especially the presence of autoantibodies that recognize and bind to AChR.

Following immunization, T cells mediate the production of AChR-specific antibodies in murine EAMG as well as human MG [88–90], and treatment with anti-CD4 or anti-Ia antibodies can block the induction or induce remission of EAMG [89, 91]. Further evidence for a role of T cells in EAMG pathogenesis was shown by the use of lymphocyte immunosuppressive agents and oral tolerance to AChR that inhibited disease onset [92–94]. Similar to that observed in EAE, the role of pro-inflammatory cytokines including IL-1, IL-12, IFN-γ, and TNF-α is also important in the onset of EAMG, with functions related to T-cell development, proliferation, and differentiation. Studies in EAMG demonstrated that treatment of EAMG rats or mice with anti-TNF-α treatments reduced EAMG development [95] and significantly improved established disease [96]. Confirmation of a role for TNF in MG was demonstrated by a trial investigating the use of a TNF inhibitor (etanercept) in MG patients that reduced muscle weakness [97].

Once the autoreactive T cells become activated, they stimulate B cells to produce and secrete anti-AChR antibodies that induce the observed clinical symptoms. EAMG studies have indicated that following the binding of AChR antibodies to NMJs, complement activation including C3 and C9 deposition and membrane attack complex might mediate the destruction of NMJ plasma membranes [98–100]. Confirmation of a role for complement in EAMG was shown in studies reporting that the blockade of the complement system by complement inhibitors protected rats against the induction of EAMG [101–103]. Importantly, the role of complement was indicated in human MG [99, 100, 104], indicating the validity of EAMG.

Clinical signs of active EAMG (tremor, hunched posture, muscle weakness, and fatigue) usually occur 3–10 days after the second immunization, and mice are observed for signs of muscle weakness by the paw-grip test at least once a week. In addition, a number of other tests have been developed to assess the extent of disease including the quantitative measurement of muscle weakness by electromyography, the evaluation of EAMG induction by the quantification of muscle AChR loss, and serum anti-AChR antibody levels by radioimmunoassay or ELISA.

Therefore, active EAMG is a useful model for myasthenia gravis and the extent of disease can be measured using fairly noninvasive techniques.

3.4.2 Passive Experimental Autoimmune Myasthenia Gravis

Another method for the induction of EAMG is the passive transfer model, where autoantibodies specific for AChR from donor AChR-immunized animals are injected daily into naïve animals, and this was first shown in a rat model [83, 105]. Another method for the passive induction of EAMG is the injection of AChR-specific antibodies isolated from the serum of MG patients [106].

Interestingly, the transfer of EAMG by autoreactive lymphocytes is less robust than that by autoantibodies [107] and indicates that autoreactive antibodies are probably the major mechanism involved in the onset of NMJ destruction.

In summary, active EAMG is useful for investigating the induction phase of disease (T- and B-cell activation and autoantibody production) including loss of self-tolerance, mechanisms of antigen-specific immune response induction, and their modulation by therapeutic agents (to induce tolerance or immunosuppression), whereas passive EAMG is useful for the study of the effector phase of disease including IgG deposition in NMJs, complement molecules, and investigating treatments using regulatory proteins to prevent the degradation of NMJs. Clinical disease in the passive model of EAMG is similar to that observed in active EAMG and therefore serves as a useful model of MG when full activation of the immune system is not required, as is seen following active immunization protocols.

3.4.3 *Experimental Autoimmune Myasthenia Gravis Induced by Musk Antibodies*

Although most MG patients develop autoreactive antibodies to AChR, approximately 20 % of MG patients are AChR antibody negative; however, 30–40 % of these MG patients have antibodies that recognize muscle-specific kinase (MuSK) [108]. MuSK is a tyrosine kinase receptor that is involved in the development of postsynaptic membranes in the NMJ. However, whether MuSK antibodies are involved in the pathogenicity of MG is unclear and it is unknown whether they contribute to muscle weakness. Therefore, the use of animal models is useful to help dissect the potential pathogenic function of MuSK antibodies. A recent study demonstrated that immunization of rabbits or mice with a MuSK ectodomain induced muscle weakness as measured by electromyographic analysis and flaccid paralysis, which was similar to that in human MG [109, 110]. However, the appearance of disease-related symptoms is longer than that for AChR-active and AChR-passive models of EAMG, and the passive transfer of MuSK antibodies is less effective than the equivalent AChR-passive model. Therefore, MuSK antibody-related MG might represent a subtype of MG or reflect differences in environmental or genetic susceptibility factors.

3.4.4 *Relationship of Experimental Autoimmune Myasthenia Gravis with Human Myasthenia Gravis*

EAMG is a very useful animal model for the study of the pathways involved in disease pathogenesis as well as investigating novel therapeutic strategies. Importantly, EAMG shares similar symptoms with MG, especially muscle weakness and fatigue. Furthermore, they share such immunopathological features as AChR-specific antibodies in the serum, muscle AChR loss, presence of complement factors such as C3 and C9 at NMJs, and a supportive role for T and B cells as

regards autoantibody production. One of the major differences between EAMG and MG is the involvement of the thymus (loss of self-tolerance) in human MG, which is absent in EAMG, where tolerance must be "broken" by immunization of the autoantigen in complete Freund's adjuvant. Although many therapeutic strategies have been demonstrated to be beneficial in EAMG, few have translated to the clinic for human MG. This might be due to the human form of disease having a more complex aetiology than EAMG. However, EAMG still has an important role to play in preclinical studies, whether to investigate pathogenic mechanisms or potential therapeutic strategies.

3.5 Conclusions

Despite the numerous beneficial advances of researchers in each of these human neuroimmune diseases, the underlying mechanisms of many of these disease processes are still unclear and this has hindered the search for curative therapies. Although the use of human tissues and samples is critical for these types of studies, often they are invasive and provide an indication of a single time point within a disease process that might have been ongoing for years or decades. Therefore, it is vital to use animal models to fill in the "gaps" that cannot be analysed by research using human tissues. However, whilst animal models of disease can provide useful information, it is important to note that no single model is a perfect model of its human counterpart and each has their advantages and disadvantages. Therefore, it is important to use the correct model for the study involved, i.e. immune cell activation, mechanisms of demyelination, immune cell trafficking into the target organ, induction vs effector phases of disease or routine drug testing. In addition, it is important to remember that each mouse strain used is genetically representative of a single human individual; therefore, multiple strains should ideally be used when developing novel treatments or investigating pathogenic mechanisms, to reduce the possibility of strain irregularities confounding the results.

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Chapter 4
Blood–Brain Barrier and Blood–Nerve Barrier

Yasuteru Sano and Takashi Kanda

Abstract Homeostasis of the central and peripheral nervous systems is a prerequisite for the proper communication of neuronal cells. To this end, blood–neural barriers, including blood–brain barrier, blood–cerebrospinal fluid (CSF) barrier, blood–spinal cord barrier, and blood–nerve barrier, tightly seal off the central and peripheral nervous systems from the continuously changing milieu within the bloodstream. These neural barriers inhibit the free paracellular diffusion of water-soluble molecules through complex tight junctions that interconnect endothelial cells and choroid plexus epithelial cells. These barriers also play a role in the influx transport of essential molecules as well as efflux transport of xenobiotics. The differences and similarities between these barrier systems have gradually been elucidated in recent years, and it is now clear that disruption of the blood–neural barrier plays a key role in a number of diseases affecting the central and peripheral nervous systems. Understanding the mechanisms that regulate these barriers under conditions of both health and disease provides important insights for treating a wide array of neurological disorders.

Keywords Blood–brain barrier • Blood–nerve barrier • Tight junction • Transporter

4.1 Introduction

The blood–neural barriers are multicellular vascular structures that separate the central and peripheral nervous systems from the circulating blood. Beyond the barrier function, the influx transport of essential molecules and efflux transport of xenobiotics are actively regulated at the blood–neural interface. This precise control of homeostasis of the nervous system allows for a proper neuronal function, and alterations of these barrier properties are an important component of the pathology and progression of various neurological diseases. Such blood–neural

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barriers include the blood–brain barrier (BBB), blood–cerebrospinal fluid (CSF) barrier (BCSFB), blood–spinal cord barrier (BSCB), and blood–nerve barrier (BNB) [1–3]. While the BBB, BSCB, and BNB are substantially localized at the level of the endothelial cells comprising microvessels within the central and peripheral nervous systems, the BCSFB is composed of choroid plexus epithelial cells. In this chapter, we describe current knowledge regarding the cellular and molecular basis of functional and dysfunctional blood–neural barriers.

4.2 Blood–Brain Barrier

The accurately regulated BBB provides stringent control of the extracellular environment in neural tissues, a critical factor for a proper neuronal function, which requires precise ionic concentrations in the surrounding areas. Furthermore, the BBB protects the CNS from injury and disease by limiting the entry of toxins, pathogens, and the body's own immune cells into the brain, which has a limited regenerative capacity. The BBB consists of a complex system composed of highly specialized brain microvascular endothelial cells (BMECs) and their basement membrane, in which a large number of pericytes are embedded, in addition to ensheathed astrocytic end feet and their associated parenchymal basement membrane (Fig. 4.1a). Features distinguishing BMECs from cells of peripheral organs include their lack of fenestration, formation of complex intercellular tight junctions (TJs) preventing passive diffusion between cells, minimal endocytosis, expression of specific transporters, and increased activity of enzymes that metabolize xenobiotics [4–7]. The interendothelial TJs in microvessels in the central nervous system (CNS) form an intricate complex of transmembrane and cytoplasmic proteins linked to the actin cytoskeleton.

Many of the molecular constituents of TJs have been identified in recent years, including occludin [8], claudin-3 [9–11], claudin-5 [12], claudin-12 [12, 13], zonula occludens (ZO)-1 and ZO-2 [14], and junctional adhesion molecule (JAM)-A [15]. Of TJ-associated proteins, claudin-5 is an essential protein for maintaining the barrier properties of the BBB under physiological conditions [12, 13], and its expression level is often deteriorated under various pathological conditions [16, 17]. Claudin-3 is also an important molecule constituting the BBB [9–11]. -Claudin-3 has been shown to be upregulated by Wnt-signaling during BBB maturation [11] and downregulated at TJs in a model of experimental allergic encephalomyelitis [9].

The specific expression of different transporters in various CNS endothelial membranes, combined with high-resistance TJs, determines the movement of molecules and ions between the blood and brain. A series of molecular transporters has been identified whose expression is enriched in CNS endothelial cells compared to non-neural tissues, both to remove potential toxins and deliver specific nutrients. Among the most significant efflux transporters located at the BBB are P-glycoprotein (P-gp) [18, 19], multidrug resistance-associated proteins (MRPs)

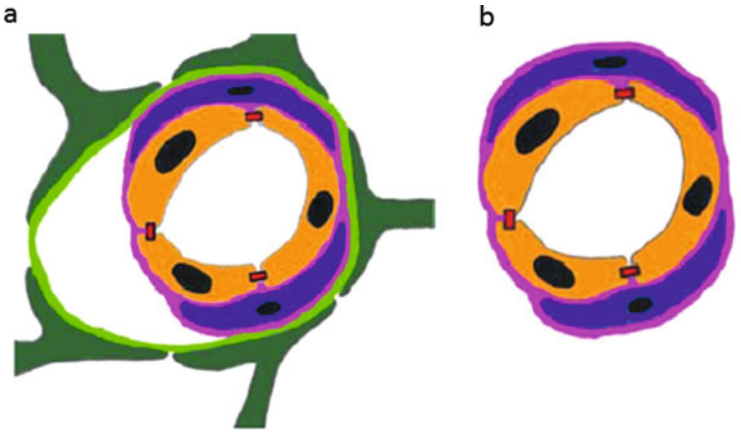

Fig. 4.1 Composition of the blood–brain barrier and blood–nerve barrier. (**a**) The blood–brain barrier is primarily formed by brain microvascular endothelial cells (*orange*), which are surrounded by the first basement membrane (*pink*), pericytes (*blue*), the second basement membrane (*light green*), and astrocytes (*dark green*). The highly specialized endothelial cells (*orange*) exhibit barrier properties due to their low pinocytotic activity and by sealing the paracellular space with complex tight junctions (*red rectangles*). These cells deposit an endothelial basement membrane (*pink*), in which many embedded pericytes (*blue*) can be found. The endothelial basement membrane (*pink*) is molecularly distinct from the parenchymal basement membrane (*light green*), which is deposited by astrocytes (*dark green*) and establishes the glia limitans perivascularis together with astrocytic end feet (*dark green*). (**b**) Endothelial cells (*orange*) of the blood–nerve barrier (BNB) are embedded in a single basement membrane (*pink*) with surrounding pericytes (*blue*). The endothelial cells forming the BNB are also highly specialized and have complex tight junctions (*red rectangles*) (Adopted from [3])

[20], and ATP-binding cassette subfamily G member 2 (ABCG2) [21], all of which are thought to prevent xenobiotics from entering the CNS. BMECs also support the blood-to-brain influx of various nutrients using influx transporters, such as glucose transporter 1 (GLUT1) [22], system L [23], monocarboxylic acid transporter 1 (MCT1) [24], and creatine transporter (CRT) [25].

Cultured BMECs constitute an important in vitro BBB model that can be used to analyze the physiological and biological functions of the BBB as well as estimate the permeability of the BBB to certain compounds. However, primary cultured human BMECs (HBMECs) generally undergo rapid senescence, even upon limited passages. Therefore, most previously established human in vitro BBB models were immortalized using tumor genes such as simian virus 40 (SV40) large T antigen. However, immortalized cells often act like tumor cells and lose the morphological and physiological properties of the parental cells. In order to overcome these disadvantages, we first established excellent human in vitro BBB models using temperature-sensitive SV40 large T-antigen genes [26–28]. One of these models, TY10 cells, is shown in Fig. 4.2a. All of the models demonstrate a spindle-shaped morphology, express TJ proteins and various transporters, and have excellent

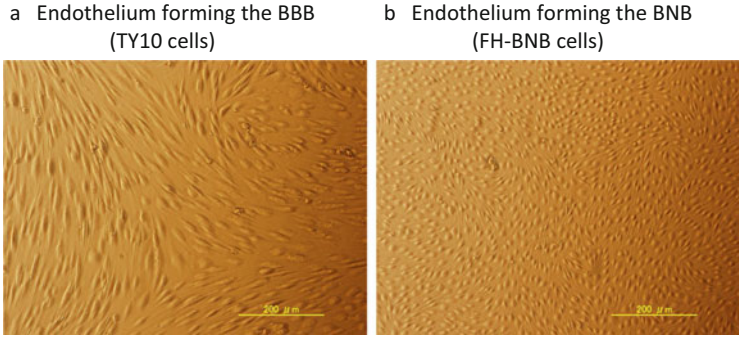

Fig. 4.2 Morphology of microvascular endothelial cells derived from the BBB and BNB. Phase contrast micrographs of the TY10 cells derived from the BBB (**a**) and FH-BNBs derived from the BNB (**b**). Both cell types exhibit a spindle-shaped morphology with contact inhibition. The *scale bars* correspond to 200 μm

barrier properties [26–28]. They grow and proliferate well under a permissive temperature of 33 °C and stop growing and subsequently display superior barrier properties compared with that observed at 33 °C under a non-permissive temperature of 37 °C. These conditionally immortalized HBMECs have been widely used for basic research of the BBB and investigations of neurological diseases [17, 29–36]. Importantly, we previously reported that the sera from patients with multiple sclerosis and neuromyelitis optica induce breakdown of the barrier properties in conditionally immortalized human in vitro BBB models via the downregulation of claudin-5 [17, 30, 32]. Another reliable human in vitro BBB model, termed hCMEC/D3, which has been reported to sustain BBB-specific properties, is widely used in analyses concerning physiological and pathological conditions of the BBB [10, 37, 38]. These excellent human in vitro BBB models, including TY10 and hCMEC/D3 cells, are expected to be valuable tools for the future development of new therapeutic methods against many CNS disorders.

Astrocytes provide nutrition for neurons, regulate the extracellular potassium balance, carry out neurotransmitter clearance, and control immune reactions [39]. Astrocytes also control water flux at BBB sites by expressing a specific water channel, namely, aquaporin-4 (AQP4), involved in the molecular composition of the orthogonal arrays of particles (OAPs) located on perivascular glial end feet and tightly coupled with the maintenance of the integrity of the BBB [40]. Moreover, astrocytes have been shown to regulate the junctional and transport properties of the BBB in the brain endothelium [11, 41–44]. In particular, astrocyte-derived sonic hedgehog has been reported to control BBB formation during development and TJ integrity [43]. In addition, the Wnt/β-catenin pathway was recently discovered as a major BBB-regulation pathway [11, 44].

Pericytes are also important cellular constituents of brain capillary and postcapillary venules. The distribution of pericytes differs from tissue to tissue and may exhibit vessel variation. The rat pericyte/endothelial ratio has been reported to be 1:3 in the retina, 1:5 in the brain, and 1:10 in the lungs [45], thus

suggesting that the largest pericyte coverage around microvessels is found in organs with barrier systems that contribute to regulating barrier properties. In addition to being regulators of the BBB, pericytes have been reported to serve as vital integrators, coordinators, and effectors of many neurovascular functions, including angiogenesis, vascular stability, and angioarchitecture maintenance as well as regulation of the capillary blood flow and clearance of toxic cellular by-products [46].

Interestingly, brain pericytes derived from rats express efflux transporters, such as P-gp, MRP1, and ABCG2, at the protein level [47], suggesting the possibility that pericytes function as an efflux pump to exclude xenobiotics by cooperating with BMECs. Pericytes also express several TJ molecules, including claudin-12, occludin, ZO-1, and ZO-2, and may control the permeability of the BBB in association with BMECs [47]. In addition, pericytes control the expression of TJ molecules in BMECs by secreting factors such as transforming growth factor β [48] and angiopoietin [49]. An in vitro study [50, 51] using pericyte cell lines derived from the human brain indicated that soluble factors, such as glial cell-derived neurotrophic factor (GDNF), released from pericytes enhance the barrier properties of TY08 cells, a human BMEC cell line [26], via the upregulation of claudin-5. Recently, Ben-Zvi et al. demonstrated that the endothelial expression of *major facilitator superfamily domain containing 2a* (*Mfsd2a*), which is regulated by pericytes, maintains the BBB integrity by suppressing transcytosis in CNS endothelial cells [52].

While pericytes influence BMECs, as indicated above, BMECs also contribute to the recruitment and maintenance of pericytes in vessels and the process of vascular maturation through platelet-derived growth factor β [53].

The basement membrane is also an essential element of the BBB and consists of four major components: type IV collagens, laminins, nidogen, and heparan sulfate proteoglycans, in addition to many other glycoproteins [54, 55]. In the CNS, blood vessels are surrounded by two layers of basal lamina: the inner endothelial BM and the outer parenchymal BM (Fig. 4.1a). The endothelial basement membrane is secreted by endothelial cells and pericytes, which are embedded within the endothelial BM [55]. The parenchymal BM is secreted by astrocytes and links astrocyte end feet to the BM through protein–protein interactions mediated in part by dystroglycan [56]. The two BMs have different molecular compositions, as the endothelial BM is rich in laminins alpha4 and alpha5, whereas the parenchymal BM is rich in laminins alpha1 and alpha2 [54]. Disruption of the BM has been reported to be strongly associated with increased BBB permeability in several pathological conditions [57–59].

Although the term “BBB” generally represents the barrier properties of microvessels within the brain parenchyma, microvessels in the leptomeninges also have such barrier properties.

Subtle differences have been noted between parenchymal and meningeal microvessels, with the parenchymal, but not meningeal, endothelium lacking the storage of certain adhesion molecules in Weibel–Palade bodies [60, 61]. In spite of these differences, meningeal microvessels also establish a functional BBB [62], and it has been suggested that this barrier should be referred to as the blood–leptomeningeal barrier [63, 64].

4.3 Blood–Cerebrospinal Fluid Barrier

The blood-CSF (BCSF) barrier is formed by the choroid plexus. The endothelium of the thoroughly vascularized choroid plexus (CP) has been found to be leaky [65]. As the CP forms an interface between the blood and the CSF, a barrier must also be present in this brain region. Instead of a fenestrated endothelium, an underlying sheet of epithelial cells expressing TJs at the apical ventricle-facing membrane takes over this barrier function, forming the BCSFB [66, 67]. Claudin-1, claudin-2, and claudin-3 are highly and selectively expressed in the CP, as compared with that observed in the brain parenchyma microvessels, and localized at epithelial junctions [67]. Other TJ-associated proteins, such as occludin, ZO-1, and ZO-2, are also expressed in the CP [67, 68]. Importantly, Wolburg et al. demonstrated that claudin-11 is a unique claudin in the BCSFB, inducing the formation of parallel tight junction strands [69].

The CP and secretary functions are aided by the expression of a variety of transporters, allowing for the precise regulation of the ion and nutrient content in the CSF, as well as the removal of waste products and limited entry of potentially neurotoxic compounds [1, 68–70]. A recent study disclosed the expressions of GLUT1, P-gp, MRP1, and ABCG2 in the choroid plexus epithelium in both in vivo and in vitro analyses [71–74]. Therefore, the BCSFB maintains homeostasis in the brain by cooperating with the parenchymal BBB.

4.4 Blood–Spinal Cord Barrier

Breakdown of the blood-spinal cord barrier (BSCB) has been reported in a number of neurological disorders, including neuromyelitis optica (NMO) [75, 76], amyotrophic lateral sclerosis (ALS) [77, 78], radiation injury to the spinal cord [79], spinal cord ischemia [80], and spinal cord trauma [81]. The BSCB is the functional equivalent of the BBB in the sense of providing a specialized microenvironment for the cellular constituents of the spinal cord. However, recent data suggest that functional differences exist between the BSCB and BBB [28, 82–85]. In a rabbit experimental model studying the permeability of the blood–CNS barrier, the transfer constant values for samples of the spinal cord exhibited a significantly increased uptake of [^{3}H]-D-mannitol and [^{14}C]-carboxyl-inulin compared to that observed in samples of the brain [82]. Pan et al. also demonstrated that the spinal cord is more permeable to cytokines than the brain [83]. In order to analyze the unique barrier properties of the BSCB, we established a conditionally immortalized rat endothelial cell line (rBSCB-1) derived from rat BSCB [28]. Interestingly, the barrier function of rBSCB-1 cells is more vulnerable than that of previously reported rodent BBB models, as well as TY10 cells used as a human in vitro BBB model [28]. Furthermore, in an in vitro study of cultured microvascular endothelial cells obtained from the murine spinal cord, Ge and Pachter

showed decreased expression levels of ZO-1 and occludin, compared to that observed in cultured brain microvascular endothelial cells [84]. These results indicate the possibility that the barrier function of the BSCB is slightly weaker than that of the BBB, providing an explanation for the vulnerability of the spinal cord noted in various neurological diseases, such as ALS and NMO.

Do astrocytes influence the barrier properties of the endothelium forming the BSCB? Importantly, treatment with astrocytic factors increases the barrier properties of rBSCB-1 cells, including their expression levels of tight junction molecules [28], which is reminiscent of the astrocytic effects on BBB-constituting endothelial cells.

Taken together, despite exhibiting similarities to the BBB, the BSCB also appears to possess unique properties that distinguish it from the BBB. Further analyses are needed to elucidate the common features and different aspects of the BSCB and BBB, as the findings of such analyses could lead to the development of novel therapies for many spinal cord-specific diseases.

4.5 Blood–Nerve Barrier

The presence of the BNB restricts the movement of soluble mediators and leukocytes from the blood to the peripheral nervous system (PNS) parenchyma [3, 86]. As the endoneurial homeostasis protected by the BNB is a prerequisite for the proper function of the PNS, the pathological breakdown of the BNB may be a key event inducing various peripheral neuropathies [87]. Indeed, the breakdown of the BNB is considered to be a key step in many autoimmune disorders of the peripheral nervous system, including Guillain–Barré syndrome, chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), and paraproteinemic neuropathy [3, 88–91]. The BNB comprises the endoneurial microvasculature and the innermost layers of the perineurium [92]. Tight junctions (TJs) between adjacent peripheral nerve microvascular endothelial cells (PnMECs) as well as between perineurial cells, in addition to the lack of vesicular transport, are responsible for the BNB function [92, 93]. Blood-borne substances can reach the endoneurial extracellular space by crossing either the endoneurial vascular endothelium or perineurium. Many studies have indicated that perineurial permeability is much lower than the permeability of endoneurial microvessels against various substances [94–97]. Hence, PnMECs constituting the bulk of microvessels in the endoneurium may be considered to constitute the real interface between blood and peripheral nerves. Because PnMECs form the structural basis of the BNB, as indicated above, understanding the biology of these endothelial cells using cell culture techniques may provide new insights into how solutes and macromolecules gain access into or are expelled from the endoneurium, providing cues to the nutritional requirements of peripheral nerves and the mechanisms underlying the regulated removal of metabolic end products. Although it has been reported that the barrier properties of the BNB are similar to those of the BBB [86], little is known about the molecular

mechanisms constituting the BNB. This is due to a lack of good in vitro BNB models. Do TJ proteins and transporters expressed at the BBB also exist and work at the BNB? In order to address these questions, we established rat [98] and human [99] in vitro BNB models and conducted basic research. Consequently, isolated rat PnMECs expressed tight junction molecules, such as occludin, claudin-5, claudin-12, ZO-1 and ZO-2, and junctional adhesion molecule 1 (JAM1) at the mRNA level [98]. Claudin-5 was also detected at the cell–cell boundaries of these cells. Furthermore, the rat PnMECs expressed blood-to-brain influx transporters, such as GLUT1, system L, and CRT, and brain-to-blood efflux transporters, such as P-gp, MRP1, and ABCG2, at the mRNA level [98]. Moreover, we confirmed the in vivo protein expressions of GLUT1, P-gp, occludin, and claudin-5 in the endoneurium of a rat sciatic nerve [98]. On the other hand, rat PnMECs did not express the efflux transporters organic anion transporter 3 (OAT3) and organic anion-transporting polypeptide 2 (Oatp2), both of which are expressed at the BBB [98]. Homovanillic acid (HVA) is a major metabolite of dopamine and thought to be excreted from the brain to blood by OAT3 [100]. Dehydroepiandrosterone (DHEAS) is a neurosteroid that interacts with GABA type A receptors and sigma receptors, increases memory and learning, and protects neurons against excitatory amino acid-induced neurotoxicity [101]. Oatp2 has also been reported to be involved in the brain-to-blood transport of DHEAS [101]. The absence of OAT3 and oatp2 in rat PnMECs may reflect the differences between the environment of the CNS and that of the PNS, i.e., the former has synapses and the latter does not. This finding supports the concept that the BNB is a distinct structure that differs from the BBB at the cellular and molecular levels.

The established human PnMEC cell line, termed FH-BNBs, appears to be closely packed and exhibits a spindle fiber-shaped morphology similar to human endothelial cell lines derived from the BBB (Fig. 4.2b). In addition, FH-BNBs express TJ proteins, including occludin, claudin-5, ZO-1, and ZO-2, at cell–cell boundaries [99]. P-gp and GLUT1 in FH-BNBs are also detected using Western blot analyses, and these cells also exhibit the functional expression of P-gp1 [99]. These results indicate that the endothelial cells forming the BNB, also present in the human PNS, express some of the same TJ proteins and efflux transporters as in the BBB to protect nerve fibers from both toxic and pathogenic agents in the blood and deliver essential nutrients using various influx transporters. These findings showing that human BNB express TJ proteins and various transporters expressed at the BBB have also been confirmed by the experiments using other primary and immortalized human PnMEC cell lines [102–104].

FH-BNB cells have been widely used for basic research on the BNB and investigations of neurological diseases [105–108]. Interestingly, the sera from patients with chronic inflammatory demyelinating polyradiculoneuropathy and multifocal motor neuropathy deteriorate the barrier properties of FH-BNBs via the downregulation of caludin-5 [105, 106].

The BBB is composed of BMECs, astrocytes, and pericytes, whereas the BNB is comprised of only PnMECs and pericytes (Fig. 4.1b). It is well known that astrocytes strengthen the barrier function of BMECs via the secretion of soluble

factors [11, 41–44]. Unlike the BBB, the BNB lacks cells corresponding to astrocytes. Therefore, we hypothesize that peripheral nerve pericytes, the only cells composing endoneurial microvessels other than PnMECs, may play a similar role to that of astrocytes in the BBB. Interestingly, soluble factors secreted from a peripheral nerve pericyte cell line derived from a human sciatic nerve reduce inulin clearance across the monolayer of PnMECs [50]. In particular, bFGF and GDNF released from peripheral nerve pericyte cell lines strengthen the barrier function of PnMECs via the upregulation of claudin-5 [50, 51]. Therefore, peripheral nerve pericytes in the BNB secrete these soluble factors and may thus play an important role in maintaining the barrier properties of PnMECs within the endoneurium, similar to astrocytes in the BBB.

It has not been elucidated which cells release various neurotrophic factors in the peripheral nervous system. Interestingly, human peripheral nerve pericyte cell lines express several neurotrophic factors, such as nerve growth factor, brain-derived neurotrophic factor (BDNF), and GDNF [50]. Many studies have demonstrated that astrocytes produce neurotrophic factors, such as BDNF and GDNF, which protect against neuronal loss in the CNS [109, 110]. Neurotrophic factors secreted from peripheral nerve pericytes may prevent axonal loss and promote axonal regeneration in the peripheral nervous system. Small hydrophobic substances capable of reaching the pericyte membrane through the endothelial monolayer and strengthening the pericytic activity, including various cytokines/chemokines that influence the endothelial barrier function and neurotropic factors that promote the repair of damaged nerve fibers, may be useful drug candidates against various intractable peripheral neuropathies.

4.6 Conclusions and Future Perspectives

Although there are differences among the BBB, BCSFB, BSCB, and BNB, all of these barrier systems maintain the homeostasis of the entire nervous system through, at least partially, common components, including TJs and transporters. Recent advances in this field of research are remarkable. However, further progression in elucidating the entire neutral barrier system is required, as new therapies modifying the barrier system may lead to breakthroughs in treating many refractory neurological diseases.

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Part II
Clinical Immunology

Chapter 5
Multiple Sclerosis: Etiology and Mechanism, with Special Reference to Asians

Jun-ichi Kira

Abstract Multiple sclerosis (MS) is a disease that targets myelin in the central nervous system (CNS). It is thought to be an autoimmune disease but this is not yet proven. Genome-wide association studies have revealed many susceptibility genes for MS, and the functions of these genes are mostly immune-related, supporting the autoimmune hypothesis. Increased numbers of T cells showing inter- and intramolecular epitope spreading against myelin proteins; increased cerebrospinal fluid (CSF) levels of interferon (IFN)-γ, interleukin (IL)-17, and downstream pro-inflammatory cytokines; exacerbation of disease following IFN-γ administration; and increased percentages of T helper (Th)1 cells secreting IFN-γ and of Th17 cells secreting IL-17 at relapse all support T-cell involvement in MS lesion formation. B-cell infiltration in the CNS parenchyma is not prominent, while B-cell follicles in the meninges appear to have a close correlation with subpial demyelination, suggesting an involvement of autoantibodies. However, no specific autoantibodies for MS have been detected, although anti-aquaporin-4 antibodies are a specific biomarker for neuromyelitis optica (NMO). We reported the extensive loss of connexins (Cxs) 43, 32, and 47 in acute lesions in patients with Baló's concentric sclerosis, MS, and NMO. Such loss of astroglial and oligodendroglial Cxs could induce widespread disruption of the glial syncytium, leading to failure of energy source transfer. Thus, loss of Cxs in acute MS lesions may contribute to extensive lesion formation. In the Japanese population, *HLA-DRB1*0405* is the most common susceptibility allele for non-NMO MS. *DRB1*0405* carriers comprise >40 % of all Japanese MS patients, and this proportion is higher in younger generations. *HLA-DRB1*0405*-positive MS patients show characteristic features, such as younger age at onset, fewer brain lesions, fewer CSF IgG abnormalities, and a slower progression. The recent increase in the number of MS patients in this subgroup may explain the overall decrease in onset age, as shown by the fourth nationwide survey of Japanese MS patients. Therefore, changes in environmental factors may have increased susceptibility to MS in Japanese populations with certain genetic backgrounds.

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5.1 Introduction

Multiple sclerosis (MS) is an idiopathic inflammatory demyelinating disease of the central nervous system (CNS) and is one of the most common causes of neurological disability among young adults worldwide. MS is regarded as an autoimmune disease mediated by T cells that target CNS myelin antigens. Recent genome-wide association studies (GWASs) have identified many susceptibility loci for MS [1, 2]. Most of these loci are intergenic, but for the identified susceptibility genes, most have immune-related functions, supporting the autoimmune nature of the disease.

MS is prevalent in the temperate regions of Europe, North America, and Australia/New Zealand, but is relatively rare in Asia. The prevalence of MS has rapidly increased in line with modernization, including in Asian countries, such as Japan. Previously, MS in Asians was characterized by selective and severe involvement of the optic nerve and spinal cord with sparing of the cerebrum and cerebellum [3]. Two distinct MS phenotypes were reported in Asians: opticospinal MS (OSMS), selectively affecting the optic nerve and spinal cord, and conventional MS involving multiple sites of the CNS including the cerebrum and the cerebellum [3]. OSMS in Asians shows similar features to the relapsing form of neuromyelitis optica (NMO) in Caucasians, and a specific marker for NMO, namely, NMO-IgG, targeting aquaporin-4 (AQP4) [4, 5], is also present [6, 7]; therefore, OSMS is now regarded as the same disease as relapsing NMO [8]. Most genetic and environmental studies in Japanese MS patients were carried out in the 1990s to early 2000s before the discovery of this specific biomarker for NMO; therefore, NMO-IgG status was not examined in these studies. Thus, studies predating the discovery of anti-AQP4 antibodies should be carefully interpreted. In this chapter, taking NMO and anti-AQP4 antibody status into account, I review clinical, epidemiological, genetic, pathological, and immunological aspects of MS and relate these to the etiology and pathomechanisms of the disease, focusing on MS in Asians.

5.2 Clinical Aspects

Most MS patients initially experience a relapsing remitting phase with a mean age of onset around 30 years of age. This is termed relapsing remitting MS (RRMS). 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 steadily progressive course from the onset, termed primary progressive MS (PPMS). Recent

GWASs found no significant differences in risk genes between RRMS and progressive MS, suggesting that these two types could be distinct manifestations of the same disease [1, 2].

Clinical relapse is often accompanied or even preceded by emergence of contrast-enhanced magnetic resonance imaging (MRI) lesions in the CNS, indicating destruction of the blood-brain barrier (BBB). Recent 7-T MRI studies clearly showed the presence of vessels in the center of MS lesions [9], 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.

However, relapses have only a weak effect on disability progression [10]. Irrespective of the initial disease course, a clinically progressive phase develops in both SPMS and PPMS patients around 40 years of age and then proceeds at a similar rate in both groups [11]. MS patient disability, determined by Kurtzke Expanded Disability Status Scale (EDSS) score [12], progresses at varying rates until the development of an EDSS score of four and, thereafter, worsens at approximately the same rate [13]. These findings suggest that common pathogenic mechanisms may underlie clinical disability progression, regardless of the clinical type. At the progressive stage of MS, none of the recently developed disease-modifying drugs (DMDs), which act on the peripheral immune system, are effective, even if they have high efficacy for reducing both annualized relapse rates and new MRI lesions. Thus, disability progression may have a distinct mechanism from relapse that is closely associated with BBB disruption induced by peripheral immunocytes.

MRI T2 lesion burdens in the white matter modestly correlate with disability [4]. The introduction of double inversion recovery MRI demonstrated that cortical lesions are present from the early stage of RRMS and become more prominent in SPMS [14–17]. The absence of MRI evidence for noticeable inflammation suggests that neurodegeneration may take place in cortical lesions. Cortical lesion loads and cortical and spinal cord atrophy are significantly associated with clinical progression [14, 15, 18, 19], whereas white matter atrophy does not correlate with increasing disability [15]. Thus, cortical lesions may play a major role in the development of both physical and cognitive disability [20]. Disease progression could be attributable to glial inflammation compartmentalized in the CNS, internal to the BBB [21].

5.3 Epidemiological Aspects

5.3.1 Overview

Prevalence of MS varies worldwide from 30–150/100,000 in high prevalence areas, to 5–30/100,000 in medium prevalence areas, to less than 5/100,000 in low prevalence

areas. There are clear geographical and racial differences in MS prevalence: high in Europeans and their descendants and low in Asians and Africans. In Asian countries, MS is more common in West Asian countries and rare in Southeast Asian countries [3] (Fig. 5.1). MS prevalence increases according to latitude; the higher the latitude, the higher the prevalence. In countries having long north to south dimensions, such as Japan, MS prevalence shows a significant positive correlation with latitude [3] (Fig. 5.2), suggesting that environmental factors related to latitude are operative in the development of MS. Migration studies also reported that migration before puberty from high prevalence regions to low prevalence regions decreases MS risk, while

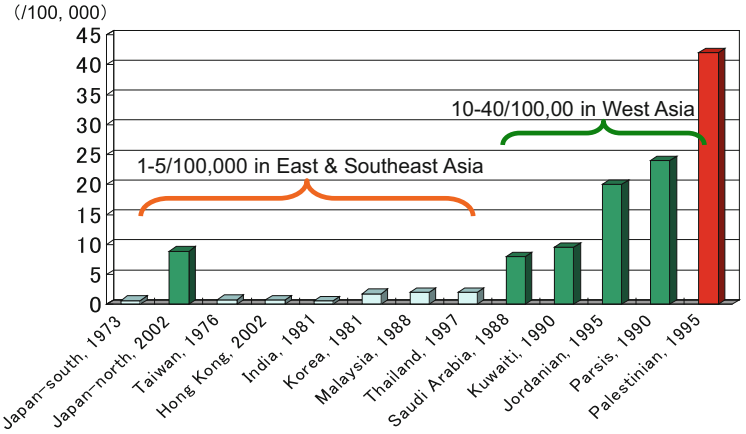

Fig. 5.1 Prevalence of MS in Asian countries. The prevalence of MS is higher in West Asian countries than in Southeast Asian countries (Modified from Ref. [3])

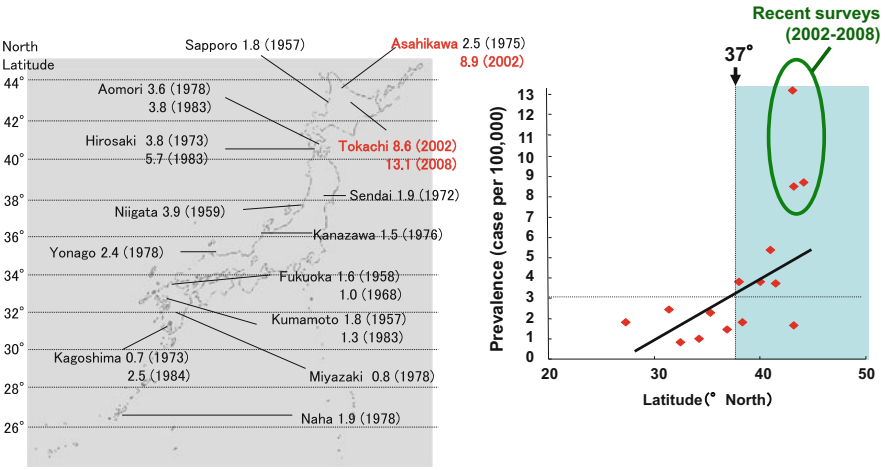

Fig. 5.2 North to south gradient of MS prevalence in Japan. MS prevalence shows a significant positive correlation with latitude (Modified from Ref. [3])

migration in the reverse direction increases MS risk [22–27]. This supports the concept that the geographical location where one is born and raised until puberty is an important factor in the occurrence of MS. However, as migrants may have genetic admixture, which may increase or decrease MS risk [28], migration study results should be carefully interpreted.

MS incidence and prevalence have increased worldwide, especially in women [29–31]. Such increase cannot be solely attributed to better diagnostic techniques such as MRI or newer diagnostic criteria. 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 [32], suggesting that changes in environmental factors associated with modernization or westernization have potentiated MS risk in younger generations. Interestingly, the recent increase of MS incidence has reduced the north to south gradient of MS prevalence in some countries [29–31]. These observations suggest that the effect of latitude and environmental changes related to modernization potentially overlap in MS inflammatory cascades. Potential environmental factors postulated to contribute to increased incidence and prevalence of MS include amount of sunlight, infectious agents, products of industry in the environment, and dietary and habitual differences [22].

5.3.2 Recent Epidemiological Changes in Japan

Nationwide surveys on MS carried out in 1972, 1982, 1989, and 2004 [32–35], using essentially identical diagnostic criteria based on Schumacher et al. [36], produced the following findings: (1) a fourfold increase in the estimated number of clinically definite MS patients in 2003 (9900; crude MS prevalence, 7.7/100,000) compared with 1972; (2) a shift in the peak age at onset from the early 30s in 1989 to the early 20s in 2003; and (3) an increase in the female-to-male ratio from 1.7:1 in 1972 to 2.9:1 in 2003. Three recent epidemiological surveys conducted in the northernmost island of Japan also revealed a marked increase of MS prevalence, 10.2/100,000 [37], 13.1/100,000 [38], and 16.2/100,000 as compared with those in the 1970s [39]. Although the increase in MS prevalence appears to be partly attributable to improved case ascertainment, the twofold increase in the proportion of females, consistent with a worldwide increase in the number of female MS patients [29–31], as well as the younger age at onset in the latest survey, cannot be fully explained by improved case ascertainment [40]. In Canada, the female-to-male ratio increases rapidly with advancing year of birth, suggesting the importance of environmental factors in childhood in the disproportionate increase of female MS patients [41, 42]. Therefore, it is possible that MS susceptibility has markedly increased among younger Japanese women that have grown up in a westernized environment, resulting in anticipation of age at onset.

5.4 Genetic Aspects

5.4.1 Human Leukocyte Antigen (HLA) Genes

The major histocompatibility complex (MHC) class II genes, that is, the *human leukocyte antigen* (*HLA*) genes, have the largest genetic effect of any gene family on MS in Caucasians as well as in Asians. In Caucasians, the *DR15* haplotype (*DRB1*1501-DQA1*0102-DQB1*0602*) and its individual alleles, especially *HLA-DRB1*1501*, are strongly associated with MS risk in people of Northern European descent [43], while the *DR3* (*DRB1*0301-DQA1*0501-DQB1*0201*) and *DR4* (*DRB1*0405-DQA1*0501-DQB1*0301*) haplotypes confer susceptibility to MS in Sardinians [44, 45].

Earlier studies performed in the 1990s on Japanese MS patients repeatedly showed that conventional MS is associated with the *HLA-DRB1*1501* allele, while OSMS is associated with the *HLA-DPB1*0501* allele [46–49]. However, these studies were carried out before the discovery of NMO-IgG. The presence/absence of NMO-IgG needs to be taken into account when considering these classifications, even though there is still some debate about whether NMO and MS have completely distinct mechanisms.

In the fourth Japanese nationwide survey, patients were classified according to the presence of MS-like brain lesions that fulfilled Barkhof criteria and longitudinally extensive spinal cord lesions (LESCLs), a hallmark of NMO/OSMS. The most common form of MS in the Japanese population according to this survey was the Barkhof(−)/LESCL(−) type [33]. In Japanese cases, Barkhof(−)/LESCL(−) MS is associated with *HLA-DRB1*0405* [50], suggesting that certain HLA alleles are influential on MRI features. After completely excluding cases with NMO/NMO spectrum disorder (NMOSD), based on an anti-AQP4 antibody assay, we also found that the frequencies of *DRB1*0405* and *DPB1*0301* were significantly higher, and those of *DRB1*0901* and *DPB1*0401* significantly lower, in non-NMO/NMOSD MS patients compared with healthy controls [51]. Thus, *DRB1*0405* and *DPB1*0301* are susceptibility alleles for non-NMOSD MS, while *DRB1*0901* and *DPB1*0401* are resistance alleles.

Carriers of the *DRB1*0405* susceptibility allele comprised 44.8 % of all non-NMO/NMOSD MS patients in a Japanese MS series [51]. Such MS patients with the *DRB1*0405* allele are characterized by an earlier age of onset, lower EDSS scores, lower progression indexes, lower frequencies of brain lesions fulfilling Barkhof criteria, and lower frequencies of CSF oligoclonal IgG bands (OBs) and/or increased IgG indexes, as compared with patients without this allele (Table 5.1) [51]. This is in accord with previous reports showing that *DRB1*04* is associated with OB-negative MS in Swedish [52] and Japanese populations [53]. The existence of MS patients with *DRB1*0405* may partly explain the low prevalence of OB (54 %) in Japanese MS patients, a unique feature compared with MS in Westerners [3, 54]. Therefore, *DRB1*0405*-positive MS patients could be a unique subgroup of Japanese MS patients that experience a relatively benign

Table 5.1 Milder disease activity of non-NMO/NMOSD MS patients with HLA-DRB1*0405

	*DRB1*0405* (+) ($n = 65$)	*DRB1*0405* (−) ($n = 81$)	p
Men:Women	22:43	29:52	0.8054
Age at onset (years)	27.22 ± 10.45	34.78 ± 13.75	**0.0014**
Disease duration (years)	12.83 ± 9.68	10.19 ± 7.23	0.1184
EDSS score	2.48 ± 2.05	3.49 ± 2.35	**0.0066**
Annual relapse rate (/years)	0.54 ± 0.45	0.70 ± 0.77	0.2079
Progression index	0.33 ± 0.42	0.65 ± 1.37	**0.0018**
OB/increased IgG index	22/42 (52.4 %)	37/53 (69.8 %)	0.0820
Barkhof criteria (+)	31/58 (53.5 %)	51/71 (71.8 %)	**0.0309**
LESCL	3/58 (5.2 %)	5/72 (6.9 %)	0.7313

Progression index = EDSS/disease duration; *OB* oligoclonal bands

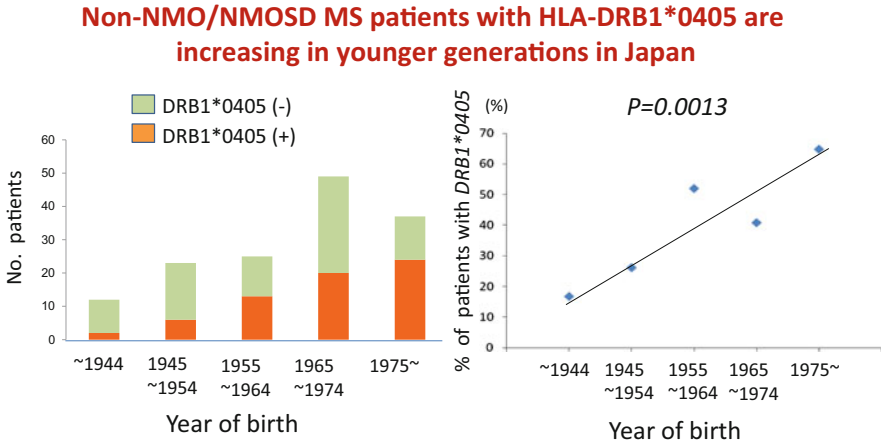

Fig. 5.3 The proportion and absolute numbers of MS patients with *DRB1*0405* according to year of birth. The proportion and absolute numbers of MS patients with *DRB1*0405* both show an increase with advancing year of birth [51]

disease course. The proportion and absolute number of MS patients with *DRB1*0405* both increase with advancing year of birth (Fig. 5.3), and this group of MS patients has a significantly younger age at disease onset [51]. Therefore, the recent increase in the number of patients in this MS subgroup may explain the recently observed decrease in age at onset among Japanese MS patients. Frequency of *DRB1*0405* 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 *DRB1*0405* is a susceptibility allele in both Japanese and Sardinian populations, both of which recently demonstrated marked increases in MS incidence [54]. A shift in the peak age at onset toward a younger age was also reported in Sardinia [55]. Hence, recent environmental changes may enhance MS susceptibility in populations carrying certain HLA alleles [32, 34].

In MS patients without *DRB1*0405*, frequency of the *DRB1*1501* allele was significantly higher, while frequency of the *DRB1*0901* allele was significantly lower compared with healthy controls (HCs) [51]. *DRB1*0405*-negative MS patients had significantly greater EDSS scores, a higher progression index, and a higher frequency of brain lesions fulfilling Barkhof criteria compared with *DRB1*0405*-negative MS patients [51]. In these subjects, the presence of *DRB1*1501* was significantly associated with CSF OB/increased IgG index [51, 56]. Because high brain MRI lesion loads and CSF IgG abnormalities are characteristic features of Caucasian MS patients, *DRB1*1501* is likely to be a susceptibility allele in Japanese MS cases with similar features to MS in Westerners.

After excluding MS cases, the *DPB1*0501* allele is associated with NMO-IgG/anti-AQP4 antibody-positive NMO in Japanese [57] and Southern Han Chinese populations [58]. In Caucasian NMO, HLA class II gene alleles other than *DPB1*0501*, such as *DRB1*03*, are also risk alleles [59, 60]. Our recent GWAS in Japanese cases demonstrated that most significant associations are found in the HLA regions on chromosome 6, not only in MS but also in NMO (unpublished data). These observations reinforce the notion that susceptibility to NMO-IgG-positive NMO is in part genetically determined, although genetic backgrounds for the production of NMO-IgG vary among races.

In contrast, the frequencies of *DRB1*0901* are significantly lower in non-NMO/NMOSD MS patients compared with healthy controls, regardless of *DRB1*0405* status, and they are also lower in NMO/NMOSD patients, irrespective of the presence or absence of anti-AQP4 antibody [51, 57, 61, 62]. This suggests that the *DRB1*0901* allele confers resistance to idiopathic demyelinating disease in Japanese cases, regardless of clinical phenotype. A recent meta-analysis of Chinese populations also indicated that this allele is protective against MS [63]. The *DRB1*0901* allele is more frequently observed in Asians than in European ethnic groups (30 % of Japanese vs. 1 % of Caucasians) [64]. Thus, the lower prevalence of MS in Asian countries may be partly attributable to the relatively high frequencies of the *DRB1*0901* allele in the region.

5.4.2 Non-HLA Genes

Recent genome-wide surveys in Caucasian MS patients have identified more than 100 non-HLA genes related to MS susceptibility [1, 2]. Among these, we observed that the *IL-7 receptor alpha chain* (*IL-7RA*) gene single-nucleotide polymorphism (SNP) rs6897932 confers susceptibility to conventional MS, but not to NMO in the Japanese population [65]. Among MS patients, rs6897932 is associated with Barkhof brain lesion-positive MS, but not Barkhof brain lesion-negative MS [66]. rs6897932, located within the alternatively spliced exon 6 of *IL-7RA*, modifies the ratio of membrane-bound to soluble IL-7Rα [67]. The CC genotype elevates

levels of the soluble form of IL-7Rα and decreases expression of membrane-bound IL-7Rα, thereby downregulating IL-7/IL-7 receptor signaling [67]. IL-7/IL-7R signaling induces thymic production of Foxp3+ regulatory T cells, which efficiently ameliorate experimental autoimmune encephalomyelitis (EAE), an animal model of MS [68]. Thus, the rs6897932 CC genotype may confer MS susceptibility through decreased production of Foxp3+ regulatory T cells owing to dampened IL-7/IL-7 receptor signaling. This effect is only apparent in Japanese patients with Barkhof brain lesion-positive MS, which is similar to MS in Caucasians, who also show an association of MS susceptibility with rs6897932 [67]. Intriguingly, we previously found that IL-7 levels in the CSF from MS patients at relapse were significantly lower than in patients with other noninflammatory neurological diseases [69]. Thus, decreased IL-7/IL-7 receptor signaling may relate to MS relapse through weakened regulatory T-cell functions.

An MHC-wide SNP study on idiopathic CNS demyelinating disease also found that the G allele of rs422951 in the *NOTCH4* gene, which is located on the short arm of chromosome 6 (approximately 0.4 Mb telomeric of *HLA-DRB1*), is protective against MS in a Japanese population [70]. Notch signaling is an evolutionarily conserved pathway that regulates cell fate decisions. In vertebrates, the pathway consists of four transmembrane receptors (Notch-1–4) and five transmembrane ligands (delta ligand-like (DLL) 1, 3, and 4, and Jagged 1 and 2) [71]. Upon ligand binding, Notch signaling plays important roles in vascular development and T-cell biology. Notch-4 is primarily expressed in the vascular endothelium of embryonic and adult mammals [72]. Activated Notch-4 inhibits angiogenesis [73, 74]. It is thus possible that Notch-4 is involved in MS through its effects on CNS vessels that constitute the BBB.

We recently conducted a GWAS of copy number variations (CNVs) using high-density single-nucleotide polymorphism microarrays [75]. Most CNVs were 5–50 kb deletions at particular T-cell receptor (TCR) gamma and alpha loci. Among them, a TCR gamma locus deletion was found in 16.4 % of MS patients [$p = 2.44\text{E-}40$, odds ratio (OR) = 52.6], while deletion at the TCR alpha locus was found in 17.3 % of MS patients ($p = 1.70\text{E-}31$, OR = 13.0) and in 13.3 % of NMO/NMOSD patients ($p = 5.79\text{E-}20$, OR = 54.6). These CNVs were observed in peripheral blood T-cell subsets only, suggesting that they were somatically acquired. MS patients are known to show a skewed TCR usage [76]. Our results are consistent with this and support a notion that certain T-cell populations targeting unknown CNS antigens are involved in MS pathogenesis. Interestingly, NMO/NMOSD patients carrying a CNV tend to be seronegative for anti-aquaporin-4 antibody or to have significantly lower titers compared with those without a CNV [75]. We previously reported that Th2 cell percentages had a significant inverse correlation with anti-AQP4 antibody titers [77]; therefore, such CNV carriers with absent or low titers anti-AQP4 antibodies are assumed to have a Th1 shift. Together with our previous findings that CSF T-cell cytokines, such as IFN-γ and IL17, were markedly increased in the CSF from NMO patients at relapse [69], high frequency of deletion-type CNVs at certain TCR regions suggests T-cell involvement in

NMO. Therefore, it is plausible that even non-HLA genes are commonly and differentially associated with MS and NMO.

5.5 Environmental Aspects

5.5.1 Infection and Immunology

Among environmental factors, infection is assumed to play significant roles in the acquisition of MS susceptibility or resistance. It is well known that frequent childhood infections reduce MS susceptibility [78, 79]. Conversely, improved hygiene in childhood is hypothesized to confer development of not only autoimmune disease but also atopic/allergic inflammation [80]. Sanitary conditions in Japan have drastically improved since World War II through rapid lifestyle westernization. Therefore, we studied the profiles of common infections in Japanese patients with MS and NMO by measuring IgG antibody responses to *Helicobacter pylori*, *Chlamydia pneumonia*, varicella zoster virus, and Epstein-Barr virus (EBV) nuclear antigen [51, 62, 81, 82]. The *H. pylori* infection rate was lower in Japanese conventional MS patients compared with healthy controls and OSMS/NMO patients [81]. The same observation was recently reported in Australian MS patients [83]. Moreover, we detected that *DRB1*0405*-negative MS patients had a higher frequency of EBV nuclear antigen IgG antibodies compared with healthy controls, *DRB1*0405*-positive MS patients, AQP4 antibody-negative NMO patients, and AQP4 antibody-positive NMO patients [51, 62]. In contrast, anti-*H. pylori* and anti-*C. pneumoniae* IgG antibodies were present at significantly higher levels in anti-AQP4 antibody-positive NMO patients compared with healthy controls, whereas anti-AQP4 antibody-negative NMO patients did not show such a trend [62]. Anti-varicella zoster virus antibody positivity rates did not differ significantly among the four subgroups. In summary, among *DRB1*0405*-negative Japanese MS patients presenting typical features common to MS in Westerners, EBV appears to potentiate MS susceptibility, while *H. pylori* infection provides resistance. In anti-AQP4 antibody-positive NMO, *H. pylori* and *C. pneumonia* infections confer risk for the disease. No such association was found in either *DRB1*0405*-positive MS or anti-AQP4 antibody-negative NMO.

H. pylori infection occurs in infancy, when the mucosal barrier of the stomach is immature, and persists for life [84]. Thus, it is conceivable that the *H. pylori* infection rate reflects sanitary conditions during childhood [85], when MS susceptibility is acquired. The "hygiene hypothesis" proposes that repeated childhood infection induces maturation of regulatory immune systems, while improved sanitation hampers its development as a result of fewer childhood infections [80]. As a result, in adulthood, regulatory immune systems cannot suppress either autoimmune or allergic inflammation [80, 86, 87]. Therefore, improved sanitary conditions in infancy, reflected by a lower *H. pylori* infection rate, may facilitate the

development of MS with typical Western MS-like characteristics [87]. It is interesting to note that the CC genotype at the *IL-7RA* rs6897932 SNP, which decreases production of Foxp3+ regulatory T cells because of dampened IL-7/IL-7 receptor signaling, confers MS susceptibility only for Barkhof brain lesion-positive MS [67]. Thus, in *DRB1*0405*-negative MS patients showing a higher frequency of Barkhof brain lesions, regulatory T-cell dysfunction may be critical for development of the full-brown disease.

In contrast, higher infection rates of *H. pylori* and *C. pneumonia* were observed only in anti-AQP4 antibody-positive NMO patients [51, 62, 81, 82]. In certain bacterial infection-associated autoimmune diseases, cross mimicry with bacterial antigens is suggested [88]. For example, regression of idiopathic thrombocytopenic purpura after eradication of *H. pylori* is due to molecular mimicry between platelets and *H. pylori* antigens [89, 90]. Certain bacteria harbor bacterial aquaporins; therefore, cross mimicry of these bacterial antigens with human AQP4 may induce anti-AQP4 antibodies. Or, alternatively, microbial infection stimulates the innate immune system, resulting in enhanced expression of co-stimulatory molecules and pro-inflammatory cytokines [88]. We observed a systemic increase in the levels of myeloperoxidase [91] and complements [92] during relapses in NMO. Increased levels of IL-17 and downstream pro-inflammatory cytokines in CSF from NMO patients at relapse, as well as increased levels of Th1 and Th17 cells in peripheral blood and CSF from these patients [69, 93–96], suggest involvement of Th17/Th1 cells, even in NMO. As *H. pylori* infection induces Th1- and Th17-type cytokine production [97], such chronic bacterial infections may potentiate activation of Th17/Th1 cells, which are supposed to trigger CNS inflammation in NMO patients [63, 93–96]. Improved sanitation in Japan may enhance susceptibility to MS presenting typical features of MS seen in Westerners, while it decreases susceptibility to NMO.

EBV infection is more prevalent in MS patients than in healthy controls in Western countries [98–100], while a more hygienic environment during childhood predisposes individuals to later EBV infection [101]. Such a delay in EBV infection increases the MS risk [101]. In the Japanese population, the EBV infection rate was increased only in *DRB1*0405*-negative MS patients who showed typical features common to MS in Westerners [51]. Thus, improved sanitation, as reflected by the lower *H. pylori* infection rate in this group, may have caused delayed EBV infection, thereby conferring MS risk, as seen in Caucasians. Indeed, increased age of EBV infection was recently observed in Japanese subjects [102], further supporting such a notion.

5.5.2 Other Environmental Factors

In Caucasians, EBV infection, latitude, vitamin D deficiency, and smoking are established risk factors for MS [86, 98]. In previous Japanese studies, only an effect of latitude on MS susceptibility has been confirmed [3], although other

environmental risk factors have not been extensively studied. Recently it has been reported that vitamin D levels were also significantly decreased in MS patients, especially in secondary progressive MS patients, irrespective of latitude; both MS patients residing in Hokkaido and Kyushu islands showed a significant reduction [103, 104]. Therefore, vitamin D deficiency may be an environmental susceptibility factor for MS in Japan and may aggravate MS by inducing secondary progression. Or, alternatively, disabled MS patients may have lowered vitamin D levels secondarily because of fewer opportunities to go outside and be exposed to sunlight.

5.6 Pathology

5.6.1 White Matter Pathology

MS predominantly affects CNS white matter where myelin is enriched. Axons are relatively preserved in the sharply demarcated plaques. Actively demyelinating lesions are featured by densely and diffusely infiltrated macrophages/activated microglia phagocytosing myelin debris [105, 106]. Such lesions are accompanied by perivascular lymphocyte cuffing. Chronic-active lesions display a rim of macrophages/activated microglia, while in chronic-inactive lesions, the density of macrophage/activated microglia shows no increase throughout the lesions. Remission results from the resolution of inflammation, redistribution of ion channels along demyelinated axons, and remyelination. A mild global inflammation characterized by microglial activation and a diffuse low-level T-cell infiltration is seen even in normal-appearing white matter and is more prominent in SPMS and PPMS than RRMS. In chronic MS, leakage from the BBB is absent, which corresponds to a paucity of gadolinium-enhanced lesions in PPMS and SPMS. Thus, compartmentalized inflammation behind the BBB is suggested as a mechanism for the progressive phase based on these pathological findings.

MS pathology shows heterogeneity. Lucchinetti et al. [107] proposed four demyelinating patterns for MS lesions and claimed that an individual only develops one pattern, suggesting that a single mechanism is operative in individual patients. All lesions have inflammatory infiltrates composed of T cells and macrophages/activated microglia, while each pattern has its own specific features as follows:

Pattern I: Active demyelination associated with the infiltration of T cells and macrophage/activated microglia in the absence of antibody and complement deposition. These lesions are centered around blood vessels.

Pattern II: Active demyelination associated with immunoglobulin and complement deposition. Prominent depositions of immunoglobulins (mainly IgG) and complement C9neo antigen are detected in association with degenerating myelin at the active plaque edge. This pattern is also centered around vessels.

Pattern III: Distal oligodendrogliopathy characterized by selective myelin-associated glycoprotein (MAG) loss. A profound loss of oligodendroglia at the

active plaque border, DNA fragmentation, and oligodendroglial apoptosis are observed with infiltration of T cells and macrophage/activated microglia but without the deposition of immunoglobulins and complement. Such lesions are not centered around vessels and the margin is ill-defined.

Pattern IV: Oligodendroglial death with DNA fragmentation but without features of apoptosis in a small rim of periplaque white matter. A near complete loss of oligodendroglia in active and inactive lesions is observed without remyelination. The border is sharply demarcated.

Immunoglobulin and complement deposits are found in lesions from about 50 % of autopsied MS patients (pattern II) [108], suggesting that antibody and complement-mediated myelin phagocytosis might become the dominant mechanism in established MS lesions [109]. However, we have observed heterogeneous demyelinating patterns even within one autopsied individual, indicating such patterns may represent stage-dependent heterogeneity but not disease heterogeneity [110]. Therefore, it is still controversial whether an individual only develops one demyelinating pattern or can develop more than one pattern.

5.6.2 Gray Matter Pathology

Gray matter lesions have gained much recent attention because they closely correlate with disability progression. Demyelination is present to varying degrees in the spinal cord gray matter, cerebral and cerebellar cortex, and in the deep gray matter, including the thalamus, basal ganglia, and hypothalamus [111, 112]. Cortical lesions are classified into three types: leucocortical lesions affecting both subcortical white matter and the lower layer of the gray matter (type I), entirely intracortical lesions (type II), and subpial gray matter regions (subpial demyelination) (type III) [112]. Frontal and temporal cortices, cingulate gyrus, and hippocampus are most frequently affected [21] and may explain the correlation between cognitive impairment and cortical pathology. Cortical demyelination does not correlate with changes in white matter pathology [111], suggesting independent mechanisms are in operation. Cortical lesions are distinct from white matter lesions in terms of inflammatory infiltrates and integrity of the BBB; cortical lesions show increased levels of activated microglia without evident inflammatory infiltrates or complement activation and lack significant leakage of plasma proteins suggesting preserved BBB [21, 111–113]. 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 [113, 114]. It is possible that secretion of pro-inflammatory cytokines into the CSF from lymphocytes in the meningeal follicles is responsible for such changes. However, such a relationship between meningeal lymphoid follicles and cortical demyelination has not been confirmed [115]. Diffuse cortical neuronal loss was also described, even in normal-appearing gray matter [113], suggesting that demyelination and neuronal loss may not be

directly linked [21]. Although neuronal apoptosis and mitochondrial damage were thought to be responsible for the neuronal loss [21, 116, 117], further studies are required to establish the mechanisms for cortical lesions.

5.6.3 Oligodendroglia Pathology and Remyelination Failure

Oligodendroglia express excitatory amino acid transporter (EAAT)-1 and EAAT-2 and are regarded as the principal cells for glutamate clearance in the white matter [118]. Oligodendroglia also possess AMPA/kainite receptors on the cell body and NMDA receptors in the processes. Magnetic resonance spectroscopy [119] and brain biopsy [120] demonstrated accumulation of glutamate in MS lesions, while EAAT-1 and EAAT-2 were reduced in oligodendroglia [121]. Oligodendroglia are thus vulnerable to glutamate toxicity and may be damaged by glutamate secreted by activated microglia. Oligodendroglia are also sensitive to oxidative stress because the cells contain a large pool of iron but have a low-capacity antioxidative system [118].

Demyelination in the MS brain and spinal cord can be followed by remyelination to a variable extent [122–124]. Remyelination is more prominent in early stages of the disease, while chronic lesions have less or no remyelination. Fewer active inflammatory lesions and more remyelination were observed in PPMS brains than in SPMS brains [21]. Oligodendroglia progenitor cells (OPCs) exist even in chronic MS lesions [125, 126]; therefore, remyelination failure is not attributable to the absence of OPCs, but rather to the blockade of OPC differentiation into myelinating oligodendroglia.

In MS plaques, extracellular inhibitors or intrinsic intracellular blocking mechanisms are seemingly operative. Expression of LINGO-1 on astrocytes and macrophages [127, 128], abnormal expression of PSA-NCAM on demyelinated axons [129], and myelin debris [130] can inhibit the differentiation of OPCs to myelinating oligodendroglia. Extracellular inhibitors include astrocyte-produced Jagged 1, which activates Notch 1 receptors on oligodendroglia to promote expression of Hes5 [131], a transcriptional inhibitor that blocks differentiation. In MS plaques, fibronectin levels are increased due to secretion by astrocytes and leakage because of BBB damage, and aggregated fibronectin inhibits oligodendroglial differentiation and remyelination [132].

5.6.4 Microglia/Macrophage Pathology

In the white matter, active and chronic-active inflammatory lesions are accompanied by macrophages and activated microglia. In the cortical gray matter, diffuse microglial activation is present without visible inflammatory infiltrates. Macrophages and resident microglia are thought to play major roles in demyelination

lesion formation through re-stimulation of T cells within the CNS and in CNS tissue damage and repair.

In the adult CNS, microglia constitute more than 10 % of all cells. Microglia are derived from extraembryonic yolk sac myeloid cells. Colony-stimulating factor 1 receptor (CSF1R) is a cell-surface receptor for the cytokines CSF1 and IL-34. CSF1R is usually expressed on monocytes and macrophages in the peripheral blood as well as on the surface of microglia in the CNS [133]. During fetal development, IL-34 signaling through CSF1R mediates the colonization of the CNS with yolk sac myeloid cells. These cells then lose surface markers characteristic of mononuclear phagocytes and are assumed to become microglia in adults [133]. CSF1 on CSF1R signaling is associated with survival, proliferation, regulation, and differentiation of microglia [134].

In the resting state, microglia have small bodies with extensively branched processes and are termed "ramified microglia." Microglia expressing CD11b, ionized calcium-binding adapter molecule 1 (Iba1), and CD68 constantly monitor the CNS environment [133]. Upon activation, the soma enlarges, processes retract, and myeloid cell markers are enhanced. These cells are termed "amoeboid microglia." Microglia are the only cells that express CX3CR1 in the CNS. CX3CR1-deficient mice develop severe EAE and increased neuron loss in a transgenic model of ALS [135]. Its ligand, CX3CL1, is produced by neurons and downregulates microglial neurotoxicity. A lack of CX3CL1 input from neurons rapidly activates microglia [133]. Furthermore, plasma fibrinogen extravasated from a disrupted BBB also can activate microglia [133]. Activated microglia produce numerous cytokines/chemokines, growth factors, reactive oxygen and nitrogen species via oxidative burst, and inducible nitric oxide synthase. Activated microglia can express major histocompatibility complex (MHC) class II molecules and co-stimulatory molecules. However, they never traffic to the draining lymph node, unlike dendritic cells in other tissues.

In the CNS, perivascular and meningeal macrophages act as major antigen-presenting cells to restimulate T cells. Without restimulation by relevant antigens, T cells do not invade into the CNS parenchyma by disrupting 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 [136]. CSF CCL2 levels are decreased in MS [66], presumably because it is taken up by infiltrating cells [137]. CCR2-deficient mice develop mild EAE with neutrophil infiltration [138]. Thus, macrophages play major roles in antigen presentation and tissue destruction, while microglia induce tissue damage but also exert neuroprotective functions through phagocytizing tissue debris and producing neurotrophic substances.

5.6.5 *Astroglial Pathology*

Astrocytes normally have neuroprotective functions; however, in inflammatory circumstances, they become neurotoxic.

5.6.5.1 Neuroprotective Aspects

Astroglia extend numerous processes, forming highly organized domains with little overlap between adjacent cells. Astroglia appose each other and interconnect via connexin 43 (Cx43) gap junction channels to form functional networks. Highly ramified protoplasmic astrocytes in the gray matter ensheath synapses, forming tripartite synapses, while fibrous astrocytes in the white matter cover the nodes of Ranvier [139]. Astrocyte end feet have close contact with parenchymal basement membrane around vessels, contributing to maintenance of the BBB through induction of tight junctions between endothelial cells [140]. Astroglia also produce components of the extracellular matrix, such as collagens, laminins, fibronectins, hyaluronan, chondroitin sulfate, and heparin sulfate [141–143], which constitute the basal lamina around vessels. Astroglia constitutively express the membrane-bound death ligand, CD95L, and can induce CD95L-mediated apoptosis of infiltrating T cells [144, 145]. Astroglia also secrete tissue inhibitors of metalloproteinases (TIMPs), thereby limiting disruption of the basement membrane and extracellular matrix by MMPs secreted by infiltrating T cells [146].

Ablation of proliferating astroglia exacerbates EAE and is associated with a massive infiltration of macrophages and T cells [147], suggesting critical roles of astroglia in preventing the expansion of inflammation. We demonstrated that cerebrospinal fluid levels of angiotensin and angiotensin-converting enzymes produced and secreted from astrocyte end feet were significantly decreased in NMO [148] and MS [149, 150] patients, suggesting that injury to astrocyte end feet and dampening of astrocytic barrier functions may occur in both MS and NMO.

Astroglia can produce a variety of growth factors that promote oligodendrocytes to form myelin [151] by influencing OPCs [152]. IL-6 and transforming growth factor (TGF)-β produced by activated astrocytes may promote neuroprotection [153]. Ablation of astroglia in glial fibrillary acidic protein (GFAP)-thymidine kinase transgenic mice using ganciclovir induced a failure of damaged myelin removal by decreasing microglial activation during cuprizone-induced demyelination [154]. Therefore, astroglia can deliver signals to microglia to clear myelin debris, thereby contributing to the regenerative process.

5.6.5.2 Neurotoxic Aspects

Activated astroglia become hypertrophic and increase expression of GFAP. Activated astroglia produce cytokines/chemoattractants as well as adhesion molecules

for lymphocyte trafficking. Astroglia produce various pro-inflammatory cytokines, such as IL-1, IL-6, IL-12, IL-15, IL-23, IL-27, IL-33, CCL2 (MCP-1), CCL5 (RANTES), CXCL8 (IL-8), CXCL10 (IP-10), and CXCL12 (SDF-1). IL-12, IL-23, and IL-27 are essential for inducing Th1 and Th17 cells [155–157], while IL-15 is crucial for the activation of encephalitogenic CD8+ T cells [158]. CCL2 is a critical chemokine that attracts peripheral blood macrophages into the CNS [138]. In addition, astroglia can express VCAM-1 and fibronectin CS-1, which are upregulated in MS lesions [159, 160]. α4β1 integrin expressed on T cells interacts with its receptors, VCAM-1 and CS-1, thereby enabling T cells to traffic from the perivascular regions to the CNS parenchyma [161]. Astroglia express MHC class II and co-stimulatory molecules, such as B7-1, B7-2, and CD40 [162], depending on the presence of IFN-γ, TNF, and IL-1β [163]. Thus, astroglia may present CNS antigens to T cells in the context of MHC class II molecules. Astroglia also produce inducible nitric oxide synthase (iNOS) via effects of endoplasmic reticulum stress chaperones [164], leading to the production of superoxide anion and peroxynitrite, which can damage oligodendrocytes with low antioxidant levels [165].

5.7 Immunology and Hypothetical Mechanism for Lesion Extension

5.7.1 T Cells

An autoimmune mechanism of MS has been hypothesized based on the following findings: (1) an increased frequency of T cells showing inter- and intramolecular epitope spreading against myelin proteins; (2) increased levels of interferon (IFN)-γ, interleukin (IL)-17, and downstream pro-inflammatory cytokines in the CSF; (3) exacerbation of disease following the administration of IFN-γ; (4) increased frequency of T helper 1 (Th1) cells secreting IFN-γ and Th17 cells secreting IL-17 at relapse [69, 94, 166].

Perivascular infiltrates mainly consist of CD4+ T cells, while clonally expanded CD8+ T cells dominantly infiltrate in the parenchyma [167]. The roles of CD8+ T cells remain to be established; however, CD8+ T cells outnumber CD4+ T cells in MS lesions. In EAE, myelin antigen-specific CD4+ T cells can transfer EAE to naïve animals, and inflammatory foci are produced by a CD4+ T-cell-mediated process, while B cells and plasma cells represent a relatively minor component of inflammatory infiltrates [168]. Thus, it is hypothesized that in MS, naïve T cells are sensitized by myelin antigens in the peripheral lymph nodes, such as the deep cervical lymph nodes, and differentiate into myelin antigen-specific Th1 or Th17 cells. These peripherally activated Th1 or Th17 cells express adhesion molecules that allow them to pass through the BBB (Fig. 5.4). In EAE, adoptively transferred

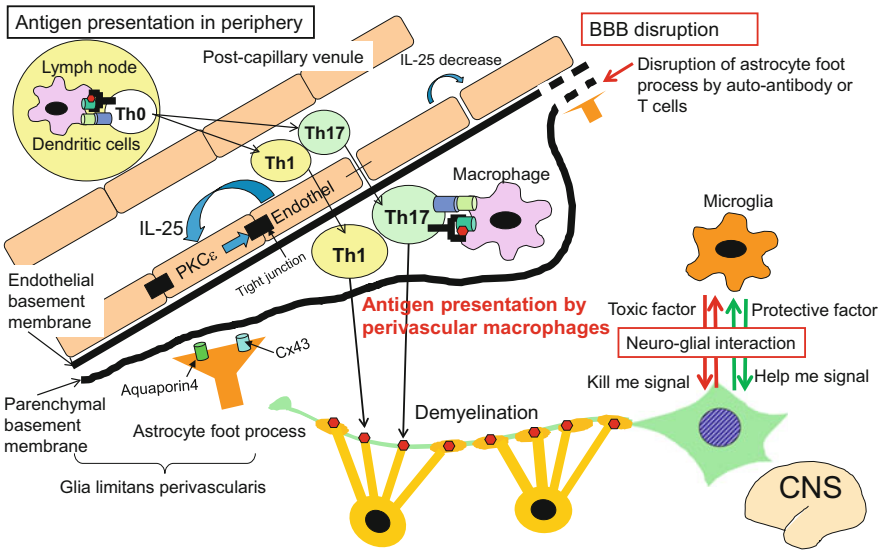

Fig. 5.4 Th1 and Th17 cell infiltration into central nervous system tissue. Naïve T cells are stimulated with relevant antigens by antigen-presenting cells, such as dendritic cells in peripheral lymph nodes, and differentiate into antigen-specific Th1 or Th17 cells. Such activated Th1 or Th17 cells expressing adhesion molecules firmly adhere to the surface of vascular endothelial cells and pass through the blood-brain barrier. These cells are restimulated by peripheral blood-borne macrophages in the perivascular space delineated by the endothelial basement membrane and the parenchymal basement membrane. Subsequently, restimulated T cells disrupt the basement membrane and deeply invade into the CNS parenchyma, secreting matrix metalloproteinase-2 and metalloproteinase-9

myelin antigen-specific T cells reside in bronchus-associated lymphoid tissue for several days and become eligible to enter the CNS [169].

T cells egress from postcapillary venules (high endothelial venules) and enter into the Virchow-Robins space (perivascular space) in the CNS. Here, activated T cells can firmly adhere to the surface of vascular endothelial cells via interactions between α4β1 integrin expressed on activated T cells and vascular cell adhesion molecule 1 (VCAM-1) on endothelial cells lining the BBB. Anti-α4β1 integrin antibody, natalizumab, effectively blocks firm adhesion of T cells, thereby markedly suppressing MS relapses [170]. Thus, peripherally activated T cells can cross endothelial cells, either transcellularly or paracellularly, to reside in the perivascular space delineated by the endothelial basement membrane and the parenchymal basement membrane, which is an extension of the subarachnoid space [171, 172]. T cells require restimulation by perivascular macrophages to further invade into the CNS parenchyma across the glia limitans perivascularis, which is composed of parenchymal basement membrane and astrocyte end feet [171, 172]. Perivascular macrophages are continuously repopulated from the peripheral blood stream and 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. The macrophages then present these antigens to T cells [173]. Subsequently, restimulated T cells secrete matrix metalloproteinase-2 and matrix metalloproteinase-9, which disrupt the basement membrane leading to destabilization of astrocyte end feet anchored to the parenchymal basement membrane, and promote T-cell entry into the CNS parenchyma [171]. Once in the CNS parenchyma, T cells secrete pro-inflammatory cytokines and chemokines that further recruit macrophages, neutrophils, and activated microglia, which then serve as effectors for tissue destruction.

The ability of natalizumab to markedly suppress relapses supports the critical importance of T cells in CNS inflammation at relapse. However, according to MS pathology, there is still considerable debate as to whether T-cell infiltration is a primary event or secondary to oligodendroglial apoptosis and subsequent microglial activation. Barnett and Prineas [174] reported oligodendroglial apoptosis without lymphocyte infiltration in autopsied cases with very early MS and proposed that oligodendroglial apoptosis preceded the formation of all MS lesions and BBB "leakiness," while microglial activation and T-cell infiltration were secondary events. However, factors causing initial oligodendroglial apoptosis remain totally unknown.

5.7.2 B Cells

Plasma cells are few in the CNS during the early stages of MS but become increasingly prominent as the disease progresses. Furthermore, as disease duration increases, the prevalence of CSF OBs also increases [175]. B cells exist in the perivascular areas and leptomeninges during all disease stages, but are rarely found in the CNS parenchyma [114]. Autoantibody and complement-mediated myelin phagocytosis are assumed to be the dominant mechanisms in established MS lesions [113]. In the leptomeninges, ectopic lymphoid follicle-like structures have been observed in approximately 40 % of postmortem SPMS cases [113, 114]. These follicle-like structures consist mainly of CD20+ B-cell aggregates interspersed with CD21+ CD35+ follicular dendritic cells, CD4+ T cells, and CD8+ T cells. They predominantly exist in the deep cerebral sulci [113, 114]. The majority of such meningeal lymphoid follicle-like structures are closely associated with large subpial demyelination [113, 114]. MS cases with meningeal lymphoid-like structures have a younger age at disease onset, a shorter time to wheelchair-bound disability, and a shorter time to progression compared with cases without meningeal lymphoid-like structures [113, 114].

The importance of B cells in MS is demonstrated by the fact that rituximab, which targets CD20 molecules expressed on B cells but not plasma cells, is highly efficacious in suppressing MS relapses [176]. In rituximab trials, B-cell numbers decreased in parallel with a reduction in the number of relapses, whereas total antibody levels did not decrease significantly. It is thus assumed that B-T cell

interactions, including antigen presentation or pro-inflammatory cytokine secretion by B cells, are the critical step inhibited by rituximab, but not the inhibition of autoantibodies themselves. Rituximab is also effective in NMO but without reducing anti-AQP4 antibody levels [177], suggesting that B-T cell interactions and B-cell cytokines may also be critical in NMO. Highly specific autoantibodies in MS pathology remain to be identified. Autoantibodies against myelin oligodendrocyte glycoprotein (MOG) have been detected in children with atypical demyelinating disease and in a fraction of anti-AQP4 antibody-seronegative NMO patients [178, 179], but not in adult MS cases. The significance of anti-glycolipid antibodies and a recently described autoantibody against KIR4.1, an ATP-sensitive inwardly rectifying potassium channel expressed in astroglial end feet and oligodendroglia [180], need further confirmation in large-scale independent cohorts.

5.7.3 Hypothetical Mechanism for Lesion Extension

T-cell infiltration initially occurs in relatively limited areas around blood vessels, while demyelination eventually evolves to encompass large areas. Lesion extension mechanisms in MS remain to be established. We focused on connexins (Cx) which form homotypic or heterotypic gap junctions between astrocytes, or between astrocytes and oligodendrocytes. Gap junctions appose two cells and form channels for direct intercellular communication through which intracellular second messengers, such as calcium ions and energy sources such as lactate, are exchanged. Astrocytic Cx43 and Cx30, oligodendrocytic Cx32 and Cx47, and astrocytic Cx43 and oligodendrocytic Cx32 double-knockout mice showed widespread demyelination [181–183]. It was demonstrated that mice lacking Cx43/Cx30 in GFAP-positive astrocytes displayed astrocyte end feet edema and a partial loss of AQP4 [184]. Thus, astrocytic and oligodendrocytic Cxs may play critical roles in maintaining CNS myelin.

Recently, we showed the extensive loss of Cxs 43, 32, and 47 in demyelinated and myelin-preserved layers of acute lesions in patients with Baló's concentric sclerosis, a rare extremely severe variant of MS [185]. In the leading edge areas, where the expression of myelin-associated glycoprotein (MAG) was partly diminished but other myelin proteins were well preserved, compatible with distal oligodendrogliopathy, astrocytic Cx43 was totally lost. We also found similar changes in extensive lesions of MS and NMO cases that died within 2 years after disease onset (Fig. 5.5) [186]. A significant reduction of Cx32 and Cx47 occurs in active lesions of MOG-induced EAE [187]. In MOG- and myelin basic protein-induced EAE [188], astrocytic Cx43 was also diminished in active lesions, suggesting that myelin antigen-specific T cells may down-modulate Cx expression in oligodendrocytes and astrocytes. In the healthy state, Cx43 on astrocytes apposes Cx47 on oligodendrocytes, forming astrocyte-oligodendrocyte (A/O) gap junctions. A/O gap junctions are important for intercellular communication through this channel. In addition, glucose and lactate are taken up from the blood stream by

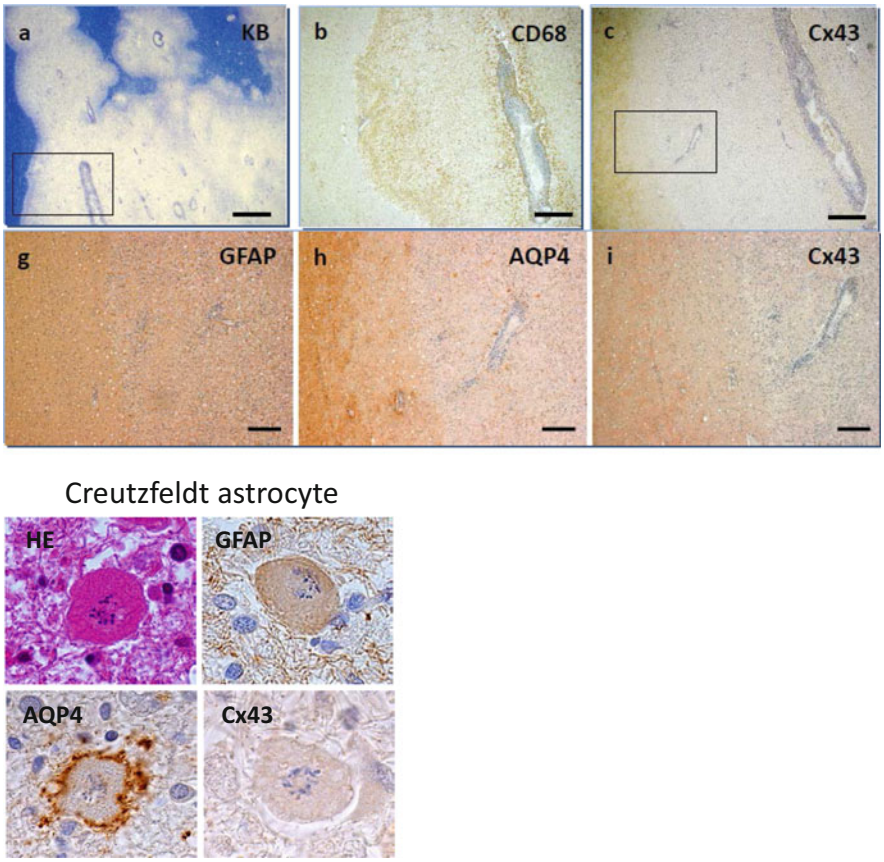

Fig. 5.5 Widespread loss of connexin 43 in extensive demyelinating lesions in MS patients. Extensive demyelinating lesions in a 29-year-old female patient with MS. She was negative for anti-AQP4 antibodies. Note that active demyelinating lesions show diffuse Cx43 loss and patchy loss of AQP4 while GFAP staining is preserved. Higher magnification (*lower panel*) of a lesion demonstrates bizarre-shaped Creutzfeldt astrocytes that express GFAP and AQP4 but not Cx43

astrocyte end feet through glucose transporter and monocarboxylate transporter (MCT) and transferred from astrocytes to oligodendrocytes via Cx gap junction channels and further from oligodendrocytes to axons via MCT1/MCT2. We observed a marked reduction of MCT4 on the astrocyte end feet in MS and NMO lesions (manuscript in preparation). Therefore, disruption of A/O gap junctions and down-modulation of MCT may destroy energy flow from the blood stream to axons by dysfunction of the glia syncytium, thereby inducing widespread oligodendroglial and axonal damage.

5.8 Conclusion

Although our understanding of the pathogenesis of MS has increased remarkably in recent years, its etiology remains to be established. Recently developed DMDs can suppress MS relapse; however, even with the most potent DMDs, such as alemtuzumab, disability still progresses. The mechanism of the progressive phase remains totally unknown, and its elucidation and the development of DMDs to control this phase are a major challenge for the future.

Conflict of Interest Statement Jun-ichi Kira is an advisory board member for Merck Serono and a consultant for Biogen Idec Japan. He has received payment for lectures from Bayer Schering Pharma, Cosmic Cooperation, and Biogen Idec Japan.

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Chapter 6
Multiple Sclerosis: Diagnosis and Treatment

Makoto Matsui

Abstract Multiple sclerosis (MS) is a demyelinating inflammatory disease of the central nervous system (CNS) with an etiology that has yet to be fully elucidated. However, progress in basic immunology and technology over the past 20 years has enabled early diagnosis and initiation of treatment. For diagnosing a patient with MS, multiplicity of lesions in the CNS (i.e., dissemination in space), recurrent disease relapses during the course of illness (i.e., dissemination in time), and the presence of immunological disturbance should be determined. Diagnostic criteria have been improved and the latest international version of McDonald's criteria in 2010, which utilizes magnetic resonance imaging (MRI) findings, has promoted earlier treatment with disease-modifying drugs (DMDs). Injectable drugs including interferon-β and glatiramer acetate still hold their position as first-line therapy, while oral fingolimod and intravenous natalizumab are second line, even though they are more effective, because the long-term safety profiles remain unknown. To achieve disease activity-free status (DAFS), which includes the combination of no clinically apparent relapses, no new MRI activity, and no progression of disability, physicians must carefully select DMDs according to the disease activity status and social circumstances of each patient. Should clinical relapse occur despite preventive treatment, therapy for ameliorating acutely exacerbated symptoms and signs (pulsed steroid therapy and/or plasmapheresis) should be started as soon as possible.

Keywords Multiple sclerosis • Diagnosis • Treatment • Disease activity • Disease-modifying drug

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6.1 Diagnosis of Multiple Sclerosis

6.1.1 General Concept

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) in which autoimmune mechanisms targeting CNS myelin components are implicated. For diagnosing a patient with MS, three different findings are required, multiplicity of lesions in the CNS, i.e., dissemination in space (DIS); recurrent disease flare-ups during the course of illness, i.e., dissemination in time (DIT); and immunological disturbance. Diagnostic criteria have been improved in association with advances in immunology and radiology. The previously well-known Schumacher's criteria [1] were based on two or more episodes of manifestations of clinically apparent symptoms and signs. Thereafter in the 1980s, Poser's criteria [2] were helpful for diagnosis by adopting electrophysiological examinations to detect CNS lesions and cerebrospinal fluid (CSF) tests for checking aberrant immunity in the CNS. The latest international version of McDonald's criteria [3] utilizes magnetic resonance imaging (MRI) findings and enables earlier diagnosis for early initiation of disease-modifying treatment (DMT). However, those still indicate the need for careful differential diagnosis. Therefore, even in this era of scientific progress that includes decoding of the entire human genome, no specific means to diagnose MS has been established and physicians remain challenged by the task.

6.1.2 History Taking

History taking is one of the most important methods available to reach a correct diagnosis. Lesions in the CNS are vulnerable to MS, some of which cause characteristic symptoms and signs, as described in the following section. Physicians should interview their patients in regard to past or present episodes of blurred vision, double vision, disturbed articulation, clumsiness with a hand, unilateral weakness or sensory disturbance of the extremities, band-like sensation in the trunk, and gait unsteadiness. Related neurologic dysfunctions tend to occur either suddenly or gradually, and then worsen for a few days or weeks to reach the peak level of disability; after which the symptoms and signs will likely disappear within several weeks. Thus, spontaneous improvement of a neurologic deficit is a feature of this demyelinating disease because of the natural remyelination process. Physicians should attempt to determine the occurrence of another episode showing different symptoms or signs that last for at least 24 h and separated from the previous one by more than 1 month. If such is noted, then the patient may have relapsing-remitting MS (RRMS) and further examinations are needed. This is how DIS and DIT can be distinguished by interview.

It is known that 30–40 % of patients with RRMS will show a progressive course over 10 years of disease duration (secondary progressive MS, SPMS) [4]. Although that study clarified the natural course of RRMS in Caucasian patients, the same type of RRMS course seems to be prevalent in Japanese patients [5]. On the other hand, approximately 10–15 % of patients show added neurological deficits during the entire course of illness (primary progressive MS, PPMS). Disease course can only be elucidated by detailed interview findings, except when long-term neurological follow-up results are available. It is also important to find red flags [6] related to a diagnosis of MS when obtaining a history of illness (Table 6.1).

Table 6.1 Differential diagnosis of MS and major red flags

		Major red flags		
	Headache	Neuropathy	Rash	Diabetes insipidus
1. Acquired demyelination of the CNS				
Inflammation				
Multiple sclerosis				
Neuromyelitis optica				○
Acute disseminated encephalomyelitis	○			
Infection				
Progressive multifocal leukoencephalopathy				
Toxic leukoencephalopathy				
Anticancer drugs (e.g., 5-FU)				
2. Impaired myelination of the CNS (leukodystrophies)				
Adrenoleukodystrophy (X-linked)		○		
Metachromatic leukodystrophy (AR)		○		
Krabbe's disease (AR)		○		
Pelizaeus-Merzbacher disease (X-linked)				
3. Multiple white and gray matter lesions in CNS				
Inflammation				
Systemic lupus erythematosus	○		○	
Sjögren's syndrome		○		
Antiphospholipid antibody syndrome				
Vasculitis of any kind	○	○	○	
Behçet's disease	○			
HTLV-I-associated myelopathy				
Infection				
Lyme disease	○	○	○	
Whipple's disease				
Sarcoidosis	○	○		○
Lymphoma	○		○	
Mitochondrial disease	○			

AR autosomal recessive, *CNS* central nervous system (Partially adapted from Katz Sand and Lublin [6])

6.1.3 *Evidence of Multiplicity of Lesions or Dissemination in Space (DIS)*

6.1.3.1 Neurological Examination

A history of recurrent episodes shown by neurologic symptoms and signs is not adequate to confirm a diagnosis of MS, as the presence of lesions localized in the CNS must be determined according to the results of a neurological examination. Although the spinal cord and visual pathways are major sites of involvement, symptoms at disease onset and those experienced during the course of illness appear to be different among races. Ataxia due to cerebellar lesions is a common feature in Western countries, while Charcot's triad, including nystagmus, intention tremor, and scanning speech, is also well known [7]. However, in Japan, less than half of affected patients exhibit ataxia over the entire course, and visual disturbance is more common at disease onset than in Western countries [8]. When performing neurological examinations, it is important to pay attention to racial differences.

Although not a contributor to MS diagnosis, physicians should be alert to any change in patient neurological status at every visit to the outpatient clinic using Kurtzke's expanded disability status scale (EDSS) [9]. This is the only way to assess the occurrence of progression of disability, which is defined as a sustained increase in EDSS scores.

6.1.3.2 Characteristic Signs and Symptoms

Relatively afferent pupillary defect (RAPD) is frequently observed in patients suffering from acute unilateral optic neuritis or its residual deficit. When an examiner stimulates the normal eye with a bright penlight, constriction of the pupil is seen on both sides. Then, rapidly moving the light from the normal eye to the other side results in paradoxical dilatation of the pupil on the affected side. Fundoscopy may be able to detect past damage to the optic nerve, characterized by a pale-colored optic disk, termed optic atrophy.

Internuclear ophthalmoplegia (INO) is highly suggestive of MS if seen bilaterally, as it indicates the presence of two or more tiny lesions in the brainstem. In a lateral gaze to one side, abduction of the eye on that side is accompanied by coarse jerky nystagmus, while neither synchronous adduction of the other eye nor nystagmus occurs. However, when the abducted eye is covered, the patient can adduct the other eye to some extent by following a target. INO is manifested when the bundle connecting paramedian pontine reticular formation (PPRF) on the side of lateral gaze and the opposite third-nerve nucleus innervating the medial rectus muscle, i.e., the medial longitudinal fasciculus (MLF), is specifically damaged. Therefore, INO is called MLF syndrome.

Lhermitte's sign is regarded as positive when the patient experiences a rapid and transient worsening of tingling sensation or an electric shock-like sensation passing

down the back to the lower extremities when the neck is flexed. This phenomenon is thought to occur because of ephaptic transmission of nerve impulses between denuded axons in the dorsal column of the cervical spinal cord, thereby indicating the presence of a demyelinating process at the site.

Band sensation, which is spontaneously noted as if a tight bandage was pulled around or a hard board inserted into the lower thorax or abdomen, sometimes afflicts patients when demyelinating lesions develop in the dorsal column of the thoracic spinal cord.

Painful tonic spasm is an annoying symptom experienced during the recovery stage of acute myelitis rather than at the peak stage of illness. The phenomenon is usually manifested as painful tonic contractions in a limb that rapidly spread to the arm or leg on the same side. These may be elicited by movement or touch sensation and last less than 2 min. Ephaptic transmission of nerve impulses in the affected spinal cord has also been implicated.

Uhthoff's phenomenon is manifested as a worsening of previous symptoms, or development of new symptoms and signs when the body temperature of the patient rises slightly in association with daily activities including exercise, a hot shower, and sun exposure. The phenomenon usually disappears when the temperature is lowered, though can be irreversible in some cases. It results from a transient conduction block of nerve impulses among demyelinated axons under the condition of elevated temperature.

6.1.3.3 Magnetic Resonance Imaging (MRI)

MRI is an epoch-making noninvasive tool that has fundamentally changed diagnostic strategies, as it is able to visualize demyelinating lesions in the CNS. T1-weighted (T1W) and T2W images, fluid-attenuated inversion recovery (FLAIR) imaging, and gadolinium-enhanced T1W imaging are helpful for assessing the nature of those lesions. Demyelinated lesions are usually detected as a high-signal area with T2W and FLAIR images, whereas signal intensity shown by T1W imaging appears to be the same as that of normal appearing white matter. However, an area of low signals shown in T1W images, termed a T1 black hole, reflects severe demyelination or substantial axonal damage [10]. The site of breakdown of the blood-brain barrier (BBB) indicating the presence of inflammatory process can be visualized as an enhanced area using gadolinium-DTPA (diethylenetriamine pentaacetic acid) by T1W MRI. The half-life of gadolinium enhancement is about 3 weeks; thus, acute inflammatory lesions can be detected when enhanced MRI is performed within 3–6 weeks of active disease. Open-ring enhancement is more likely to be due to active demyelination than the presence of a neoplasm or infection [11]. It has been reported that only about 10–20 % of active lesions in the brain detected by MRI cause clinical relapse [12], whereas about 50 % of cases with those in the spinal cord develop clinical symptoms.

Important features of demyelinating lesions are visualized by MRI on the basis of their location and shape, which serve as a clue to a diagnosis of MS. Since

demyelination tends to occur near the ventricles, periventricular lesions are frequently seen. Typical ovoid lesions are oval shaped and project from periventricular regions with the long axis parallel to vertical to the rim of the lateral ventricles (Fig. 6.1). Juxtacortical lesions that are located in the subcortical area but not extending to the cortex (U-fiber sparing) will support the diagnosis, as that finding is included in McDonald's criteria. The presence of lesions in the corpus callosum

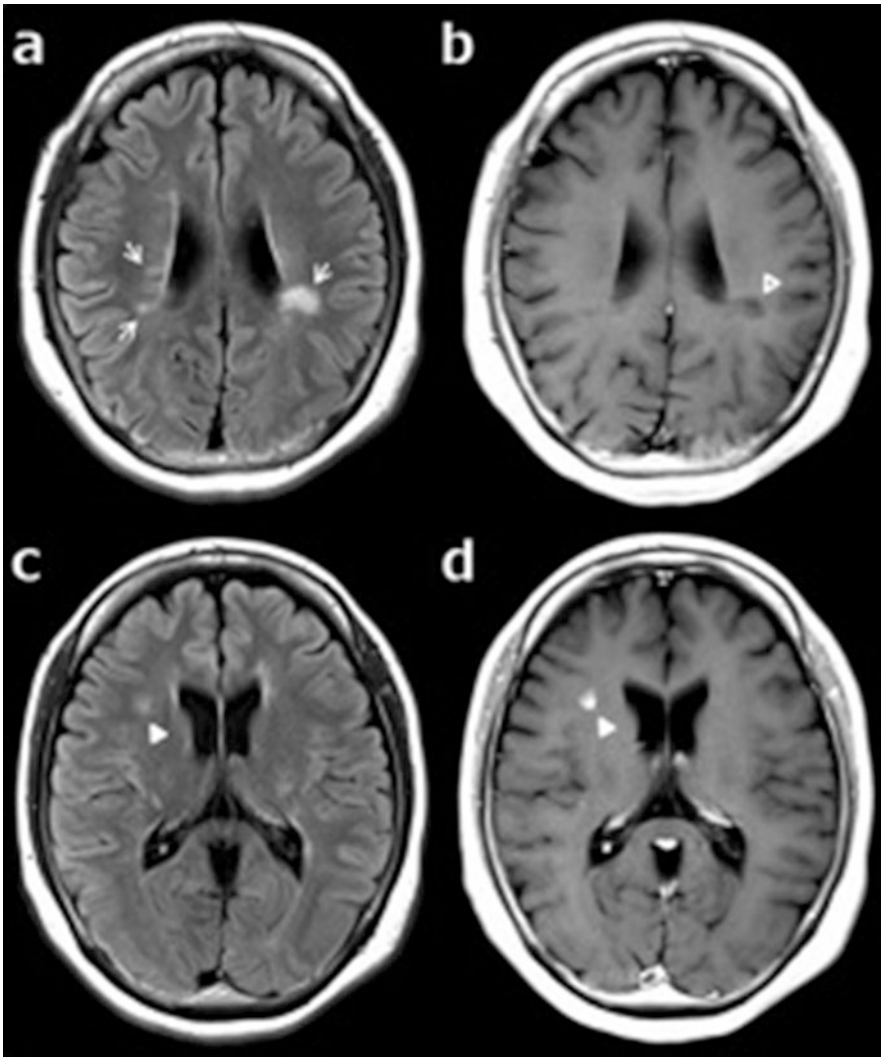

Fig. 6.1 MRI profile of MS plaque. (**a**) *Arrows* show ovoid lesions in a FLAIR image, (**b**) one of which has formed a T1 black hole (open *arrowhead*) shown by T1-weighted imaging. (**c**) High-signal lesion (*arrowhead*) in a FLAIR image showing (**d**) gadolinium enhancement

is helpful for differential diagnosis, as that portion is rarely involved in vascular disease.

Spinal cord lesions are better detected with T2W imaging using a 1.5 Tesla as compared to a 3.0 Tesla MRI scanner. Typical MS lesions are visualized as a round, oval, or wedge-shaped high-signal area in axial sections [13]. A central lesion location and lesions extending over three or more vertebral segments suggest the presence of neuromyelitis optica (NMO) pathology [14], though not exclusively [13].

6.1.3.4 Evoked Potentials

The clinical relevance of evoked potential techniques including visual evoked potentials (VEPs), somatosensory evoked potentials (SEPs), and brainstem auditory evoked potentials (BAEPs) has been surpassed by findings provided by MRI. However, they still have importance for MS based on their potential to detect the presence of clinically unsuspected demyelinating lesions in visual, spinal sensory, and auditory pathways [15]. The principle of these tests is based on the assumption that nerve impulses are delayed at the site of demyelination. Therefore, the presence of lesions is judged from the delay of negative (upward) or positive (downward) peaks to the estimated time of latency.

A VEP examination should be routinely performed for diagnosis of MS. Pattern reversal stimulation is the most sensitive method for detecting a substantial delay of the positive peak that usually appears with a latency of 100 ms (P100), as shown in Fig. 6.2. The criterion for prolonged latency should be determined by each institution using data obtained from normal subjects.

6.1.4 Evidence of Immunological Abnormality

Immunological tests specific for MS have yet to be established, though autoimmune mechanisms against CNS myelin components including myelin basic protein (MBP) are considered to play a key role (see Chap. 5). Therefore, immunological measurements cannot be used to determine a final diagnosis of MS, though are helpful to assess disease activity [16]. An elevated MBP level in the CSF is also useful for detection of ongoing demyelinating processes in the CNS during an acute neurological relapse.

The presence of oligoclonal IgG bands (OBs) in the CSF serves as one of the criteria required for diagnosis of MS [2, 3]. Positive results reflect expansion of a few plasma cell clones secreting a substantial amount of IgG in the CNS. The nature of those IgGs is restricted, and the recommended method of isoelectric focusing with IgG immunodetection for CSF samples yields a few bands of IgG, whereas employment of the same method for serum samples only shows a monotonous pattern [17]. OB thus indicates B cell activation in the CNS. In this regard, an

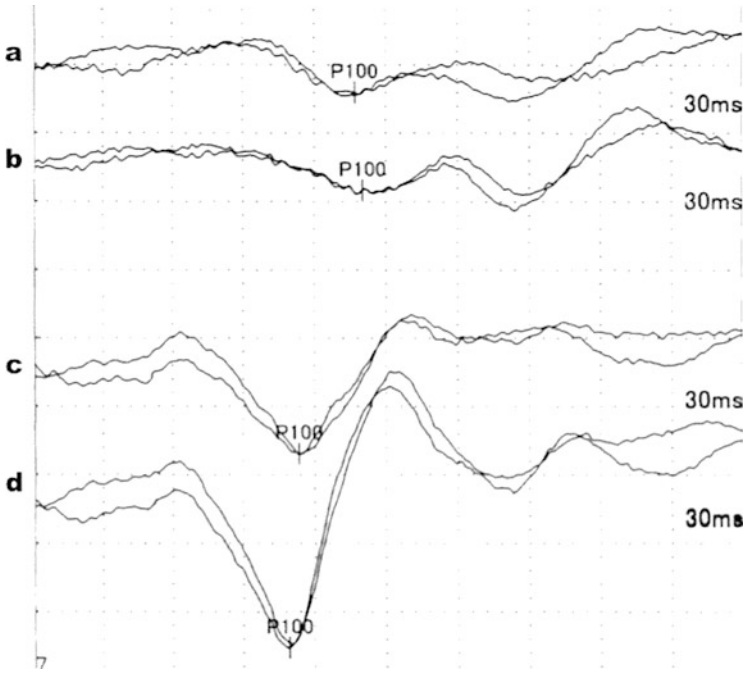

Fig. 6.2 Visual evoked potentials caused by pattern reversal stimulation in representative patient with right optic neuritis. Stimulation to the right eye revealed a delay in P100 latency (approximately 138 ms) when recorded at the (**a**) *left* and (**b**) *right* occiput, while that to the left eye yielded a normal P100 response (approximately 110 ms) in recordings from the (**c**) *left* and (**d**) *right*

elevated IgG index can be substituted for OB, which is calculated by the following formula: $IgG_{csf}/IgG_{serum} \times Albumin_{serum}/Albumin_{csf}$. It is of note that while OB is highly diagnostic in Western countries, physicians may not necessarily be able to rely on this test in Japan and likely in other Asian countries as well, because of its low sensitivity [18].

6.1.5 Diagnostic Criteria

In summary, diagnostic procedures for MS verification of DIS as well as DIT are mandatory. Patient history provides important clinical information regarding both DIS and DIT if the symptoms result from a typical acute inflammatory demyelinating event in the CNS. For example, if the patient can recall a past event in which acute blurring of vision in one eye accompanied by retro-orbital pain lasted for 2–4 weeks and then gradually improved, that serves as evidence of a single attack (optic neuritis). Results of currently available neurological and evoked potential examinations can provide firm evidence for DIS but not DIT. MRI is a powerful

tool for visualizing demyelinated lesions in at least two of four areas of the CNS (periventricular, juxtacortical, or infratentorial areas in the brain or spinal cord), thus confirming DIS. In addition, CSF test results can reveal immunological abnormalities and ongoing demyelination, supporting a diagnosis of MS.

In this context, for determining a diagnosis of MS for patients who have experienced their first demyelinating event (clinically isolated syndrome, CIS), there is no technique that can provide evidence of DIT except for a history of multiple attacks or waiting for the next clinically apparent event to occur. However, during the period prior to confirmation of diagnosis, irreversible pathological processes (axonal damage) will progress [19] and render patients at risk for accumulated subclinical disability. To address difficulties with making an early diagnosis, the revised version of McDonald's criteria has been proposed and is widely used (Table 6.2). With the latest version [3], DIT can be verified when a new T2 or gadolinium-enhanced lesion is shown by follow-up MRI as compared to the baseline images. Even results of a single MRI session can be used to diagnose MS if they show the simultaneous presence of asymptomatic gadolinium-enhanced and nonenhanced lesions, which are now regarded as a reflection of DIT, thus fulfilling the criteria for DIS described above. However, it should be noted that differential diagnosis remains a difficult task for physicians, as the purpose of McDonald's criteria is early recruitment of CIS patients in whom the etiology of the first event has already been attributed to demyelination for early therapy for MS.

6.1.6 Differential Diagnosis

Considering the various events related to development of illness and also focusing on disorders affecting myelin or white matter in the CNS, diseases to be differentiated from MS are listed in Table 6.1. It is important to determine whether the patient possesses an NMO background, especially members of Asian and Latin American populations, in whom NMO is more prevalent than in Western populations. The revised McDonald's criteria recommend that patients with the following features be tested for serum anti-aquaporin-4 antibodies: (1) myelopathy associated with spinal cord lesions extending over three or more vertebral segments in MR images, (2) bilateral or severe optic neuritis with a swollen optic nerve or chiasm lesion or an altitudinal scotoma, and (3) intractable hiccough or nausea/vomiting lasting for more than 2 days with the presence of periaqueductal lesions in medulla oblongata in MR images [3].

Table 6.2 2010 McDonald's criteria for diagnosis of MS

Clinical presentation	Additional findings needed to confirm MS diagnosis
≥2 attacks, objective clinical evidence of ≥2 lesions or objective clinical evidence of one lesion with reasonable historical evidence of a prior attack	None
≥2 attacks, objective clinical evidence of one lesion	Dissemination in space, demonstrated by ≥1 T2 lesion in at least two of four MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord) or awaiting a further clinical attack implicating a different CNS site
One attack, objective clinical evidence of ≥2 lesions	Dissemination in time, demonstrated by the simultaneous presence of asymptomatic gadolinium-enhanced and nonenhanced lesions at any time, new T2 and/or gadolinium-enhanced lesion(s) shown by follow-up MRI irrespective of its timing with reference to a baseline scan, or awaiting a second clinical attack
One attack, objective clinical evidence of one lesion (clinically isolated syndrome)	Dissemination in space and time, demonstrated by the following
	For DIS: ≥1 T2 lesion in at least two of four MS-typical regions of the CNS or awaiting a second clinical attack implicating a different CNS site
	For DIT: the simultaneous presence of asymptomatic gadolinium-enhanced and nonenhanced lesions at any time, new T2 and/or gadolinium-enhanced lesion(s) shown by follow-up MRI irrespective of timing with reference to a baseline scan, or awaiting a second clinical attack
Insidious neurological progression suggestive of MS (PPMS)	One year of disease progression (retrospectively or prospectively determined), plus two of the following three criteria
	1. Evidence for DIS in the brain based on ≥1 T2 lesions in MS-characteristic (periventricular, juxtacortical, infratentorial) regions
	2. Evidence for DIS in the spinal cord based on ≥2 T2 lesions in the cord
	3. Positive CSF (isoelectric focusing evidence of oligoclonal bands and/or elevated IgG index)

6.2 Treatment for Multiple Sclerosis

6.2.1 General Concept

The major purpose of treatment for MS is to achieve disease activity-free status (DAFS). The concept of DAFS is based on the assumption that activities of daily living (ADLs) will be maintained to keep a good quality of life (QOL) if the patient with MS is able to remain with that status throughout life [20]. DAFS is comprised of at least three aspects: no clinically apparent relapses, no new MRI activity (no new or enlarging T2 hyperintense lesions, no new gadolinium-enhanced lesions), and no progression of disability. In a phase III trial [21], administration of natalizumab, one of the most potent disease-modifying drugs (DMDs), kept only 37 % of treated patients free of relapse and MRI activity over a period of 2 years; thus, additional therapies are needed for accomplishing DAFS. Furthermore, it remains uncertain whether the absence of clinical relapse and MRI activity will lead to complete prevention of disability progression.

In clinical settings, physicians are usually engaged in adjustment of DMD therapy to the most favorable one according to disease activity in each patient. If clinical relapse occurs despite such effort, treatments for ameliorating acutely exacerbated symptoms and signs should be started as soon as possible.

6.2.2 Treatment During Acute Stage

6.2.2.1 Treatment Strategies

The main purpose of treatment during acute disease relapse is early termination of ongoing inflammation in the CNS and suppression of immune reaction. Although autoimmune responses are initiated in peripheral lymph nodes, further events leading to demyelination and subsequent destruction of adjacent CNS tissue require infiltration of immune cells including T cells, B cells, and macrophages. After breakdown of the BBB from inflammation, autoantibodies and complements have easy access to the CNS and are involved in propagation of immune reactions (see Chap. 5). Immune response related to CNS tissue damage has two paths, cell-mediated and humoral (antibody-mediated) immunity, and treatment options may be selected based on consideration of these aspects of the immunopathogenesis of MS lesion formation.

6.2.2.2 Corticosteroids

Corticosteroids have both anti-inflammatory and immunosuppressive effects. For acute-stage disease, the former effect is utilized by intravenous (iv) administration

of high-dose (500–1000 mg) methylprednisolone for 3–5 days (pulsed therapy), with or without a subsequent short-term tapering course of oral corticosteroids. This treatment strategy is considered to be effective for ongoing inflammation by suppressing the function of immune cells and blocking their cell entry into the CNS through the BBB.

Short-term effects of this therapy are usually evident, while adverse effects are minor, such as insomnia, gastric discomfort, and transient elevation of blood sugar level. Patient symptoms and signs begin to recover within a few days after starting this treatment, followed by further improvement after 3 or 4 weeks. However, when the attending physician notices that the initial treatment was less effective than expected, then pulsed therapy with iv methylprednisolone may be repeated after approximately 2 weeks in cases of acute illness. It is important to note that pulsed corticosteroid therapy is likely to facilitate recovery from acute exacerbation of the disease, though in light of results from a study of that treatment for optic neuritis, it is uncertain whether that therapy is able to reduce the peak neurological deficits [22]. If repeated pulsed therapy fails to substantially improve neurological disturbances in the patient, the physician should consider alternative treatment using plasmapheresis.

6.2.2.3 Plasmapheresis

The purpose of therapeutic plasmapheresis for MS is to remove large molecular weight particles such as IgG from the blood. Plasmapheresis is mainly performed using one of three techniques; simple plasma exchange, double filtration plasmapheresis, and immunoadsorption plasmapheresis. However, only the efficacy of simple plasma exchange has been verified by results of a randomized controlled trial [23]. The beneficial effects of this therapy are rendered via removal of circulating autoantibodies, immune complexes, and pro-inflammatory cytokines and chemokines [24]. Plasmapheresis is theoretically most efficient for patients in whom antibody-mediated immunity targeting CNS tissue components are predominant; thus, patients experiencing fulminant MS attacks showing an antibody-/complement-associated pathology in biopsy specimen findings [25] exhibit a good response.

Simple plasma exchange may be effective when performed within 3 months of acute relapse [23]. It is usually performed every 2 days or three times per week by processing 2–3 l of plasma each time until neurological improvement is achieved, with the reference exchange number being 7.

6.2.3 Prevention of Relapse and Progression of Disability

6.2.3.1 Treatment Strategies

There is no consensus about escalation and induction (de-escalation) therapy in regard to which to choose for securing a better prognosis in patients with MS [26, 27]. Escalation therapy has a long history in the field of autoimmune disorders, with interferon-β (IFN-β) and glatiramer acetate (GA) each used as first-line treatment for RRMS [26, 28]. If a particular patient shows a suboptimal response to one of those treatments, switching to another option may have favorable effects [29]; otherwise, escalation to second-line treatment such as fingolimod or natalizumab will be necessary [28]. Although controversy remains regarding the criteria used to judge suboptimal response to any particular therapy in individual patients, the following conditions showing disease activity despite current treatment may indicate a suboptimal responder: (1) $\geq$1 clinical relapse within 1 year of therapy; (2) worsening of EDSS score by 1 or more points during 2 years of therapy; and (3) detection of 2 or 3 changes in MRI images with any interval, including new gadolinium-enhanced, new T2, enlarged T2, new T1 hypointense, and enlarged T1 hypointense lesions [30].

In contrast, induction or de-escalation therapy using the potent immunosuppressive drug mitoxantrone or a monoclonal antibody preparation, such as alemtuzumab or natalizumab, is given in the very early phase of MS with the aim of resetting the immune system [26, 27], which hopefully prevents development of secondary progressive phase MS (SPMS). Once a patient has reached that stage, there is no reliable treatment option that can halt the disease process [31]. No biomarker has been established for assessment of axonal damage in the CNS that will likely be linked to actual patient disability [16]; thus, consensus about the superiority of those therapeutic concepts is lacking. At present, escalation therapy is generally accepted from a long-term risk/benefit point of view [28]. A useful panel has been proposed to assist physicians when prescribing MS therapeutic drugs according to reported risk/benefit profiles (Fig. 6.3) [32]. Brief profiles of some of those relevant drugs are described in the following sections. It must be noted that therapeutic evidence for these drugs has only been obtained from patients with RRMS, while those showing a progressive course (SPMS, PPMS) are waiting for future development of new drugs that can prevent neurodegenerative processes [31].

6.2.3.2 Interferon-β

Interferon-β (IFN-β) was the first DMD found to provide substantially preventive effects (30 % reduction) on MS relapse as compared to a placebo group and remains as a first-line treatment option because of its long-term safety profile [33]. Three types are available, with the route of administration being either subcutaneous

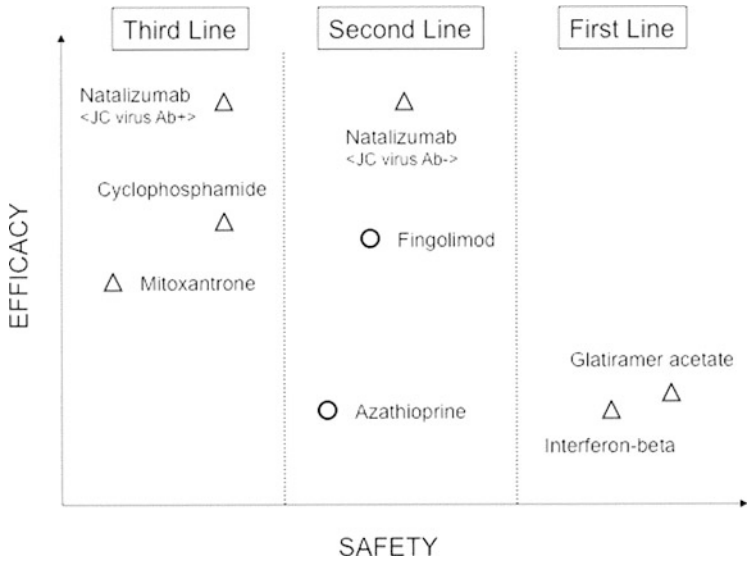

Fig. 6.3 Positions of MS therapeutic drugs (Adapted from Hauser et al. [32]). As off-label drugs differ in different countries, the drugs shown here are not categorized in this context. *Open triangles* show drugs administered by injection and *open circles* show oral drugs

(every 2 days or three times a week) or intramuscular (once a week) injection. Blockade of inflammatory cell entry to the CNS and shifting Th1-dominant immune responses to Th2 type have been postulated as its mechanisms of action. IFN-β exerts prominently suppressive effects on MRI activity. However, approximately one-third of patients with MS exhibit a suboptimal response to this therapy; thus, other treatment options are definitely required in clinical settings.

6.2.3.3 Glatiramer Acetate

Glatiramer acetate (GA), also known as copolymer 1, is a polypeptide with a molecular weight of 4700–13,000 Da that comprises randomly sequenced L-type alanine, L-glutamate, L-lysine, and L-tyrosine in molar ratios of 4.2, 1.4, 3.4, and 1.0, respectively [33]. GA is regarded as an immunological modifier to inhibit autoimmunity to MBP via cross-reactivity to that protein. The drug also seems to induce Th2 shift in immune responses. Its effects on annualized relapse rate (ARR) and MRI activity are nearly equivalent to those exerted by IFN-β, and GA remains as a first-line treatment choice for MS. The drug may have some advantages for patients exhibiting neutralizing antibodies to IFN-β or those who want to become pregnant [34], though daily subcutaneous injections are necessary.

6.2.3.4 Fingolimod

Fingolimod is a compound isolated from *Isaria sinclairii* that exerts its function as a modulator of sphingosine-1-phosphate (S1P) receptors. When fingolimod occupies the S1P receptors expressed on T and B cells, resultant internalization of those receptors inhibits egression of lymphocytes from secondary lymphoid organs. This seemingly simple mechanism of action has had a large impact on MS clinics, as fingolimod can be administered orally and results indicate a 60 % reduction in ARR [35]. However, the drug remains as second-line therapy, because of the many clinical requirements before and after the initial dose, as well as an unclear long-term safety profile [28]. Furthermore, special attention may be required for Asian patients, as those with an NMO background might develop severe symptoms with this therapy [35].

6.2.3.5 Natalizumab

Natalizumab is a monoclonal antibody specific to α4 integrin. Lymphocytes and monocytes enter the CNS by way of interaction of that surface molecule with vascular cell adhesion molecule-1 expressed on endothelial cells of vessels in the brain and spinal cord. With use of this drug, the entry of activated effector memory T cells and monocytes involved in inflammatory reactions in the CNS is blocked at the level of the BBB [31]. Although the drug may be able to provide DAFS in some patients [21] and has been reported to remarkably reduce ARR by more than 80 % [36], there is a risk of progressive multifocal leukoencephalopathy as a serious and life-threatening adverse effect. Therefore, natalizumab is regarded as a second-line treatment option for patients negative for the JC virus antibody and third line for those positive for that antibody [32].

6.2.3.6 Immunosuppressive Drugs

Mitoxantrone is a topoisomerase II inhibitor that reduces lymphocyte proliferation, and has been approved in the United States as therapy for patients with aggressive RRMS or SPMS. The drug has also been used for induction therapy [26], with some effects seen on clinical attacks, MRI activity, and disease progression [37]. However, in this era of promising treatment options including targeted immunosuppression using monoclonal antibody preparations, this drug is much less frequently chosen [28], because of a substantial risk for developing cardiotoxicity and acute leukemia [38].

6.2.4 Symptomatic Treatment

Symptomatic treatments have an important position in total care of patients with MS for better QOL. However, the amount of established evidence for routinely recommended treatments is low. Spasticity sometimes afflicts patients and can be treated with oral or intrathecal baclofen, tizanidine hydrochloride, or dantrolene sodium. Dysesthesia/paresthesia may be ameliorated with clonazepam, tricyclic antidepressants, or pregabalin, while trigeminal neuralgia and painful tonic spasms may be responsive to carbamazepine. Also, pollakiuria due to an uninhibited urinary bladder is sometimes improved with anticholinergic drugs including oxybutynin chloride. Furthermore, fatigue, an annoying symptom, may become less frequent by the use of amantadine hydrochloride or modafinil.

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Chapter 7
Neuropsychological Aspects of Multiple Sclerosis

Masaaki Niino

Abstract Many patients with multiple sclerosis (MS) suffer from neuropsychological impairments such as cognitive dysfunction, apathy, fatigue, and depression. However, physical disabilities in MS remain the main focus of daily clinical practice and of research into symptoms of the disease. In patients with MS, cognitive impairment typically involves domain-specific deficits rather than global cognitive decline, and sustained attention and information-processing speed are prominently affected. On the other hand, cognitive impairment in MS patients is often associated with other neuropsychological disorders, and its pathophysiology is complex. Neuropsychological impairments can influence activities of daily living and social activities in patients with MS. It has been suggested that cognitive impairment begins in the very early stages of the disease, but no established treatments are currently available. Urgent investigation into the treatment of neuropsychological impairments in MS is warranted, and challenges to conquer these widely neglected symptoms of the disease are continuing.

Keywords Apathy • Cognition • Depression • Fatigue • MRI • Multiple sclerosis • Quality of life

7.1 Introduction

As a result of widespread lesions in the brain, many patients with multiple sclerosis (MS) suffer not only from physical disability but also from neuropsychological impairments such as cognitive dysfunction, depression, apathy, and fatigue. However, it is not easy to evaluate the neuropsychological aspects of MS in daily clinical practice, and these dysfunctions are usually underestimated. Neuropsychological impairments can affect quality of life and activities of daily living in patients with MS, and cognitive impairment is considered to be a predictor of the need to reduce work responsibilities or to leave the workforce [1]. This chapter presents a review

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of neuropsychological impairments, especially in terms of cognitive function, in patients with MS.

7.2 Cognitive Function in MS

The prevalence of cognitive dysfunction in MS has been historically underestimated. This is because it is difficult to detect cognitive impairment during brief office visits without a formal neuropsychological assessment and because there is a widespread belief that cognitive dysfunction occurs rarely and only in the advanced stages of the disease [2]. Furthermore, in routine clinical practice, there tends to be a focus on physical disability, evaluated by scales such as the expanded disability status scale (EDSS), and little attention is paid to the evaluation of cognitive dysfunction. However, recent research suggests that cognitive impairment is a common symptom of MS [3].

Many studies have been performed to investigate cognitive impairment in patients with MS. However, the results have been mixed and not necessarily consistent. One of the reasons for the diverse findings is that MS lesions can occur in a number of different areas of the brain in individual patients and MS lesions vary widely among patients. Furthermore, because features of cognitive impairment in MS differ from those in neurodegenerative dementia diseases such as Alzheimer disease, it is important to use an appropriate assessment tool. Although it remains a difficult challenge to improve cognitive impairment in patients with MS, several trials have been conducted.

7.2.1 Batteries to Evaluate Cognitive Function in MS

The reported prevalence of cognitive impairment in MS ranges from 40 % to 80 %. Two factors that potentially influence prevalence rates are the sample composition and the neuropsychological assessment tools used [4]. Cognitive ability in MS is not readily observable on routine neurological examination. Other domains of cognition, such as language, visual or spatial processing, memory, mental processing speed and flexibility, and executive function, need to be assessed to evaluate neuropsychological impairments in MS [3]. To identify cognitive impairment in patients with MS, screening batteries such as the Mini-Mental State Examination (MMSE) are not appropriate. Several batteries have been developed to specifically evaluate cognitive functions in patients with MS. The international consortium recommends the use of current standardized test batteries for MS, i.e., the Brief Repeatable Battery of Neuropsychological Tests (BRB-N), the Minimal Assessment of Cognitive Function in MS (MACFIMS), and the Brief International Cognitive Assessment for MS (BICAMS), to evaluate cognitive function (Table 7.1) [4].

Table 7.1 Comparisons of the neuropsychological tests commonly used in multiple sclerosis

	MACFIMS	BRB-N	BICAMS
Expected examination duration	90 min	45 min	15 min
Domain			
Visual processing speed and working memory	SDMT	SDMT	SDMT
Verbal memory	CVLT-II	SRT	CVLT-II[a]
Visual/spatial episodic memory	BVMTR	10/36 SPART	BVMTR[b]
Auditory processing speed and working memory	PASAT	PASAT	
Verbal fluency	COWAT	COWAT	
Spatial processing	JLO		
Executive function	DKEFS		
Reference	[15]	[5, 6]	[17]

MACFIMS Minimal Assessment of Cognitive Function in Multiple Sclerosis, *BRB-N* Brief Repeatable Battery of Neuropsychological Tests, *BICAMS* Brief International Cognitive Assessment for Multiple Sclerosis, *PASAT* Paced Auditory Serial Addition Test, *SDMT* Symbol Digit Modalities Test, *CVLT-II* California Verbal Learning Test-II, *BVMTR* Brief Visuospatial Memory Test-Revised, *DKEFS* Delis–Kaplan Executive Function System, *JLO* Judgment of Line Orientation, *COWAT* Controlled Oral Word Association Test, *SRT* Selective Reminding Test, *SPART* Spatial Recall Test

[a]For the BICAMS, only the first five recall portions of the CVLT-II are included

[b]For the BICAMS, only the first three recall portions of the BVMTR are included

The BRB-N was originally developed in English [5, 6] and has been translated into other languages including Dutch [7], Italian [8], Hebrew [9], German [10], Spanish [11], and Japanese [12]. Test scores of the BRB-N are influenced by variables such as age, gender, and level of education [7, 13]. The BRB-N has been shown to have a sensitivity of 71 % and a specificity of 94 % in discriminating between MS patients with and without cognitive impairment [14]. In 2002, the MACFIMS was developed by an expert panel of neuropsychologists and psychologists working in the field of cognitive function in MS. This test battery was determined to be a minimal neuropsychological examination to assess the principal features of cognitive impairment in patients with MS [15]. The BRB-N and MACFIMS have comparable sensitivity among patients with MS [16]. However, the BRB-N and the MACFIMS require time-consuming expert evaluation using special materials, which is not routinely available outside specialist centers. Recently, the BICAMS, a brief battery to evaluate cognitive impairment in MS that takes 15 min to complete and requires no specialist equipment or specialist expertise, has been developed. The BICAMS focuses on measures of processing speed and visual–spatial and verbal memory and has been recommended by an international expert consensus committee of neurologists and neuropsychologists, based on extensive research and clinical experience of MS cognition [17].

7.2.2 Characteristics of Cognitive Impairment in MS

Cognitive impairment has been detected in all subtypes of MS, but most studies have demonstrated that patients with relapsing–remitting MS (RRMS) have lower degrees of cognitive impairment compared with patients with chronic progressive MS (CPMS) [2]. In the majority of studies, the category CPMS included subjects with both primary progressive MS (PPMS) and secondary progressive MS (SPMS). However, a number of studies have shown that cognitive impairment is most frequent and severe in SPMS [2, 18]. On the other hand, it has been reported that some cognitive impairment occurs in 20–50 % of patients with clinically isolated syndrome (CIS), a very early stage of MS [19, 20], and even in some patients with radiologically isolated syndrome (RIS) [21, 22]. Furthermore, benign MS (BMS), which is usually defined based on physical disability and disease duration, may not in fact be benign because 45 % of patients with BMS (defined as a disease duration $\geq$15 years and an EDSS score $\leq$3.0) had some cognitive impairment [23]. These findings suggest that a comprehensive assessment of different disease-related dimensions, not only physical disability, is needed to evaluate patients with MS.

In patients with MS, cognitive impairment typically involves domain-specific deficits rather than global cognitive decline. Although the profile of cognitive deficits varies among patients, memory (long term, explicit, and episodic), complex attention, information-processing speed, and executive functions are most commonly involved, while language, semantic memory, and attention span are rarely involved [18, 24].

7.2.3 Association of Magnetic Resonance Images with Cognitive Dysfunction in MS

It has been reported that patients with a greater lesion burden, evaluated based on frequency and volume, have significantly higher cognitive impairment than those with a smaller lesion burden [25–29]. More robust associations have been found with quantitative magnetic resonance parameters of brain tissue damage and atrophy [26]. Brain atrophy may also be associated with cognitive impairment. There is increasing research interest in the evaluation of the whole-brain atrophy using fully automated semiautomated parenchyma volume measurements or brain volume-to-cranial space ratios [3]. Furthermore, the pathological substrate of cognitive impairment appears to be closely related to gray matter damage, such as cortical atrophy [30–32] and cortical lesions [33, 34]. Magnetic resonance imaging (MRI) studies have been performed to assess whether lesion location within the brain is related to specific patterns of cognitive decline in patients with MS. Some studies suggested that third-ventricle width was the strongest predictor of cognitive dysfunction [35, 36]. A recent longitudinal study demonstrated that corpus callosum atrophy was strongly associated with decreased performance in cognitively demanding

information-processing tasks [37]. The overall relationship between cognitive impairment and lesion load was found to be weak to moderate. While MRI studies have been crucial to dissecting the contribution of gray and white matter pathology to cognitive impairment in MS, findings have been limited by inadequate resolution to detect disease in brain areas critical for cognition [38].

7.2.4 Cognitive Impairment and Depression, Apathy, and Fatigue in MS

Several factors are considered to contribute to cognitive dysfunction in MS, including depression, fatigue, and apathy. Depression is a common symptom of MS, and recent studies suggest that information-processing speed, working memory, and executive cognitive functioning may indeed be affected in patients with moderate to severe depression [39]. Other studies also reported that depression influences cognitive performance [12, 40], although depression was not found to correlate with cognitive function in another study [25].

Apathy has been defined as lack of motivation not attributable to a diminished level of consciousness, cognitive impairment, or emotional distress, and three domains of apathy include deficits in goal-directed behavior, a decrement in goal-related thought content, and emotional indifference with flat affect [41]. Apathy is one of the major neuropsychiatric symptoms in MS [42], and research has shown that apathy has a negative correlation with cognitive function in patients with MS [12].

Fatigue, which is a frequent complication of MS, could lead to unemployment in MS patients and to a reduction in quality of life [43]. Patients with MS often report a correlation between self-reported fatigue and their perception of poor performance on cognitive tests [44]. However, the relationship between fatigue and cognitive performance is complex and inconsistent [45], and a number of studies found no relationship between fatigue and cognitive impairment [12, 46, 47]. Some assessments of cognitive function, such as the MACFIMS, take a long time to complete, and this may cause fatigue and contribute to a low score in those assessments in patients with MS. It is important to note which battery is used to evaluate cognitive impairment in patients with MS.

7.2.5 Treatment of Cognitive Dysfunction in MS

In patients with MS, cognitive impairment affects many aspects of quality of life such as maintaining employment, daily living activities, social life, and the ability to drive [4]. The natural history of MS suggests that cognitive impairment is progressive, with little evidence of improvement once the impairment is apparent

[38]. The development of methods to improve cognitive impairment in patients with MS is of critical importance. There have been several reports on pharmacological treatments for cognitive impairment in MS, but in most of the studies, the number of patients treated was less than 50 [3].

Drugs for Alzheimer disease, such as donepezil [48], rivastigmine [49], and memantine [50], have been assessed for cognitive impairment in patients with MS. However, most of these studies did not demonstrate positive findings. Modafinil, a memory-improving and mood-brightening psychostimulant, had some positive effects on cognitive impairment in patients with MS in one study [51], but another study found no convincing effects on cognitive dysfunction [52]. One small study identified positive effects of l-amphetamine for cognitive impairment in patients with MS [53], but those effects were not replicated in a continuous-dosing, large-sample study [54].

As discussed above, most studies of drugs typically prescribed for dementia did not demonstrate positive findings for cognitive impairment in patients with MS. However, disease-modifying drugs (DMDs) could have the potential to prevent occurrence or progression of cognitive impairment or to improve cognitive dysfunction in patients with MS. Based on the results of assessment batteries to evaluate cognitive function, it has been suggested that subcutaneous IFNβ-1b [55], intramuscular IFNβ-1a [56], subcutaneous IFNβ-1a [57], and glatiramer acetate [58] could have positive effects on cognitive impairment in patients with MS. In two large clinical trials, the Natalizumab Safety and Efficacy in Relapsing–Remitting MS (AFFIRM) study and the Safety and Efficacy of Natalizumab in Combination with Interferon β-1a in Patients with Relapsing–Remitting Multiple Sclerosis (SENTINEL) study, the efficacy of natalizumab to treat cognitive dysfunction evaluated using the Paced Auditory Serial Addition Test (PASAT) was not consistent between the two studies [59]. However, a recent small, short-term clinical study suggested that natalizumab was effective for cognitive impairment in patients with MS [60, 61]. The Trial Assessing Injectable Interferon versus FTY720 Oral in Relapsing–Remitting Multiple Sclerosis (TRANSFORMS) study demonstrated that fingolimod reduced brain volume loss over 12 months as compared with intramuscular IFNβ-1a [62] and suggested that fingolimod could improve cognitive impairment.

Cognitive rehabilitation is a potential nondrug treatment for cognitive impairment in patients with MS. Effective cognitive rehabilitation programs could improve specific domains of cognitive function as well as related factors such as behavioral and personality difficulties. However, cognitive rehabilitation in MS is not an established treatment, and there is limited evidence to support the effectiveness of such programs. This is because placebo-controlled, double-blinded, or even single-blinded research into cognitive rehabilitation in MS is very difficult to implement, and levels of support from industry for this type of clinical research are not the same as for pharmacological treatments [3].

Based on current evidence, it is not easy to measure cognitive outcomes in MS. As discussed above, DMDs and cognitive rehabilitation could prevent occurrence or progression of cognitive impairment or improve cognitive dysfunction in

patients with MS. However, most of the studies of these treatment modalities were conducted with small numbers of subjects and were of short duration. Assessments of cognitive outcomes should be sensitive and reliable and should correlate with clinically meaningful change [18].

7.3 Conclusion

Cognitive impairment tends to occur to progress at different rates and within the context of large inter-patient variability, during long-term follow-up [63]. Such a condition could limit various activities of daily life in patients with MS, independently of the degree of physical disability. The following risk factors are suggested to predict cognitive dysfunction in MS: early age of onset, male sex, secondary progressive course, neurodegeneration as indicated by gray matter atrophy, and low average or inferior intelligence [3]. Currently, there are no established drug treatments for cognitive impairment in patients with MS. However, it is important for patients and neurologists to recognize what kinds of cognitive impairment can occur in patients with MS and for neurologists to advise patients with MS on how best to deal with cognitive impairment. In the management of patients with MS, routine clinical practice should address both physical disability and neuropsychological impairments.

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Chapter 8
Neuromyelitis Optica: Diagnosis and Treatment

Yuji Nakatsuji, Makoto Kinoshita, Tatsusada Okuno, Kazushiro Takata, Toru Koda, Josephe A. Honorat, Saburo Sakoda, and Hideki Mochizuki

Abstract Neuromyelitis optica (NMO) is an inflammatory disorder of the central nervous system (CNS) characterized by optic neuritis and transverse myelitis. NMO is also known as Devic's disease, after a case report by French neurologist Eugène Devic and his colleagues in the late nineteenth century. NMO has been considered a variant of multiple sclerosis (MS) and called opticospinal MS in Japan, where its prevalence is much higher than in Western countries. In 2004 however, an autoantibody, NMO-IgG (anti-aquaporin 4 antibody), was detected in the serum of patients with NMO, but not in patients with MS, indicating that NMO is independent of MS.

Recent studies of NMO have contributed to a growing understanding of the disease that includes NMO spectrum disorders (NMOSD). In this chapter, we describe the clinical and laboratory characteristics of NMO/NMOSD and their treatments. While corticosteroids and/or plasmapheresis are treatments for NMO/NMOSD, novel therapeutic approaches are being developed through research elucidating the pathomechanisms of NMO.

Keywords NMO • NMOSD • Diagnosis • Treatment

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8.1 Diagnosis of NMO and NMO Spectrum Disorder

While the relationship between NMO and MS has long been debated, the discovery in 2004 of the autoantibody NMO-IgG, which is present in the serum of patients with NMO, but not in patients with MS, established the belief that NMO was independent of MS [1]. The target of the autoantibody was identified as the water channel protein aquaporin 4 (AQP4) expressed in CNS astrocytes [2].

8.1.1 Clinical Characteristics of NMO

Many patients with NMO exhibit relapse, while Devic's disease is considered as predominately monophasic. The incidence of optic neuritis and myelitis is usually more severe and remission is poorer in patients with NMO than in those with MS, and may cause disability if appropriate treatment is not provided.

With regard to optic neuritis, optic nerve lesions in patients with NMO tend to be more extensive than in those with MS and may affect the optic chiasm resulting in bilateral visual disturbance. Visual acuity can be severely impaired without recovery [3, 4]. Young patients with NMOSD tend to present with optic neuritis, whereas older patients tend to exhibit spinal cord lesions and show significant rates of permanent motor disability [5].

Myelitis presents typically as severe gait disturbance with bladder and bowel dysfunction. The typical spinal cord lesion appears as longitudinal extensive transverse myelitis (LETM) that is situated centrally in the gray matter and extends over three vertebral segments in length. This LETM, as detected by MRI, is the imaging hallmark of NMO and is in contrast to the short and peripherally located (white matter) lesions observed in patients with MS (Fig. 8.1). The acute LETM lesion is edematous involving both gray and white matters [6]. Patchy and inhomogeneous contrast enhancement is observed often during the acute phase of NMO, whereas the enhancement is less common compared to that observed in MS [7]. The spinal cord lesions tend to become short or often fragmented into multiple shorter lesions during the course of NMO along with atrophy of the spinal cord. T1 hypointensity and cavity formation can be observed, both of which are uncommon in MS [8, 9].

In the past, brain lesions were thought to be rare in cases of NMO. However, they are detected in 60 % of patients with NMO [10, 11]. Most are nonspecific and do not conform to the diagnostic criteria of Paty et al., which indicate the presence of four or more lesions or the presence of three lesions when one is periventricular [12]. Those lesions are usually asymptomatic. Medullary lesions, which correlate with intractable hiccupping or vomiting, bilateral hypothalamic lesions, and periaqueductal lesions are relatively characteristic features that suggest NMO [11]. Contrast enhancement is less common in brain lesions, as in spinal cord lesions, although few studies have examined the frequency of contrast enhancement. Matsushita et al. reported enhancement in one of five patients [13], while Ito

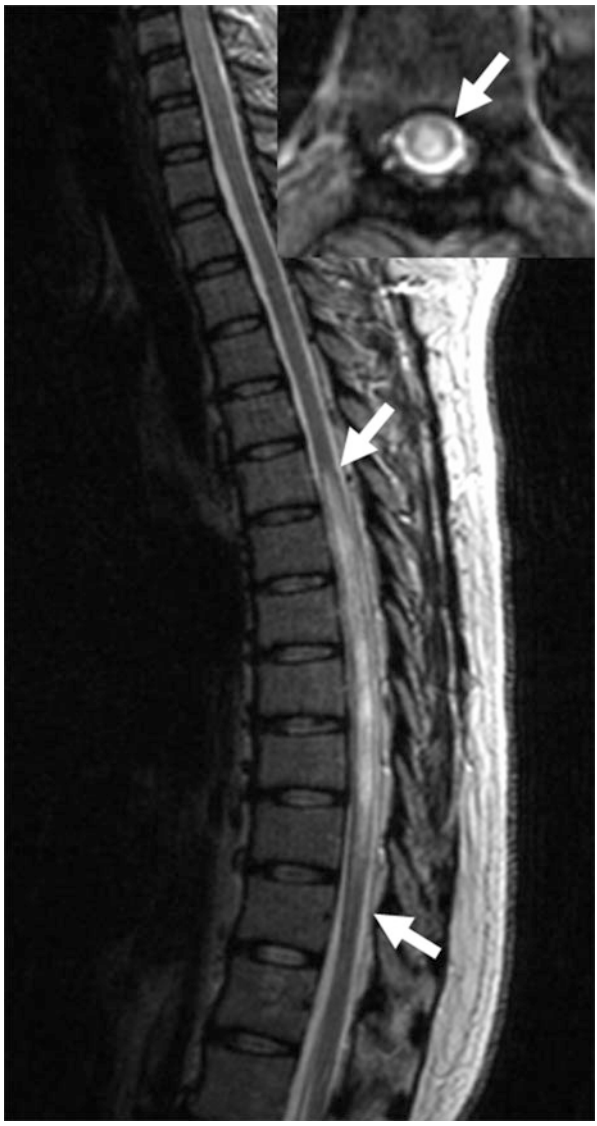

Fig. 8.1 Longitudinal extensive transverse myelitis (LETM) in NMO

et al. reported multiple patchy enhancing lesions with blurred margins (cloud-like enhancement) in 90 % of patients with NMO [14]. Cognitive impairment in NMO has been investigated to a lesser extent than in MS because the brain has been recognized as relatively spared in patients with NMO. However, Saji et al. reported that 57 % of patients with NMOSD show impaired performance on at least three cognitive tests included in Rao's Brief Battery of Neuropsychology Test (BRBN) [15].

There is a higher ratio of female patients in the NMO patient population than observed in the MS patient population, and the female to male ratio has been reported as high as 9 to 1 [16]. The median age at the onset is 39 years, which is older than that of MS [17]. Patients with NMO tend to have comorbid autoimmune diseases such as Sjögren's syndrome, myasthenia gravis, systemic lupus erythematosus, and Hashimoto disease [18].

8.1.2 Laboratory Characteristics

The cerebrospinal fluid (CSF) findings in patients with NMO and in those with MS are different. Pleocytosis at $>50/mm^3$ is observed frequently in NMO, and neutrophils and eosinophils, which are usually absent in MS, are present in addition to mononuclear lymphocytes. Elevated protein levels are also common in NMO. The positivity of the oligoclonal band (OCB) in NMO is 10–30 %, which is much lower than the 70–90 % observed in MS [12, 19]. Levels of inflammatory cytokines and chemokines, such as IL-17 and IL-6, in CSF are increased in NMO over that in MS, although these increases are not NMO-specific features [20, 21].

Presence of the anti-AQP4 antibody (AQP4-Ab) in the serum of patients with NMO is the most useful diagnostic biomarker for NMO. Several methods of tissue-, cell-, and protein-based assays are currently available to detect AQP4-Abs. While the specificity of each method is nearly 100 %, the sensitivity varies between 50 and 90 % [22–26]. HEK293 cells transfected with recombinant full-length human AQP4 are used in the cell-based assay. AQP4 is present in two major isoforms, the longer M1 and shorter M23 isoforms, produced by alternative splicing. A cell-based assay using HEK293 cells transfected with the M23 isoform provides the highest sensitivity [27]. AQP4-Ab testing using an enzyme-linked immunosorbent assay (ELISA) system for the diagnosis of NMO is a convenient quantitative method that recently received insurance approval in Japan; however, the sensitivity is approximately 20 % lower than that of the cell-based assay. Therefore, a seronegative status reported by ELISA in highly suspected cases of NMO should be reexamined using the cell-based assay. In addition, because the titers of AQP4-Ab are higher during the relapse phase and tend to decline in accordance with treatments and during remission [28], assessment for the presence of AQP4-Ab in serum obtained prior to initiation of therapy is recommended.

8.1.3 Diagnostic Criteria

According to the progress of our understanding of NMO, the diagnostic criteria originally proposed by Wingerchuk et al. in 1999 were revised in 2006 [4, 29]. The main revised points are the introduction of brain lesions and AQP4-Ab seropositivity. The revised criteria enable a diagnosis of NMO by both optic neuritis and

acute myelitis with at least two of the following three supportive criteria: (1) a contiguous spinal cord MRI lesion extending over three vertebral segments, (2) brain MRI not meeting diagnostic criteria for multiple sclerosis, and (3) an NMO-IgG (AQP4-Ab)-seropositive status.

These revised criteria for NMO are the most widely used, and the disease specificity is as high as 90 %. However, a small group of patients considered as cases of NMO does not exhibit AQP4 seropositivity, even when examined by the method with the highest sensitivity. Seronegative NMO or NMO-like disorders can be explained. A false-negative positivity due to very low titer of the antibody or the existence of unknown antibodies may result in a seronegative NMO status. In addition, many patients diagnosed as NMO exhibit either isolated optic neuritis or myelitis and do not have simultaneous lesions at the disease onset [5]. Thus, the number of NMO-like patients who do not fulfill the Wingerchuk's criteria of 2006 at the onset but develop NMO later is increasing. For these patients to be included in a broader entity, the concept of NMO spectrum disorder (NMOSD) was proposed using the following criteria [17]: (1) identification of NMO; (2) limited forms of NMO, such as idiopathic single or recurrent events of longitudinally extensive myelitis ($\geq$3 vertebral segment spinal cord lesions observed on MRI) and recurrent or simultaneous bilateral optic neuritis; (3) Asian optic-spinal MS; (4) optic neuritis or longitudinally extensive myelitis associated with systemic autoimmune disease; and (5) optic neuritis or myelitis associated with brain lesions typical of NMO (hypothalamic, corpus callosal, periventricular, or brainstem).

This concept of NMOSD has been accepted widely. It is useful to avoid a misdiagnosis as MS when the patients do not meet the NMO criteria and to avoid inappropriate therapies. About 90 % of patients with NMO and more than half of those with NMOSD are positive for AQP4-Ab [26]. Thus, NMOSD may be an expanding and broader clinical entity based on the pathogenic AQP4 antibody. However, autoantibodies against myelin oligodendrocyte glycoprotein (MOG) have been reported in patients with NMOSD [5, 30]. These MOG Ab-positive patients with NMOSD comprise 7 % of patients with NMOSD and tend to have a more restricted phenotype (optic neuritis), bilateral simultaneous optic neuritis, and a single attack and demonstrated better functional recovery after an attack.

8.1.4 Differential Diagnosis

LETM usually extends over three vertebral segments in length and is a cardinal imaging feature of NMO. However, not all patients with LETM have NMO. Therefore, the differential diagnosis of LETM is important. Other causes should be investigated including spinal cord infarction, spinal dural arteriovenous fistula, metabolic causes such as vitamin B12 deficiency and copper deficiency, Alexander disease, spinal cord neoplasia, paraneoplastic causes, acute disseminated encephalomyelitis (ADEM), infectious myelitis, and MS [31].

8.2 Treatment

Over the past several years, the core pathogenic mechanism of NMO has been clarified. At initial development and during relapse of the disease, the autoantibody AQP4-Ab plays a critical role in the formation of lesions in a complement-dependent manner. Thus, therapy at the acute phase of the disease is focused primarily on the elimination of the serum autoantibody. On the other hand, various immunosuppressants have proven efficacious for the prevention of future relapses. In this regard, the pivotal roles of both inflammatory T cell subsets and autoantibody-producing B cells may promote disease activity. This section discusses therapeutic approaches that are currently applied to the management of patients with NMO and NMOSD in the light of previous studies and case series.

8.2.1 Treatment of Acute Relapses

At the initial attack or during acute relapses, the primary aim of therapy is to eliminate serum AQP4-Ab and avoid subsequent complement activation. However, because of the elaborate preparation required for treatment with plasmapheresis, intravenous methylprednisolone (IVMP) is applied as the first-line therapy in the majority of patients. When the response to IVMP is not sufficient, plasmapheresis can be chosen as a second-line therapy. Plasmapheresis has been shown to be effective at the acute phase in several reports. Although more detailed analysis should be performed, some reports also show beneficial effects of intravenous immunoglobulin (IVIG), lymphocyte apheresis, and cyclophosphamide infusion for the treatment of NMO and NMOSD at relapses [32, 33].

8.2.1.1 Corticosteroids

Although IVMP therapy seems beneficial in clinical settings, its efficacy for patients with NMO has not yet been demonstrated in a large cohort. A recent report analyzed 345 patients with either multiple sclerosis (MS) or NMO and showed that methylprednisolone pulse therapy is beneficial in approximately 80 % of the patients [34]. The difference in treatment response between patients with MS and those with NMO remains to be demonstrated in a future study.

Based upon empiric application used for the treatment of MS, IVMP is given at a dose of 1000 mg daily for 3–5 days in most cases. Depending on the response of each patient to this therapy, another dosage regimen of IVMP may be added or an alternative therapeutic approach such as plasmapheresis may be performed.

In cases where patients show significant recovery after the IVMP therapy, oral steroids are usually prescribed for prevention of future attacks, and this treatment will be described in more detail in a later section.

8.2.1.2 Plasmapheresis

Several studies and case reports suggest that plasmapheresis is effective in patients who are refractory to steroid therapy at acute phases [35]. Typically, plasmapheresis is applied in a form of plasma exchange (PE), where patients receive five to seven sessions daily or every other day. A retrospective study examining the response to PE of various demyelinating diseases of the CNS demonstrated that 60 % of patients with NMO showed moderate or marked recovery [36].

Case reports and a retrospective study also demonstrated the efficacy of double-membrane filtration plasmapheresis (DFPP) instead of PE for the treatment of NMOSD. Serum AQP4-Ab are removed efficiently by DFPP, and this procedure is followed by substantial neurological improvements [37, 38]. In addition to PE and DFPP, immunoadsorption plasmapheresis (IAPP) is a well-recognized therapy for the treatment of MS, especially in Japan. The effectiveness of IAPP using a tryptophan-linked polyvinyl alcohol adsorber in patients with NMO has also been reported [39]. Considering that the tryptophan-linked adsorber efficiently removes immunoglobulins of the IgG1 subclass, to which AQP4-Ab belongs, its rationale is to consider IAPP as a second-line therapy for the treatment of acute attacks in NMO. Thus, the American Society for Apheresis has accepted plasmapheresis as a second-line therapy for the treatment of NMO [40].

8.2.1.3 Combination Therapy of Steroids and PE

From the results described above, it is reasonable to consider that a combination therapy of IVMP and PE may provide a better outcome. Indeed, a recent report showed that patients with NMO who presented with optic neuritis responded better to a combination therapy with IVMP and PE, compared to monotherapy with IVMP [41]. Another retrospective study investigated the efficacy of add-on therapy of PE in 43 patients with spinal attacks of NMOSD. Interestingly, the improvement by PE was observed irrespective of the seropositivity of AQP4-Ab [42]. Studies of larger cohorts with a variety of symptoms are warranted to provide stronger evidence of the efficacy of combining several therapeutic approaches in the treatment of acute attacks in patients with NMOSD.

8.2.1.4 Patients Resistant to IVMP and Plasmapheresis

Although the efficacy of novel therapeutic drugs including monoclonal antibodies is now being investigated, drugs currently used for other autoimmune diseases have also been applied for the treatment of acute attacks in patients with NMO resistant to steroid and PE therapies.

Since IVIG has comparable effects as PE in other autoimmune diseases such as Guillain-Barré syndrome, a case series demonstrated that patients with myelitis

who respond unsuccessfully to steroid therapy showed significant improvement after IVIG therapy [43].

Interestingly, there is a case report indicating that patients refractory to both IVMP and PE responded successfully to cyclophosphamide infusion [44]. The efficacy of cyclophosphamide for patients with idiopathic transverse myelitis has also been previously reported [45].

Whether these novel approaches can be beneficial to other patients with NMO and NMOSD in general awaits future investigations.

8.2.2 Treatment for the Prevention of Future Relapses

In addition to the treatment of acute attacks, it is crucial to prevent future relapses in the management of NMO. Patients with NMO rarely show secondary progression in the absence of apparent attacks in contrast to patients with MS. Therefore, appropriate preventive therapies directly influence the functional morbidity of the patients. Oral steroid therapy was shown to be beneficial for the prevention of relapses in several studies. However, a steroid dose of <10 mg/day may not be sufficient, as suggested previously [46]. Thus, to avoid various side effects arising from long-term steroid therapy at high dosage, several types of immunosuppressants have been used either as mono- or concomitant therapies.

8.2.2.1 Corticosteroids

After IVMP treatment for the acute attacks, oral steroid (5–25 mg/day) administration is the usual course of treatment. According to a retrospective study analyzing the preventive effects of oral steroid for future attacks in nine patients with NMO, the annual relapse rate (ARR) decreased from 1.48 to 0.49 with oral steroid therapy. Moreover, patients receiving steroid at <10 mg/day showed a higher relapse rate, which suggests the ideal dose of oral steroid for relapse prevention [46].

8.2.2.2 Immunosuppressants

Azathioprine

Among various types of immunosuppressants, azathioprine has been used most widely for the treatment of NMO in Japan. Azathioprine is converted to a purine analogue and interferes with purine biosynthesis, thereby inhibiting the proliferation of inflammatory lymphocytes. Generally, the effect of azathioprine takes a few months to be exerted after the initiation of treatment. Therefore, concurrent therapy with oral steroid is required at the beginning of the preventive therapy.

A retrospective study of 99 patients with NMOSD showed that the ARR reduced from 2.20 to 0.52 in patients receiving azathioprine more than 2 mg/kg/day. Less improvement was noted when the dose of azathioprine was less than this, where the ARR was decreased from 2.09 to 0.82 [47].

A beneficial effect of azathioprine in relapse prevention is demonstrated in other studies. However, one should consider the adverse effects caused by long-term azathioprine administration, such as leukopenia, liver dysfunction, and the development of cancers. Monitoring thiopurine methyltransferase activity may be useful for predicting the risk of azathioprine toxicity [48].

Mycophenolate Mofetil

Although mycophenolate mofetil (MMF) is currently not approved for treatment of NMO in Japan, several reports have demonstrated its efficacy for relapse prevention in other countries. MMF interferes with guanosine nucleotide synthesis, resulting in the inhibition of lymphocyte proliferation.

A retrospective study of 24 patients with NMOSD showed that the ARR reduced from 1.28 to 0.09 with MMF treatment. However, 24 % of the patients showed mild to moderate adverse effects such as diarrhea and leukopenia [49].

Methotrexate

In addition to azathioprine and MMF, several studies report the effectiveness of methotrexate for the treatment of NMO. Methotrexate hinders purine and thymidylate synthesis by inhibiting dihydrofolate reductase and is commonly used for various autoimmune diseases, such as rheumatoid arthritis and psoriasis. A retrospective study of 14 patients with NMO and NMOSD showed that the ARR was reduced from 1.39 to 0.18 over a median treatment duration of 21.5 months. Of those patients, 43 % remained free of relapse, and none had to stop the treatment due to adverse effects [50].

Other Oral Immunosuppressants

Compared to azathioprine, MMF, or methotrexate, there is less evidence regarding the efficacy of other immunosuppressants. Some case reports have indicated that cyclosporine or tacrolimus may be effective as a preventive therapy in patients with NMO. In contrast, a previous report of seven patients with NMO demonstrated an insufficient effect of cyclophosphamide on relapse prevention [51]. The reason for this failure is not clear, but in addition to the possibility of inappropriate dosage or interval of administration, there may be a distinct mode of action of effective immunosuppressants, as mentioned in previous sections.

Mitoxantrone

Mitoxantrone is an anthracenedione antineoplastic drug that inhibits topoisomerase II, thereby suppressing lymphocyte development. Some evidence indicates a specific inhibitory effect of mitoxantrone on B-cell activation. Although the number of reports analyzing the effect of mitoxantrone on NMO is limited, one study that analyzed 20 patients with highly relapsing NMO showed a significant reduction of both the ARR and the Expanded Disability Status Scale (EDSS). Over an average treatment duration of 17 months, the ARR reduced from 2.8 to 0.7 and the mean EDSS decreased from 5.6 to 4.4 [52]. Caution should be paid to the development of severe adverse effects of mitoxantrone, such as leukemia and cardiotoxicity.

8.2.2.3 Intermittent Plasmapheresis

Other than the effect of plasmapheresis for acute relapses of NMO, one case report described two patients who received intermittent plasmapheresis that successfully prevented relapses. In the report, one patient discontinued immunosuppressant therapy due to adverse effects and received intermittent DFPP with oral steroid for relapse prevention. The other patient had intermittent PE therapy after an insufficient effect of a concomitant approach of oral steroid and azathioprine. The recurrence was almost completely prevented by inducing intermittent PE in the latter case [53]. These observations suggest beneficial effects of intermittent plasmapheresis for patients refractory to currently applied immunosuppressant therapies. To confirm the efficacy of plasmapheresis for relapse prevention, the accumulation of case series and studies of larger cohorts is warranted.

8.2.3 Treatments That Have the Potential of Exacerbating Disease Activity

In contrast to the significant improvement demonstrated in the treatment of MS, interferon-beta, fingolimod, and natalizumab have been reported to be less beneficial or even exacerbate NMO [54–56]. A retrospective study of 40 patients with NMOSD receiving interferon-beta showed that the ARR increased from 1.49 to 2.38 and the mean EDSS increased from 2.72 to 4.78 [57]. Supporting these findings, one case report showed that the serum anti-AQP4 Ab titer in patients with NMO increased after the initiation of interferon-beta treatment [58]. As a hypothetical mechanism of this harmful effect of interferon-beta on NMO, interferon-beta has been shown to cause the activation of Th17 cell-mediated autoimmunity, which is suggested to play a pivotal role in the pathogenesis of NMO [48].

In addition to interferon-beta, patients who received natalizumab, a monoclonal antibody directed against very late activation antigen-4, fail to respond to the therapy. A study of five case series showed that even during natalizumab treatment, all patients continued to suffer from relapses, and four patients showed increased disability [59]. The precise mechanism remains to be elucidated. However, inflammatory T cells sequestered at the peripheral immune system are thought to promote the activation of antibody-producing B cells under natalizumab therapy [48].

Lastly, in high contrast to the beneficial effect of oral fingolimod on MS treatment, several cases of NMO reportedly developed fulminant relapses within 2 weeks after the initiation of treatment [60]. The mechanism for the cause of this adverse effect is unclear, but the egress of eosinophils promoted by fingolimod therapy could be a factor facilitating disease activity [48].

8.3 Recent Advances in the Treatment of NMO

Recent advances in the research to clarify the pathomechanisms of NMO have led to the development of novel treatments. Since AQP4-Ab, complements, inflammatory cytokines including IL-6, and neutrophils have been shown to play important roles in the formation of NMO lesions, each molecule may be a potent therapeutic target. Thus, we stress the potential for drug repositioning of approved therapeutics in NMO, some of which are undergoing clinical trials.

8.3.1 Rituximab

Rituximab is a chimeric monoclonal anti-CD20 antibody that depletes B cells via antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Rituximab has been approved for B-cell-mediated diseases including lymphomas, leukemias, transplant rejections, and autoimmune diseases. Because CD20 is expressed exclusively on B cells, and not on plasma cells, it directly depletes the intermediate B-cell stage and does not affect pre-B cells or long-lived plasma cells. Although multiple regimens have been tested, rituximab was administered at regular 6-month intervals beginning with four weekly doses of 375 mg/m^2 followed by two biweekly doses of 1 g in most studies [61–64]. In those studies, patients treated with rituximab showed a significant reduction in the ARR and a stabilization of disability. Notably, the EDSS scores stabilized or even improved in majority of the patients [62, 63, 65, 66].

Jarius et al. reported that AQP4-Ab titers and CD19$^+$ B-cell counts increased before relapse and fell with remission [67]. However, another study suggests that the suppression of disease activity by rituximab correlates with the extent of B-cell depletion, but not with the serum AQP4-Ab titer [68]. Further investigations are required to determine whether the effect of rituximab is mediated by a reduction in

AQP4-Ab production or through modulation of proinflammatory B-cell functions [66].

With regard to the detrimental effects of rituximab, infusion reactions have been observed, especially at 30–120 min after the first infusion. In addition, severe infections and cardiovascular failure have been reported. Progressive multifocal leukoencephalopathy (PML) has been reported in rituximab-treated patients with non-Hodgkin's lymphoma and rheumatoid arthritis, while it has not been reported in patients with NMO. These facts suggest a potential limitation in therapy, especially in combination therapies [69].

8.3.2 Tocilizumab

Tocilizumab is a humanized murine anti-IL-6 receptor monoclonal antibody approved for rheumatoid arthritis, systemic juvenile idiopathic arthritis, and Castleman's disease. Accumulating evidence suggests that IL-6 is involved in the pathogenesis of NMO. Several reports demonstrate that the levels of IL-6 and soluble IL-6 receptor in the cerebrospinal fluid (CSF) are elevated in patients with NMO during an attack [70, 71] and that plasmablasts in the blood and CSF expand during the relapse phase [72]. In vitro, IL-6 enhanced the survival of these cells and increased AQP4-IgG secretion, whereas blockade of the IL-6 receptor reduced their survival [73]. Recent case reports demonstrated a reduced relapse rate in patients with NMO treated with tocilizumab [74–77]. In one pilot study, patients were administered a monthly intravenous injection of tocilizumab (8 mg/kg) with their current therapy for a year. After 1 year of treatment with tocilizumab, the ARR was decreased significantly from 2.9–0.4. Unexpectedly, neuropathic pain and general fatigue declined concurrently with the improvement assessed by EDSS. Recently, another anti-IL-6 receptor monoclonal antibody, SA237, an alternative formulation for subcutaneous injection with a fourfold longer duration of action than tocilizumab, entered a clinical trial of NMO.

8.3.3 Eculizumab

Activation of the complement system is critical for NMO pathogenesis. AQP4-Ab can cause astrocyte necrosis by binding AQP4, followed by the activation of the classical complement pathway in cell cultures [78]. In spinal cord slice cultures and in vivo animal models, complement activation is required for AQP4-Ab to reproduce the pathological features of NMO, including the loss of AQP4 and glial fibrillary acidic protein [79, 80].

The C5 complement inhibitor eculizumab, which has been approved for paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome, has been tested in patients with NMO in an open-label trial [81]. Eculizumab is a

recombinant humanized IgG2/4 antibody that binds C5 and inhibits its conversion into C5a and C5b. Patients with NMOSD and positive for AQP4-Ab, who had at least two attacks before enrollment, received 600 mg intravenous eculizumab weekly for 4 weeks, 900 mg in the fifth week, and then 900 mg every 2 weeks for 48 weeks. A significant reduction of the relapse rate and stabilized or improved neurological disabilities were observed. Despite its efficacy, the main disadvantage of eculizumab is its high cost (approximately US$400,000 per patient-year) and risk of meningococcal sepsis, which was observed in one case during the trial.

8.3.4 Immunoglobulins

Intravenous immunoglobulins (IVIg), which consist of pooled human IgG from more than 1000 blood donors, have been used to treat various immune-mediated neurological diseases including myasthenia gravis, polymyositis, Guillain-Barré syndrome, chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, and multifocal motor neuropathy [82]. Although the mechanism of IVIg action is incompletely understood, it exerts a beneficial effect through pleiotropic actions on the immune system, including blockade of antibody-target binding, clearance and neutralization of autoantibody, inhibition of leukocyte migration, inhibition of complement activation, and blockade of Fcγ receptors [83].

Several lines of evidence support a beneficial effect of IVIg in preventing relapse, as well as improving acute attacks [32, 84, 85]. In one study, IVIg was efficient in preventing relapses in eight patients with NMO, with a reduction in the mean ARR from 1.8 in the year before IVIg treatment to 0.006 after IVIg treatment [86]. Consistently, human immunoglobulin G significantly reduced the size of damaged brain lesions in the rat model of NMO induced by intracerebral injection of AQP4-Ab [87]. Results of in vitro studies suggest that the inhibition of AQP4-IgG-mediated CDC and ADCC are the primary mechanisms mediating the beneficial effects observed. However, the data supporting the clinical benefit of IVIg in NMO are limited. Further investigations are needed to establish the efficacy of IVIg.

8.3.5 Other Potential Drugs Under Investigation

The presence of perivascular neutrophils in the CNS is a pathological feature of patients with NMO and of the NMO animal model [88]. In addition, the number of neutrophils in the CSF was reported to be elevated by about 60 % in patients with NMO than in untreated patients during relapse [19], suggesting a detrimental role of neutrophils in the acute phases of NMO. Consistent with these observations, tissue damage accompanying the increased neutrophil infiltration was ameliorated by the elimination of the cells in animal models of NMO [88].

8.3.5.1 Sivelestat

Sivelestat, a small-molecule inhibitor of neutrophil elastase, was originally developed to treat acute respiratory distress syndrome (ARDS) and is approved for treatment in Japan. Neutrophil elastase is involved in neutrophil migration and neutrophil-mediated tissue damage and reduced NMO pathology in a mouse model of NMO [88, 89]. Studies in animal models of NMO suggest that neutrophils enter the CNS at the early stage, whereas macrophages became predominant in the lesions at later stages [88]. The experimental data and pathological observations suggest the availability of sivelestat during the acute phase of NMO attack. Based on this hypothesis, this drug is being tested in a small clinical trial in Japan.

8.3.5.2 Aquaporumab

Since AQP4-Ab has high pathogenicity in NMO, approaches to block the binding of AQP4-IgG to AQP4 have been shown to reduce almost all of the pathogenic process, including CDC and ADCC. Tradtrantip et al. developed a nonpathogenic human monoclonal antibody against AQP4, aquaporumab, which binds to AQP4 with high affinity. To suppress its CDC and ADCC effector functions, mutations were made in the Fc region of the antibody [90]. Aquaporumab competitively displaces AQP4-IgG in the serum of patients with NMO. Aquaporumab markedly blocked AQP4-Ab-dependent cytotoxicity and astrocyte damage in animal models of NMO [90]. Because aquaporumab selectively targets AQP4, but not the immune system itself, immunosuppression should not occur, although further investigations are needed to exclude off-target effects and immunogenicity. Aquaporumab is currently in preclinical development.

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Chapter 9
Guillain-Barré Syndrome: Epidemiology, Diagnosis, and Treatment

Susumu Kusunoki

Abstract Guillain-Barré syndrome (GBS) is an acute monophasic motor-dominant peripheral neuropathy. A multicenter prospective survey of Guillain-Barré syndrome (GBS) revealed that the axonal types of GBS and Miller Fisher syndrome (MFS), a variant of GBS characterized by ophthalmoplegia and ataxia, are more frequent in Japan than in Western countries. Bickerstaff brainstem encephalitis (BBE) is an acute self-limited disease, characterized by ophthalmoplegia, ataxia, and impaired consciousness and/or pyramidal signs. BBE is considered to form a continuous spectrum with GBS and MFS. A recently performed nationwide survey showed that the annual onset of BBE in Japan was roughly estimated as 100 cases. Diagnosis of GBS can be made by characteristic history and neurological signs, including preceding infection, rapidly progressive symmetric weakness, and decreased tendon reflexes. Nerve conduction studies, cerebrospinal fluid examination, and anti-glycolipid antibody assay are useful for the diagnosis of GBS. Although plasmapheresis and intravenous immunoglobulin (IVIg) are effective therapies for GBS, there are still severe or intractable cases. New therapies such as the second course of IVIg and agents interfering with complement activation are among candidates used for severely affected patients. Prediction of the outcome of each case is important for determining indications of such therapies.

Keywords Guillain-Barré syndrome • Miller Fisher syndrome • Bickerstaff brainstem encephalitis • Anti-glycolipid antibody • Complement

9.1 Introduction

Guillain-Barré syndrome (GBS) is an acute monophasic motor-dominant peripheral neuropathy with or without sensory disturbances, frequently preceded by some infection. Autoimmunity may be involved in the pathogenesis. The clinical course is monophasic, the nadir being within 4 weeks of the neurological onset, after which

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patients recover gradually. GBS had long been considered as a demyelinating disease in the peripheral nervous system (acute inflammatory demyelinating polyneuropathy, AIDP) before the recognition of the subtype primarily affecting axons (acute motor axonal neuropathy, AMAN) [1]. In addition, there are several subtypes including Miller Fisher syndrome (MFS), characterized by acute monophasic ophthalmoplegia and ataxia without prominent limb weakness [2].

In recent years, the importance of the autoantibodies including anti-glycolipid antibodies in the pathogenesis of GBS has been reported [3]. Those antibodies are also useful diagnostic markers of GBS. In this chapter, epidemiology, diagnosis, and treatment of GBS are described.

9.2 Epidemiology

9.2.1 Epidemiology of GBS

The annual incidence of GBS has been reported to be 0.62–2.66 per 100,000, the incidence increasing with age [4]. In a systematic review and meta-analysis, the incidence is shown to be higher in men than in women in the ratio of 1.78:1. In the Japanese study on the period between March 1993 and February 1998, the annual incidence was 1.15 per 100,000, and the ratio of men to women was about 1.5 [5]. It has been reported that AIDP is frequent but AMAN is rare in Europe and North America [6]. In contrast AMAN is not so rare in Asia [7] and South America.

The onset of GBS is often preceded by some prodromal events, such as respiratory or gastrointestinal infections. Infectious agents are usually not determined. But in the case of gastrointestinal infections, most of them are caused by *Campylobacter jejuni*. Cytomegalovirus, Epstein-Barr virus, *Mycoplasma pneumoniae*, and *Haemophilus influenzae* also are known infectious agents of the antecedent infections of GBS [8].

9.2.2 The Incidence of Subtypes of GBS in Japan

Recently, we performed a multicenter prospective survey of GBS and examined the electrophysiological subtypes in a large prospective cohort of Japanese population [9]. Data from patients with GBS were collected from 23 university hospitals or tertiary hospitals in the Japan GBS study group between August 2007 and July 2010. In the same period, the number of patients with MFS was also surveyed. It was sponsored by the Research Committees for Neuroimmunological Diseases of the Ministry of Health, Labor and Welfare of Japan.

In the above period, 222 patients with GBS were treated in the hospitals of the study group. Among those patients, informed consent for utilization of the data was

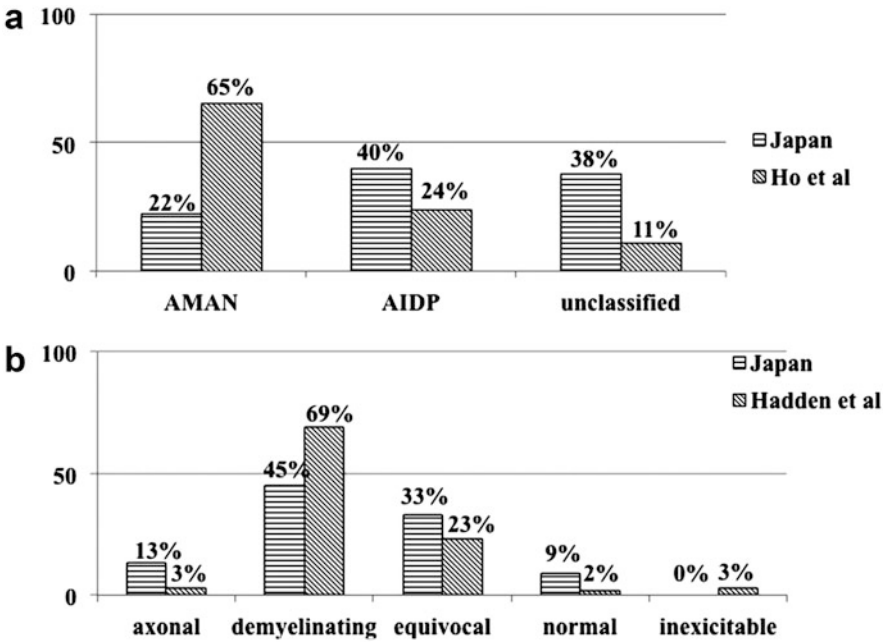

Fig. 9.1 Rate of the subtypes in GBS (Cited from Ref. [9]). (**a**) Comparison between the data in Japan and that in China (Ho et al. [7]). (**b**) Comparison between the data in Japan and that in Western countries (Hadden et al. [6])

obtained from 184 patients. Analysis of nerve conduction studies showed that 73 patients (40 %) were classified as AIDP, 40 (22 %) as AMAN, and 71 (38 %) as unclassified according to Ho's criteria [7]. By the use of Hadden's criteria [6], 83 patients (45 %) were classified as demyelination, 24 (13 %) as axonal, 61 (33 %) as equivocal, 16 (9 %) as normal, and none as inexcitable. Therefore, although demyelinating type of GBS is also more common than axonal type in Japan, the incidence of axonal type is higher in Japan than in Western countries (Fig. 9.1).

During the same period, a total of 79 patients with MFS were seen in those hospitals of the study group. The percentage of MFS cases out of all cases (222 (GBS) + 79 (MFS) = 301) was 26 %. In the study of an Italian cohort, it was reported that MFS cases accounted for only 3 % of the cases of both GBS and MFS [10]. It is therefore suggested that the incidence of MFS is higher in East Asia than in Europe. Regional study in Taiwan [11] and in Japan [12] also showed compatible results.

9.2.3 The Incidence of Bickerstaff Brainstem Encephalitis in Japan

Bickerstaff brainstem encephalitis (BBE) is an acute self-limited disease characterized by ophthalmoplegia, ataxia, and impaired consciousness and/or pyramidal signs. As described in the following section of Diagnosis, anti-GQ1b IgG antibody is frequently present in the acute-phase sera from BBE patients. BBE has a feature in common with MFS and, therefore, forms a continuous spectrum with GBS and MFS.

A nationwide survey of BBE in Japan was recently performed [13]. The results indicated that the annual onset of BBE in Japan was roughly estimated as 100 cases.

9.3 Diagnosis

9.3.1 Diagnosis of GBS

Diagnosis of GBS is made by characteristic history and neurological signs, including preceding infection, rapidly progressive symmetric weakness, and hypo- or areflexia (Table 9.1). There are several diagnostic criteria of GBS [2, 14, 15]. The criteria by Asbury and Cornblath [14] are most frequently used.

Table 9.1 Clinical features of typical GBS

Features required for diagnosis
(1) Progressive motor weakness of more than one limb
(2) Areflexia or hyporeflexia[a]
Features strongly supportive of the diagnosis
(1) Progression. Symptoms and signs of motor weakness develop rapidly but cease to progress by 4 weeks from the neurological onset
(2) Relative symmetry of symptoms
(3) Mild sensory symptoms of signs
(4) Cranial nerve involvement
(5) Recovery. It usually begins 2–4 weeks after progression stops
(6) Autonomic dysfunction
(7) The absence of fever at the onset of neuritic symptoms
Features casting doubt on the diagnosis
(1) Marked, persistent asymmetry of weakness
(2) Persistent bladder or bowel dysfunction
(3) Bladder or bowel dysfunction at onset
(4) Sharp sensory level

Based on the diagnostic criteria of GBS [14] with modifications
[a]Normal tendon reflexes can be observed in some cases of AMAN

9.3.2 Laboratory Examinations

Some examinations are also useful for the diagnosis of GBS. Cerebrospinal fluid (CSF) examination shows increased protein level with normal cell count. It should be kept in mind that CSF protein level is often within the normal range in the first week but mostly elevated in the second week. Nerve conduction study is also very important for the diagnosis of GBS. Nerve conduction studies (NCSs) show conduction block, decreased compound muscle action potentials, temporal dispersion, decreased conduction velocities, increased distal latency, and abnormality in F waves. NCS is also useful for discriminating between subclasses such as AIDP and AMAN [6, 7].

It has been recognized that examination of autoantibodies against glycolipids, especially gangliosides, is useful for the diagnosis of GBS [3]. Anti-glycolipid antibodies are positive in about 60 % of the acute-phase GBS sera. Therefore, negative results do not deny the diagnosis of GBS, but if positive, the results strongly suggest that the patient is affected with GBS.

Binding specificities of the antibodies are associated with a certain clinical features. It indicates that the anti-glycolipid antibodies may play some roles in the pathogenesis of GBS. However the association is not always so rigid. IgG antibodies to such gangliosides as GM1, GM1b, GD1a, and GalNAc-GD1a are known to be associated with the pure motor or axonal type of GBS (AMAN) preceded by *C. jejuni* infection [3]. However, 14 % of AIDP patients also had anti-GM1 antibody in the above-mentioned epidemiological study [9]. Antibodies against glycolipids localized in myelin, such as galactocerebroside [16] and LM1 [17], are mainly detected in the acute-phase sera from AIDP patients. But some patients with AMAN also had such antibodies [18]. The association between anti-Gal-C antibody and preceding infection with *Mycoplasma pneumoniae* has been reported [19].

IgG antibodies to GQ1b ganglioside are shown to be detected in 80–90 % of the acute-phase sera from MFS patients [20]. Most of the anti-GQ1b antibodies cross-react with GT1a ganglioside because both gangliosides have the terminal disialosyl residues [20]. The antibodies may bind to the regions where GQ1b is densely localized, such as paranodal regions of the cranial nerves innervating ocular muscles [20], some dorsal root ganglion neurons [21], and muscle spindles [22], to cause ophthalmoplegia and ataxia. Anti-GQ1b IgG antibodies are also present in the acute-phase GBS patients [20] with ophthalmoplegia and/or ataxia. Anti-GQ1b IgG antibodies are sometimes detected in the GBS variants with bulbar palsies, such as acute oropharyngeal palsy and pharyngo-cervical-brachial variant of GBS. In such cases, the antibody activity to GT1a may be involved in the pathogenesis [3]. Anti-GQ1b antibodies are also frequently detected in BBE, showing CNS involvement as well as characteristic features of MFS, including ophthalmoplegia and ataxia [13]. A diagnostic criteria of BBE used in the survey mentioned above (in 9.2.3) was shown in Table 9.2 [13]. Groups of those diseases are sometimes called anti-GQ1b antibody syndrome.

Table 9.2 Diagnostic criteria for Bickerstaff brainstem encephalitis

"Definite" Bickerstaff brainstem encephalitis is defined when (1), (2), and (4) are satisfied
"Probable" Bickerstaff brainstem encephalitis is defined when (1) and (4) or when (2), (3), and (4) are satisfied
(1) Acute progressive external ophthalmoplegia[a], ataxia, and impaired conscious level by 4 weeks, followed by spontaneous recovery within 12 weeks after onset
(2) Positive for serum IgG anti-GQ1b antibodies
(3) Incomplete agreement on (1) because of one or more of the following reasons[b]
It is impossible to evaluate ataxia because of severe limb weakness or consciousness disturbance
Unconfirmed recovery of the symptoms
Remarkable laterality of external ophthalmoplegia
Long tract sign (hemisensory disturbance, pyramidal sign, or spasticity) instead of impaired level of consciousness
(4) Other conditions are excluded in laboratory and image tests
The excluded conditions are Wernicke encephalopathy, cerebrovascular disorder, multiple sclerosis, neuromyelitis optica, neuro-Behçet syndrome, neuro-Sweet disease, pituitary apoplexy, viral brainstem encephalitis, myasthenia gravis, brainstem tumor, vasculitis, botulism, and Hashimoto encephalopathy

Cited from Ref. [13]
[a]Lateral symmetry is the rule but mild laterality is also permitted
[b]Features other than the incomplete item(s) must meet (1)

It has been shown that the patients with GBS who are positive for anti-GQ1b IgG antibodies more frequently need artificial ventilation than the antibody-negative patients [23]. This may be associated with the blocking activity of the anti-GQ1b antibodies on the neuromuscular transmission model using mouse diaphragm [24]. Thus, anti-GQ1b IgG antibodies can be used as a prognostic marker of GBS (discussed later).

Some GBS patients have antibodies to ganglioside complexes, novel epitopes formed by two different gangliosides [25]. Those antibodies may bind to the rafts on the plasma cell membrane of the peripheral nervous system. Antiganglioside complex antibodies also are useful as diagnostic markers of GBS.

The autoantibodies in GBS are described in more detail in the next chapter.

9.3.3 Differential Diagnosis

There are many diseases that should be considered in the diagnosis of GBS (Table 9.3).

Among them, it is sometimes difficult to discriminate acute-onset chronic inflammatory demyelinating polyneuropathy (CIDP) from GBS. The use of artificial ventilation, severe pain, severe autonomic disturbance, and cranial nerve palsy are less common in CIDP, and those clinical features can be used for differential diagnosis.

Table 9.3 Differential diagnosis of GBS

Intracranial or spinal cord abnormalities
Acute compression of spinal cord, transverse myelitis
Meningeal carcinomatosis, cerebral infarction
Anterior spinal artery syndrome, brainstem encephalitis
Anterior spinal cord abnormalities
Poliomyelitis, West Nile encephalitis
Spinal nerve root lesion
Spinal root compression
Inflammation of spinal root (CMV, herpes simplex, etc.)
Peripheral nerve abnormalities
Lyme disease, diphtheria, HIV, acute intermittent porphyria
Vasculitic neuropathy, sarcoid neuropathy, neuralgic amyotrophy
Critical illness polyneuropathy, lymphoma
Paraneoplastic neuropathy, alcoholic neuropathy, beriberi
Drug-induced neuropathy, metabolic disturbances
Toxic neuropathy, CIDP
Neuromuscular junction abnormalities
Myasthenia gravis, botulism, organophosphate poisoning
Muscular abnormalities
Polymyositis, dermatomyositis, acute rhabdomyolysis
Periodic paralysis, critical illness myopathy
Others
Conversion reaction, etc.

The presence of some IgG antiganglioside antibodies also may suggest the diagnosis of GBS.

Deterioration after initial improvement or stabilization with IVIg therapy is observed in about 5–10 % of GBS patients and is called as treatment-related fluctuation (TRF). It is considered that if patients who were initially diagnosed with GBS deteriorate three or more times or if they have deterioration after 9 weeks from onset of GBS, the diagnosis of CIDP should be suspected [26]. Recurrence of GBS is rare and it is reported to be seen in about 2–5 % of the cases [27].

9.4 Treatment of GBS

9.4.1 General Care

As GBS is a monophasic disease, the pathogenetic mechanism is no longer active after 1 month from the neurological onset. However, as such life-threatening problems as respiratory insufficiency and severe autonomic disturbances can develop in the acute phase, careful control of the systemic condition is necessary.

Monitoring of vital capacity, respiration frequency, heartbeat frequency, blood pressure, etc. is needed. The use of intensive care unit is often necessary to be considered. Rehabilitation is important both in the acute and the recovery phases. Prevention of infection and deep vein thrombosis is also required.

9.4.2 Immunotherapy

To control the immunopathogenesis, immunotherapy should be performed for patients who cannot walk independently. The positive effect of either plasmapheresis or intravenous immunoglobulin (IVIg) has been confirmed by several randomized controlled trials (RCTs). Plasmapheresis and IVIg have been shown to have equivalent efficacy [28].

Plasmapheresis includes plasma exchange (PE), double filtration plasmapheresis (DFPP), and immunoadsorption plasmapheresis (IAPP). RCTs of large scale have been performed for PE. It has been reported that the efficacy of DFPP and IAPP is comparable to PE [29, 30].

IVIg in a regimen of 0.4 g/kg (body weight)/day for 5 days has been shown to be as effective as PE. As IVIg is available in most of the hospitals and is more convenient and less harmful than plasmapheresis, IVIg is more frequently used for GBS patients in Japan.

Steroids alone, either oral steroids or intravenous methylprednisolone pulse therapy, are not effective in GBS. Superiority of the combined therapy of IVIg and intravenous methylprednisolone to IVIg alone was not confirmed [31]. However, as there might be a short-term effect of this combined therapy, it should be investigated whether this combined therapy is effective for the treatment of severe cases in the future.

Typical clinical course of GBS is shown in Fig. 9.2.

As for the treatment of MFS, case reports have been published on the effectiveness of IVIg and plasmapheresis for MFS, but there is no RCT. An observational study indicated that IVIg and PE did not influence the outcome of MFS because patients with MFS usually show good natural recovery [32]. Some patients are diagnosed as MFS but later develop GBS. In such cases, patients more frequently need artificial ventilation than the other GBS patients [33]. Therefore, it is necessary to follow a clinical course carefully in the acute phase even if a patient shows clinical features of MFS.

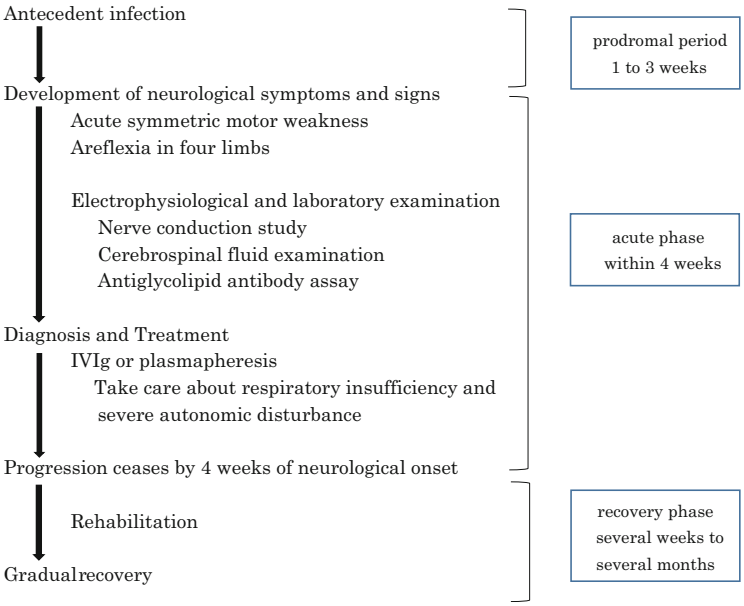

Fig. 9.2 Typical clinical course of GBS

9.4.3 Possible Novel Therapies for GBS

9.4.3.1 Second Course of IVIg

When delta IgG (the increase in serum IgG level at 2 weeks after IVIg treatment) was investigated, GBS patients with low delta IgG recovered significantly more slowly [34]. This may indicate that patients with low delta IgG may benefit from the second course of IVIg.

In addition, in four patients with severe unresponsive GBS, the effect of a second course of IVIg has been reported [35].

Therefore, the efficacy and the indication of the second course of IVIg should be investigated in future studies.

9.4.3.2 Interference with Complement Activation

In the pathological study of human peripheral nerve, the role of the complements in the pathogenesis of GBS has been reported [1]. The importance of complement system has also been shown in in vitro and in vivo model of blocking activity of the anti-GQ1b antibodies on the neuromuscular transmission model of mouse diaphragm [36]. This may be an animal model of GBS with respiratory insufficiency associated with anti-GQ1b antibodies [23]. When the efficacy of the humanized monoclonal antibody eculizumab, which blocks the formation of human C5a and

C5b-9, was investigated, eculizumab prevented electrophysiological and structural lesions in in vitro model. In addition, in in vivo mouse model of respiratory paralysis generated by intraperitoneal injection of anti-GQ1b antibody and normal human serum, intravenous injection of eculizumab effectively prevented respiratory paralysis [36]. Therefore, eculizumab may be a candidate for the novel therapy of GBS, especially for severe cases.

9.5 Prediction of Prognosis

Such factors as old ages, preceding *C. jejuni* infection, and severity at the nadir are reported to be associated with intractable GBS. Research group in the Netherlands reported clinical scores such as Erasmus GBS Outcome Score (EGOS) [37] and modified EGOS [38] for predicting the disability at 6 months from the onset. The same group also reported Erasmus GBS Respiratory Insufficiency Score (EGRIS) for predicting respiratory insufficiency [39]. Those clinical scores are based on the data available in the daily clinical practice and may be useful prognostic markers.

As described above, anti-GQ1b IgG antibody, if positive in GBS but not in MFS, is also a marker of cases requiring artificial ventilation [23].

As prognosis of GBS in general is not so bad, it is necessary to discriminate cases with severe or intractable course to determine the indication of the novel therapies for GBS. Thus, the above-mentioned clinical scores and antibodies may be useful markers for the clinical research in the future.

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Chapter 10
Autoantibodies in Guillain-Barré Syndrome (GBS)

Kenichi Kaida

Abstract Autoantibodies contribute to the pathogenesis of Guillain-Barré syndrome (GBS) and variants such as Fisher syndrome (FS) and partly explain the pathophysiology of antibody-mediated nerve injury in GBS. The major target antigens in GBS and FS are sialic acid-containing glycolipids, gangliosides such as GM1 or GQ1b. The frequency of IgG antiglycolipid antibodies (subclass IgG1 or IgG3) is approximately 60 %. The heterogeneity of ganglioside expression in the peripheral nervous system (PNS) explains the differential clinical manifestation of GBS variants. Antiganglioside antibody-mediated complement activation plays a key role in development of GBS, but some in vitro studies suggest that antibody-mediated nerve dysfunction can emerge in a complement-independent manner, such as through inhibition of voltage-gated Ca^{2+} channel currents and changes in the integrity of lipid rafts. Pathogenic actions of antiglycolipid antibodies are regulated by the antibody avidity, which is influenced by (1) the specific distribution of target glycolipids in the PNS, (2) the glycolipid environment surrounding target antigens, (3) the amount of targeted glycolipids in specific regions, (4) the steric microstructure of sialic acids in target gangliosides, and (5) conformations of glycoepitopes. The discovery of antibodies to complexes of two gangliosides has raised the detection rate of antiglycolipid antibodies in GBS and introduced a new concept of antibody-antigen interactions through clustered carbohydrate epitopes. Peripheral nerve proteins at the nodes of Ranvier, such as neurofascin (NF) 155 and moesin, are candidates as GBS-associated antigens. Most target molecules in the demyelinating variant of GBS are unclear.

Keywords Antibody • Ganglioside • Ganglioside complex

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10.1 Introduction

In the last two decades, much evidence has emerged to indicate that humoral immunity plays a primary role in the pathogenesis of an acute immune-mediated polyradiculoneuropathy, Guillain-Barré syndrome (GBS). The probable targets for autoantibodies in GBS are glycolipids, especially *N*-acetylneuraminic acid (sialic acid)-bearing glycosphingolipids, which are referred to as gangliosides. As described below, circumstantial evidence suggests that autoantibodies play a pathogenic role in development of immune-mediated disorders: as clinical evidence, (1) patients with certain antibodies have homogeneous clinical features, and (2) there is a relationship between antibody activity and features such as symptoms and severity; and as pathophysiologic evidence, (3) target antigens have been identified in specific tissue of relevance to pathognomonic symptoms, (4) disease models in animals can be induced by sensitization with target antigens (active immunization), and (5) passive transfer models in animals can be induced by administration of autoantibodies from active immunization models. From this viewpoint, antiglycolipid antibodies are the most pathogenic in development of GBS, but recent studies suggest that peripheral nerve proteins surrounding nodes of Ranvier are also targets for autoantibodies in GBS.

In this chapter, all types of autoantibodies in GBS are covered, with an emphasis on the clinical and immunobiological aspects of the pathogenic action of antiglycolipid antibodies.

10.2 Antiglycolipid Antibodies and Clinical Phenotype of GBS

Since the first report of antiglycolipid antibodies in the 1980s [1], much progress has been made in unraveling the pathogenesis of GBS. The predominant targets discovered to date are gangliosides, which aggregate and form clusters in neuronal membranes with display of oligosaccharides on the cell surface [2]. Approximately 50 configurationally distinct gangliosides are synthesized through stepwise addition of monosaccharides by Golgi glycosyltransferases. Gangliosides are enriched in neural tissues and account for 10–12 % of total lipid weight in the neuronal membrane and for 20–25 % of lipids in the outer layer of the lipid bilayer in neurons. The gangliosides reside in membrane microdomains referred to as lipid rafts or detergent-resistant membranes, together with other sphingolipids, cholesterol, and glycosylphosphatidylinositol (GPI)-anchored proteins [3], where they are available for antiganglioside antibody binding. These microdomains form platforms and facilitate a variety of membrane-mediated functions, including signal transduction.

Gangliosides in the peripheral nervous system (PNS) are targeted by serum antibodies in approximately 60 % of patients with GBS [4] and in 90 % of patients

with Fisher syndrome (FS), a GBS variant characterized by ophthalmoplegia, ataxia, and areflexia [5]. Screening for antiganglioside antibodies is usually conducted by enzyme-linked immunosorbent assay (ELISA) and thin-layer chromatogram (TLC) immunostaining. Gangliosides purified from bovine brain are generally used as antigens. Clinically, GBS has different subtypes and each subtype is associated with specific antiganglioside antibodies (Table 10.1). A recent collaborative study in Japan and Italy showed that 83 % of patients with acute motor axonal neuropathy (AMAN) had IgG antibodies to GM1, GD1a, N-acetylgalactosaminyl GD1a (GalNAc-GD1a), or GM1b [6]. On the contrary, there is no definitive association with antiglycolipid antibodies in acute inflammatory demyelinating polyneuropathy (AIDP), the prevalent form of GBS in Western countries, although antibodies to galactocerebroside (Gal-C), LM1, or GD1b are present in some AIDP patients [7–9]. Diversity in ganglioside expression in the PNS is thought to influence development of the subtype and symptomatology of GBS. The association of antiglycolipid antibodies with clinical phenotypes in GBS is discussed in the following sections.

10.2.1 Pure Motor Variant of GBS

The pure motor variant of GBS is characterized by an absence of sensory loss and by electrodiagnostic findings of primary axonal dysfunction, virtually synonymous with AMAN. IgG antibodies to gangliosides such as GM1, GalNAc-GD1a, GD1a, or GM1b are associated with this variant [10–13].

10.2.1.1 Anti-GM1 Antibody

Anti-GM1 IgG antibodies are the most common in AMAN and are associated with clinical features of sparing of cranial nerves and distal limb weakness [10]. However, some studies have shown no correlation of anti-GM1 antibodies with neurophysiological results indicative of axonal neuropathy [14, 15]. GM1 is located on myelin of motor nerves and dorsal root ganglia (DRGs), as well as axolemmae at the nodes of Ranvier [16–18]. In a rabbit model of AMAN inoculated with lipooligosaccharide (LOS) from *C. jejuni* or a ganglioside mixture containing GM1, target molecules for pathogenic anti-GM1 IgG antibodies were distributed in the axonal membrane at the nodes of Ranvier of motor nerves [19, 20]. In β1,4-N-acetylgalactosaminyltransferase (GalNAc-T; GM2/GD2 synthase) knockout or wild-type mice, abundant GM1 is present in the lipid raft fraction at paranodes and is required for preservation of the paranodal architecture and clusters of voltage-gated sodium channels (Nav) [21]. In a rabbit AMAN model with GM1-specific antibodies, nodes of the ventral roots were lengthened at locations where Nav channel clusters were disrupted in a complement-mediated manner [22]. Paranodal axoglial junctions, nodal cytoskeleton, and Schwann cell microvilli

Table 10.1 Target antigens of antiglycolipid antibodies, clinical features, and localization in human peripheral nerves

Antigens	Ig subclass	Frequency (%)	Antecedent infection	Clinical features	Localization in PNS
GM1	IgG	30–40	*C. jejuni*, *H. Influenzae*	AMAN	ND (node?)
GD1a	IgG	20–30	Unidentified	AMAN	ND
GalNAc-GD1a	IgG	10–20	*C. jejuni*	AMAN	Axonal membrane of motor nerves at node and paranode, axolemma of small fibers in sural nerves
GM1b	IgG	20–30	*C. jejuni*	Pure motor GBS	ND
GM2	IgG, IgM	4	*Cytomegalovirus*	Facial palsy, sensory loss	ND
GD1b (specific)	IgG	2	Unidentified	Ataxia in GBS, sensory deficits	Large neurons in DRG, paranodal myelin
GD3	IgG	Unknown	Unidentified	AIDP?	ND
GQ1b	IgG	80–90 in FS	*H. Influenzae*, *C. jejuni* (uncommon)	FS, GBS with ophthalmoplegia	Paranodal myelin of oculomotor, trochlear, and abducens nerves, large neurons in DRG, nerve terminals inside muscle spindles and in the vicinity of intrafusal fibers
GT1a (specific)	IgG	Unknown	Unidentified	Bulbar palsy in GBS, PCB-GBS	ND
LM1	IgG	Unknown	Unidentified	AIDP?	Myelin
Galactocerebroside	IgG	Unknown	*Mycoplasma*	AIDP?	Myelin

Ig immunoglobulin, *PNS* peripheral nervous system, *C. jejuni Campylobacter jejuni*, *H. influenzae Haemophilus influenzae*, *GBS* Guillain-Barré syndrome, *AMAN* acute motor axonal neuropathy, *AIDP* acute inflammatory demyelinating polyneuropathy, *FS* Fisher syndrome, *DRG* dorsa root ganglion, *PCB* pharyngeal-cervical-brachial, *ND* not determined

were also disrupted. Taken together, these results show that anti-GM1 IgG antibodies react with GM1 epitopes at the nodes, leading to alteration and destruction of nodal function and structure through complement activation.

The presynaptic axonal membrane is also a target of anti-GM1 IgG antibodies [23]. Presynaptic sites in the neuromuscular junction are outside the blood-nerve barrier, rich in gangliosides, and function as receptors for toxins such as botulinum toxin, from which it is inferred that presynaptic membranes are susceptible to antiganglioside antibody attack.

10.2.1.2 Anti-GalNAc-GD1a Antibody

GalNAc-GD1a, a minor ganglioside in human brain and peripheral nerves [24, 25], is a target for serum antibodies in GBS [26]. IgG anti-GalNAc-GD1a antibody-positive GBS is a pure motor variant characterized by infrequent cranial nerve deficits and distal-dominant weakness [12, 27]. An immunohistochemical study in human peripheral nerves showed that anti-GalNAc-GD1a IgG antibodies bind to nodal and paranodal axolemmae in ventral roots and intramuscular nerves [28]. A biochemical study also showed that GalNAc-GD1a is rich in ventral spinal roots [29]. IgG anti-GalNAc-GD1a antibody is likely to cause conduction failure of motor nerves in axolemmae at nodes or nerve terminals. The above immunohistochemical study also indicated that sensory small fibers can be targeted by the anti-GalNAc-GD1a antibody [28], and antibody accessibility should be kept in mind in the pathogenesis of antibody-mediated neuropathy.

10.2.1.3 Anti-GD1a and Anti-GM1b Antibodies

IgG anti-GD1a antibodies seem to be associated with the AMAN subtype of GBS, rather than anti-GM1 antibodies [11], although GD1a is expressed in human motor and sensory nerves. The precise cellular and subcellular localization of GD1a in motor nerves is known, and structural and chemical differences of glycoepitopes of GD1a between motor and sensory nerves may explain the predisposition of motor nerves for selective dysfunction, as discussed below. The anti-GD1a antibody inhibits regeneration of damaged peripheral nerves, which may be associated with delayed or poor recovery in anti-GD1a-positive AMAN [30].

Kusunoki et al. first reported that a minor ganglioside, GM1b, can be a target antigen for serum antibodies in GBS [4]. Among GBS patients with IgG anti-GM1b antibodies, 36 % had IgG anti-GalNAc-GD1a antibodies and 32 % had anti-GM1 antibodies. Another study in Japan and the Netherlands showed that 56 % of anti-GM1b-positive GBS patients had anti-GM1 antibodies and suffered from pure motor neuropathy, but there was no correlation between the presence of anti-GM1b antibodies and electrodiagnostic findings indicative of axonal neuropathy [13]. The tissue localization of GM1b in human PNS also remains to be determined. A recent study [31] showed that the real target for anti-GM1b antibodies is likely to

be a new glycoepitope resulting from *cis*-interaction of GM1 and GD1a in the cell membrane. That is, a GM1/GD1a complex may be a candidate target for the anti-GM1b antibody.

10.2.2 Other Variants of GBS

Miller Fisher syndrome (MFS), a variant of GBS, is characterized by a clinical triad of ophthalmoplegia, ataxia, and areflexia. IgG anti-GQ1b antibody, which frequently cross-reacts with GT1a, is an excellent diagnostic marker and a pathogenic factor for MFS and plays a pathophysiological role in development of ophthalmoplegia and ataxia in MFS and GBS [5, 32–34]. An immunohistochemical study indicated that GQ1b is densely localized in the paranodal regions of cranial nerves innervating the extraocular muscles and in a subpopulation of large DRG neurons. Nerve terminals inside muscle spindles can also be targeted by anti-GQ1b antibodies [35]. Therefore, GQ1b is likely to be a prime antigen in FS and the IgG anti-GQ1b antibody may cause ophthalmoplegia and ataxia through specific binding to these regions.

The pharyngeal-cervical-brachial (PCB) variant of GBS is defined by acute oropharyngeal and cervicobrachial weakness, often accompanied by areflexia in the upper limbs. Approximately 50 % of patients with PCB have IgG anti-GT1a antibodies, which often cross-react with GQ1b [36]. Therefore, PCB belongs to a continuous spectrum consisting of GBS, MFS, and Bickerstaff brainstem encephalitis [36]. A monospecific anti-GT1a antibody without GQ1b reactivity may be required for development of bulbar palsy [37]. Human glossopharyngeal and vagal nerves contain GT1a, as well as GQ1b [37], with unknown localization in these nerves.

Some patients with GBS suffer from severe ataxia, but not ophthalmoplegia, at disease onset. Case reports have described a close association of IgG anti-GD1b antibodies with ataxia of the sensory or cerebellar type [38, 39]. A model of ataxic neuropathy in rabbits sensitized with GD1b proves this association and indicates that monospecific anti-GD1b IgG antibodies are critical for development of ataxic neuropathy [40, 41]. A study of anti-GD1b activity in response to a mixture of GD1b and another ganglioside in GBS patients with and without ataxia also supports the importance of GD1b-specific antibodies in development of ataxia in GBS [42]. GD1b has been proven immunohistochemically to be localized on human DRG neurons and especially on neurons of large diameter [43].

Acute inflammatory demyelinating polyneuropathy (AIDP) accounts for almost 90 % of cases of GBS in Western countries. Antibodies to myelin glycolipids can cause demyelinating neuropathy, as shown in rabbits immunized with galactocerebroside (Gal-C) [44]. Glycolipids such as LM1 and GD1b are also putative target antigens for AIDP [7–9, 45], but without definitive evidence. The pathology of AIDP partially resembles the experimental allergic neuritis (EAN) produced by immunizing susceptible rodents with adjuvant in peripheral nerves.

10.2.3 Immunoglobulin Subclass of Antiglycolipid Antibodies

The IgG class of antiglycolipid antibodies is more pathogenic than IgM class antibodies. IgG antiglycolipid antibodies in GBS and MFS are divided into IgG1 and IgG3 subclasses, which may be associated with clinical features [46–48]. The IgG1 and IgG3 subclasses of anti-GM1 antibodies are associated with slow and rapid recovery, respectively [49]. Among IgG antibodies to GM1, GM1b, GD1a, or GalNAc-GD1a, IgG1 antibodies have been linked to antecedent diarrhea, positive *Campylobacter* serology, cross-reactivity to *C. jejuni* LOS, and a poor outcome, whereas IgG3 antibodies are associated with upper respiratory infections, cross-reactivity to *Haemophilus influenzae* LOS, and a better outcome [50]. The type of antecedent infectious agents precipitating GBS or FS may govern the IgG subclass.

10.3 Antibodies to Ganglioside Complexes in GBS and MFS

Conventional measurement of antiglycolipid antibodies was conducted only for purified single glycolipid antigens by ELISA or TLC immunostaining prior to discovery of antibodies to mixtures of two different gangliosides in GBS sera. Glycolipids tend to cluster and function as platforms for cell functions in the plasma membrane, where glycoepitopes of each glycolipid gather and bind tightly to form complex glycoepitopes in the membrane. Antibodies specific to mixtures of two different gangliosides were found in sera from GBS and MFS patients and named antiganglioside complex (GSC) antibodies [51, 52]. The activity of these antibodies is optimal at a 1:1 (w/w) ratio of the two gangliosides. The anti-GSC antibodies often have little or no reactivity with each constituent ganglioside, but bind to clustered glycoepitopes formed in the GSC. Since the discovery of anti-GSC antibodies, ELISA conducted in a grid manner with horizontal and vertical mixing lines has been utilized for antiganglioside antibody-screening. In this section, the clinical and pathophysiological significance of anti-GSC antibodies in GBS and MFS are discussed.

10.3.1 Anti-GSC Antibodies in GBS

Anti-GSC antibodies in GBS and its variants are shown in Table 10.2. A survey of antibodies to GSCs consisting of two of the four major gangliosides (GM1, GD1a, GD1b, and GT1b) in GBS showed that 17 % of GBS patients had more than one IgG anti-GSC antibody, such as GM1/GD1a, GD1a/GD1b, GD1b/GT1b, and GM1/GT1b and that antibodies to GD1a/GD1b or GD1b/GT1b were closely

Table 10.2 Frequency and clinical association of antibodies to ganglioside complexes in GBS and its variants

Ganglioside complex antigens	Frequency	Antecedent infection	Clinical features
GM1/GD1a	GBS (17 %)	GI (*C. jejuni*)	
GD1a/GD1b	GBS (7 %)	GI (*C. jejuni*)	Lower cranial nerve deficits, severe disability (artificially ventilated)
GD1b/GT1b	GBS (6 %)	GI (*C. jejuni*)	
GM1/GalNAc-GD1a	GBS (3–5 %)	URI	Pure motor variant, AMCBN
GM1/GQ1b, GM1/GT1a, GD1b/GQ1b, GD1b/GT1a	41 % of FS, 28 % of GBS with ophthalmoplegia		Ophthalmoplegia
GD1a/GQ1b, GD1a/GT1a, GT1b/GQ1b, GT1b/GT1a	6 % of FS, 19 % of GBS with ophthalmoplegia		Ophthalmoplegia
GA1/GQ1b, GA1/GT1a	Unknown		Ophthalmoplegia (FS, GBS, BBE)
GM1/LM1	GBS (7.5 %)		AIDP?

GI gastrointestinal infection, *URI* upper respiratory infection, *AMCBN* acute motor conduction block neuropathy, *BBE* Bickerstaff brainstem encephalitis. Other abbreviations are described in the footnotes to Table 10.1

associated with lower cranial nerve deficits and severe disability requiring artificial ventilation [53]. An immunoabsorption study of the four anti-GSC antibodies above indicated that an epitope formed by a combination of [Galβ1-3GalNAc] and [NeuAcα2-3Galβ1-3GalNAc] in terminal moieties of ganglio-*N*-tetraose structures is essential for antibody binding (Fig. 10.1a, b). More multivalent glycoepitopes in GSCs and tighter interactions between anti-GSC antibodies and GSC antigens can induce stronger antibody-mediated immunoreactions, explaining the association of anti-GSC antibodies with severe disability.

An antibody to a GM1/GalNAc-GD1a complex consisting of GM1 and GalNAc-GD1a is found in about 5 % of GBS patients and is associated with development of pure motor GBS [54, 55]. GBS with anti-GM1/GalNAc-GD1a antibodies is characterized by a preceding respiratory infection (66 %) and infrequent disturbance of cranial and sensory nerves. In half of cases positive for anti-GM1/GalNAc-GD1a, electrophysiological findings show early conduction block (CB) at intermediate nerve segments of motor nerves, but not at common compression sites such as the wrists and elbows. Serial nerve conduction studies show rapid recovery of CB and no findings indicative of remyelination and axonal degeneration. Therefore, CB is likely to result from impairment of axonal membrane properties at nodes of Ranvier [56], where voltage-gated sodium channels (Nav) are densely clustered. Nav dysfunction may cause reversible conduction failure in AMAN. Temporary blockade of Nav can provoke CB with rapid normalization within days, as often observed in poisoning by the Nav-blocking toxins, saxitoxin and tetrodotoxin [57]. GM1 and

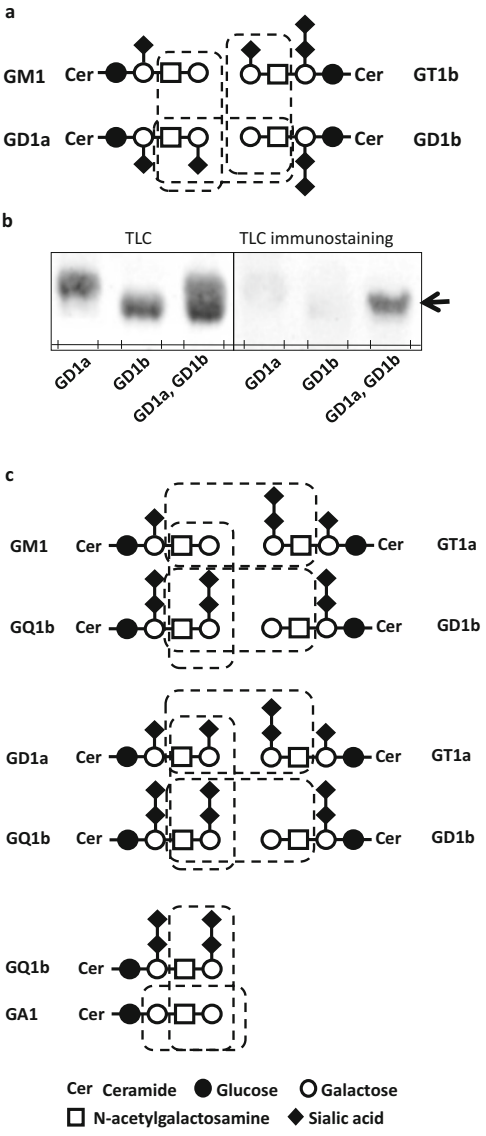

Fig. 10.1 Antibodies to ganglioside complexes (GSCs) in GBS. (**a**) Pattern diagrams of GSCs: GM1/GD1a, GD1a/GD1b, GM1/GT1b, and GD1b/GT1b. *Squares* with *dotted lines* illustrate putative antigenic epitopes for anti-GSC antibodies. (**b**) Results from thin-layer chromatography (TLC) using GD1a and GD1b. The *left panel* shows TLC bands visualized with orcinol. The *right panel* shows TLC immunostaining using a representative anti-GD1a/GD1b-positive serum. The overlapping region between GD1a and GD1b is clearly stained (*arrow*). Serum is diluted 1:100. Developing solution: chloroform, methanol, and 0.2 % $CaCl_2 \cdot 2H_2O$ (50:45:10, v/v). The experimental procedure is described in detail in the GlycoScience Protocol Online Database (http://jcggdb.jp/GlycoPOD/protocolShow.action?nodeId=t122). (**c**) Pattern diagrams of GSCs containing GQ1b or GT1a. *Squares* with *dotted lines* indicate putative antigenic epitopes for anti-GSC antibodies

GalNAc-GD1a are likely to form a GM1/GalNAc-GD1a complex on axolemmae at the nodes of Ranvier in motor nerves, where the binding of anti-GM1/GalNAc-GD1a antibodies may provoke direct or indirect alteration of regulatory functions of Nav.

10.3.2 Antibodies to Ganglioside Complexes in MFS and GBS with Ophthalmoplegia

IgG antibodies to GSCs containing GQ1b or GT1a are detected in half of patients with MFS [52]. Anti-GM1/GQ1b antibodies react with GD1b/GQ1b, GM1/GT1a or GD1b/GT1a, and anti-GD1a/GQ1b antibodies react with GD1a/GT1a or GT1b/GQ1b, indicating specific binding of anti-GM1/GQ1b-reactive sera to GSCs with a combination of [Galβ1-3GalNAc] and [NeuAcα2-8 NeuAcα2-3Galβ1-3GalNAc] in terminal residues of ganglio-N-tetraose structures. On the other hand, anti-GD1a/GQ1b-reactive sera bind to GSCs with [NeuAcα2-3Galβ1-3GalNAc] and [NeuAcα2-8 NeuAcα2-3Galβ1-3GalNAc] in terminal residues (Fig. 10.1c) [52, 58]. Anti-GSC antibodies associated with MFS can be subdivided into (1) GQ1b-specific, (2) GM1/GQ1b-reactive, and (3) GD1a/GQ1b-reactive antibodies. Antibodies to GSCs containing GQ1b or GT1a are also found in 47 % of GBS patients with ophthalmoplegia [58]. The clinical triad of ophthalmoplegia, ataxia, and areflexia in MFS is independent of the variety of anti-GSC antibodies, implying that GQ1b, GM1, and GD1a are commonly colocalized in the nerve membrane in targeted tissues.

IgG antibodies to glycolipid complexes consisting of GA1 and GQ1b or GA1 and GT1a are found in some patients with MFS or GBS with ophthalmoplegia [59]. The terminal residues of GA1/GQ1b are similar to those of GM1/GQ1b or GD1b/GQ1b (Fig. 10.1c). However, 70 % of antibodies to GA1/GQ1b or GA1/GT1a do not bind to GM1/GQ1b or GD1b/GQ1b. Sialic acids attached to internal galactose or those on terminal residues may influence the avidity of antibodies to GSC containing GQ1b or GT1a.

10.3.3 Induction of Anti-GSC Antibodies

Molecular mimicry between *C. jejuni* LOS and GSCs targeted by serum antibodies from GBS patients is associated with induction of anti-GSC antibodies [60]. Antibodies to GM1/GD1a, GD1a/GD1b, GD1a/GQ1b, and GD3/GQ1b from a GBS patient reacted with LOS from autologous *C. jejuni* strains, whereas strains isolated from GBS patients with anti-GD1a/GQ1b antibodies expressed a homogeneous LOS with only a GD1c-like structure, suggesting that the structures of target glycolipids may differ from that expected based on serum anti-GSC antibodies [59].

10.4 Antiglycolipid Antibody-Mediated Pathophysiology in GBS and MFS

Recent studies indicate that complement activation plays a pathophysiological role in antiglycolipid antibody-mediated nerve damage [61]. Some in vitro studies suggest that a complement-independent pathophysiology also has a role in nerve damage. The discovery of anti-GSC antibodies has allowed a better understanding of the pathophysiological action of antiganglioside antibodies in GBS. In this section, an updated overview of the pathogenic roles of these antibodies in GBS and MFS is given.

10.4.1 Complement-Mediated Nerve Injury

Complement activation on nerve cell membranes plays a key role in antiganglioside antibody-mediated nerve injury in GBS and its variants [62–65], as shown in vitro and ex vivo [65]. Deposits of a membrane attack complex (MAC) are present at motor nerve terminals together with antiganglioside antibodies in experimental models of GBS or MFS [66–68], along with destruction of perisynaptic Schwann cells and neurofilaments. Induction of antiganglioside antibody-mediated complement activation is Ca^{2+} dependent, indicating that the classical pathway is critical [67].

In an anti-GM1 antibody-positive rabbit model of AMAN, complement-mediated impairment of paranodal axoglial junctions, nodal cytoskeleton, and Schwann cell microvilli are observed with destruction of clusters of Nav, which gradually improves in the late recovery phase [22]. In an anti-GD1b antibody-positive rabbit model of acute sensory ataxic neuropathy (ASAN), rabbit IgG, activated complement (C3), and MAC are deposited at nodes of Ranvier in dorsal roots, where clusters of nodal and paranodal molecules such as Nav and contactin-associated protein (Caspr) are disrupted [69]. Axon diameters increase at nodes with C3 deposition, indicating that larger axons in dorsal roots may be preferentially attacked by IgG anti-GD1b antibodies, consistent with sensory ataxia [69]. These animal models provide convincing evidence that complement activation plays a primary role in the pathogenesis of antiganglioside antibody-mediated nerve dysfunction in GBS. The hypothesis that nerve injury in GBS patients with antiganglioside antibodies is induced through complement activation is also supported by the observation that the IgG subclass (IgG3 or IgG1) of antiganglioside antibodies in GBS and MFS has the ability to fix complement.

In a recent ex vivo study in a mouse phrenic/diaphragm muscle model, anti-GSC antibodies from GBS patients had a neurophysiological blocking effect at motor nerve terminals [70], where deposits of C3 and MAC, as well as human IgG, coexist, accompanied by loss of neurofilaments. Thus, anti-GSC antibodies are

also likely to induce complement-mediated nerve damage through an antigen-antibody interaction.

10.4.2 Complement-Independent Nerve Injury

10.4.2.1 Antibody-Mediated Dysfunction of Ion Channels

Recent in vitro studies suggest that a complement activation-independent mechanism can cause nerve dysfunction in GBS. Rabbit IgG anti-GalNAc-GD1a antibody binds to motor nerve terminals and blocks neurotransmitter release through a presynaptic inhibitory effect on voltage-gated Ca channel currents, independent of complement activation [71]. IgG antibodies to GM1, GalNAc-GD1a, or GD1a from AMAN patients also inhibit the Cav2.1 voltage-gated Ca channel current in cerebellar Purkinje cells [72]. Anti-GM1 and anti-GD1a mouse monoclonal antibodies inhibit presynaptic-transmitter release in a complement-independent manner, probably because depolarization-induced calcium influx is inhibited [23]. Finally, antiganglioside antibodies may cause a complement-independent functional blockade at motor nerve terminals through dysregulation of synaptic transmitter release by decreasing entry of Ca^{2+} at the presynaptic membrane. However, neuromuscular transmission failure has not been observed in clinical electrophysiological tests in GBS patients with antiganglioside antibodies.

10.4.2.2 Alteration of Functional Molecules in Lipid Rafts

Antiganglioside antibodies bind to clustered gangliosides located on microdomains, referred to as lipid rafts, in biological membranes, and may directly exert a harmful effect on cell function without complement activation. This hypothesis is supported by the following intriguing study of biological effects of antiganglioside antibodies on PC12 cells. IgG anti-GM1 antibodies from GBS sera inhibited nerve growth factor (NGF)-associated neurite outgrowth and reduced NGF-induced Trk autophosphorylation through displacement of Trk protein from the lipid raft fraction to the non-lipid raft fraction [73]. Thus, antiganglioside antibodies potentially have a direct influence on the integrity of membrane lipid rafts and provoke complement-independent nerve dysfunction.

10.4.2.3 Apoptosis

In a rabbit model of anti-GD1b-positive ataxic neuropathy, apoptotic changes occur in large-diameter DRG cells [74]. This suggests that activation of an apoptotic cascade plays a key role in development of ataxia in anti-GD1b-positive GBS.

10.5 Putative Factors Influencing Pathophysiological Action of Antiglycolipid Antibodies

The pathophysiological effect of antiganglioside antibodies on nerves is chiefly regulated by antibody-antigen interactions, which are influenced by various factors, as discussed below.

10.5.1 Antibody Specificity and Distribution of Gangliosides in the PNS

Antibody specificity and the distribution of target gangliosides in the PNS are important factors in antiganglioside antibody-mediated nerve dysfunction (Table 10.2) [75]. As described above, anti-GQ1b antibody-associated ophthalmoplegia in GBS and MFS is principally associated with specific localization of GQ1b on paranodal myelin in human oculomotor, trochlear, and abducens nerves [5]. Antibodies specific to GD1b precipitate ataxia in GBS [42] and induce ataxic neuropathy in rabbits sensitized with GD1b, correlated with the location of GD1b in subsets of large DRG cells [40]. GalNAc-GD1a, a target ganglioside in AMAN, is densely located on axolemmae at nodes of Ranvier and motor nerve terminals [28].

10.5.2 Glycolipid Environment and Antibody Avidity

Complex glycolipid environments in the cell membrane influence accessibility and avidity of antiganglioside antibodies for target gangliosides [42, 76, 77]. This is an important concept that has emerged from studies of anti-GSC antibodies. In an ELISA of the specificity of IgG anti-GD1b antibody, the binding of antibodies specific to GD1b was strongly inhibited in a well in which gangliosides with two or more sialic acids were added to GD1b [42], indicating that colocalization of gangliosides with two or more sialic acids masks or modifies target epitopes of GD1b and influences accessibility of the anti-GD1b antibodies. *Cis*-interaction of the sugar chain of gangliosides in membranes may modify the conformations of glycoepitopes and regulate the accessibility and avidity of antiganglioside antibodies for target gangliosides (Fig. 10.2). An in vitro and ex vivo study in GalNAc transferase-deficient (GalNAc-T$^{-/-}$) and GD3 synthase-deficient (GD3s$^{-/-}$) mice showed that the avidity of anti-GM1 antibodies to GM1-like epitopes can be governed by which glycolipids neighbor GM1 on the cell membrane [76]. In another study using monoclonal antibodies to GA1 or GQ1b [78], the anti-GQ1b antibody could access GQ1b epitopes in a GA1/GQ1b complex, but the anti-GA1 antibody could not access GA1 epitopes in the same complex, suggesting that an

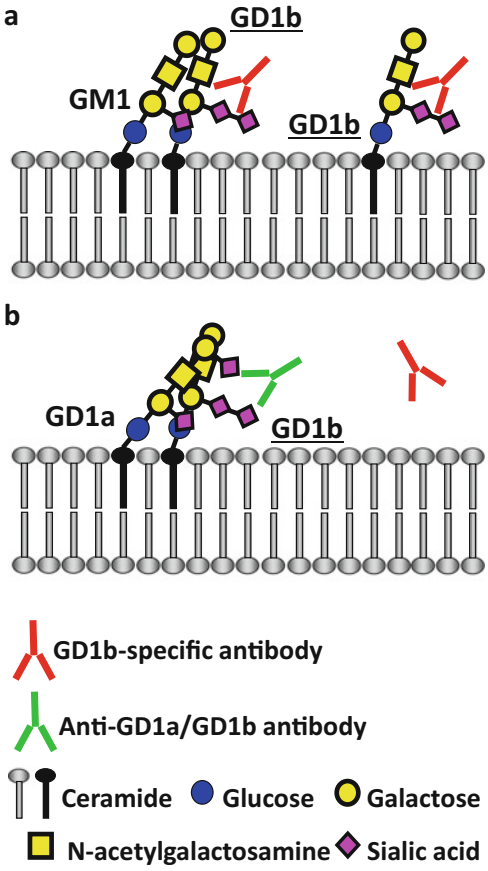

Fig. 10.2 Schematic diagram depicting the influence of the glycolipid environment on antigen-antibody interactions in the nerve cell membrane. (**a**) The GD1b-specific antibody binds to antigenic epitopes of GD1b that are exposed and unmasked in the cell membrane (*right side of the figure*). When GD1b and GM1 colocalize and *cis*-interact together in the membrane, the GD1b-specific antibody can access antigenic epitopes of GD1b in the GM1/GD1b complex (*left side of the figure*). Binding of the GD1b-specific antibody to GD1b is not interrupted by colocalization and *cis*-interaction between GD1b and monosialogangliosides. (**b**) GD1b and GD1a colocalize and *cis*-interact in the membrane. The GD1b-specific antibody cannot bind to antigenic epitopes of GD1b in the GD1a/GD1b complex, while the anti-GD1a/GD1b antibody can bind to glycoepitopes formed in this complex

epitope targeted by a monoclonal anti-GA1 antibody is masked in the GA1/GQ1b complex, whereas that targeted by a monoclonal anti-GQ1b antibody is preserved. Whether glycoepitopes are masked or exposed in clusters of glycolipids is clearly important for antibody binding. In addition to gangliosides, phospholipids may influence binding of antiganglioside antibodies to glycoepitopes. In GBS, anti-GM1 antibody activity against a mixture of GM1 and phospholipids such as phosphatidic acid, phosphatidylinositol, or phosphatidylserine is higher than that against GM1

alone, but anti-GM1 antibody activity against a mixture of GM1 and sphingomyelin is lower [79]. Enhancement of activity by a phospholipid such as phosphatidic acid is likely to depend on the presence of a negatively charged disialosyl residue in the ganglioside antigen [80], rather than that of a particular phospholipid within the pathogen for the antecedent infection. Thus, it should be borne in mind that the local glycolipid environment in the plasma membrane influences the avidity of antiganglioside antibodies.

10.5.3 Amount of Targeted Gangliosides in Specific Regions

A large amount of target gangliosides in specific regions of peripheral nerves predisposes these regions to antiganglioside antibody-mediated damage. Anti-GD1a antibody-mediated nerve damage at motor terminals is evident in GD3-synthase knockout mice that overexpress GD1a, as well as GM1, whereas there is little damage in normal mice [81]. Similarly, a harmful effect of anti-GM1/GD1a antibodies is more evident in the GD3-synthase knockout mice than in wild-type mice [70]. These studies indicate that specific loci with a high level of target gangliosides may be predisposed to attack by antibodies to those gangliosides.

10.5.4 Steric Microstructure of Gangliosides

Avidity of antiganglioside antibodies can also be influenced by the steric microstructure of gangliosides. In experiments using GD1a derivatives with chemically modified sialic acid residues, anti-GD1a monoclonal antibodies that preferentially stained motor axons bound specifically to GD1a-1-ethyl ester, GD1a-1-alcohol, and GD1a-1-methyl ester, whereas another anti-GD1a monoclonal antibody that stained both motor and sensory axons did not react with any of these GD1a derivatives [82]. Anti-GD1a antibodies from patients with AMAN showed the same response to the GD1a derivatives as the motor-specific anti-GD1a monoclonal antibodies.

10.5.5 Conformation of Glycoepitopes

The binding of antiganglioside antibodies to target gangliosides is influenced by the conformation of the glycoepitopes. In human motor and sensory nerves, the amount of GM1 and GD1a is biochemically equal, but the ceramide composition is different [83]. Gangliosides from sensory nerves contain abundant long-chain fatty acids, compared with those from motor nerves. A binding assay using derivatives of GD1a bearing very long-chain fatty acids revealed that the length of ceramide fatty acids of GD1a affects the binding ability of anti-GD1a antibodies and that the ceramide

composition regulates the steric structure of glycoepitopes outside the lipid bilayer [84]. The difference in ceramide composition between motor and sensory nerves may partly explain the preferential binding of anti-GD1a antibodies from AMAN patients to GD1a in motor nerves [18].

10.6 Antibodies to Peripheral Nerve Proteins

Compact myelin proteins such as P0, P2, or peripheral myelin protein 22 (PMP22) can induce experimental allergic neuritis (EAN) in rodents [85, 86]. However, these myelin proteins have yet to be proved to play a pathogenic role in development of GBS, despite evidence from studies of EAN [87]. Recent studies also indicate that nodal, paranodal, or juxtaparanodal proteins can be target antigens in some GBS cases, especially in AIDP.

Neurofascin (NF) 155 is the glial isoform of the cell adhesion molecule NF, which exists in paranodes and is a constituent of an axoglial complex, together with contactin-1 and contactin-associated protein 1 (Caspr 1). NF is palmitoylated and is found with GM1 in lipid rafts at nodes [88]. Two studies using an ELISA or cell-based assay showed that 3–14 % of patients with AIDP had IgG antibodies to NF 155 [89, 90]. The neuronal isoform of NF, NF 186, is located on the nodes of Ranvier and is a constituent of an axoglial complex, together with gliomedin and neuron glia-related cell adhesion molecule. IgG antibodies to NF 186 are found in 3 % of patients with AIDP [89]. In EAN immunized with peripheral myelin, serum antibodies to NF 186, NF 155, or gliomedin were found and voltage-gated sodium channel clusters at nodes were disrupted [91]. However, the pathogenic role of antibodies to NF 155 or NF 186 in GBS remains to be determined.

Moesin is a member of the ezrin-radixin-moesin (ERM) family of proteins and plays structural and regulatory roles in the rearrangement of plasma membrane flexibility. Moesin is located on the membrane in Schwann cells, especially in microvilli at nodes. An IgG antibody to moesin was recently identified in acute serum samples from AIDP patients with antecedent cytomegalovirus (CMV) infection [92]. The mechanism of production of anti-moesin antibodies and their pathogenic role in GBS remain unsettled.

10.7 Perspectives

The main mechanism of antiganglioside antibody-mediated nerve injury in GBS is complement activation. A recent study in mice, however, has shown that activating Fc-gamma receptor-mediated inflammation may also play a role in antiganglioside antibody-mediated axonal injury, independent of complement activation [93]. It is important to consider such complement-independent mechanism, which is able to develop novel therapeutic strategy.

Approximately 40 % of GBS patients have no autoantibodies in their serum. In cases in which only IgM antiganglioside antibodies are detected in routine clinical tests, IgG autoantibodies against unknown glycolipid antigens may play a role in development of GBS [94]. Discovery of unknown target antigens, especially surrounding the nodes of Ranvier, may more clearly unveil the pathophysiology of antibody-mediated nerve injury in GBS.

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Chapter 11
Fisher Syndrome

Atsuro Chiba

Abstract Fisher syndrome (FS) is a regional variant of Guillain–Barré syndrome (GBS) characterized by acute ophthalmoplegia, ataxia, and areflexia. It has a male predominant sex difference with a M:F ratio of ~2:1, and 70 % of FS patients have episodes of antecedent respiratory infection. The annual incidence varies geographically and is higher in Far East countries. As a variant of GBS, immunotherapies are sometimes applied to FS, but the efficacies and benefits of these treatments have not been established. The most important diagnostic marker is serum IgG anti-ganglioside GQ1b antibody, which is present in 80–95 % of FS patients. Using this antibody as a nosological marker has revealed a presumed relationship between FS and other conditions, including Bickerstaff brainstem encephalitis. Anti-GQ1b antibody may be actively involved in the pathogenic process of FS. The localization of the GQ1b epitope in the human ocular motor nerves, muscle spindle, and dorsal root ganglia could reasonably explain all of the core clinical features and physiological observations of this disease. GQ1b-mimic oligosaccharide structures have been identified in bacterial lipooligosaccharides, and immunization with these lipooligosaccharides produces anti-GQ1b antibodies. Siglec-7, a sialic acid-binding Ig-like lectin expressed on monocytes and macrophages, strongly binds to GQ1b and may be involved in antigen-presentation and the induction of anti-GQ1b antibody.

Keywords Fisher syndrome • Anti-GQ1b antibody • Ophthalmoplegia • Ataxia • Bickerstaff brainstem encephalitis

11.1 Introduction

Fisher syndrome (FS) is a regional variant of Guillain–Barré syndrome (GBS). It is characterized by three clinical symptoms, ophthalmoplegia, ataxia, and areflexia, which are known as the triad of FS. In 1956, Miller Fisher was the first to report a

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complete clinical description of this syndrome with insight into its nature [1]. In subsequent reports, the terms "Fisher syndrome," "Fisher's syndrome," and "Miller Fisher syndrome" have been widely used for this striking syndrome. Although Dr. Fisher described that the manifestations of this disease include characteristics of central nervous system (CNS) involvement, he concluded that its main pathology is in the peripheral nervous system. He claimed that this syndrome is a variant of GBS in which there are very selective attacks on the ocular motor nerves and on the sensory neurons that are needed for postural adjustments and stretch reflexes. This conclusion was mainly based on the polyneuropathic motor and sensory symptoms and the albuminocytological dissociation in the cerebrospinal fluid (CSF) of one patient and on the fact that a similar ophthalmoplegia develops in GBS. However, the mechanisms of this selectivity were unknown at that time.

Serum IgG antibody against ganglioside GQ1b was initially discovered in FS, but it has also been detected in other conditions that share some common clinical features with FS, such as GBS with ophthalmoplegia, acute ophthalmoplegia, or ataxia following infection, and so-called Bickerstaff brainstem encephalitis (BBE), which presents with the triad of FS along with CNS manifestations [2–7]. This antibody has become the most important diagnostic biomarker for FS and these related conditions. Possible pathogenic roles for the IgG anti-GQ1b antibody in FS have been suggested, mainly based on the distribution of the antigenic epitope, the GQ1b epitope, in the human nervous system, which is consistent with the clinical features of FS [3, 8].

11.2 Clinical Aspects

11.2.1 Epidemiology

To date, there has not been a survey on the annual incidence of FS specifically, but the FS/total GBS syndromes (including FS) ratio has been reported from several regions. In local studies, these ratios were 3 % in Italy [9], 3 % in Spain [10], 7 % in Thailand [11], 7 % in southwest China [12], 9 % in Hong Kong [13], 18 % and 19 % in Taiwan [14, 15], and 27 % and 34 % in Japan [16, 17]. A nationwide multicenter study in Japan reported this ratio was 26 % [18]. In the eastern hemisphere, there is a trend toward higher FS ratios at higher degrees of longitude. The annual incidence of GBS is relatively similar among regions: 0.81–1.89 per 100,000 members of the population in Europe and North America [19] and 1.15 per 100,000 in Japan (unpublished data, Japanese Research Group of Neuroimmunological Disease). These facts suggest that the incidence of FS varies geographically, with a higher incidence in Far East countries.

There is a sex difference as in GBS; males are more frequently affected than females at a male/female ratio of approximately 2:1. The sex difference is not

different between children and adults [20]. FS occurs at all ages (14 months to 80 years), and the mean age of onset is 43 years [20, 21].

Approximately 80 % of FS patients have preceding infectious episodes: 72–74 % of FS patients have a prior respiratory infection, and 5–6 % of FS patients have prior gastroenteritis. The typical incubation period between the preceding infection and FS onset is 8–10 days [21]. A case-control serological study revealed that FS development has significant relationships with *Campylobacter jejuni* and *Haemophilus influenzae* [22]. Other unusual conditions that were related to FS development include *Escherichia coli* infection, TNFα inhibitor treatment, antiretroviral treatment, influenza vaccination, lymphoproliferative disorders, and cancer [23].

11.2.2 Clinical Manifestations

Ophthalmoplegia, ataxia, and areflexia, known as the triad of FS, are the core clinical manifestations of FS. An analysis of 50 consecutive series in Japan found that the disease started more frequently with diplopia alone (44 %) than with a combination of diplopia and ataxia (34 %) or with ataxia alone (12 %) [17]. The median interval between the onset of diplopia and that of ataxia is 1 day (range 0–4 days). Dysesthesia in the limbs, blepharoptosis, photophobia, and dysphagia can also be present at FS onset. The median time from disease onset to nadir is 6 days (range 2–21 days) [17].

The ophthalmoplegia during FS is relatively symmetrical and generally predominant in the abducentes during the onset period. However, some asymmetricity can be also observed, and unilateral external ophthalmoplegia has been reported in extreme cases [24, 25]. During FS, blepharoptosis is observed as a part of external ophthalmoplegia in approximately 60 % of patients, and internal ophthalmoplegia also develops in approximately 40 % of patients [21]. At its nadir, the severity of external ophthalmoplegia can be complete, with the eye fixed in a mesial position (30–100 % of patients). In some patients, findings suggesting supranuclear or internuclear ocular palsy, dissociation between voluntary and reflexive eye movement, and gaze-directional nystagmus, among other conditions, have also been observed [17].

The ataxia during FS has been described as “cerebellar-like” because patients show an intentional oscillation of their limbs in coordinated movements without a disturbance of their joint position sense. Truncal ataxia, which results in an unsteadiness of gait, is usually more prominent than limb ataxia, and about half of FS patients cannot walk independently at the nadir of their disease course [21]. Even in such severe cases, ataxic speech disturbance is unusual.

Patients with the typical FS features sometimes also show additional clinical manifestations, and as long as they are peripheral in origin, these patients are generally still regarded as having FS. Facial palsy and bulbar palsy are commonly observed, each appearing in approximately 40 % of FS cases. Dysesthesia, a

decrease in superficial sensation, and minimal limb weakness (Medical Research Council scale for muscle strength: grade 4) each occur in around 20s% of cases, and a decrease in deep sensation and autonomic abnormality are each seen in approximately teen percentage of cases [21]. The decrease in vibratory sensation during FS is sometimes proximal dominant, and in a test with a tuning fork, some patients feel a weaker vibratory sensation at the first touch in the sternum than they do at the first touch to the distal end of the forearm.

Optic neuritis has been also reported in patients with otherwise typical FS as another rare additional manifestation [26, 27]. Optic neuritis is a CNS pathology, but FS with optic neuritis is not normally regarded as a form of BBE.

11.2.3 Related Conditions

Other conditions related to FS are listed in Table 11.1. FS and its related conditions are divided into three categories: (1) typical FS (the prototype of these conditions), which makes up the majority of this group; (2) FS-minus, an incomplete or limited form of FS in which ataxia or ophthalmoplegia is absent throughout the entire clinical course (acute ophthalmoplegia and acute ataxia, respectively); and (3) FS-plus, in which motor weakness of the spinal nerve area or CNS symptoms as a consciousness disturbance and/or pyramidal tract signs is/are present (GBS with ophthalmoplegia and BBE, respectively). The relationships between FS and these other conditions had not been well understood. However, IgG anti-GQ1b antibodies have been detected in all of these conditions, so they are considered to be anti-GQ1b antibody-related conditions. Acute isolated blepharoptosis and acute isolated internal ophthalmoplegia are subtypes of acute ophthalmoplegia.

The incidence of GBS with ophthalmoplegia is about 10 % (range 2–28 %) of all GBS cases [28], and the annual incidence of BBE in Japan has been roughly estimated as 100 cases [29]. The incidences of the FS-minus conditions are unknown, but acute ophthalmoplegia is more common than acute ataxia. Interestingly, ophthalmoplegia is more common than ataxia as the initial symptom of FS,

Table 11.1 Fisher syndrome-related conditions

	Popular diagnostic term	Plus or Minus feature
FS-plus	Bickerstaff encephalitis	+ CNS signs [a]
	Guillain-Barré syndrome with ophthalmoplegia	+ Motor weakness in limbs
Prototype	Fisher syndrome (FS)	
FS-minus	Acute ataxia	- Ophthalmoplegia
	Acute ophthalmoplegia	- Ataxia
	(subtype) Acute isolated ptosis	
	Acute isolated internal ophthalmoplegia	

[a] Alteration in consciousness and/or corticospinal tract sign

which may suggest that the ocular motor system is more vulnerable than the ataxia-related system to FS pathogenic factors.

The concept of BBE originally included a postulation that the acute ophthalmoplegia, ataxia, and areflexia in FS are all due to lesions of the CNS, especially the brainstem, and that the syndrome is a kind of brainstem encephalitis [30]. However, now this designation is generally used for any atypical FS presenting with some clinical features of CNS involvement, without specifying the responsible lesions of the triad. IgG anti-GQ1b antibody is detected in approximately 70 % of BBE cases [7, 31], which is a lower percentage than that for FS cases. A nationwide study in Japan revealed that BBE patients who had atypical neurological findings or lacked anti-GQ1b antibodies had clinically features different from those with typical neurological findings and the antibodies; specifically, the former group had a significantly longer duration from disease onset to nadir, significantly higher frequencies of brain MRI abnormalities, and higher CSF protein levels than the latter group. Even though it was not statistically significant level, the frequency of marked CSF pleocytosis over 50/mm^3 and gait inability without aid 3 months after onset were also higher in the former group [29]. These findings suggest that BBE is caused by heterogeneous pathomechanisms and that BBE patients with anti-GQ1b antibodies are probably the true variant of FS with CNS involvement.

11.2.4 Examinations

11.2.4.1 Anti-Glycolipid Antibody

Serum IgG antibody against ganglioside GQ1b is detected in 80–95 % of patients with FS [3, 31, 32]. FS patients also have a relatively high incidence of IgG anti-GT1a antibody (70–80 %) [3, 31, 32]. These antibodies often coexist, and the same IgG molecule reacts with both GQ1b and GT1a [3], suggesting that their common oligosaccharide structure, a disialosyl residue in the nonreducing end, is important as the epitope. IgG antibodies against ganglioside complexes containing either GQ1b or GT1a are also detected in FS patients, and some cases only have antibodies against these complexes, lacking antibodies against the isolated antigens [32]. IgG antibodies against other gangliosides, specifically GD3, GM1b, GalNAc-GD1a, and GD1b, are also detected in a small percentage of FS patients [3, 31]. IgG anti-GQ1b/GT1a antibody is highly disease specific, except for in cases of the pharyngeal-cervical-brachial type of GBS, in which IgG anti-GT1a antibody is detected, and reaches a nearly peak titer right at the onset of neurological symptoms. Therefore, this antibody has been established as a useful early diagnostic biomarker for FS and FS-related conditions.

11.2.4.2 Cerebrospinal Fluid (CSF)

Albuminocytological dissociation in the CSF with a increased protein concentration and a white blood cell count $<10/mm^3$ is observed in 40–60 % of FS patients [21, 33]. Detection of this dissociation depends on the timing of the test, and it is detected more frequently after the first week following disease onset. Pleocytosis is present in 4–7 % of FS patients [21, 31].

11.2.4.3 Electrophysiological Study

The most common electrophysiological abnormal finding in FS is a reduced or absent soleus H-reflex in combination with normal results from motor conduction studies [34–37]. In sensory nerve conduction studies, decreased sensory nerve action potentials are also often observed, but the incidence of this is much lower than that of the H-reflex abnormality. In the largest series of electrophysiological studies on FS patients, the incidences of abnormalities were 32 % in the sensory nerve conduction studies and 67 % in the H-reflex studies [37]. These findings suggest a predominant involvement of group Ia muscle spindle fibers, and this may be related with the ataxia and areflexia in FS.

11.2.4.4 Neuroimaging

The results from brain magnetic resonance imaging (MRI) are essentially normal in FS patients, and this test is usually performed to exclude other disorders. In a large study of FS and BBE patients, abnormal MRI findings were only reported in 1 % of the 353 FS patients and in 11 % of the 47 BBE patients [31]. Using a special methodology, cranial nerve enhancement has been observed via MRI in patients with FS and acute ophthalmoplegia [38–40].

11.2.5 Diagnosis

Some diagnostic criteria have been proposed [41–43]. The diagnostic principle and the core of these criteria are almost identical, and they are described below:

1. Essential features for the diagnosis:
   (1) Clinical manifestations: relatively symmetrical ophthalmoplegia, ataxia, and reduced or absent tendon reflexes
   (2) Clinical course: monophasic, self-limiting course with a progressive period within 4 weeks after disease onset
   (3) No, or minimal if present, motor weakness in the limbs
   (4) No alterations in consciousness or corticospinal tract signs

Table 11.2 Differential diagnosis of Fisher syndrome

Brainstem and/or cerebellum	Peripheral nerve
Wernicke encephalopathy	Lesion in/around the orbital fossa-cavernous sinus
Multiple sclerosis	Vascular neuropathy[b]
Neuromyelitis optica	Tolosa–Hunt syndrome
Behçhet disease	Ramsay Hunt syndrome
Neuropsychiatric SLE	Cerebral arterial aneurysm
Acute disseminated encephalomyelitis	Meningitis carcinomatosa
CLIPPERS[a]	Neoplasm in skull base
Sarcoidosis	Idiopathic intracranial hypertension
Neoplasm	Neuromuscular junction
Vascular disease	Myasthenia gravis
Hypertensive encephalopathy (brainstem type)	Botulism

[a]Chronic lymphocytic inflammation with pontine perivascular enhancement responsive to steroids
[b]Including diabetes mellitus neuropathy, autoimmune vasculitis, and others

2. Features supportive of the diagnosis:
   (1) A history of infectious symptoms within the 4 weeks prior to the onset of neurological symptoms
   (2) Presence of serum IgG anti-GQ1b antibody
   (3) Albuminocytological dissociation in the CSF
   (4) Normal results from a nerve conduction study, or abnormal findings, if any, only in the sensory nerve conduction
3. Exclusion of alternative diagnoses (see Table 11.2)

The items in parts 1 and 3 above are all essential for a diagnosis of FS, but the item 1(2) is used for the final confirmation. Of the items listed in part 2 above, 2 (2) is the most FS disease specific.

11.2.6 Treatments and Prognosis

FS usually takes a monophasic self-limiting clinical course that ends with complete recovery. Patients with FS sometimes receive immunotherapies, such as intravenous immunoglobulin therapy (IVIg) or plasmapheresis, on the theoretical basis that FS is a variant of GBS, but the efficacy of these treatments against FS has not been confirmed for either short- or long-term recovery. To date, there have been no randomized control studies, and only retrospective analyses are currently available.

A retrospective study of 92 patients who had been treated with intravenous immunoglobulin therapy (IVIg; n = 28), plasmapheresis (n = 23), or did not receive any immunotherapies (n = 41) showed limited benefits of IVIg treatment [44]. For

both ophthalmoplegia and ataxia, the periods between the symptom onset and the start of recovery were significantly shorter in the IVIg treatment group than in the group that did not receive immunotherapy. However, there were no significant differences among these three groups in the periods between the onset and the disappearance of ophthalmoplegia or ataxia. The average recovery times for ataxia and ophthalmoplegia were approximately 1 month and 3 months, respectively.

In the Cochrane Review, after excluding the atypical cases that were managed with a ventilator, of the patients who did not receive immunotherapies, were treated with plasmapheresis, or received IVIg, 95 %, 95 %, or 89 %, respectively, experienced complete clinical recovery at 6 months [45]. The recovery rate at 6 months in limited FS (FS-minus) patients was 100 % regardless of treatment. As for BBE, the 6-month recovery rate was 61 % with no clear benefit for any tested treatments.

About 6 % of patients with FS as their initial clinical diagnosis progress to having a GBS diagnosis, and such cases are more likely to be treated with immunotherapies. Additionally, GBS patients with IgG anti-GQ1b antibody more frequently develop respiratory failure requiring ventilator support than those lacking this antibody [46].

FS recurrences have been also reported. The worldwide recurrence incidence is not clear, but two case series in Japan reported recurrence incidences of 14 % (4/28) and 10.8 % (4/37) [47, 48]. In a study in the Netherlands, the FS recurrence rate was 17 % (4/23), which is higher than that for GBS in that study (6 %; 28/485) [49]. Given that the GBS recurrence rate has been estimated as 2–5 % [50], FS may recur more frequently than GBS. The clinical features of recurrent cases are as follows [47, 48, 51]. The number of recurrences for most cases is one or two, but in some exceptional cases, up to seven recurrences have been reported. The interval between attacks ranged from 3 months to 44 years (average: 9.5 years). Most recurrences took the same clinical forms as the initial episode of FS, but some patients also showed additional features during their recurrence, including facial and bulbar weakness, autonomic disturbance, and motor weakness (overlapping GBS and FS). IgG GQ1b/GT1a antibodies were present in all episodes in which they were tested.

11.3 Pathomechanisms

11.3.1 Autoantibodies

Serum IgG anti-GQ1b antibody is elevated during the acute phase in 80–95 % of FS patients [3, 31, 32]. The IgG subclasses of these antibodies are usually IgG1 and IgG3, and these can occur together or separately [52]. The anti-GQ1b antibodies also cross-react with ganglioside GT1a, but they do not usually cross-react with ganglioside GT1b, suggesting that the disialosyl residue in the nonreducing terminals of GQ1b and GT1a oligosaccharides is important as the epitope [3] (Fig. 11.1).

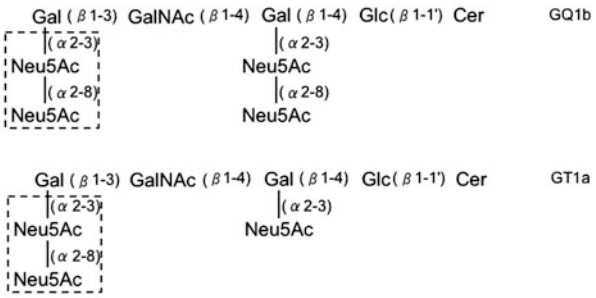

Fig. 11.1 Structures of gangliosides GQ1b and GT1a. Both gangliosides GQ1b and GT1a have a disialosyl residue in their nonreducing ends (*dotted line square*). *Neu5Ac N*-acetylneuraminic acid (sialic acid), *Gal* galactose, *GalNAc N*-acetylgalactosamine, *Glc* glucose, *Cer* ceramide

The antibody titers are usually the highest in the first serum sample, and, in one detailed serial study, they decreased with time even when the clinical manifestations progressively got worse [3]. In the rare instances in which serum samples were obtained from the patients just before the neurological onset of FS, the IgG anti-GQ1b antibodies were already detectable in the samples. These observations suggest that this antibody likely does not result from the damage to GQ1b/GT1a-containing tissues, but instead is involved in the initiation of the damage process.

IgM or IgG anti-GQ1b antibodies against other gangliosides, such as GD3, GM1b, GalNAc-GD1a, and GD1b, are detected infrequently, but they are usually detected simultaneously with IgG anti-GQ1b antibody [3, 31]. IgG antibodies against ganglioside complexes, which react more strongly to mixtures of two different gangliosides than to either isolated ganglioside alone, were detected in about half of the tested FS patients [32]. In these cases, the recognized complex antigens contained either GQ1b or GT1a. As for the relationship between the anti-ganglioside complex antibodies and FS clinical features, FS patients with antibodies against the ganglioside complexes with two sialic acids in their complexed nonreducing terminals showed less frequent sensory disturbances than those with the other FS-associated antibodies. In 5–20 % of FS patients, IgG anti-GQ1b antibody is not detected in enzyme-linked immunosorbent assays that use a isolated ganglioside as the antigen (GQ1b-seronegative FS). In such patients, about 30 % have antibodies against other isolated gangliosides (GM1b, GD1a, and/or GT1a) or against the ganglioside complex (GM1/GT1a) [53]. There were no clinical differences between GQ1b-seropositive and GQ1b-seronegative FS patients, except that males and antecedent gastrointestinal illnesses were more predominant in the latter group.

11.3.2 Autoantibody Targets

The most common clinical feature in IgG anti-GQ1b-positive groups is ophthalmoplegia. The results from an immunohistochemical study with an anti-GQ1b monoclonal antibody that cross-reacts with GT1a and possesses the same fine specificity as those detected in FS patient sera showed a paranodal accumulation of GQ1b antigen in the three ocular motor cranial nerves (oculomotor, trochlear, and abducens nerves) [3] (Fig. 11.2). This staining pattern was only rarely detected in the other cranial nerves and spinal nerve roots. Results from an immunoelectron microscopy study using the same antibody suggested that the plasma membranes of the Schwann cells at the paranodal myelin loop were positively stained by this antibody (Fig. 11.3a). Additionally, scarce, patchily stained spots were also observed on both the outer side and the adaxonal side of the plasma membrane of the Schwann cells around compact myelin (Fig. 11.3b, c). Gangliosides are generally distributed on the cell plasma membrane, forming lipid rafts with cholesterol and other functional molecules. These observations from immunoelectron microscopy experiments suggest that GQ1b-containing lipid rafts are present on the plasma membrane of the Schwann cells of the ocular motor nerves, and they accumulate at high levels in the paranodal myelin loops. Another study also demonstrated staining by an anti-GQ1b/GT1a antibody of the neuromuscular junctions in human extraocular muscles [8]. In a biochemical analysis of the ganglioside composition of human tissues, the proportions of GQ1b in these three ocular motor nerves ranged between 11 % and 13 % in lipid-bound sialic acid. These percentages are significantly higher than those in the other cranial nerves and peripheral nervous tissues, except for that in the optic nerve [54].

Another unique clinical feature of FS is a “cerebellar-like” ataxia. Physiological studies of FS patients revealed abnormalities in their soleus H-reflexes with normal motor conductions [34–37] and abnormalities in their postural body sway analyses [37, 55], suggesting the involvement of Ia afferent fibers. An immunohistochemical

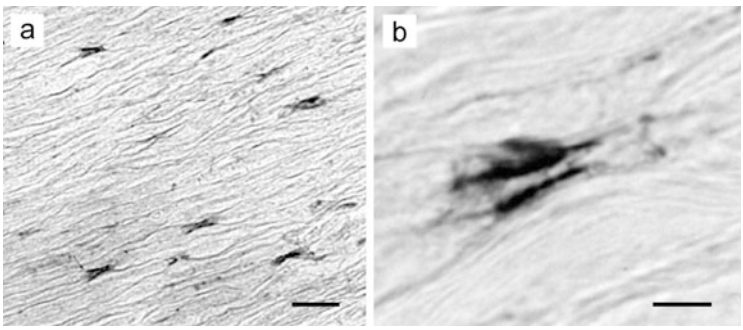

Fig. 11.2 Immunostaining of the human oculomotor nerve by an anti-GQ1b monoclonal antibody. (**a**) Ribbon-like stainings are scattered in the lower magnification view. Scale bar = 50 μm. (**b**) In the high magnification view, the paranodal region, especially the paranodal loops, of the Schwann cell appears to be stained. Scale bar = 5 μm

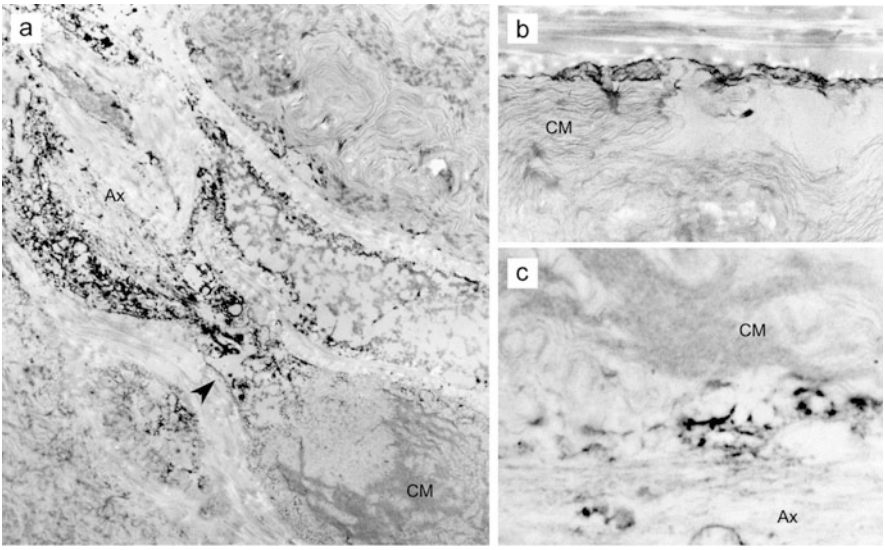

Fig. 11.3 Immunoelectron microscopy of a human oculomotor nerve stained with an anti-GQ1b monoclonal antibody. (**a**) Membranous stainings aggregate at the paranodal region (*arrow head*: node of Ranvier). (**b–c**) Scarce, patchily stained spots are also observed on both the outer side (**b**) and the adaxonal side (**c**) of the plasma membrane of the Schwann cells around compact myelin. *Ax* axon, *CM* compact myelin

study on human muscle spindles found that the entire surface of each individual intrafusal fiber was positively stained by an anti-GQ1b/GT1a monoclonal antibody [8]. The region stained by this antibody is usually abundant in group Ia sensory nerve terminals. Another study showed that some large neurons in the human dorsal root ganglia (DRG) were immunostained by an anti-GQ1b/GT1a monoclonal antibody [5]. In the DRG sensory neurons, neuronal somata with Aα/β nerve fibers, which include group Ia fibers, are large neurons [56, 57]. These observations suggest that anti-GQ1b antibody may be involved in ataxia and areflexia by causing group Ia fiber dysfunction at muscle spindle or DRG as the possible target site.

An anti-GQ1b antibody-related animal model of FS has not been established yet, and the pathological significance of anti-GQ1b antibody has not been completely proven at this point. However, finding a unique distribution of the GQ1b-antigen that is consistent with clinical features of FS patients would support a hypothesis that a combination of antigenic specificity of the antibody and antigen localization determines the clinical feature of GBS.

11.3.3 Molecular Mimicry of Infectious Agents

Although preceding infectious agents have not been identified in the majority of FS patients, some FS cases develop following a *C. jejuni* or *H. influenzae* infection [22].

These pathogens are both gram-negative bacteria, and they have lipooligosaccharides (LOSs) in their outer membranes. Some strains have LOSs containing oligosaccharide structures that mimic gangliosides in their outer core portion. In biochemical analyses on strains isolated from FS patients, a GT1a-mimicking trisialosyl oligosaccharide structure was identified in *C. jejuni* [22], and a disialosyl–galactose structure, which is common to the nonreducing terminals of GQ1b and GT1a, was found in an *H. influenzae* strain from another FS patient [58]. The exact GQ1b-mimicking tetrasialosyl oligosaccharide structure in LOSs has not yet been identified, but immunization of animals with the GT1a-mimicking LOS from *C. jejuni* induced antibodies that react with both GQ1b and GT1a [59, 60]. In human disease, those mimicking molecules might induce anti-GQ1b/GT1a antibodies.

Campylobacter sialyltransferase-II (cst-II) is involved in the biosynthesis of ganglioside-mimics in *C. jejuni* [61]. Because of cst-II gene polymorphisms, there are two products with a substitution at amino acid position 51: Cst-II (Asn51) and Cst-II (Thr51). Cst-II (Asn51) has both α2,3- and α2,8-sialyltransferase activities, whereas Cst-II (Thr51) only has α2,3-sialyltransferase activity. Therefore, Cst-II(Asn51) can synthesize the disialosyl–galactose structure common to the nonreducing terminals of gangliosides GQ1b and GT1a, but Cst-II (Thr51) can only synthesize the monosialosyl structure seen in GM1- and GD1a-mimics. In clinically isolated strains, the presence of a GQ1b-mimicking epitope in the LOS and the clinical presentation of FS were associated with *cst-II*(Asn51), whereas the presence of GM1- or GD1a-epitopes and the clinical presentation of GBS were associated with *cst-II*(Thr51) [62].

11.3.4 Mechanism of Autoantibody Production

The details of the mechanism responsible for the autoantibody production are still unclear. Among immune-related cell surface molecules, the specific HLA type is not linked to anti-GQ1b IgG production [63]. Sialic acid-binding Ig-like lectins (siglecs) are candidate molecules for the recognition of sialylated oligosaccharide antigens. They are I-type lectins, expressed mainly on hematopoietic cells. Fifteen members have been identified in human, and each member is unique in terms of its ligand specificity and which cells express it. Among them, siglec-7 strongly binds to GQ1b- and GT1a-mimic-bearing LOSs from *C. jejuni*, and binding to a disialo-oligosaccharide, especially one with an α2-8 linkage, causes a dramatic conformational shift in siglec-7 [64–66]. In a study using clinical material, binding between siglec-7 and *C. jejuni* LOSs isolated from GBS and FS patients was significantly higher in the presence of anti-GQ1b antibody and ophthalmoplegia [67]. Siglec-7 expressed on monocytes and macrophages may bind to *C. jejuni* with disialylated LOSs, after which the cells process these pathogens, present the resulting antigens, and therefore induce antibodies against terminal α2-8 disialylated residues.

The production of autoreactive antibodies in GBS and FS is thought to be mainly triggered by antecedent infections. In this case, the reaction itself is purposeful for biological defense, and it self-limitingly withdraws after achieving that aim. This is a likely reason for the important features of GBS and FS, specifically the presence of an immunological incubation period and a monophasic clinical course. Because of this, GBS and FS could be considered as partial autoimmune disorders.

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Chapter 12
POEMS Syndrome

Satoshi Kuwabara and Sonoko Misawa

Abstract POEMS (polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes) syndrome is a rare cause of demyelinating and axonal neuropathy and multi-organ involvement, associated with plasma cell dyscrasia and upregulation of serum levels of vascular endothelial growth factor (VEGF). The pathogenesis of the disorder is not well understood, but increased vascular permeability and neovascularization mediated by VEGF and other inflammatory cytokines are likely to play an important role in most of the characteristic symptoms. However, the pathogenesis of peripheral neuropathy is unclear; VEGF may affect the blood-nerve barrier and allow neurotoxic substances to access the nerve parenchyma, resulting in nerve structural damages. POEMS syndrome is a potentially fatal disease, and there is no established treatment regimen for this syndrome. In appropriate candidates, high-dose chemotherapies with autologous peripheral blood stem cell transplantation are recommended, because this treatment can lead to obvious and rapid clinical improvement in neuropathy as well as other symptoms, with a significant decrease in serum VEGF levels. The indication for this treatment has not yet been established, and the long-term prognosis is unclear. Potential future therapies include the administration of thalidomide, lenalidomide, bortezomib, and anti-VEGF monoclonal antibody (bevacizumab). Prognosis of POEMS syndrome has been substantially improved by these novel interventions, whereas randomized control trials are required to establish a proper therapeutic guideline. This review focuses on recent advances in diagnosis and treatment of POEMS syndrome and discusses future perspectives of therapeutic strategy.

Keywords POEMS syndrome • Plasma cell • Autologous stem cell transplantation • Thalidomide • Vascular endothelial growth factor

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12.1 Introduction

POEMS (polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes) syndrome is a rare multi-system disorder with monoclonal plasma cell proliferation and overproduction of cytokines such as vascular endothelial growth factor (VEGF) [1–3]. POEMS syndrome has also been called the Crow-Fukase syndrome, Takatsuki syndrome, and PEP (plasma cell dyscrasia, edema, and polyneuropathy) syndrome. The prevalence of POEMS syndrome is unclear, but a national survey conducted in Japan showed a prevalence of approximately 0.3 per 100,000. The average age of onset is around 40–50s and men are more preferentially affected. The disease was initially thought to be more common in Japan given that the largest initial reports were from Japan [2]. However, large series have also been reported from France, the United States, China, and India, and the disorder has been increasingly recognized in many areas of the world.

POEMS syndrome is a potentially fatal disorder due to refractory pleural effusion/ascites, renal failure, and thromboembolic events, unless treated appropriately. Moreover, polyneuropathy becomes progressively worse and patients often develop disabling muscle weakness leading to tetraplegia [4].

Approximately 50 % of POEMS patients have polyneuropathy as an initial symptom/sign. Largely because electrophysiologic (nerve conduction studies) and pathological (nerve biopsy) showed peripheral nerve demyelination, POEMS syndrome patients are often misdiagnosed as suffering chronic inflammatory demyelinating polyneuropathy (CIDP) [5]. The clues for differentiating POEMS syndrome from CIDP are (1) the lack of response to therapies that are usually effective for CIDP, such as intravenous gamma globulin, corticosteroids, and plasmapheresis; (2) the presence of other features, particularly monoclonal protein, thrombocytosis, papilledema, ascites, new endocrine issues, skin changes, or sclerotic bone lesions; and (3) the presence of monoclonal plasma cell proliferation found in the bone marrow.

12.2 Pathophysiology

The pathogenesis of POEMS syndrome is not well understood, but overproduction of VEGF and other cytokines (interleukin-6 and interleukin-12, tumor necrosis factor-α), possibly secreted by plasmacytomas, is likely to be responsible for many of the characteristic symptoms [6, 7]. Almost all patients with POEMS syndrome have highly elevated serum VEGF levels, and disease activity appears to correlate with VEGF levels. VEGF is a potent multifunctional cytokine that induces prominent angiogenesis and microvascular hyperpermeability and therefore could cause many of the symptoms of POEMS syndrome such as edema/effusion, organomegaly, and skin angioma. However, mechanisms for the peripheral neuropathy are still unclear, but VEGF may affect the blood-nerve barrier and allow

some neurotoxic substances in the serum to access the nerve parenchyma, resulting in nerve demyelination. Moreover, microangiopathy due to proliferative endothelial cells and hypercoagulability may contribute to the development of neuropathy [7].

To date, VEGF is the cytokine that correlates best with disease activity [8]; VEGF induces an increase in vascular permeability and prominent angiogenesis. It is expressed by osteoblasts, in bone tissue, macrophages, plasma cells, and megakaryocytes/platelets. IL-12 has also been shown to correlate with disease activity [7]. Little is known about the plasma cells in POEMS syndrome except that more than 95 % of the time, they are lambda light chain restricted with restricted immunoglobulin light chain variable gene usage.

12.3 Diagnosis and Clinical Features

The diagnosis of POEMS syndrome is established by the combination of clinical and laboratory features. In the advanced stage of the disease, when many systemic manifestations already develop, the diagnosis of POEMS syndrome is not difficult. However, in the early phase, patients show a part of the symptoms or laboratory abnormalities; the disease is often underdiagnosed. Skin changes include angiomata, skin thickening, pigmentation, and hypertrichosis. Important clinical features other than the five cardinal symptoms of "POEMS" include peripheral edema, pleural effusion, ascites, sclerotic bone lesions, Castleman disease, papilledema, polycythemia, and thrombocytosis [3]. Endocrinopathy is a central but poorly understood feature of POEMS. Approximately 80 % of patients have a recognized endocrinopathy, with hypogonadism as the most common endocrine abnormality, followed by thyroid abnormalities, glucose metabolism abnormalities, and lastly by adrenal insufficiency. The majority of patients had evidence of multiple endocrinopathies in the four major endocrine axes (gonadal, thyroid, glucose, and adrenal). For male patients, gynecomastia is frequently present [8].

12.3.1 Diagnostic Criteria

Proposed diagnostic criteria are shown in Table 12.1. Whereas several diagnostic criteria have been published [3, 8], the presented criteria show the highest sensitivity and specificity. Polyneuropathy is present in all patients, and the diagnosis is made by a combination of characteristic symptoms and laboratory findings. Serum VEGF level is a useful diagnostic biomarker [9].

The first issue is whether "monoclonal plasma cell proliferative disease" is defined as a mandatory criterion. Patients in the very early phase of the disease could lack M-protein even by the use of sensitive immunofixation method, and detection of plasmacytoma is sometimes difficult when sclerotic bone lesions or

Table 12.1 Criteria for the diagnosis of POEMS syndrome

Major criteria
(a) Polyneuropathy (mandatory)[a]
(b) Monoclonal plasma cell proliferative disorder
(c) Elevation of serum or plasma VEGF levels
Minor criteria
(d) Sclerotic bone lesions
(e) Castleman disease[b]
(f) Organomegaly (hepatosplenomegaly or lymphadenopathy)
(g) Edema (edema, pleural effusion, or ascites)
(h) Endocrinopathy (adrenal, thyroid, pituitary, gonadal, parathyroid, or pancreatic)[c]
(i) Skin changes (hyperpigmentation, hypertrichosis, plethora, cyanosis, hemangiomata, or white nails)
(j) Papilledema
(k) Thrombocytosis and/or polycythemia

1. Definite POEMS syndrome: three major criteria and at least one minor criterion
2. Probable POEMS syndrome: two major criteria, with at least one minor criterion

[a]Defined by M-protein or monoclonal plasma cell proliferation in bone marrow biopsy or biopsy of a plasmacytoma or sclerotic lesion

[b]There is a Castleman disease variant of POEMS syndrome that occurs without evidence of a clonal plasma cell disorder that is not accounted for in this table list. This entity should be considered separately

[c]Because of the high prevalence of diabetes mellitus and thyroid abnormalities, this diagnosis alone is not sufficient to meet this minor criterion

tumors are present focally or multifocally. There are actually patients who show typical POEMS syndrome features except for demonstration of monoclonal plasma cell proliferative disease [2]. Although plasma cell dyscrasia would play an essential role in pathophysiology of POEMS syndrome, it seems to be helpful for earlier diagnosis that monoclonal plasma cell proliferation is not regarded as a mandatory criterion. Evaluation of serum free light chain ratio may be useful to detect monoclonal plasma cell proliferation in the future.

The second problem is whether Castleman disease or bone sclerotic lesion should be one of the major criteria. The relationship between POEMS syndrome and Castleman disease is still unclear. However, because POEMS syndrome associated with Castleman disease is different from typical POEMS syndrome from the point of view of neuropathy severity, frequency of clonal plasma cell disorder, and cytokine profiles [2], Castleman disease does not appear to be as a major finding to diagnose "typical" POEMS syndrome. Conversely, osteosclerotic lesions are characteristic feature of POEMS syndrome. However, histopathology of osteosclerosis usually reveals diffuse infiltration of light chain-restricted plasma cell [10], and therefore sclerotic bone lesion is almost equivalent to plasma cell proliferative disease. It may be practical to recognize only "plasma cell proliferative disease" as one of the major criteria and to define "sclerotic bone lesions" as one of the minor criteria. From these points, we suggest modified diagnostic criteria (Table 12.1).

Further investigation and discussion is necessary to establish appropriate diagnostic criteria with high sensitivity and specificity.

12.3.2 Diagnostic Procedures

Polyneuropathy is a mandatory criterion of POEMS syndrome. Neurological signs, such as paresthesia or weakness, are invariably symmetric and distal dominant. Evidence of demyelinating neuropathy and axonal degeneration (presumably secondary) predominant in the legs is demonstrated by nerve conduction studies. Electrodiagnostic evaluation should be performed in all patients suspected as suffering POEMS syndrome. Terminal latency index in the median nerve is known as a useful nerve conduction parameter to distinguish POEMS syndrome from CIDP. Careful evaluation can differentiate between POEMS syndrome and CIDP [5].

Monoclonal plasma cell proliferative disease is confirmed by the presence of M-protein (usually lambda light chain), histopathology of osteosclerotic bone lesion, or plasma cell dyscrasia.

Serum or plasma VEGF levels markedly elevate and correlate with disease activity in POEMS syndrome [6, 7, 11, 12]. Serum VEGF levels increase 10–50 times higher than plasma levels.

Bone lesions are common features of POEMS syndrome. Whereas densely sclerotic lesions are typical for POEMS syndrome, osteolytic lesions may be present [8, 13]. The systemic bone survey using PET-CT or bone scan is suitable to screen the whole body, but it could miss small lesions. The windows of bone level on CT scan are informative and practical to detect small lesions in vertebrae, ribs, or pelves [13]. However, it is important to note that benign bone islands, fibrous dysplasia, or aneurysmal bone cysts can be confused with bone sclerosis.

Castleman disease is a rare lymphoproliferative disease. Clinical presentation ranges from asymptomatic localized lymph node swelling to multicentric Castleman disease with systemic general constitutional symptoms such as fever and malaise. Castleman disease or Castleman-like histopathology is complicated by 11–30 % of POEMS syndrome [8]. Lymph node biopsies reveal either hyaline vascular type or plasma cell type.

Previous reports on endocrinopathy in POEMS syndrome have shown that 84 % of the patients had endocrinopathy. The frequent endocrine abnormalities are hypogonadism, hypothyroidism, glucose intolerance, and adrenal insufficiency [8, 10, 14].

Skin changes consist of hyperpigmentation, hypertrichosis, hemangiomata, or white nails. Other features of POEMS syndrome include pulmonary hypertension, restrictive lung disease, respiratory muscle weakness, renal dysfunction, and cardiomyopathy. These are not included in diagnostic criteria but should be evaluated, because they substantially affect treatment course and prognosis.

12.4 Treatment

12.4.1 Overview

POEMS syndrome is a potentially fatal disease, and patients' quality of life deteriorates because of progressive neuropathy, massive peripheral edema, pleural effusion, or ascites. Serious complications such as multi-organ failure from capillary leak syndrome, restrictive lung disease, pulmonary hypertension, and thromboembolic events may occur, contributing to the poor prognosis [3, 8].

There are no randomized controlled trials for POEMS syndrome, presumably because of the rarity of the disorder and therefore no established treatment regimen. Previous case reports and series have described patients with POEMS syndrome who have been treated with irradiation, resection of plasmacytomas, chemotherapies, corticosteroids, plasmapheresis, and intravenous immunoglobulin infusion. Irradiation has usually been proposed for patients with a solitary plasmacytoma. If patients have widespread osteosclerotic lesions, systemic chemotherapy is necessary. In appropriate candidates, high-dose chemotherapy with autologous peripheral blood stem cell transplantation (auto-PBSCT) is recommended. This treatment resulted in obvious improvement in neuropathy as well as other symptoms, with a significant decrease in serum VEGF levels. From data of published experience, the transplant-related mortality was initially reported to be 7.4 %, but a recent update in 2012 suggested a lower transplant-related mortality (3.3 %) with better peritransplant supportive care [3, 10]. Indications for this treatment have not yet been established, and long-term prognosis is unclear. Treatments that may be considered in the future include lenalidomide or thalidomide, anti-VEGF monoclonal antibody (bevacizumab), and bortezomib [3, 10].

12.4.2 Current Treatment

The prognosis of POEMS syndrome substantially improved after introduction of new therapeutic options. The algorithm at present is that high-dose chemotherapy with autologous peripheral stem cell transplantation (auto-PBSCT) is the first-line therapy [3, 9, 10], and patients, who are not indicated for transplantation due to high age (over 65 years) or poor general condition, are treated with thalidomide or lenalidomide with dexamethasone. Radiation therapy is indicated for patients with single or limited numbers of bone lesions. However, small bone lesions can be missed, even if careful systemic survey is conducted. Biopsy of the iliac crest [10] or bone windows of CT scan can help to detect disseminated monoclonal plasma cells [13].

Long-term outcomes of new treatment options and recent increasing numbers of further new agents raise several new clinical questions. The first one is whether all patients under 66 years old should undergo auto-PBSCT, if their condition is

eligible for the treatment. A previous report of long-term outcome of transplantation demonstrated substantial rate of recurrence: a progression-free survival at 5 years was 75 % [11]. Given that transplant-related death, engraftment syndrome, or recurrence, transplantation may not be proper for patients with mild disability. High-dose chemotherapy is a sole intervention to improve advanced polyneuropathy and should be performed in patients with moderate to severe muscle weakness caused by polyneuropathy.

As for mild cases, auto-PBSCT might be kept as the sheet anchor if there is later progression of the disease. In such situation, thalidomide/lenalidomide or proteasome inhibitor can be the first-line therapy, and thalidomide seems to be preferable from the regards of effects on stem cell collection or neurotoxicity.

12.4.3 Perspectives

In the near future, there will be increasing numbers of therapeutic choices for multiple myeloma, such as proteasome inhibitor, signal transduction inhibitors, and molecular targeting drugs, and they can be applied to POEMS syndrome. Clinicians should efficiently choose an appropriate agent from multiple options, and therapeutic algorithms must progress. There is no randomized control trial for POEMS syndrome [3]. To establish an evidence-based therapeutic guideline, well-designed clinical trials are required. Treatment goal has already shifted from short-term survival to long-term management of the disease. Separately, there are considerable numbers of patients, who are undiagnosed until the advanced stage. For the further prognosis improvement, not only development of novel therapeutic approach but also earlier diagnosis is particularly important.

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Chapter 13
Treatment of Myasthenia Gravis After the 2014 Japanese Clinical Guideline

Masakatsu Motomura, Ruka Nakata, and Hirokazu Shiraishi

Abstract Treatments for MG, which is an autoimmune disease, are based on immunotherapy by steroids and immunosuppressants. In Japan, treatments include cholinesterase inhibitors, thymectomy, steroids, immunosuppressants, plasma exchange, and intravenous immunoglobulin therapy. However, even when utilizing all of these treatments, the proportion of MG patients who enter complete stable remission is less than 10 %. The current goal of MG treatment is firstly to treat MG patients at onset to pharmacological remission as rapidly as possible using the above therapies; the second is reducing oral prednisolone to 5 mg/day or less to minimize disease manifestation and symptoms in daily life. Japanese MG clinical guidelines were revised in 2014, with proposed novel MG diagnostic criteria and new standard treatments. Currently, reviewing MG treatments which had been done previously is being done in each facility. Here, the authors describe the therapeutic protocol according to onset age, disease type, and pathogenic autoantibodies conducted in Nagasaki University Hospital. We have combined the MG clinical guidelines for 2014 with our experiences to date and are treating MG patients using this protocol.

Keywords Myasthenia gravis • Pathogenic autoantibodies • Acetylcholine receptor • Muscle-specific receptor tyrosine kinase • Clinical guideline • Treatment

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13.1 Introduction

Myasthenia gravis (MG) is the most common autoimmune disease affecting neuromuscular junctions. MG patients produce pathogenic autoantibodies against acetylcholine receptors (AChRs), muscle-specific receptor tyrosine kinase (MuSK), and low-density lipoprotein receptor-related protein 4 (Lrp4) which are localized on the postsynaptic membranes of neuromuscular junctions [1] (Fig. 13.1a). Since these functional synaptic proteins involved in synaptic transmission from nerves to muscles are impaired, muscle weakness with easy fatigability is observed. This clinical feature is useful for diagnosis; thus, MG is a disease for which the skills of the neurologist are required. MG patients develop muscle weakness with easy fatigability and diurnal variation in their extraocular muscles, swallowing muscles, and muscles of the trunk and extremities, with remission and exacerbation and rapidly progressive respiratory failure, the so-called crisis, which is clinically important.

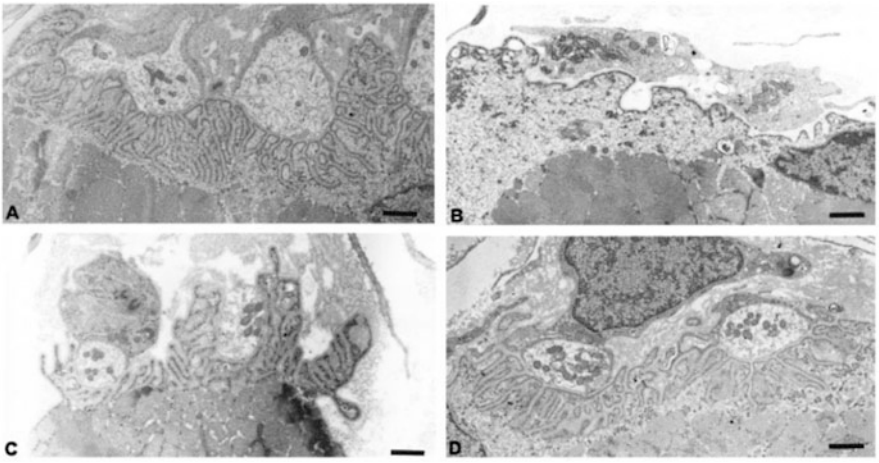

Fig. 13.1 Electron microscopic observations of the motor end-plates are shown. In control (**a**), the postsynaptic membrane is well preserved, and the folds are thick. In (**c**, **d**) muscle-specific receptor tyrosine kinase (MuSK) antibodies (Abs), the postsynaptic membrane is also well preserved. The primary and secondary synaptic cleft showed normal width and the folds are slightly thin. However, the primary and secondary postsynaptic fold in **b** acetylcholine receptor (AChR) Ab-positive myasthenia gravis (MG) patients showed widening and simplification by the destruction of postsynaptic membrane by complement. Bar 1 μm

13.2 Classification

There are some disease classifications based on pathogenic autoantibodies and clinical features. They are based on the type of pathogenic autoantibodies present: (1) AChR antibody-positive MG, (2) MuSK antibody-positive MG, (3) Lrp4 antibody-positive MG, and (4) those where these antibodies are not detected, being seronegative MG [1]. The autoantibody seropositivity of patients is estimated by routine radioimmunoassay (RIA), being 85 % AChR antibody-positive MG patients, less than 5 % MuSK antibody-positive MG patients, less than 1 % Lrp4 antibody-positive MG patients, and the remaining 10 % comprising seronegative MG patients.

Instead of the Osserman classification of 1971, up to now, the most widely accepted clinical classification is the Myasthenia Gravis Foundation of America (MGFA) clinical classification which has been widely used as a measure of severity rather than disease type. In Japan, depending on the presence of thymoma and age of onset of MG, early-onset MG (EOMG, age at onset $\leq$49 years), late-onset MG (LOMG, age at onset $\geq$50 years), or thymoma-associated MG (TAMG), the so-called E-L-T classification [2] has been recommended in the 2014 MG clinical guideline.

13.3 Epidemiology and Guidelines

In the 2006 Japanese nationwide epidemiological survey, the estimated number of MG patients in Japan was 15,100, giving a prevalence of 11.8 per 100,000. The male-to-female ratio was 1:1.7 and mean $\pm$ standard deviation of onset age was 42.7 ± 21.2 years of age. In particular, late-onset MG (LOMG) ($\geq$65 years) accounted for 7.3 % in 1987 (adjusted for population in 2005), but this had increased to 16.8 % in 2006 [3]. Therefore, standard treatment guidelines for LOMG were published by the Japanese Society of Neurological Therapeutics in 2010. Thereafter, MG clinical guidelines were revised in 2014. In these guidelines, the up-to-date estimated number of MG patients was 20,000; MuSK antibodies were recognized as a second type of pathogenic autoantibodies, and therapeutic strategies were also significantly changed as described below (Fig. 13.2).

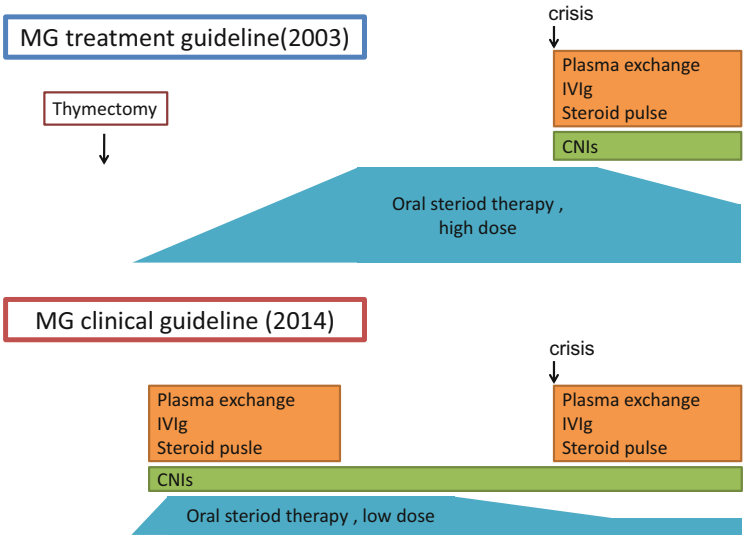

Fig. 13.2 Comparison between MG treatment guideline 2003 and MG guideline 2014

13.4 Pathogenic Autoantibodies and Pathomechanism

The history of autoantibody studies in MG began from that of Lindstrom et al. who discovered AChR antibodies in the serum of MG patients in 1976. The antibody assay was based on immunoprecipitation by patient IgG antibodies of detergent-solubilized human muscle AChRs labeled with ^{125}I-α-bungarotoxin, a snake toxin. This assay is still in use today. The pathogenicity of AChR antibodies was demonstrated by AChR-immunized animal models and passive transfer by AChR monoclonal antibody such as mAb35. In its pathogenesis, the binding of AChR antibodies to AChR leads to loss and/or inactivation of AChRs in the motor end-plates (Fig. 13.1b). AChR loss is caused by complement-mediated destruction of the postsynaptic membrane and increased internalization and degradation of the AChRs (antigenic modulation). Human AChR antibodies are polyclonal and predominantly IgG1, but the majority of anti-AChR antibodies appear to bind to a region known as the main immunogenic region (MIR) which is located on the α-subunit N-terminal region including amino acid residues 67–76 [4]. On the other hand, in 2001, Hoch et al. reported MuSK antibodies to be detected in the sera of generalized MG patient which were AChR antibody negative. Anti-MuSK antibodies are predominantly IgG4 which does not activate complement. Shiraishi et al. reported no complement-mediated destruction at the motor end-plates in MuSK antibody-positive MG (Fig. 13.1c, d), unlike that caused by the IgG1 subclass in AChR antibody-positive MG [5]. Thereafter, a lot of clinical studies including transient neonatal MuSK antibody MG and successive MuSK animal model studies demonstrated their pathogenicity. However, the MIR for antibody binding and the mechanism of MuSK antibody-mediated damage still remains unknown. In 2011,

Higuchi et al. reported Lrp4 antibodies from patients in Japan [6]. After that, there have been subsequent clinical studies and success in inducing Lrp4 antibody-mediated MG in animal models. Lrp4 antibodies are being recognized as the third type of pathogenic autoantibodies in addition to AChR/MuSK antibodies in MG.

13.5 Clinical Manifestation

Although there are some characteristic symptoms and signs of MG caused by each type of pathogenic autoantibodies, it is difficult to distinguish clinically which AChR antibodies and MuSK antibodies MG patients have. In AChR antibody-positive MG patients, the extraocular muscles are likely to be impaired. In most cases, the first noticeable symptoms and signs are diplopia and blepharoptosis. In others, difficulty in swallowing and slurred speech may be the first signs.

Eye symptoms such as double vision (diplopia) and drooping of the upper eyelid (blepharoptosis) can be seen in most of the cases during the entire course of the disease. In addition to eye symptoms, dysarthria and dysphagia are often seen as early symptoms as well as limb weakness. Demonstrating muscle weakness (easy fatigability) following repeated exercises and improvement after rest as well as symptoms being more evident in the evening than in the morning (diurnal variation) is characteristic of AChR antibody-positive MG. In myasthenic crisis, paralysis of the respiratory muscles occurs, necessitating assisted ventilation to sustain life. In patients whose respiratory muscles are already weak, crises may be triggered by infection, fever, adverse reaction to medication, or emotional stress.

MuSK antibody-positive MG patients have no thymoma, and muscle atrophy and dysphagia tend to be symptoms of crisis in a subject [7] (Tables 13.1 and 13.2).

Table 13.1 Patient characteristics in MuSK Ab-positive and AChR Ab-positive MG

Clinical factors	MuSK MG, $n = 83$	AChR MG, $n = 83$	P value
Female (%)	75.9	68.7	0.30
Mean onset age ± SD (years)	43.5 ± 16.1	42.5 ± 20.7	0.73[a]
Thymoma (%)	0	25.3	0.00000094[**]
EOMG (≤49 years) (%)	60.2	43.4	0.03[*a]
LOMG (≥50 years) (%)	39.8	31.3	0.26[a]
MGFA classification at maximum severity (%)			
I	2.4	25.3	0.00002[**]
IIa, IIIa, IVa	20.5	32.5	0.11
IIb, IIIb, IVb	48.2	26.5	0.0039[**]
V	28.9	15.7	0.04[*]

Source: European Journal of Neurology, Sep 2013, Vol. 20 Issue 9, p1272–1276
MuSK muscle-specific receptor tyrosine kinase, *AChR* acetylcholine receptor, *MG* myasthenia gravis, *EOMG* early-onset myasthenia gravis, *LOMG* late-onset myasthenia gravis, *MGFA* Myasthenia Gravis Foundation of America
[a]Statistics by $\mathcal{X}^2$ test or student's t-test, [*]<0.05, [**]<0.001

Table 13.2 Thymus histology in MuSK Ab-positive and AChR Ab-positive MG

Thymectomized	MuSK MG, $n = 24$	AChR MG, $n = 43$	P value
Thymoma (%)	0	48.8	0.000036*
Hyperplasia (%)	12.5	32.6	0.07
Normal/atrophy (%)	87.5	18.6	0.000000048*

Source: European Journal of Neurology, Sep2013, Vol. 20 Issue 9, p1272-1276
MuSK muscle-specific receptor tyrosine kinase, *AChR* acetylcholine receptor, *Ab* antibody, *MG* myasthenia gravis
Statistics by $\mathcal{X}^2$ test, *<0.001

13.6 Diagnosis

In 2014, Japanese MG clinical guidelines proposed novel MG diagnostic criteria. Using these criteria, we can diagnose MG when one or more of the myasthenic symptoms and signs and seropositivity of AChR or MuSK antibodies are confirmed (Table 13.3). On the other hand, when these pathogenic autoantibodies are negative, we need one or more of the following positive laboratory findings; easy fatigability test of the eyelid, positive ice pack test, positive edrophonium test, positive repetitive stimulation test, single fiber EMG, and further tests may be required to distinguish other diseases mimicking MG.

13.7 Treatment

Treatments for MG, which is an autoimmune disease, are based on the immunotherapy by steroids and immunosuppressants. In Japan, treatments include cholinesterase inhibitors, thymectomy, steroids, immunosuppressants, plasma exchange, and intravenous immunoglobulin (IVIg) therapy. However, even when utilizing all of these treatments, the proportion of MG patients who enter complete remission is less than 10 % [8]. Therefore, it is important to explain to MG patients that immunotherapy will be a long-term treatment. The current goal of MG treatment is firstly that we treat MG patients at onset to pharmacological remission as rapidly as possible using the above therapies; the second is reducing oral prednisolone to 5 mg/day or less to minimize disease manifestation and symptoms in daily life. Thymectomy was commonly performed in the past for non-thymoma MG patients, but its lack of effectiveness lessened its use recently in late-onset MG patients. Recently, since late-onset MG has increased, adverse effects of steroid therapy such as diabetes, infections, psychiatric symptoms, and osteoporosis have become a problem. MG clinical guidelines were revised in 2014 and the new standard treatments are presented. Currently, reviewing MG treatments which had been done previously is being done in each facility. Here, the authors describe the therapeutic protocol according to onset age, disease type, and pathogenic

Table 13.3 Proposed diagnostic criteria for myasthenia gravis

(A) Symptoms and signs
1. Blepharoptosis
2. Eye movement disorder (diplopia, ophthalmoplegia)
3. Facial muscle weakness
4. Dysarthria
5. Dysphagia
6. Mastication disorder
7. Cervical muscle weakness
8. Limb muscle weakness
9. Respiratory disorder
Note: These symptoms and signs show easy fatigability and diurnal variation
(B) Pathogenic autoantibody
1. Positive acetylcholine receptor antibodies
2. Positive muscle-specific receptor tyrosine kinase antibodies
(C) Neuromuscular junction disorders
1. Positive on eyelid easy fatigability test
2. Positive on ice pack test
3. Positive on edrophonium chloride (Tensilon) test
4. Jitter increase on single fiber electromyography test
(D) Determination
Diagnosis of myasthenia gravis made if either of the below is true
1. One or more items from A are true and any item of B is true
2. One or more items from A are true and any item of C is true and other diseases can be ruled out

autoantibodies conducted in Nagasaki University Hospital. We have combined the MG clinical guidelines for 2014 with our experiences to date and are treating MG patients using this protocol (Fig. 13.3).

13.8 Cholinesterase Inhibitors

Cholinesterase inhibitors are indicative for all MG patients. The authors recommend that patients who have dysphagia should be administered with them 1 h before meals. It is most important for cholinesterase inhibitors to be used to prevent aspiration pneumonia which is a risk factor of crisis. First of all, pyridostigmine, Mestinon®, which acts for 3–4 h, is used before meals. Then, long-acting ambenonium chloride, Mytelase®, may be used before bedtime. After starting steroids, we try to reduce the dose of cholinesterase inhibitors while evaluating MG symptoms and signs with the aim to stop them.

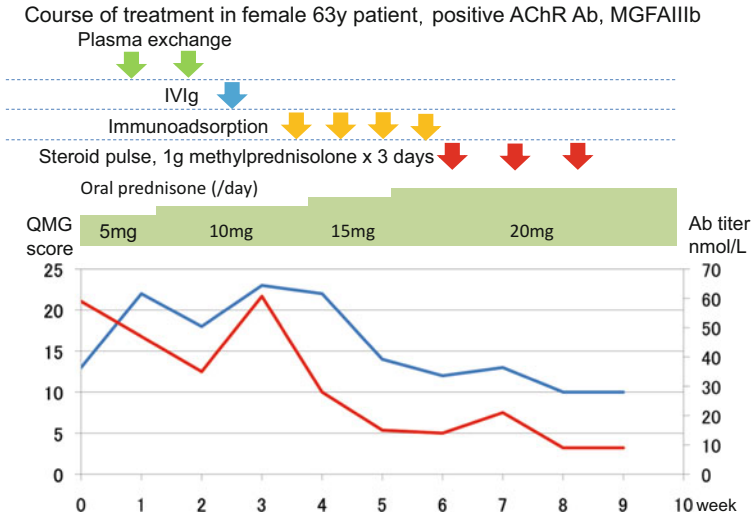

Fig. 13.3 This 63-year-old female patient had a progressive dysphagia which will be able to cause myasthenic crisis. Considering the initial exacerbation by steroids, a small amount of oral prednisolone treatment, two plasma exchanges, and immune globulin IV were administered. However, MG symptoms, QMG score, and acetylcholine receptor-binding antibody titers did not improve. So, oral prednisolone was gradually increased while performing immunoadsorption. MG symptoms and QMG score improved more than 4 weeks after the start of treatment. After making sure that the dysphagia was almost eliminated, steroid pulse therapy was commenced. As a result, MG symptoms entered pharmacological remission

13.9 Thymectomy

Thymectomy is defined here as Masaoka's extended thymic resection. In thymoma cases, thymectomy is the first choice for MG treatment. In Japan, thymectomy in non-thymoma and generalized MG cases has been the first choice in the past, but there has been no proven evidence that this strategy is effective [9]. Currently, a worldwide clinical trial (MGTX study) in order to verify any evidence is in progress. The authors do not recommend this surgical treatment in LOMG patients since there seems to be a low frequency of thymic abnormalities (MG practice guidelines 2014: see Clinical Questions 4-2).

13.10 AChR Antibody-Positive Generalized Patients: Age at Onset Is Less Than 70 Years

Severe MG patients with more than MGFA2b should be administered pyridostigmine 1 h prior to each meal. After making sure of its effect, the authors would commence therapy with a small amount of prednisolone orally. Since some

generalized MG patients may develop a crisis due to initial worsening caused by steroid therapy, we start with 5 mg of oral prednisone and gradually increase to 20 mg prednisone over 1–2 weeks. If patients still have dysphagia, the authors perform plasma exchange two to three times. The plasma exchange method is 2 liter at once using a substituted solution of 5 % albumin, two to three times a week. Most patients have no dysphagia with steroid and plasma exchanges. If patients still have dysphagia after receiving plasma exchange, we will add IVIg treatment whose regimen is 0.4 g/kg/day for 5 continuous days.

After checking that patients do not have dysphagia, we will start steroid pulse therapy and repeat three to five times. Specifically, a drip of 1 g of methylprednisolone is started from the beginning of the week for 3 days. Most patients develop a temporary initial worsening of MG signs 2–3 days following the first infusion. It is clinically important to explain initial worsening of MG signs to the patients. The second worsening in the next week becomes less acute than the initial one. In most severe MG patients, repeating this steroid pulse leads to pharmacological remission. After that, it is outpatient treatment with oral 20 mg prednisolone. The outpatient doctor reduces treatment by 5–10 mg prednisolone/year aiming to finally stop its administration. If we cannot reduce the dose of prednisolone, we will add oral immunosuppressive drugs such as calcineurin inhibitors.

In general, when the dose of prednisolone is reduced to below 10 mg, we often see worsening ocular signs. In this case, the authors will maintain the minimum dose of prednisolone that maintains the MG symptoms in remission. When we see our outpatients developing progressive dysphagia, we have to hospitalize them as soon as possible and repeat this treatment policy. Additional immunosuppressive drugs should be considered in case of repeated exacerbation.

13.11 AChR Antibody-Positive Generalized Patients: Age at Onset Is More Than 70 Years

In general, many of the patients in this group are ocular and mild MG and are treated from onset with low dose (10–20 mg) prednisolone and immunosuppressants. If we treat the patients in this group with steroid pulse therapy, we will have a lot of problems and complications such as compression fracture of the spine, dementia, and infection. When MG signs enter remission, we adjust treatment to maintain the least dose of prednisolone and immunosuppressive drugs possible (prednisolone 5 mgMM).

13.12 Ocular MG Patients

There is no standard therapy for the treatment of ocular MG [10]. The authors recommend that we treat ocular MG patients who developed extraocular muscle paralysis at onset with steroid pulse therapy. We use high dose intravenous steroids with 1 g of methylprednisolone given daily, for 3 days in accordance with generalized MG, and usually repeat three to five times. There are no initial worsening MG signs due to steroid pulse in ocular MG patients. For this reason, we can treat ophthalmoplegia safely and completely. After steroid pulse therapy, we maintain oral prednisolone 10–20 mg in the outpatient clinic. After that, we will reduce to the minimal dose of prednisolone that can maintain the MG symptoms in remission.

13.13 MuSK Antibody-Positive MG Patients

Compared with AChR antibody-positive MG, thymectomy in MuSK antibody-positive MG cannot be the first choice due to its poor efficacy. The side effects of cholinesterase inhibitors are likely to be similar to that in AChR antibody-positive MG. Although reactivity to steroids is generally good, the combination with immunosuppressants is recommended when steroids alone do not obtain the amelioration of MG symptoms. Plasma exchange can be remarkably effective in severe cases. The efficacy of immunoadsorption and IVIg is inferior to plasma exchange.

13.14 Seronegative MG Patients

Its pathogenesis is heterogeneous, but there are two possibilities which are that AChR, MuSK, and Lrp4 antibodies are present but below detection sensitivity threshold or other unknown autoantibodies are involved. In general, thymectomy should be performed in MG patients with thymoma. Patients with non-thymoma cases should be treated similarly to those with an AChR antibody-positive type. To diagnose seronegative MG, a neuromuscular junction biopsy and passive transfer to an animal of patient immunoglobulin will be considered.

13.15 Crisis

Crisis in myasthenia gravis is defined as rapidly progressively respiratory and bulbar muscle weakness. MuSK antibody-positive patients are more likely to develop myasthenic crisis than AChR antibody-positive patients (Nakata et al.) [7]. Treatment of crisis for both types of MG is the same; the first step is airway management

by intubation and mechanical ventilation, and the second is to stop all cholinesterase inhibitors. Considering the causes of crisis, plasma exchange, IVIg, and steroid pulse should be started. In the authors' experiences, compared with AChR antibody-positive patients, MuSK antibody-positive patients rapidly improve following plasma exchange; therefore, there are few cases for which tracheotomy is needed.

13.16 Prognosis

MG prognosis is significantly improved by immunotherapy but not in every patient as some die due to the MG itself. Currently, half of MG patients receiving immunotherapy have minimal manifestation in which the patient has no symptoms or functional limitations from MG but has some weakness on examination of some muscles. However, MG patients with complete stable remission (CSR) are less than 10 %, and it is necessary for most MG patients to continue immunotherapy, such as with steroids and immunosuppressants. Long-term steroid therapy can cause complications such as osteoporosis and infection, which lead to worsen the QOL of the MG patients. Although there are rare case reports [11] with voltage-gated potassium channel antibodies and patients with thymoma who experienced sudden death considered to be due to myocarditis, it is necessary to pay attention to this.

Conflict of Interest The authors declare that no competing interests exist.

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Chapter 14
N-Methyl-D-Aspartate Receptor Antibody-Associated Autoimmune Encephalitis

Keiko Tanaka

Abstract The categorization of autoimmune encephalitis associated with antibodies against cell surface or synaptic proteins has progressively widened with the discovery of new antibodies. Many patients suffering from autoimmune encephalitis show core symptoms such as psychosis, seizures, and abnormal movement, which usually respond to the immune therapies. The clinical spectrum of autoimmune encephalitis has been expanding, following several atypical cases that require specific antibody tests. Among the antibodies, those against the N-methyl-D-aspartate receptor (NMDAR) and voltage-gated potassium channel complex (leucine-rich glioma-inactivated protein 1) have attracted considerable attention. Some patients have an underlying tumor, and the occurrence of an ovarian teratoma has been highly reported in young female patients with anti-NMDAR encephalitis. These diseases are often treatable, and the good outcomes have been reported in patients who have had an early resection of the teratoma and received extensive immune therapies.

In this review, anti-NMDAR antibody-associated encephalitis will be discussed with a focus on the underlying cellular and synaptic mechanisms of the disease considering the antibody roles on its pathogenesis.

Keywords Autoimmune encephalitis • Autoantibodies • Anti-NMDA receptor • Pathogenesis

14.1 Introduction

The development of autoantibody-detection methods has expanded the categorization of autoimmune encephalitis. These developments have been particularly important for discovering autoantigens located in the plasma membrane of neurons

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and glial cells. These autoantigens include the metabotropic glutamate receptor 1 (mGluR1) [1], voltage-gated potassium channel (VGKC) complex (common antigen: leucine-rich glioma-inactivated protein 1:LGI-1) [2, 3], N-methyl-D-aspartate receptor (NMDAR) [4, 5], α-amino-3-hydroxy-5-methy-4-isoxazolepropionate receptor (AMPAR) [6, 7], and γ-aminobutyric acid B receptor (GABABR) [8]. Among these, the anti-NMDAR autoantibody is one of the most frequently detected antibodies in autoimmune encephalitis along with anti-LGI-1 antibody [9–13]. These particular antibodies with the binding capacity to the cell surface antigens have direct roles in the pathogenesis of the disease and tend to respond well to immunotherapy.

In this review, anti-NMDAR antibody-associated encephalitis will be discussed with a focus on the underlying cellular and synaptic mechanisms of the disease considering the antibody roles on its pathogenesis.

14.2 Anti-NMDAR Encephalitis

14.2.1 Anti-NMDAR Antibody Detection and the Clinical Features of Antibody-Positive Patients

The anti-NMDAR antibodies are detected using human embryonic kidney 293 (HEK) cells that express NMDARs on the surface of the HEK cell. The cells are co-transfected with NMDAR subunit genes, either GluN1/Glu2A or GluN1/GuN2B. Notably, the receptors are spatially distributed along the cell. Either cerebrospinal fluid (CSF) or serum from the patient is applied to the cells, and bound antibodies are visualized using immunofluorescence staining (Fig. 14.1).

Using this cell-based assay in samples of Japanese population from 2009 to 2014, 384 patients who tested positive for the presence of anti-NMDAR autoantibodies were observed (Table 14.1). Ninety-two percent of the antibody-positive patients were female, and their mean age at onset was 23 ± 7.1 (range, 3–60) years. They commonly presented with prodromal headaches, with the majority (85 %) presenting psychiatric symptoms similar to those observed in schizophrenia. These symptoms included delusional thinking, hallucinations, agitation, confusion, and catalepsy. Based on the abovementioned symptoms, patients were commonly diagnosed with malignant catatonia. Seizures were observed in 47 % of the patients. Of those, 45 % had generalized seizures, 10 % had partial seizures, and 3.6 % were status epileptics. Involuntary movement was observed in 71 % of patients (orofacial, 55 %; choreoathetosis, 47 %; and abnormal catatonic posture, 47 %) along with autonomic instability (59 %) and hypoventilation (59 %). These symptoms usually last few weeks to months. On electroencephalography (EEG), abnormal findings were observed in many patients: diffuse slow waves were observed 81 % of patients and epileptic spikes were observed in 19 % of patients. Brain MRI revealed abnormal findings in 62 % of patients, with 36 % showing abnormalities in

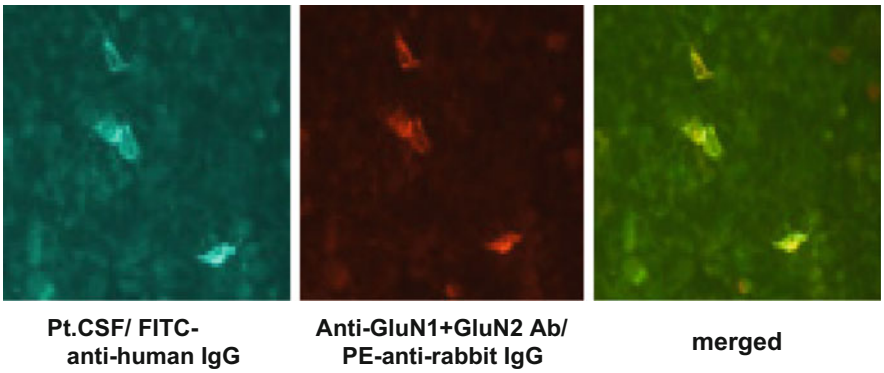

Fig. 14.1 Anti-NMDAR antibody detection (cell-based assay using GluN1 and GluN2 co-transfected HEK cells) (in-house assay at Kanazawa Medical University). Immunostaining of HEK cells expressing NMDARs following incubation with CSF from the patients and a mixture of rabbit anti-GluN1 and anti-GluN2B antibodies with FITC-anti-human IgG (*left*), with PE-anti-rabbit IgG (*middle*), and with both channels merged (*right*)

the medial temporal area. In the CSF, pleocytosis, increased protein content, and oligoclonal bands were observed in 56 %, 24 %, and 27 % of patients, respectively. Ovarian cysts and teratoma were detected in 54 % of patients. Good response to immunotherapy was observed in 48 % of patients, with recurrence observed in 21 %.

Titulaer et al. reported that approximately half of the 472 patients improved within 4 weeks following first-line immunotherapy using steroids, intravenous immunoglobulins, plasmapheresis, or tumor removal. Of the 221 patients who showed no improvement following these treatments, 125 (57 %) received second-line immunotherapy using rituximab, cyclophosphamide, or other immunosuppressing agents and then showed good outcomes [12]. Despite using these extensive therapies, some patients remained bedridden under respiratory assistance for several years.

14.2.2 Clinical Variants

14.2.2.1 Autoimmune Epilepsy with Anti-NMDAR Antibodies

In a series of studies by Dalmau et al. of 100 patients with anti-NMDAR encephalitis, 76 had seizures [5, 9]. They also investigated the frequency of epilepsy with unknown cause among children. Niehusmann et al. [10] identified 19 female patients aging 15–45 years with unexplained new-onset epilepsy. Among them, five patients had antibodies against NMDAR and had diffuse cerebral dysfunction and seizure origins. Psychiatric symptoms and pleocytosis in their CSF were significantly associated, and they showed episodic, in part a relapsing-remitting

Table 14.1 The clinical and laboratory features of anti-NMDAR encephalitis patients in a Japanese population (acquired at Kanazawa Medical University from 2009 to 2014)

Men: women	31: 353
Median age, range (years)	23 ± 7.1 (3–60)
Symptom presentation	
Psychiatric symptoms	85 %
Seizures	
Any type	47 % (generalized 45, partial 10, status epileptics 3.6)
Movement disorders	
Any type	71 % (orofacial 55, choreoathetosis 47, catatonia 47)
Autonomic instability	59 %
Central hypoventilation	59 %
EEG (information for 92 patients)	
Total with abnormal findings	92 % (slow activity 81, epileptic activity 19)
Brain MRI	
Total with abnormal findings	62 % (medial temporal lobes 36, cerebral cortex 15)
CSF	
Total with abnormal findings	84 % (pleocytosis 56, increased protein content 24, OCB 27)
Tumor	
Teratoma	54 %
Response to treatments	
(Tumor resection/ immunotherapy)	Improved 48 % (recurrence 24 %)

course, with full recoveries either spontaneously or after immunotherapies. Ekizoglu et al. investigated serum from 81 patients with epilepsy. They detected NMDAR antibodies in two patients: one patient had an explosive onset of seizures, memory problems, and poor response to antiepileptic drugs, whereas the second patient experienced only seizures [14]. Suleiman et al. investigated the frequency of epilepsy in pediatric patients with new-onset seizures [15]. They observed that 11 of 114 patients (9.7 %) were positive for one or more autoantibodies: four tested positive for VGKC complex autoantibody, three for Caspr2 autoantibody, two for NMDAR autoantibody, and two for both the VGKC complex and NMDAR autoantibodies. There were no significant differences in the demographic or clinical features between antibody-positive and antibody-negative patients.

Patients typically respond poorly to antiepileptic drugs, but they respond well to immunotherapy [16]. Toledano et al. studied 29 patients who tested positive for at least one autoantibody and had frequent and intractable seizures. Treatment with high-dose methylprednisolone pulse therapy, high-dose immune globulin, or both, reduced seizures in 62 % patients and eradicated seizure in 34 % of the patients. Of the 13 patients followed for more than 6 months after initiating long-term oral immunosuppression, the results of the treatment persisted in 11 (85 %) [17].

Some of the patients with anti-VGKC complex antibodies show characteristic seizures named as faciobrachial dystonic seizures, prior to the onset of amnesia and disorientation [18]. One-third of the anti-NMDAR encephalitis patients showed extreme delta brush (EDB: continuous δ waves superimposed with β-burst) in the EEG. EDB was not induced by external stimulation. The patients were usually unresponsive, presenting stereotypic movement or catatonia and memory loss during the EDB period [19].

14.2.2.2 Herpes Simplex Encephalitis (HSE) Patients With or Without Anti-NMDAR Antibodies

Prüss et al. reported that NMDAR antibodies were detected in serum or CSF of 13 of 44 (30 %) patients with HSE [20]. They did not observe any significant clinical differences between the antibody-positive and antibody-negative patients or between groups containing a given class of immunoglobulins (IgG, IgM, or IgA). The relationship between these two disorders remains unclear; however, it is suggested that the subgroup of patients with HSE produce anti-NMDAR antibodies secondary to the nervous tissue destruction and this subsequently causes anti-NMDAR encephalitis. These conditions may benefit from immunotherapy.

14.2.2.3 Schizophrenia-Like Symptoms in Patients with Anti-NMDAR Antibodies

Schizophrenia is characterized by hallucinations, delusions, disorganization, and lack of motivation, and individuals with schizophrenia commonly display executive function/reasoning deficits related to dysfunction in the dorsolateral prefrontal cortex and possibly dysfunction in glutamatergic transmission [21, 22]. Brain samples of individuals with schizophrenia with NMDAR genetic polymorphisms show decrease in the obligatory GluN1 subunit at both the mRNA and protein levels. These findings support the idea that hypofunction of NMDARs in the prefrontal cortex is associated with schizophrenia [23]. In addition, low doses of NMDAR antagonists, such as ketamine, cause psychosis, anxiety, agitation, memory disturbance, and decreased responsiveness to pain, whereas high doses produce dissociative anesthesia, a state of profound unresponsiveness with catatonic features, orofacial and limb dyskinesias, autonomic instability, and seizures [9, 24, 25]. This finding supports the theory that anti-NMDAR antibody binding causes hypofunction of endogenous NMDARs, resulting in schizophrenia-like symptoms in patients with anti-NMDAR encephalitis.

In our series of studies on patients treated in the psychiatric division, we investigated a total of 61 patients aging 15–61 years and observed that ten expressed anti-NMDAR IgG antibodies. Of these ten patients, three showed typical clinical features of anti-NMDAR encephalitis, whereas the others showed features of a broader range of psychiatric disorders [26].

Steiner et al. investigated the prevalence of anti-NMDAR antibodies in the serum from 459 patients with acute schizophrenia, major depression (MD), and borderline personality disorder (BLPD). Anti-NMDAR antibodies were identified in 15 patients, primarily those with an initial schizophrenia diagnosis (9.9 %) as opposed to those with MD (2.8 %), BLPD (0), and controls (0.4 %). Two patients initially classified as having catatonic or disorganized schizophrenia were reclassified as having NMDAR encephalitis. In all other seropositive cases, the antibodies consisted of classes IgA and/or IgM, which may have different antibody-binding sites than IgG antibodies [27]. Based on our experience and reports from others, the anti-NMDAR antibodies should be examined in CSF as opposed to serum because CSF is more specifically related to the anti-NMDAR encephalitis. In summary, evidence suggests that autoimmune-mediated atypical psychosis may have been misdiagnosed as psychosis of unknown etiology; thus, patients missed the opportunity to be appropriately treated.

14.2.2.4 Demyelinating Disorders

The presence of white matter lesions on the MRI along with psychotic symptoms or epilepsy has shown significant associations with anti-NMDAR antibody seropositivity [28, 29]. Titulaer et al. reported the prevalence of demyelinating disorders with or without anti-aquaporin (AQP) 4 antibodies or anti-myelin oligodendrocyte glycoprotein (MOG) antibodies in 691 NMDAR encephalitis patients [28]. Twenty-three of 691 patients with anti-NMDAR encephalitis had prominent features of demyelination as revealed on MRI or clinically. Twelve patients with anti-NMDAR encephalitis had, at some point, experienced independent episodes of neuromyelitis optica spectrum disorder (five cases, with four anti-AQP4-antibody positive) or either brainstem or multifocal demyelinating syndromes (seven cases, with all anti-MOG-antibody positive). In 11 patients, anti-NMDAR encephalitis occurred in association with symptoms and MRI findings compatible with demyelinating diseases (five AQ4 positive, two MOG positive). Symptoms of most patients were alleviated with immunotherapy, but compared with anti-NMDAR encephalitis, the demyelinating episodes required more intensive therapy and resulted in more residual deficits.

Because NMDARs are present on oligodendrocytes, the white matter may be a target for antibodies following prior or recurrent neurologic insults such as post-HSV encephalitis or frequent relapses of demyelination [30].

14.2.3 Pathogenesis of Anti-NMDAR Antibodies

14.2.3.1 Pathogenicity of Autoantibodies on the Synaptic Receptors

The synaptic receptors at variable locations interact with each other to disseminate their signals to achieve various functions in the nervous system. Binding of antibody to these receptors prevents active expression of these receptors on the cell surface resulting in the disruption of synaptic interactions. If the antibody binding continues for a long time period, it could cause irreversible damage to the CNS. If the binding occurs transiently, then recovery should occur after a certain time period.

Witebsky et al. provided criteria for direct evidence for the pathogenicity of antibodies: (1) antibodies have to be present in bodily fluids or be bound to the site of the pathologically altered tissue, (2) antigenic target of the autoantibody should be known, and (3) direct injection of patient's IgGs or immunization with a known antigen should clinically and pathologically reproduce the disorder in experimental animals [31]. One additional criterion was added by Drachmann et al. in 1990: a reduction in antibody titer should co-occur with an improvement in clinical symptoms [32]. Considering these, sound evidence for antibody pathogenicity and understanding of the underlying molecular mechanisms remains limited for several groups with autoantibody-related neurological diseases. Antibodies against neuronal surface antigens are often directed at conformational epitopes. Furthermore, it is more likely that patients possess a heterogeneous antibody population that target an immunogenic region rather than an antibody that targets a single common epitope. It is possible that the heterogeneous antibody binding to different epitopes and IgG subtypes results in multiple pathophysiological effects at the same time and in the same brain regions; this could explain the various clinical symptoms. Of all the autoantibodies known to result in disease, those targeting the NMDAR have been studied most thoroughly.

14.2.3.2 NMDAR

NMDAR is composed of four subunits: two obligatory GluN1 (NR1) subunits and two GluN2 (NR2: NR2A–2D) subunits and/or GluN3 (NR3: NR3A and NR3B) subunits. NMDAR is expressed at the cell surface where both GluN1 and GluN2/3 are present and form a tetrameric structure of receptor. The binding sites of the patients' antibodies are on the extracellular region of the GluN1 subunit [33]. The binding of antibodies is shown to dimerize NMDARs and trigger their internalization in the postsynaptic site, thereby suppressing NMDAR-mediated transmission [34]. To investigate whether the antibody-induced dysfunction of NMDARs underlies the symptoms of anti-NMDAR encephalitis, we determined the disease-produced antibody effects on NMDAR-dependent long-term potentiation (LTP), which would attribute to amnesia, a main symptom of this disease. The results

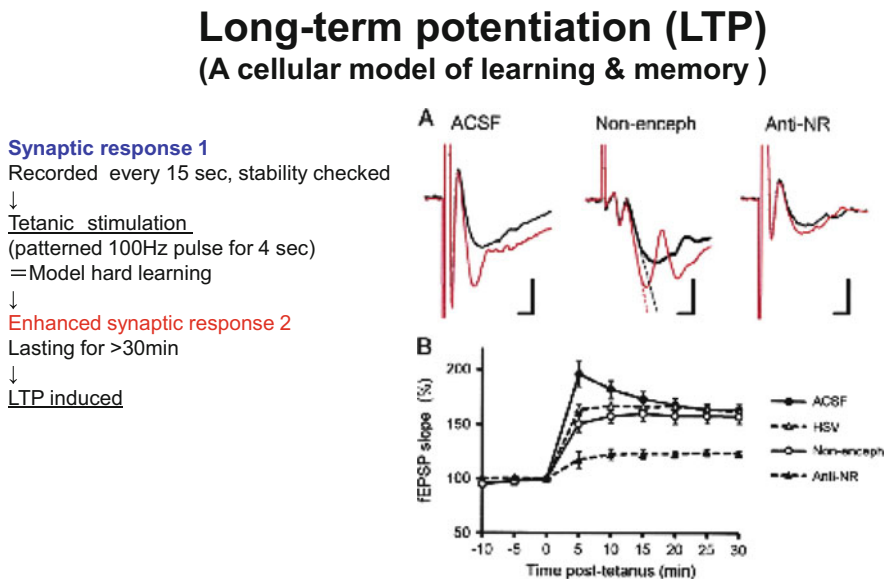

Fig. 14.2 Suppression of LTP by CSF obtained from an anti-NMDAR encephalitis patient. (**a**) Superimposed field EPSPs (fEPSPs) recorded at Schaffer collateral-CA1 synapses immediately before (*black*) and 30 min after TBS (*red*). For assessment of the magnitude of LTP, the initial slope of post-tetanic fEPSP (*red*) was expressed as percent of the pre-tetanic slope (*black*). The slope was measured as indicated in the middle panel (*dotted lines*). LTP was suppressed by bath application of the CSF from the patients (anti-NR). LTP was induced with either artificial CSF (ACSF) or CSF from a non-encephalitis control patient (Nonenceph) in the bath in experiments that used the same procedures. Scale bars = 2 ms and 0.5 mV. (**b**) Averaged time courses of LTP amplitude. The slope of fEPSPs was expressed as a percent of those recorded immediately before TBS. The *lines* represent data from the ACSF control (*solid line with filled circles*), Nonenceph (*solid line with open circles*), HSV (*chain line with open triangles*), and NMDAR antibody-positive case (*chain line with filled triangles*) (Figure adapted from Ref. [35])

showed that anti-NMDAR antibodies from the patients specifically suppressed CA1 LTP in mouse hippocampal slices (Figs. 14.2 and 14.3) [35]. LTP at the Schaffer collateral-CA1 synapse is NMDAR dependent and postsynaptic in origin [36, 37], and LTP is well established as a cellular basis of learning and memory. NMDARs are internalized after a 24-h incubation of NMDAR-expressing HEK cells with the CSF from the patients. However, regarding the suppression of LTP induction, it is conceivable that the antibody acts as an NMDAR antagonist, considering the 5-min application of the antibodies and subsequent washout during the LTP experiments [35].

NMDAR is essential for both memory formation and synaptic plasticity, and the involvement of this receptor in cognition has been suggested by drugs with NMDAR-blocking activity in humans and by NMDAR subunit knockout mice [38, 39]. In mice deficient in GluN2A subunits, LTP induction at the CA1 synapses is impaired and spatial memory is defective, suggesting that the NMDAR is

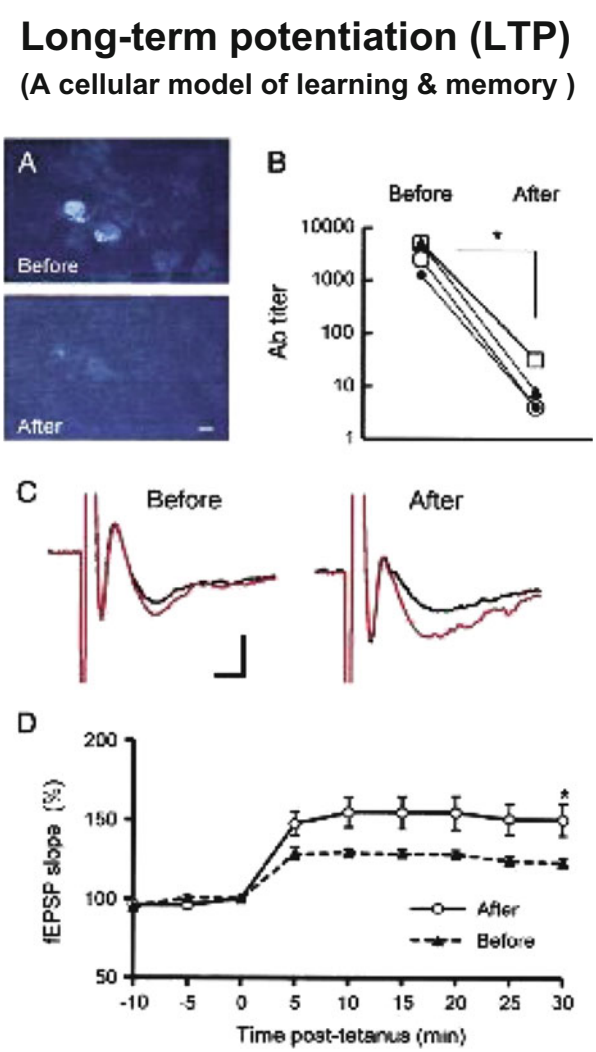

Fig. 14.3 Antibody absorption with HEK cells expressing NMDARs and its LTP-recovering effect. (**a**) Staining of NMDARs expressed on HEK cell surface with CSF before and after absorption. Scale bar = 10 μm. (**b**) Pairwise comparison of the antibody titer before and after absorption for each of the four samples on a logarithmic scale ($\log_{10}$). The titer was defined as the extent of the sample dilution up to which the staining with the diluted sample was still detected microscopically. After absorption, the antibody titer was significantly reduced ($^*P > 0.035$). (**c**) LTP at the Schaffer collateral-CA1 synapse with the CSF from the patients before and after the absorption. Each diagram consists of superimposed representative recordings taken immediately before TBS (*black*) and 30 min after TBS (*red*). Scale bars = 2 ms and 0.5 mV for all traces. (**d**) Reduction of LTP amplitude due to the antibody absorption. In five slices, LTP induction was attempted under application of a sample before absorption. In another set of five slices, LTP was induced with the same sample after the absorption. The slope of fEPSPs was expressed as a percent of the pre-TBS control. The magnitude at 30 min post-TBS was significantly larger after absorption ($^*P > 0.019$)

involved in these phenomena [40]. In addition, spatial memory is impaired in mice whose hippocampal NMDARs are conditionally knocked out [41, 42].

To confirm that the patients' antibodies actually impair memory formation in mice, we attempted to reproduce the clinical features observed in the patients with anti-NMDAR encephalitis using mice chronically exposed to the CSF from the patients through administration into the lateral ventricles using transplanted osmotic pump for 28 days. Mice treated with the CSF from the patients showed poor performance in the Morris water maze test, indicating that these mice had a spatial learning impairment [43, 44]. Recently, Planaguma et al. conducted similar experiments and observed that mice treated with the CSF from the patients showed impairments on an object recognition test and that performance recovered after the CSF administration was terminated [45]. In our experiments, the mice infused with the CSF of the patients did not show neuronal loss, inflammatory cells infiltration, or glial cell proliferation. These learning impairments observed in the mice without CNS tissue destruction suggested the anti-NMDAR antibodies were purely affecting the synaptic functions during the disease period and thus providing good recovery from the disease after the appropriate immunotherapy.

14.3 Conclusions

Recognition of autoimmune encephalitis with antibodies against cell surface channels/receptors has been expanding with discovery of new antigens. For clinicians, it is difficult to examine all of these antibodies for individual patients. Each antibody is generally linked to a certain set of clinical features; however symptoms of different conditions overlap considerably. Furthermore, in cases where multiple autoantibodies are detected, understanding the pathogenesis of these antibodies can be very difficult. Despite these challenges, the detection of these antibodies can result in the proper immunotherapy being given to the patient, which is the most important aspect of the testing.

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Chapter 15
Hashimoto's Encephalopathy

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Abstract Hashimoto's encephalopathy (HE) has been recognized as a new clinical disease based on an autoimmune mechanism associated with Hashimoto's thyroiditis (HT), and steroid treatment has been successfully administered. Recently, we discovered that the serum autoantibodies (Abs) against the NH_2-terminal of α-enolase (NAE) can serve as a highly specific diagnostic biomarker for HE. We reviewed the clinicoimmunological features of 80 cases of HE with serum anti-NAE Abs from institutions throughout Japan. The mean age of the patients was 62 years, but the ages were widely distributed, with two peaks (20–30 and 60–70 years). Most patients with HE were in euthyroid states. All patients had anti-thyroid Abs, and, in rare cases, anti-TSH receptor Abs. The common neuropsychiatric symptoms were consciousness disturbances and psychosis, followed by cognitive dysfunction, involuntary movements, seizures, and ataxia. EEG abnormalities and decreased blood flow on the brain SPECT were common, whereas abnormalities on the brain MRI were rare. Patients with HE show various clinical phenotypes such as acute encephalopathy and chronic psychiatric forms including limbic encephalitis, followed by progressive cerebellar ataxia and Creutzfeldt-Jakob disease-like forms. The cerebellar ataxic form of HE clinically mimics spinocerebellar degeneration and is characterized by an absence of nystagmus, absent or mild cerebellar atrophy, and lazy background activities on the EEG. Although steroids have been the first-choice and favored medication for the treatment of patients with HE, some patients show resistance to them. In such a case,

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plasmapheresis or intravenous administration of immunoglobulin is recommended. We should pay attention to the possibility of HE as a treatable disease.

Keywords Hashimoto's encephalopathy • Anti-NAE autoantibodies • Steroid-responsiveness

15.1 Introduction

In 1912, more than 100 years ago, a Japanese surgeon, Hakaru Hashimoto, proposed a new clinical disease, "struma lymphomatosa," pathologically characterized by lymphocyte infiltration, fibrosis, and eosinophilic change with follicular cells in the thyroid gland [1]. This disease has been widely recognized worldwide and was named Hashimoto's thyroiditis (HT) after the discoverer. HT is now considered an autoimmune disease as well as an endocrine disease, based on experimental studies and observations on serum autoantibodies in patients with HT [2, 3]. Encephalopathy occasionally occurs in association with HT, but most of these occurrences are treatable. This encephalopathy includes a neuropsychiatric disorder associated with hypothyroidism, called myxedema encephalopathy.

In 1966, more than 50 years after the discovery of HT, Lord Brain et al. published a report on a 49-year-old male patient who had experienced 12 episodes, including confusion, irritability, aphasia and strokes, in a euthyroid state. Those authors offered speculations regarding the autoimmune mechanisms underlying this condition [4]. In 1991, Shaw et al. described five cases of encephalopathy associated with HT and proposed a new clinical entity called Hashimoto's encephalopathy (HE), characterized by neuropsychiatric disturbance, steroid-responsiveness and serum anti-thyroid autoantibodies (Abs) [5]. Steroid treatment was successfully administered to all of the patients.

The clinical entity and nosology of HE have been debated and the reality of the illness has been questioned because of the wide variety of clinical symptoms and the lack of a specific diagnostic marker until recently. A diagnostic marker for HE has long been sought. Accumulated case reports support the proposal that this autoimmune treatable disease, HE, is distinct from myxedema encephalopathy [6–9]. Recently, we discovered that serum autoantibodies against the NH_2-terminal of (α) alpha-enolase (hereafter termed NAE) are a highly specific diagnostic marker for HE based on a proteomic analysis [10, 11].

In this chapter, we review the clinical and immunological features of HE based on our study and on references to the literature.

15.2 Clinical Manifestations

15.2.1 Prevalence, Age, and Gender

HE has been considered a rare disorder, and an estimated prevalence of 2.1/100,000 has been reported in the literature [8]. However, considering the extremely high prevalence of HT (anti-thyroid Abs, 5–15 % in males, 10–25 % in females in Japan), HE would be expected to be more prevalent, and some cases may be underdiagnosed or misdiagnosed, as suggested in the literature [12]. Such a treatable condition is very important in the differential diagnosis of encephalopathy after the proper exclusion of other possible causes of encephalopathy, such as infections, tumors, vascular disorders, deficiencies, and intoxication. We analyzed serum anti-NAE Abs, a specific diagnostic marker for HE, and examined the clinical profiles of 80 patients with HE from our institute and other institutes in Japan. All patients fit the criteria for HE, which consist of neuropsychiatric symptoms, presence of anti-thyroid Abs and steroid-responsiveness [5], and they also had anti-NAE Abs in their serum.

The clinical profiles of these 80 patients are summarized in Table 15.1. The mean age was 62 years, but the ages of the patients were widely distributed, with two peaks (20–30 and 60–70 years). In all, 72 % of the HE patients were female, whereas more than 90 % of the HT patients in Japan are female, an extremely strong predominance. In Europe and the USA, the mean age of HE patients tends to be much younger (44–49 years), and the predominance of females among HE patients is more marked (76–92 %) [7–9].

15.2.2 Neurological Symptoms and Signs

Common neuropsychiatric features of HE found in our study included consciousness disturbance (66 %) and psychosis (53 %), followed by dementia (38 %), involuntary movements (31 %) (i.e., tremors, myoclonus, chorea, and athetosis), seizures (29 %), and ataxia (28 %). In the literature, the prevalence of consciousness disturbance (30–100 %) and psychosis (30–36 %) varied among the studied patients but were relatively predominant symptoms, as well as cognitive impairment (46 %) and seizures (52–66 %) [5, 7–9]. HE often presents with dementia and psychosis and can thus be considered "a treatable dementia." Parkinsonism, autonomic dysfunction, sensory disturbance, and peripheral neuropathy were very rare in association with HE, both in our study and in the literature [5, 7–9].

Table 15.1 Clinical characteristics in Hashimoto's encephalopathy with serum anti-NAE Abs

Characteristics	(N = 80)
Age	62 years (20–87 years)
Gender (female)	72 %
Neurological symptoms	
Consciousness disturbance	66 %
Psychiatric symptoms	53 %
Cognitive impairment	38 %
Involuntary movements	31 %
Seizures	29 %
Cerebellar ataxia	28 %
Laboratory and radiological findings	
Elevated protein in CSF/IgG	45 %
Slow waves in EEG	80 %
Normal findings in brain MRI	36 %
Decreased CBF in SPECT	76 %
Responsiveness to immunotherapies (excellent to moderate)	78 %
Clinical phenotypes	
Acute encephalopathy form	58 %
Chronic psychosis form	17 %
Cerebellar ataxic form	16 %
CJD-like form	3 %
Others	6 %

15.2.3 Clinical Phenotypes

Our cases were categorized as acute encephalopathy (58 %), chronic psychosis (17 %), ataxia (16 %), Creutzfeldt-Jakob (CJD)-like forms (3 %), and other forms (6 %) according to their clinical phenotype. Limbic encephalopathy was observed in some cases of acute encephalopathy and chronic psychosis [13]. Especially, the cerebellar ataxic form, mimicking spinocerebellar degeneration (SCD), is an important treatable condition in HE [14–16]. Notably, the cerebral ataxic form of HE is characterized by the absence of nystagmus, absence or mild cerebellar atrophy on MRI, and lazy background activities on the electroencephalogram (EEG) [15]. A very rare case with CJD-like presentation was also reported as a treatable condition [17].

Some clinicians have proposed two subtypes of HE – a vasculitic type, characterized by strokelike episodes, and a diffuse progressive type, characterized by dementia and psychiatric symptoms, based on studies of patients in Europe [18]. Both forms are accompanied by consciousness disturbance, seizures, tremors, or myoclonus [18]. In the 80 cases of HE in our study, such a vasculitic type was very rare, due presumably to the difference in immunological backgrounds (e.g., HLA typing) between Japanese and European people.

15.2.4 Laboratory and Radiological Findings

15.2.4.1 Laboratory Findings

Most HE patients were in euthyroid states, and all patients had anti-thyroid (TG) Abs and/or anti-thyroid peroxidase (TPO) Abs with a variety of combinations. Anti-TSH (TSH-R) Abs were observed in some cases. The presumed specificity of anti-NAE Abs was 90 % in HE, whereas the sensitivity was ~50 %. This result suggests that anti-NAE Abs are a useful diagnostic marker, but other Abs or immunological backgrounds can be associated with HE. Further immunological investigation is required.

Protein and immunoglobulin G (IgG) were frequently found to show increases in cerebrospinal fluid (CSF) (45 %), but pleocytosis was rare or mild compared with its occurrence in neuroinfectious diseases such as viral encephalitis.

15.2.4.2 EEG Findings

Abnormalities, especially slow-wave background EEG activity, were common features in our 80 cases of HE. Slow-wave background EEG activity was reported as a common phenomenon in HE in the literature [5, 7–9] and has been emphasized in the diagnosis of HE since this disease entity was first proposed [5].

15.2.4.3 MRI and SPECT Findings

Although the HE patients showed various neuropsychiatric symptoms, organic changes were rarely detected (36 % of all patients) on brain magnetic resonance imaging (MRI). Most of these changes involved limbic lesions or leuko-encephalopathic lesions. Continuous arterial spin labeling (CASL) imaging is a more sensitive technique for detection of the cerebral lesions in HE in comparison with conventional images such as the T_2-weighted (T2WI) and diffusion-weighted image (DWI) in brain MRI [13]. Decreased cerebral blood flow (CBF) was frequently observed (76 %) on brain single photon emission computed tomography (SPECT). We have performed a three-dimensional stereotactic surface projection (3D-SSP) analysis of regional cerebral blood flow (rCBF) in seven untreated patients with HE and 10 age-matched healthy controls using [^{123}I]IMP-SPECT and have demonstrated that rCBF was significantly decreased in the bilateral anterior cingulate areas and the left prefrontal cortex in comparison with that observed in the healthy controls. The decreased blood flow in these two cerebral regions can contribute to emotional changes and cognitive dysfunction in patients with HE [19].

Overall, only few or mild organic changes were detected in conventional brain MRI images in patients with HE, whereas functional changes frequently appeared

on EEG or SPECT evaluation, suggesting that changes that are reversible by appropriate immune treatments occur in the brain of HE patients.

15.3 Treatments

15.3.1 Steroids

Steroids such as prednisolone, dexamethasone, and methylprednisolone were the first-choice and favored medications for the treatment of patients with HE, as described in the paper in which the nosology of HE was first proposed [5]. Most of the 80 cases investigated in our study showed excellent or good responsiveness to steroids, and the response was especially good in patients with the anti-NAE Abs positive, acute encephalopathic form presenting with consciousness disturbance and psychosis.

15.3.2 Other Immunological Treatments

Because relapses of neurological symptoms often occur in patients with HE, continuity of low-dose oral steroids and supplementation of immunosuppressants (e.g., azathioprine) are recommended [20]. Despite the administration of steroids, some patients with HE (less than 20 %) showed resistance to steroids in our study. In such a situation, plasmapheresis or intravenous administration of immunoglobulin (IVIg) was effective in a limited number of cases [21, 22].

15.4 Pathogenesis

15.4.1 Pathology

Autopsy in a very few cases of HE revealed lymphotic infiltration of the veins of the brain, suggesting vasculitis [23]. Decreased rCBF was demonstrated in patients with HE. These two findings suggested that disturbance in the cerebral microcirculation contributes to the pathogenesis of HE.

15.4.2 Autoantibodies

We discovered a specific Abs against NAE in serum from patients with HE, based on a proteomic analysis, as described in the first part of this text [10] (Fig. 15.1a). These anti-NAE Abs were not detected in serum from other neuropsychiatric disorders including collagen diseases, infections, and autoimmune neurological diseases such as multiple sclerosis and myasthenia gravis. These findings demonstrate a high degree of specificity to HE [11]. The NH_2-terminal portion of

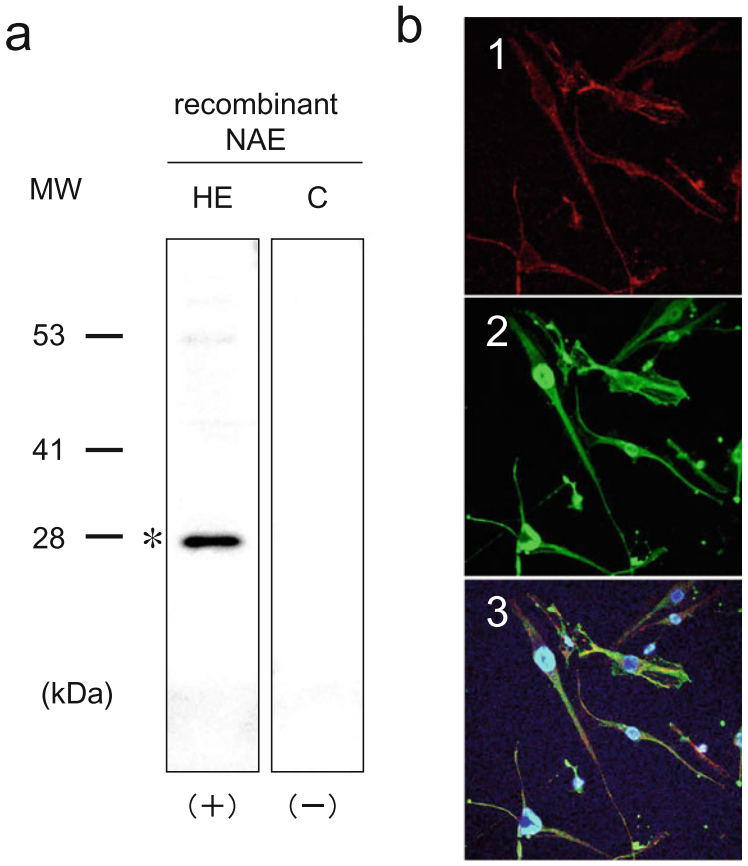

Fig. 15.1 Detection of anti-NAE Abs and their expression in cultured human endothelial cells of the blood vessels. (**a**) Immunoblot of a recombinant protein expressed in HEK293 human cultured cells with sera from a patient with HE. *MW* molecular weight. *HE* sera from a patient with Hashimoto's encephalopathy. *C* control sera. (**b**) Anti-NAE Abs from a patient with HE immunologically reacts with α-enolase in TY10, cultured human endothelial cells of the blood vessels (Adapted in part from Yoneda, Clin Neurosci (Japanese) 2015;33, 104–107 with permission). *1* The first abs, sera from a patient with HE; the second abs, anti-human IgG alexa 594. *2* The first abs, anti-mouse entire α-enolase abs; the second abs, anti-mouse IgG alexa 488. *3* Merged image

α-enolase was highly specific, whereas the COOH-terminal portion reacted abundantly with serum from HE, HT, and normal individuals, implying that the reaction with the COOH-terminal portion is nonspecific to HE [10]. Abs against whole α-enolase have been reported in various autoimmune disorders such as systemic lupus erythematosus (SLE), multiple sclerosis (MS), and rheumatoid arthritis (RA) [24]. Note, however, that only the NH_2-teminal portion of α-enolase reacted specifically to serum from patients with HE [10].

Although the original function of α-enolase is as an enzyme in the glycolytic pathway of the cytoplasm in a cell, this protein has multiple functions in association with plasminogen activation, the immune response or the regulation of gene transcription [25, 26]. In addition, α-enolase is located not only in the cytoplasm of the cell but also on the surface, especially in the endothelial cells of the blood vessels, implying an association with vasculitis [25]. The sera from a HE patient successfully immunologically reacted with an immortal human endothelial cell line of the blood vessels (TY10, courtesy of Prof. Takashi Kanda, Yamaguchi University) and merged with α-enolase in a cell (Fig. 15.1b). Apart from the vasculitic involvement of α-enolase, the CSF from a patient with HE who developed cerebellar ataxia with anti-NAE Abs successfully suppressed presynaptic transmission in a patch-clump study in a sliced rat specimen [27], suggesting a possible role in the pathogenesis of HE.

The other Abs were reported in a limited number of cases of HE, including Abs against an unknown 35 kDa protein [28], dimethylargininase-1 [29], and aldehyde reductase-I [30]. All of these Abs were not commonly found in serum from patients with HE, and their specificity to HE is still ambiguous.

15.4.3 Other Immunological Backgrounds

Histocompatibility antigen profiles in HE were reported as positive for the HLA B8DR3 haplotype, a profile common to patients with myasthenia gravis in Europe [31]. In additional immunological data, several surface antigens, such as OKT3, OKT4, and OKT8, were detected in peripheral T lymphocytes in patients with HE [31].

15.5 Conclusions

We should direct attention to the possibility that HE is a treatable disease associated with HT. The pathogenesis of this disease should be further clarified by future studies.

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Chapter 16
Paraneoplastic Neurological Syndrome

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Abstract Paraneoplastic neurological syndrome (PNS) is considered to involve humoral or cellular immunity. Although high titers of onco-neural antibodies against intracellular antigens are generally not pathogenic, cytotoxic T-cell mechanisms are believed to play a role in PNS. In such cases, immunotherapy is only marginally effective. These antibodies are useful diagnostic markers of PNS and related occult cancers, because neurological symptoms often precede discovery of tumors, and there is some relationship among the specific kind of antibody, neurological syndromes, and cancers. New means of detecting antibodies have revealed anti-neural antibodies against cell-surface or synaptic antigens in PNS, and also in many cases of non-PNS, which are generally pathogenic. They easily access the molecules comprising receptors or channels, as well as synaptic antigens, and consequently alter neuronal transmission and function. Immunotherapy for antibody reduction, and/or tumor removal, is often effective in this group. The recent discovery of new antibodies for cell-surface antigens has given rise to a paradigm shift in PNS where it should be reconsidered in the immune-mediated neurological syndromes.

The clinical neurologists should be alert for PNS and examine the presence and kind of anti-neural antibodies in patients with well-known PNS phenotypes and in cases of rapidly progressive neurological symptoms of unknown origin. Cases of suspected PNS should be examined with repeated malignancy survey using PET/CT, if a tumor is not found on initial scans.

Keywords Onco-neural antibody • Cytotoxic T-cell • Occult cancer • Cell-surface antigen • Cell-based assay

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16.1 Introduction

Paraneoplastic neurological syndrome (PNS) is caused by the so-called remote effects of tumors, which are generally cancers, and is considered to be immune mediated. Immune responses to a tumor might cross-react with self-antigens in the nervous system or muscle tissue [1, 2]. Infiltration, metastasis, and compression by a tumor, metabolic and nutritional disorders, coagulopathy, opportunistic infections, and side effects of tumor therapies should be excluded. As PNS may affect any part of the nervous system and muscles, various other mimetic neurological diseases must also be excluded. PNS is considered to be a rare disorder, affecting less than 1 % of patients with cancer except in the case of small-cell lung cancer (SCLC) where it occurs in less than 5 % of cases. The detection of PNS may increase in the future, with the advent of sophisticated detection equipment revealing new antibodies and with the use of improved PET/CT scan surveys.

Our increased awareness of PNS arose from case reports describing clinical and pathological signs of a relationship between neurological factors and tumors [3–6]. Since the 1980s, several specific onco-neural antibodies mainly against intracellular molecules that are essential to a neuron's survival have been reported [7–9]. Significant efforts to elucidate the neuronal effects of these antibodies on neurons have mostly been unsuccessful, although a cytotoxic T-cell mechanism, rather than a humoral mechanism, appears to mediate the neuronal damage. The presence of onco-neural antibodies is at least a useful diagnostic marker of PNS and related occult tumors. Antibodies for synaptic proteins were also reported in the 1990s [10, 11]. Several clinical neurological phenotypes with specific kinds of tumors and specific antibodies have been established as classical paraneoplastic syndrome [12]. Antibodies for receptors or channels on the surface of neurons were reported one after another in the 2000s [13–18]. Various neuropsychiatric syndromes, including disturbance of memory and behavior, autonomic dysfunction, epilepsy, paramyotonia, etc., associated with these antibodies, have been described, and these are largely non-PNS syndromes. Control of antibodies against cell-surface or synaptic antigens, but not intracellular antigens, by immunotherapy and/or tumor removal is often effective in this group. Tables 16.1, 16.2, 16.3, and 16.4 show the known associations among kind of antibody, clinical syndromes, and type of tumors [19]. In the present chapter, we review the pathogenesis, diagnosis, and treatment of PNS.

16.2 Pathogenesis

Although onco-neural antibodies in serum and cerebrospinal fluid (CSF) against intracellular antigens, nuclear or cytoplasmic proteins, are often seen at high titers, attempts to determine their effects on passive transfer in rodents or in vitro have been unsuccessful. For example, antigens Yo and Hu are considered to be crucial

Table 16.1 Paraneoplastic and autoantibody disorders of the peripheral nervous system [19]

Disease	Antigen(s)	Clinical syndromes	Tumors
Myasthenia gravis	Acetylcholine receptor (AChR)	Fatigable muscle weakness including bulbar muscles (diplopia, dysphagia, dysarthria, neck weakness, ptosis) and respiratory muscles	10 % of anti-AChR positives have thymoma
	Muscle-specific tyrosine kinase (MuSK) (10 % of systemic myasthenia gravis is seronegative)		
Lambert-Eaton myasthenic syndrome (LEMS)	Voltage-gated calcium channel (VGCC) (85 %)	Fatigable weakness that often resembles a myopathy, dry mouth, sexual dysfunction, constipation	50 % have tumors, mostly small-cell lung cancer; thymoma is rarely reported
	Unknown in 15 %		
Autoimmune autonomic neuropathy	Ganglionic acetylcholine receptors (containing α3β4 subunits)	Subacute pandysautonomia (hypotension, anhidrosis, dry mouth/eyes, sexual dysfunction, gastrointestinal dysmotility, fixed pupils, fixed heart rate)	10 % thymoma
Isaacs syndrome	Voltage-gated potassium channel (VGKC) complex (contactin-associated protein-like 2 [Caspr2] or unknown subtype)	Diffuse myokymia, cramps, hyperhidrosis, stiffness, muscle hypertrophy	Thymoma in a few patients
Inflammatory myopathy	Most patients with malignancy do not have Jo-1 antibodies	Muscle pain, weakness, elevated muscle enzyme levels	29 % of dermatomyositis patients have breast, bladder, lung, or hematologic cancer
		Dermatomyositis patients also have rash, Gottron papules, skin and nail bed inflammation	

molecules for neuronal survival, playing essential roles in transcription and translation, respectively. Immunization of mice with recombinant Hu or Yo produced high titers of each antibody, but no neurological symptoms [20–22]. Although pinocytosis has been observed in neurons, it is likely to be insufficient for the transport of a high volume of antibodies. However, cytotoxic T lymphocyte activity has been detected in peripheral mononuclear cells isolated from PNS patients with anti-Yo or anti-Hu antibodies [23–25]. These patients have common human leukocyte antigen (HLA)-class motifs. T-cell receptor usage of infiltrated T cells in the affected CNS tissue and tumor has been shown to be oligoclonal [26]. Therefore, a cytotoxic T-cell mechanism with HLA is suggested to be mainly responsible for the neuronal damage seen in cases with antibodies against intracellular antigens. Each antibody against Ri, Ma, Ma2(Ta), or CV/CRAM5 also targets an intracellular antigen. Onco-neural antibodies against synaptic antigens, such as the 65-kD

Table 16.2 Classic paraneoplastic disorders with autoantibodies to intracellular antigens [19]

Antibody (and alternative name) and antigen	Patient demographics	Clinical syndromes	Tumors
ANNA-1 (Hu)	75 % male, median age 63 years	Sensory neuropathy/ neuronopathy, encephalomyelitis, cerebellar degeneration, autonomic dysfunction	83 % have tumors; small-cell lung cancer most common
Target HuD and related nuclear proteins			
ANNA-2 (Ri)	66 % female, mean age 65 years	Cerebellar degeneration, encephalomyelitis, opsoclonus/myoclonus	86 % have cancer, especially lung or breast
Target the neuro-oncological ventral antigen (NOVA) proteins			
ANNA-3	Male and female, ages 8–83 years	Often multifocal and including neuropathy, myelopathy, brainstem or limbic encephalitis	Cancer very common, especially lung
PNMA1 (Ma)	Males and females, middle aged	Encephalitis, cerebellar ataxia, ophthalmoplegia, dementia	High risk of diverse tumors (lung, breast, colon, renal)
Target PNMA1 and PMNA2, expressed in brain/testes and also in tumors			
PNMA2 (Ma2) (also known as Ta)	Mostly males (median age 34 years) with fewer females (median age 64 years)	Limbic encephalitis, brainstem encephalitis, cerebellar degeneration, neuropathy	Young men with Ma2 often have germ cell tumors
Target PNMA2			
PCA-1 (Yo)	Almost all female, young adult to elderly	Cerebellar degeneration	>90 % have breast or ovarian cancers
Target cdr2, a cytoplasmic protein expressed in brain and in tumors			
PCA-2	Unknown	Cerebellar degeneration, encephalitis, autonomic dysfunction, motor neuropathy	Small cell cancers
Bind a cytoplasmic protein in neurons, especially cerebellar neurons			
CRMP-5	Men and women, older adults	Often multifocal and may involve dementia, ataxia, myelopathy, chorea, seizures, cranial neuropathies, peripheral neuropathy, and/or retinopathy	Lung cancer, thymoma
CRMP-5 is a neuronal protein critical for growth cone function			

ANNA-1 antineuronal nuclear antibody type 1, *ANNA-2* antineuronal nuclear antibody type 2, *ANNA-3* antineuronal nuclear antibody type 3, *PNMA1* paraneoplastic Ma antigen family-like 1, *PNMA2* paraneoplastic Ma antigen family-like 2, *PCA-1* Purkinje cell antibody-1, *PCA-2* Purkinje cell antibody-2, *CRMP-5* collapsin response mediator protein-5

Table 16.3 Antibodies to intracellular synaptic antigens [19]

Antibody and antigen	Patient demographics	Clinical syndromes	Tumors
Glutamic acid decarboxylase 65 (GAD65) is a synaptic enzyme that synthesizes the neurotransmitter γ-aminobutyric acid (GABA)	82 % female, 33–80 years [7]	Cerebellar degeneration, stiff-person syndrome (type 1 diabetes mellitus)	Usually none
		May coexist with other autoantibodies	
Amphiphysins regulate the recycling of synaptic vesicles	60 % male, mean age 64 years [8]	Stiff-person syndrome (when present in isolation)	Breast and ovarian cancers are common
		May coexist with other autoantibodies	

glutamic acid decarboxylase (GAD65) and amphiphysin, can access and affect those molecules during synaptic vesicle fusion and reuptake. GAD65 is a synaptic protein which produces the inhibitory neurotransmitter GABA. Antibodies against GAD65 are rarely paraneoplastic and are often detected with other antibodies in patients with cerebellar ataxia, stiff-person syndrome, limbic encephalitis, and other neurological symptoms [27]. Although anti-GAD antibodies from some human patients have shown effects in animal and tissue models, there is still concern over the T-cell mechanism [28]. Because low titers of anti-GAD antibodies have been detected in many patients with diabetes mellitus, its role must be interpreted with caution. Anti-amphiphysin antibodies are detected in women with stiff-person syndrome and various other neurological symptoms [29]. Amphiphysin plays a role in the recycling of synaptic vesicles. Injection of IgG from anti-amphiphysin antibody-positive stiff-person syndrome reproduced the pathological condition in mice where the blood-brain barrier was destroyed [30]. The passive transfer of IgG from Lambert-Eaton myasthenic syndrome (LEMS) with anti-P/Q-type voltage-gated calcium channel (VGCC) antibody associated with SCLC caused a similar pathological condition in rodents [31]. Recently IgG in LEMS has been shown to reduce the rate of acetylcholine release as a direct consequence of binding to P/Q-type channels [32]. Inducing a reduction in these antibodies generally results in improvement in neurological symptoms. Ten percent of LEMS cases show paraneoplastic cerebellar degeneration. IgG from LEMS has been shown to change the calcium voltage in cultured Purkinje cells. Infusion of IgG containing anti-mGluR1 antibody from cerebellar ataxia with Hodgkin disease in the cerebellum of mice resulted in reversible cerebellar ataxia [13]. Along with recent technical advancements in the detection of antibodies, such as in proteomics and cell-based assays, several antineuronal antibodies against cell-surface antigens have been discovered in PNS and in also many cases of non-PNS. Of particular value has been the ability of cell-based assays to preserve the tertiary structure of molecules on the cell surface and to detect the binding antibodies. These findings have led PNS to be reconsidered in the immune-mediated neurological syndrome. Among these syndromes, encephalitis beyond the limbic system has been classified

Table 16.4 The synaptic and other neuronal surface autoantibody disorders [19]

Antigen	Patient demographics	Clinical syndromes	Tumors
N-Methyl-D-aspartate (NMDA) receptor is an ionotropic glutamate receptor important for a form of synaptic plasticity	80 % female, mostly children, teens, and young adults	Early hallucinations, delusions, bizarre behaviors; evolves into seizures, abnormal movements, coma, dysautonomia, respiratory arrest	Ovarian teratoma
α-Amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA) receptor is an ionotropic glutamate receptor	90 % female, adult to elderly	Limbic encephalitis, psychiatric manifestations	Lung, breast, thymus
γ-Aminobutyric acid B (GABA-B) receptor is a metabotropic inhibitory receptor	60 % male, mean age 62 years but younger in nonparaneoplastic cases	Limbic encephalitis, often with severe seizures or status epilepticus	50 % have small-cell lung cancer
		More rarely opsoclonus-myoclonus or ataxia	
γ-Aminobutyric acid A (GABA-A) receptor is the main ionotropic inhibitory receptor in the adult brain	5 of 6 male, median age 22 years	Refractory status epilepticus or epilepsia partialis continua	One of six high-titer patients had Hodgkin lymphoma
		Low titer responses may be associated with diverse syndromes and other autoantibodies	
Leucine-rich glioma-inactivated 1 (LGI1) is a secreted synaptic protein that organizes AMPA receptors and Kv1 channels at CNS synapses	65 % male, median age 60 years	Encephalitis, relatively less severe compared with anti-NMDA receptor encephalitis	Usually none
		Faciobrachial dystonic seizures may precede encephalitis	
		Myoclonus (40 %) may initially suggest Creutzfeldt-Jakob disease	
		LGI1 antibodies usually can be detected with voltage-gated potassium channel radioimmunoassay	
Contactin-associated protein-like 2 (Caspr2) organizes Kv1 channels on myelinated CNS and	85 % male, median age 60 years	Neuromyotonia, encephalitis, or Morvan syndrome CNS and peripheral nervous	Thymoma

(continued)

Table 16.4 (continued)

Antigen	Patient demographics	Clinical syndromes	Tumors
peripheral nervous system axons		system symptoms may occur in either order	
		About 20 % may have comorbid myasthenia gravis	
Dipeptidyl-peptidase-like protein 6 (DPPX) is a regulatory subunit of Kv4.2 potassium channels	50 % male, ages 45–76 years	Encephalitis with CNS hyperexcitability (tremor, seizures, myoclonus, agitation, progressive encephalomyelitis with rigidity and myoclonus)	None so far
		Prodrome of unexplained diarrhea is common (due to dipeptidyl-peptidase 6 in myenteric plexus)	
Glycine receptor (GlyR) is the main inhibitory ionotropic receptor in the spinal cord	Six of 11 female and ages 5–69 years in one series	Stiff-person syndrome, progressive encephalomyelitis with rigidity and myoclonus	One of 11 had Hodgkin lymphoma
		Many patients have coexisting glutamic acid decarboxylase 65 (GAD65) antibodies	
Metabotropic glutamate receptor 5 (mGluR5) mediates certain types of synaptic plasticity in the hippocampus	So far one 15-year-old male and one 46-year-old female	Ophelia syndrome	Hodgkin lymphoma
Metabotropic glutamate receptor 1 (mGluR1) mediates synaptic transmission in the cerebellum	Males and females 19–69 years (total of 5 cases since year 2000)	Paraneoplastic cerebellar degeneration	Hodgkin lymphoma, prostate adenocarcinoma
Homer-3 organizes mGluR1s at cerebellar synapses	Single case of 38-year-old man	Paraneoplastic cerebellar degeneration	None reported to date
Delta/notch-like epidermal growth factor-related receptor (DNER) is a notch ligand on cerebellar Purkinje neurons	50 % males, ages 12–73 years in one series	Paraneoplastic cerebellar degeneration. Anti-DNER was previously known as anti-Tr, and commercial testing often uses that name	90 % have Hodgkin lymphoma

CNS central nervous system

based on the types of associated antineuronal antibodies against cell-surface molecules. The most common of these are anti-N-methyl-D-aspartate receptor (NMDAR) [33], anti-alpha-amino-3-hydroxy-5-methyl-4-isoxazol epropionic acid receptor (AMPAR) [16], anti-gamma-aminobutyric acid receptor-B receptor (GABABR) [17], and anti-voltage-gated potassium channel (VGKC) complex antibodies [14]. Of relevance to PNS is the fact that anti-GABABR antibody is most frequently detected in patients with SCLC who are negative for anti-Hu antibody [34]. The VGKC complex was later reported to be a composition of leucine-rich glioma-inactivated protein 1 (LGI1) [35], contactin-associated protein-like 2 (Caspr2) [36], and other related proteins [37]. Anti-Caspr2 antibody is associated with Morvan syndrome and peripheral excitability rather than encephalitis. The binding of antibodies changes synaptic transmission, plasticity, and mimetic pharmacological effects on the receptor or channel. For example, the binding of anti-NMDAR or anti-AMPAR antibody with a unit of its receptor causes internalization of the receptor [33]. Anti-GABABR antibody inhibits presynaptic neurotransmitter release and postsynaptic hyperpolarization. LGI1 is a secreted synaptic protein that organizes AMPAR and Kv1 channels at CNS synapses, while Caspr2 organizes Kv1 channels on myelinated CNS and peripheral nervous system axons. Anti-cell-surface antibodies associated with cerebellar ataxia, except anti-mGluR1, are anti-Homer3 [38], and delta/notch-like epidermal growth factor-related receptor (DNER) antibodies [39], the latter of which was previously known as Tr. Homer3, organize mGluR1s at cerebellar synapses, and DNER is a notch ligand on cerebellar Purkinje cells. However, our understanding of the underlying molecular mechanisms of these surface antigens remains limited.

16.3 Diagnosis

The main clinical manifestations of PNS are encephalomyelitis; limbic encephalitis; subacute cerebellar degeneration; opsoclonus-myoclonus; subacute sensory neuronopathy; chronic gastrointestinal pseudo-obstruction, such as due to autonomic neuropathy; LEMS; and dermatomyositis, which are considered to be classical syndromes. However, there are many other clinical presentations of PNS, and these often progress rapidly. The diagnosis of PNS requires the exclusion of many kinds of complications of tumors and of other mimetic neurological diseases. While the relationship among onco-neural antibodies, clinical features, tumor types, and immunotherapy responses has been reported, the absence of these antibodies is not sufficient to exclude PNS. Further, the presence of antibodies in tumor patients is not sufficient to diagnose PNS. To clarify the diagnostic criteria for PNS, an international panel of experts has suggested two levels of evidence to qualify diagnosis of a neurological syndrome as paraneoplastic: “definite” and “possible.” Each level can be reached by combining a set of criteria based on the presence or absence of cancer and the definitions of a “classical” syndrome and a “well-characterized” onco-neural antibody (Fig. 16.1, [12]). For example, antibodies

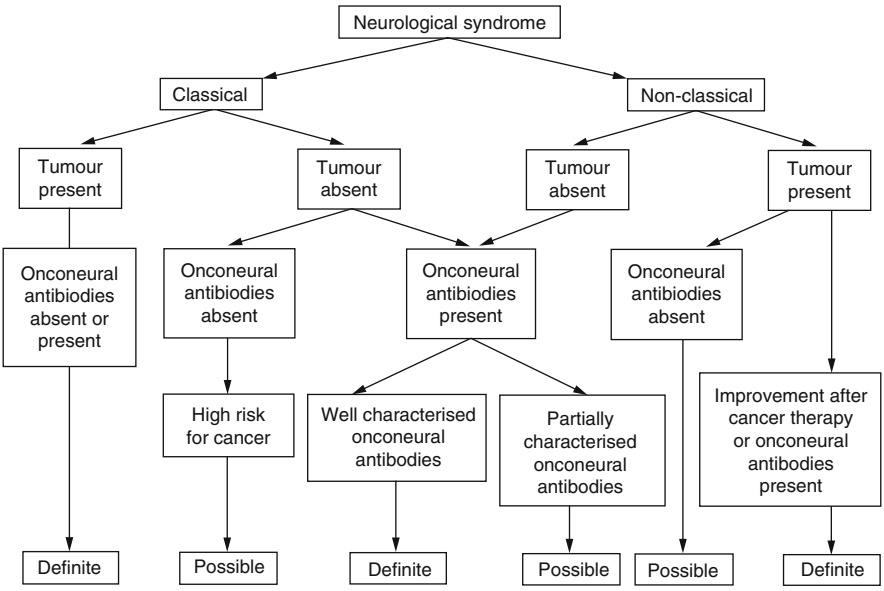

Fig. 16.1 Flow chart showing the level of diagnostic evidence of the neurological syndrome according to the criteria defined by the panel [8]

against Hu, Yo, Ri, Tr, CV2/CRMP5, Ma proteins (Ma1, Ma2), amphiphysin, and recoverin are classified as well-characterized antibodies, while anti-Zic4, mGluR1, ANNA-3, PCA2, and bipolar cells in the retina are classified as partially characterized antibodies. These antibodies, except mGluR1, can recognize intracellular antigens. An antibody is classified as well characterized if (1) it shows recognizable patterns on routine immunohistochemistry, and for which immunoblotting on recombinant proteins must be used to confirm their specificities; (2) it is found in a minimum number of cases to be associated with tumors; (3) it is seen in well-characterized neurological syndromes; (4) it is unambiguously identified across different studies; and (5) its frequency in patients without cancer is relatively low. Detection kits for these well-characterized antibodies are now commercially available. The criteria for definite PNS are (1) a classical syndrome and cancer that develops within 5 years of the diagnosis of the neurological disorder; (2) a nonclassical syndrome that resolves or significantly improves after cancer treatment without concomitant immunotherapy, provided that the syndrome is not susceptible to spontaneous remission; (3) a nonclassical syndrome with onconeural antibodies (well characterized or not) and cancer that develops within 5 years of the diagnosis of the neurological disorder; and (4) a neurological syndrome (classical or not) with well-characterized onco-neural antibodies and no cancer [12]. However, it should be emphasized that this criteria is operational, such that nominally definite PNS should not be taken as true PNS. The identification of molecules on the cell-surface membrane has traditionally been attempted using

immunoblot analysis. However, a radioimmunoassay was required to detect anti-VGCC antibody, and a patch clamp method was used to detect VGKC. More recently, a combination of immunohistochemical staining and proteomics was used to detect NMDAR antibodies, and cell-based assays expressing the N1 and N2 unit, or the N1 unit alone, have been used in clinical applications [15]. These techniques make possible the detection of antibodies against molecules comprising receptors on the neuronal cell surface. However, the clinical criteria for antibody-positive status should include its detection not only in the serum but also in the CSF [34]. It is also important to understand that the reliability of antibody detection varies both among laboratories and among individual trials within the same laboratory. Given this variability within laboratories, the recent commercial proliferation of detection kits for some antibodies should include emphasis on the need for both positive and negative controls to ensure validity. Tumor detection is also an important factor in the diagnosis of PNS. In addition to MRI with/without Gd enhancement, echogram, endoscopic examination, and analysis for various cancer markers in serum, PET/CT scans are very useful for detecting malignant tumors [40], except in the case of ovarian teratoma associated with NMDAR antibodies. Cases of suspected PNS should be examined with repeated malignancy survey every 6 months for 4 years, if a tumor is not found on initial survey.

16.4 Treatments and Prognosis

In any case of PNS, it is essential to detect and treat any associated tumors, followed by aggressive immunotherapy. Antitumor therapies include tumor resection, chemotherapy, and radiological treatments to remove the antigen source. The success of immunotherapy depends on the location of the antigens, i.e. whether they are intracellular or on the membrane surface. A limited number of cases of PNS have been successfully treated, by early and aggressive immunotherapy, when antibodies against intracellular antigen were detected. The therapies can be exceptionally effective in anti-Ma2 antibody-positive limbic encephalitis in young men with testicular cancer [41]. However, in such cases there is still the possibility that antibodies other than anti-Ma2 are responsible for the neurological damage, which are currently difficult to detect. Of course, treatment failures may also be due to irreversible neuronal damage that occurred prior to diagnosis and treatment. Generally, cases with antibodies against cell-surface antigens are responsive to immunotherapy. For example, in LEMS, either plasma exchange or intravenous immunoglobulin is usually effective in at least in the short term. Immunotherapies for PNS may follow the strategy of those for anti-NMDAR encephalopathy, which was developed based on a large number of cases [33]. First-line therapies include single or combined use of pulse therapy with methylprednisolone, intravenous immunoglobulin, or plasma exchange. It has yet to be determined whether plasma exchange can effectively reduce antibodies in CSF. First-line therapies are effective

in approximately 50 % of cases within 4 weeks [33]. Rituximab or cyclophosphamide is recommended as the second-line therapy for nonresponsive cases. Rituximab has been reported to be effective for PNS with antibodies against intracellular antigen [42]. However, since Rituximab is not effective on mature plasma cells, its full effects cannot be determined until several weeks post-administration. Cyclophosphamide can cause infertility; one should consider the value of banking gametes along with gonadotropin-releasing hormone treatment [43]. Further studies are required to identify the exact mechanisms underlying neuronal damage in PNS, which may lead to the development of more rational therapies, as well as a greater understanding of immunology in the nervous system.

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Chapter 17
Autoimmune Autonomic Ganglionopathy

Shunya Nakane, Osamu Higuchi, and Hidenori Matsuo

Abstract Autoimmune autonomic ganglionopathy (AAG) is a rare disorder of antibody-mediated impairment of transmission across the autonomic ganglia resulting in severe autonomic failure, in which autoantibodies to ganglionic nicotinic acetylcholine receptors (gAChRs) may play a central role. Here, we developed luciferase immunoprecipitation systems (LIPS) to diagnose AAG based on immunoglobulin G antibodies to both the α3 and β4 gAChR subunits in patient serum. We reviewed the serological and clinical data of 50 Japanese patients who were diagnosed with AAG. With the LIPS testing, we detected anti-α3 and anti-β4 gAChR antibodies in 48 % (24/50) of the patients. A gradual mode of onset was more common in the seropositive group than in the seronegative group. Patients with AAG frequently have orthostatic hypotension and upper and lower gastrointestinal tract symptoms, with or without anti-gAChR. The occurrence of autonomic symptoms was not significantly different between the seropositive and seronegative group, with the exception of achalasia in three patients from the seropositive group. In addition, we found a significant overrepresentation of autoimmune diseases in the seropositive group and endocrinological abnormalities and central nervous system involvement as an occasional complication of AAG. Our results demonstrated that the LIPS assay is a useful novel tool for detecting autoantibodies against gAChR in patients with AAG.

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17.1 The Autonomic Nervous System and the Ganglionic Nicotinic Acetylcholine Receptor

The autonomic nervous system has a unique anatomic structure. Unlike the somatic motor and sensory systems, the autonomic system is composed of groups of neurons (ganglia) with extensive synaptic connections outside the central nervous system (CNS). Like the somatic motor nerves, peripheral autonomic nerves originate with cholinergic motor neurons in the brainstem and spinal cord that project to the periphery [1]. These preganglionic nerves synapse with neurons in autonomic ganglia. The peripheral autonomic neurons, especially in the case of the intrinsic enteric autonomic nervous system, also synapse extensively with each other. Fast synaptic transmission within autonomic ganglia is mediated by acetylcholine acting on nicotinic acetylcholine receptors (AChRs). The ganglionic nicotinic acetylcholine receptor (gAChR) mediates fast synaptic transmission in all ganglia (sympathetic, parasympathetic, and enteric ganglia) in the peripheral autonomic nervous system. Every nicotinic AChR is formed by the association of five subunits of which at least two are α-subunits. On autonomic neurons, AChRs are typically composed of two α3 subunits in combination with three other AChR subunits [2]. The α-subunit contains important binding sites for acetylcholine. Transgenic mice lacking the α3 subunit have profound autonomic failure with prominent bladder distention, gastrointestinal dysmotility, and lack of pupillary light reflexes indicating that the α3 subunit is required for ganglionic neurotransmission. Although neurons of the autonomic ganglia can express numerous neuronal AChR subunits, including α3, α4, α5, α7, β2, and β4, the properties of the AChR at mammalian ganglionic synapses are most similar to AChRs that are formed by α3 and β4 subunits [3].

17.2 Anti-gAChR Antibodies

Autoimmune autonomic ganglionopathy (AAG) is an acquired immune-mediated disorder that leads to autonomic failure. The disorder is associated, at least in part, with autoantibodies to the gAChR. Antibodies to the gAChR are found in the serum of 50 % of patients with the acute or subacute form of AAG in concentrations that correlate with disease severity and have been shown to be pathogenic [4, 5]. Several studies have reported that these autoantibodies induce the internalization of cell-surface nicotinic gAChRs and thereby impair synaptic transmission (Fig. 17.1) [6, 7]. Although these antibodies are useful serological markers for AAG, they

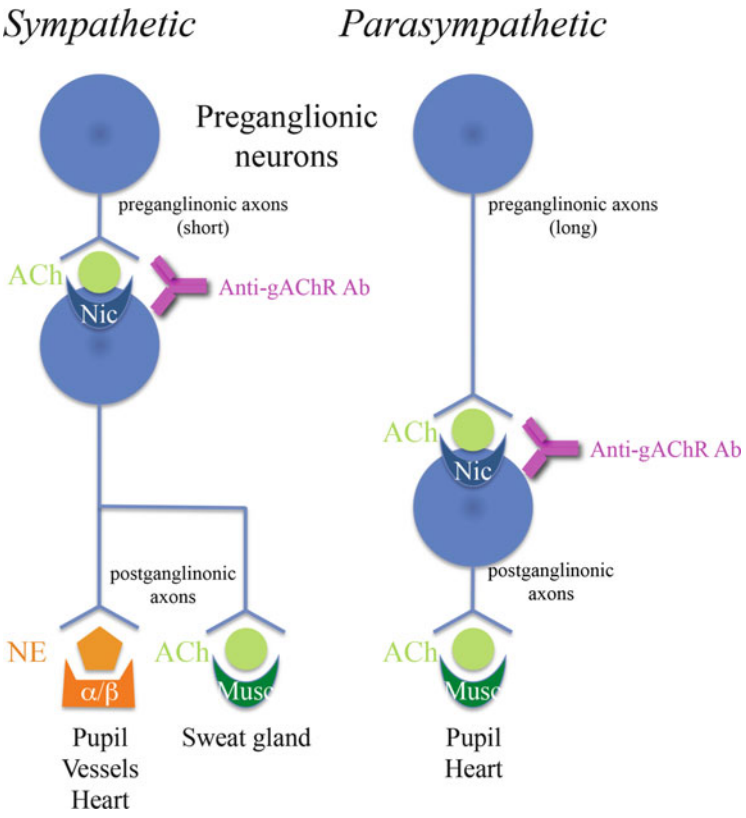

Fig. 17.1 A schematic showing the anatomy of the peripheral autonomic nervous system. The autonomic ganglia receive input from cholinergic motor neurons in the brainstem or spinal cord. Fast ganglionic synaptic transmission is mediated by acetylcholine acting on neuronal nicotinic acetylcholine receptors. The postganglionic fibers extend to innervate numerous target organs (a few examples are shown) and release acetylcholine acting on muscarinic receptors (m) or norepinephrine acting on alpha and beta adrenergic receptors (α and β)

are still not universally detected. Patients with high levels of ganglionic AChR antibodies usually have a subacute onset of disabling symptoms over a few weeks followed by spontaneous but incomplete recovery. Patients with lower antibody levels may have a chronic insidious presentation or milder, limited forms of autonomic failure. Higher ganglionic AChR antibody levels correlate with greater clinical severity and with greater severity of laboratory measures of autonomic failure. In some cases, patients treated with plasma exchange or other immunomodulatory treatments to reduce antibody levels can show dramatic improvement in autonomic function. Additionally, in vitro studies show that serum immunoglobulin G (IgG) isolated from patients with AAG reduces whole-cell neuronal AChR current in cultured human IMR-32 cells [4, 5, 8]. These clinical and experimental findings indicate that the gAChR antibodies in patients with AAG cause

physiologic changes in ganglionic AChR function and confirm that AAG is an antibody-mediated disorder.

17.3 Detection of Subunit-Specific Autoantibodies to the gAChR

Since the gAChR was identified as an autoantigen that is associated with the pathogenesis of AAG, it has been a challenge to search for gAChR-specific autoantibodies. RIA and cell-based assays (CBA) have been available for the detection of autoantibodies to the gAChR in the sera of patients with AAG [4, 8]. Cases of idiopathic pure autonomic neuropathy have been reported since 1975 in Japan, and 29 cases of AAG have been reported [9]. However, no assays that detect the autoantibodies to gAChR are available in Japan, and this has caused difficulty in the diagnosis of AAG. Furthermore, antibodies to non-α3 subunits, including the β4 gAChR subunit, have not been identified in AAG to date.

We attempted to develop a novel technique to detect the subunit-specific antibodies of gAChR without the use of a radioisotope. The radioimmunoprecipitation (RIP) assay is a very useful tool for obtaining information about the total amount of antibodies to gAChR, but it cannot distinguish subunit-specific antibodies [4]. Here we established luciferase immunoprecipitation systems (LIPS) using GL^{8990} that can detect antibodies that bind to the α3 or β4 gAChR subunits with high sensitivity. The RIP assay using [^{125}I]-labeled epibatidine has been used as a convenient method to detect autoantibodies to the gAChR [1]. In the RIP, a subunit-specific antibody cannot be detected because of the epibatidine binding to the pentamer form of the gAChR. In contrast, LIPS, which is a powerful diagnostic technique for the serological testing of antibodies that are associated with many different human pathogens, is suitable for detecting a subunit-specific antibody [10–13]. In order to provide higher performance on the LIPS, we selected a *Gaussia* luciferase (GL) mutant, called GL^{8990}, for use in this study. GL is the smallest marine luciferase that has been discovered [14]. GL generates a greater signal intensity from cells in culture (1000-fold) compared with the *Renilla* luciferase [15]. GL^{8990} (featuring a phenylalanine-to-tryptophan substitution at residue 89 and a isoleucine-to-leucine substitution at residue 90) is a GL mutant that is generated by site-directed mutagenesis and emits bioluminescence that is ten times stronger and/or prolonged than intact GL [16]. Here we performed the LIPS with the α3 or β4 gAChR subunit fused to a luciferase to detect the respective autoantibodies in human sera.

17.3.1 LIPS Assay: The Detection of Autoantibodies to gAChR

LIPS is a user-friendly assay to examine a protein-protein interaction as described elsewhere [10, 12]. To generate luciferase reporters for the gAChR α3 and β4 subunits (termed gAChRα3-GL and gAChRβ4-GL, respectively) of the human gAChR, full-length human AChR α3 (P32297, Promega Corporation, Madison, WI, USA) or β4 (P30296, Promega Corporation) was fused to a GL mutant (GL^{8990}) (Fig. 17.2). Human embryonic kidney (HEK) 293F cells (Life Technologies Corporation, Grand Island, NY, USA) were transfected with the expression plasmid encoding the gAChRα3-GL or the gAChRβ4-GL with FuGENE6 (Promega Corporation). Two days later, the transfected cells were solubilized with a Tris-based saline containing 1 % Triton™ X-100. To detect the α3 or β4 gAChR antibodies, 100 μL of the soluble fraction, containing gAChR α3-GL or gAChR β4-GL, was incubated with 15 μL of human serum for 1 h at 4 °C. Subsequently, the fraction was mixed with 15 μL of protein G-sepharose (GE Healthcare, Little Chalfont, Buckinghamshire, UK) and 600 μL phosphate-buffered saline (PBS) with 3 % bovine serum albumin and 0.05 % Tween® 20 and incubated for several hours at 4 °C. Following centrifugation and two washes with PBS containing 0.05 % Tween® 20, the bioluminescence activities of the luciferase reporters in the protein G-sepharose were measured with a BioLux™ GL assay kit (New England Biolabs, Ipswich, MA, USA) and a Lumat LB 9507 luminometer (BERTHOLD TECHNOLOGIES GmbH & Co. KG, Bad Wildbad, Germany) (Fig. 17.3). The luminometer output was measured in relative luminescence units (RLU). In order to confirm the accuracy of the LIPS assay for the gAChR antibodies, we used commercially available antibodies to human gAChR α3 and β4 (H-100 and S-15; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) as positive controls.

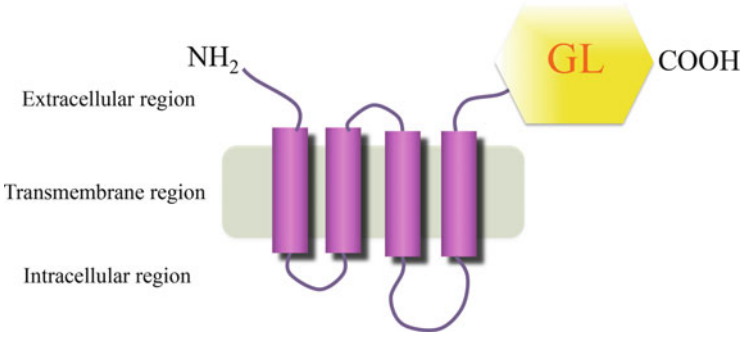

Fig. 17.2 Schematic representation of the ganglionic acetylcholine receptor (*gAChR*) α3/β4-*Gaussia* luciferase (*GL*)^{8990}. For the gAChR luciferase precipitation system (gAChR-LIPS) assay, human embryonic kidney (*HEK*) 293 cells were transfected with an expression plasmid for the gAChRα3 or β4-GL reporter

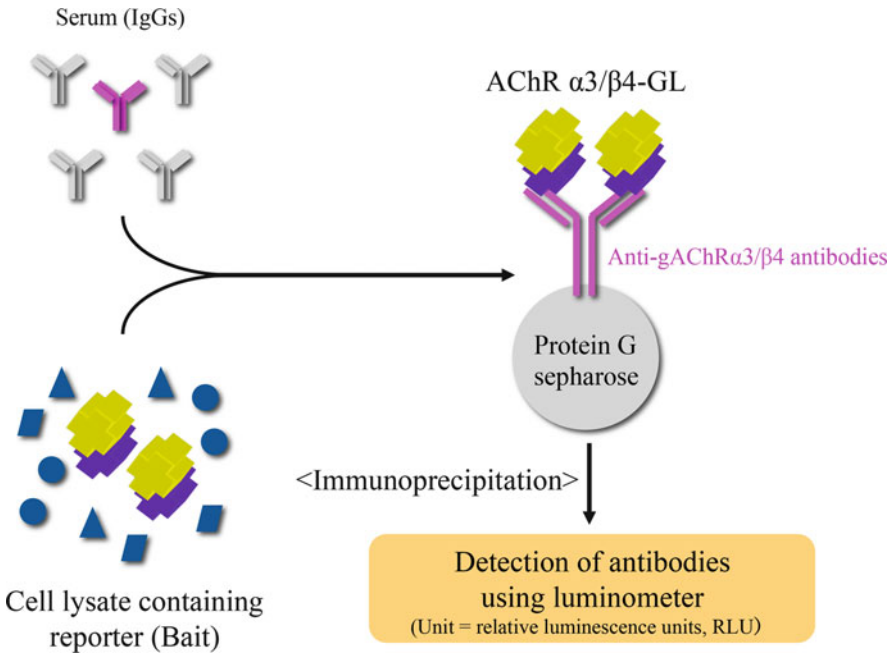

Fig. 17.3 The luciferase immunoprecipitation systems (*LIPS*). The soluble fractionated component from the solubilized HEK 293F cells (the bait), including the ganglionic nicotinic acetylcholine receptor (gAChR) α3- or β4-GL, reacted with human serum, and the specific luciferase activities of the gAChRα3- or β4-GL were found with the luminometer. The in vitro LIPS assay can quantitatively evaluate an interaction between an antigen and an antibody with high sensitivity and without a radioisotope

Based on the data for anti-gAChRα3 and β4 antibodies from the 73 HC, the cut-off values were calculated as the mean plus three standard deviations (SD) from the mean. In this study, the antibody levels were expressed as an antibody index (A.I.) that was calculated as follows:

$$\text{A.I.} = [\text{measurement value of the sample serum (RLU)}]/[\text{the cut} - \text{off value(RLU)}].$$

The normal value that was established in this study from healthy individuals was <1.0 A.I. As such, serum samples with an A.I. $>= 1.0$ were considered positive for the relevant antibody.

17.3.2 LIPS Assay: Results in the Patients with AAG

In order to confirm that these subunit-specific luciferase reporters work as bait in the LIPS, we examined a LIPS assay that used ready-made gAChR subunit-specific

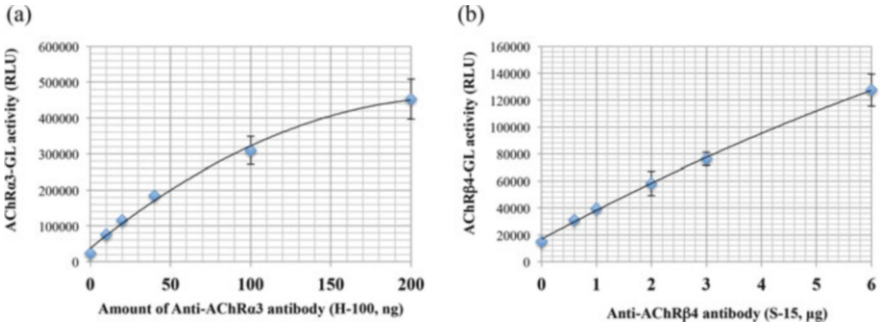

Fig. 17.4 Confirmation of the luciferase immunoprecipitation systems (*LIPS*) assay system for the ganglionic nicotinic acetylcholine receptor (gAChR) α3 or β4 with ready-made antibodies. The anti-gAChRα3 antibody (H-100) and the anti-gAChRβ4 antibody (S-15) bound the gAChRα3-GL and the gAChRβ4 reporters, respectively, in a dose-dependent manner (**a**, **b**). The *X-axis* indicates the amount of ready-made gAChRα3 or β4 antibody used. The *Y-axis* indicates the gAChRα3- or β4-GL activity. The *line* with closed diamonds shows the results that were obtained in this experiment

antibodies. As shown in Fig. 17.4, a dose-dependent response was observed between the amount of ready-made gAChR subunit-specific antibody and the gAChR subunit-specific luciferase reporter activity. These data demonstrate that each gAChR subunit-specific antibody properly bounds gAChRα3-GL or gAChRβ4-GL.

The series of subjects in our study was comprised of the groups of patients with AAG, healthy controls (HC), and other disease controls (DC). Serum samples from 50 patients with AAG were obtained from general and teaching hospitals throughout Japan between January 2012 and February 2014 (mean age, 52.5 ± 19.0 years old, 26 males and 24 females, Table 17.1). The clinical diagnoses were made in each hospital and the patients' clinical data were provided at the time of diagnosis. The control groups consisted of 73 HC (mean age, 38.3 ± 11.1 years old, 31 males and 42 females) and 34 subjects with other neurological diseases (DC group: detailed clinical characteristics of DC patients; mean age, 56.3 ± 20.4 years old, 19 males and 15 females). All subjects gave their written, informed consent to participate in the present study. The study was approved by the Ethics Committee of Nagasaki Kawatana Medical Center (Nagasaki, Japan).

Both the anti-gAChRα3 and anti-gAChRβ4 antibodies were determined by the LIPS assay to be present in 0.0 % (0 of 73) of the HC. In contrast, 48 % (24 of 50) of the sera from patients with AAG were positive for autoantibodies ($p < 0.001$, Fig. 17.5a). The anti-gAChRα3 antibodies were detected in 23 samples and the anti-gAChRβ4 antibodies were detected in seven samples (14.0 %), as shown in Fig. 17.4b ($p < 0.001$). Both of the antibodies were detected in six samples. The mean anti-gAChRα3 antibody levels in the HC and the DC were 0.305 A.I. and 0.336 A.I., respectively. These levels were significantly lower than the mean level in the AAG samples of 1.210 A.I. ($p < 0.001$, Fig. 17.5a). Similarly, the mean anti-gAChRβ4 antibody level in HC was 0.367 A.I. and DC was 0.302 A.I., respectively.

Table 17.1 Clinical features of patients with AAG

	Patients with AAG	Patients with AAG Anti-gAChR Ab positive	Patients with AAG Anti-gAChR Ab negative	P value
Number of patients	50	24	26	
Age (year)	52.5 ± 19.0	51.9 ± 20.4	53.0 ± 18.1	0.838
Age at onset (year)	48.8 ± 20.1	46.8 ± 20.8	50.7 ± 19.7	0.495
Sex (female, %)	24 (48.0)	13 (54.2)	11 (42.3)	0.413
Duration of the autonomic symptoms (year)	3.7 ± 6.9	5.1 ± 8.8	2.3 ± 4.1	0.344
Onset (%)	Subacute: 25 (50.0)	Subacute: 9 (37.5)	Subacute: 16 (61.5)	0.095
	Gradual: 25 (50.0)	Gradual: 15 (62.5)	Gradual: 10 (38.5)	
Antecedent event (%)	11 (22.0)	4 (16.7)	7 (26.9)	0.212
Orthostatic hypotension and/or orthostatic intolerance (%)	42 (84.0)	20 (83.3)	22 (84.6)	0.915
Sicca complex (%)	28 (56.0)	14 (58.3)	14 (53.8)	0.760
Coughing episodes (%)	8 (16.0)	4 (16.7)	4 (15.4)	0.915
Heat intolerance and/or anhidrosis (%)	34 (68.0)	15 (62.5)	19 (73.1)	0.435
Pupil abnormality (%)	20 (40.0)	11 (45.8)	9 (34.6)	0.281
Gastrointestinal tract symptoms (%)	46 (92.0)	22 (91.7)	24 (92.3)	0.951
Bladder dysfunction (%)	29 (58.0)	16 (66.7)	13 (50.0)	0.242
Sexual dysfunction[a] (%)	15 (57.7)	7 (63.6)	8 (53.3)	0.628
Other clinical features[b] (%)	15 (30.0)	8 (33.3)	7 (27.0)	0.633
Complication: endocrine disorder[c] (%)	5 (10.0)	3 (12.5)	2 (7.7)	0.588
Complication: autoimmune disease[d] (%)	11 (22.0)	9 (37.5)	2 (8.0)	0.012
Complication: tumor[e] (%)	5 (10.0)	4 (16.7)	1 (3.8)	0.140

[a]We reviewed the 26 male patients only
[b]Numbness, mental symptom, dementia, character change, and back pain
[c]Amenorrhea, eating disorder, SIADH (syndrome of inappropriate secretion of antidiuretic hormone), and panhypopituitarism
[d]Still disease, PBC (primary biliary cirrhosis), Hashimoto's disease, PMR (polymyalgia rheumatica), SLE (systemic lupus erythematosus), SS (Sjögren's syndrome), Graves' disease, RA (rheumatoid arthritis), fibromyalgia, and other autoantibodies positive
[e]Ovarian tumor, pancreas cancer, mediastinal tumor, and paranasal cancer

Those were significantly lower than the mean level in the AAG samples of 0.618 A.I. ($p < 0.001$, Fig. 17.5b). In the DC group, we detected anti-gAChRα3 antibodies in the serum of a patient with a suspected case of amyloid neuropathy.

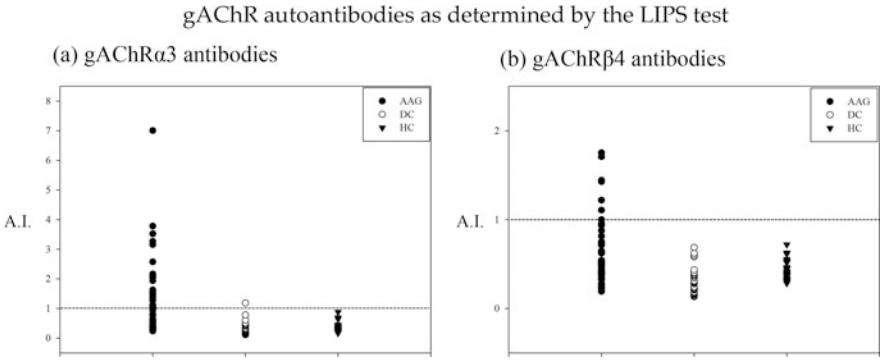

Fig. 17.5 Luciferase immunoprecipitation systems (*LIPS*) for the ganglionic nicotinic acetylcholine receptor (*gAChR*) in the sera from patients with autoimmune autonomic ganglionopathy (*AAG*) sera and controls. We tested the sera from patients with AAG, disease controls (*DC*), and healthy controls (*HC*). (**a**) Anti-gAChRα3 antibodies were detected in 23 samples. The mean anti-gAChRα3 antibody level in the HC was 0.305 antibody index (A.I.), which was significantly lower than the AAG samples with a mean level of 1.210 A.I. ($p < 0.001$). (**b**) Anti-gAChRβ4 antibodies were also detected in seven samples, as shown in Fig. 17.4b ($p = 0.005$). The mean anti-gAChRβ4 antibody level in the HC was 0.367 A.I., which was significantly lower than the mean level of 0.618 A.I. in the AAG samples ($p < 0.001$)

17.3.3 LIPS Assay: Rapid, Simple, Quantitative, Highly Sensitive and Safety

Sera from eight of the 50 patients with AAG had been examined previously for antibodies to the gAChR with conventional RIP in the laboratory of Dr. Vernino. These assays for antibodies against the AChR were performed as previously described. In brief, the antibodies were detected with an immunoprecipitation assay in which the AChR antigen was solubilized from a human neuroblastoma cell line (IMR-32) and complexed with iodine I^{125}-labeled epibatidine [4, 5, 8]. Our assay for the anti-gAChR antibodies may have a different sensitivity and specificity compared with the RIP assay; therefore, we were able to compare the results of the two different assays on these eight samples. Seventy-five percent (6/8) of the samples demonstrated perfect agreement on both assays. Anti-gAChRα3 antibodies were detected in patient 14 by the LIPS assay, but this sample was seronegative in the RIP (Table 17.2). Another sample demonstrated the reverse pattern. We further evaluated the correlation between the level of the anti-gAChRα3 antibody that was measured by LIPS and the level of the anti-gAChR antibody that was measured by the RIP in four seropositive samples. Some of the samples tracked each other well between the two assays. These results suggest that screening by the LIPS test with gAChRα3 and β4 as antigens offers an efficient quantitative approach for the evaluation of the antibody responses in patients with AAG. In addition, we need to compare the sensitivity and specificity of LIPS with the RIP for the measurement of autoantibodies to gAChR in spiraling numbers.

Table 17.2 Autonomic function tests at baseline of anti-gAChR Ab-positive AAG patients

Patient	Age	Sex	Anti-gAChRα3 Ab (A.I., LIPS)	Anti-gAChRβ4 Ab (A.I., LIPS)	Anti-gAChR Ab (nmol/L, RIP)	OH in HUTT	Decreased CV R-R	Decreased H/M ratio	Residual urine in urodynamic study	Abnormality of TST	Reduction of resting plasma NE	Abnormality of pupillary response	Albuminocytologic dissociation in CSF
1	75	M	1.626	1.000	0.060	+	+	+					–
2 [37]	60	M	2.163	0.389	1.220	+	+		+		+	+	
3	39	F	1.000	0.638			–				–		
4	26	F	3.520	0.376		+	+			+	+	+	
5	68	M	2.046	1.219		+	–	+	+				+
6	37	F	1.352	0.636									
7	45	M	7.005	1.443	42.160	+	+		+		+	+	–
8	60	M	1.008	0.494		+	+			+	+		
9	79	F	1.027	0.935		+	+	+					–
10	78	M	1.419	0.737		+	+	+					–
11	67	M	3.265	1.107		+	+	+					+
12	49	M	0.937	1.428		+							
13	73	F	1.103	0.877		+	+	+					+
14	16	M	1.488	0.713	Negative	+	+	+	+		–	+	+
15	56	M	3.781	1.708		+	+						+
16	54	F	2.084	0.525									
17	84	F	2.573	1.752		+	+	+					
18	37	F	1.054	0.523		+	–	+			+		+
19	38	F	2.101	0.488		+	+				+		–
20	68	F	1.542	0.952		+	+						
21	46	F	1.113	0.478		–	–	–		–			
22	49	F	3.154	0.336	0.100	–		–	–				
23	6	F	1.275	0.456		+	–						+
24	36	M	1.936	0.323		+	+						

Anti-gAChRα3 Ab = ganglionic acetylcholine receptor α3 antibody; anti-gAChRβ4 Ab = ganglionic acetylcholine receptor β4 antibody
A.I. antibody index, *OH* orthostatic hypotension, *HUTT* head-up tilt test, *CV R-R* CV = coefficient of variation R-R interval, *H/M ratio* heart-to-mediastinum ratio in ^{123}I-MIBG myocardial scintigraphy, *TST* thermoregulatory sweat test, *NE* norepinephrine, *SSR* sympathetic skin response, *QSART* quantitative sudomotor axon reflex test, *CSF* cerebrospinal fluid

Ching et al. have reported using the LIPS method to detect a subunit-specific autoantibody of the AChR in patients with myasthenia gravis [17]. Myasthenia gravis (MG) is an autoimmune channelopathy that is caused by autoantibodies to AChRs located in the neuromuscular junction. In about 80 % of the patient sera, these autoantibodies to the muscle type of the nicotinic AChR are detected [18]. The AChR is a pentamer that is composed of four subunits (α1, β1, γ or δ, and ε). Ching et al. have demonstrated that using the α1 AChR subunit fused to *Renilla* luciferase in LIPS was partly useful for the detection of subunit-specific antibodies of the AChR. In our study, gAChR autoantibodies were detected by LIPS in about 48 % of the patients with AAG, which indicated that our data on the frequency of the gAChR autoantibodies matched the results of the previous study that used the RIP. Furthermore, the concentrations of the gAChR antibody that were estimated by LIPS (the current group) and the RIP (reported by studies from the Steven Vernino Laboratory and Mayo Medical Laboratories [patients 1, 2, 7, 14, and 22, as shown in Table 17.2]) showed a similar trend. Together, these results demonstrated that the LIPS exhibits a similar performance to the RIP, at least in the detection of autoantibodies to gAChR. However, there is still a slight possibility that a pentamer-specific antibody exists in the AAG patient serum, because these results matched incompletely. In addition, we have identified a subunit-specific antibody for the gAChR, the anti-AChRβ4 antibody, for the first time.

The LIPS provides a powerful new approach to serological testing for antibodies associated with many different human pathogens in neuroimmunological diseases. The LIPS is based on the fusion of protein antigens to a light-emitting enzyme reporter, *Gaussia* luciferase, and then the use of these antigen fusions in immunoprecipitation assays with serum samples and protein G-sepharose. After the protein G-sepharose is washed, the level of light production is measured, yielding highly quantitative antibody levels. Taken together, these reports suggest that autoantibody detection by the LIPS will be useful for enhancing the clinical management of autoimmune diseases.

17.4 Clinical Features of AAG in Japan

17.4.1 Clinical Assessment of Autonomic Function

We extensively reviewed the histories and ongoing clinical and laboratory evaluations of 50 Japanese patients who had been diagnosed with AAG and measured their levels of antibodies to gAChR with the LIPS. All of the patients with AAG had dysfunction in at least one autonomic domain, and they underwent a baseline assessment, which included a determination of the gAChR α3 and β4 antibody levels. Subacute onset was defined as reaching peak autonomic failure within 3 months, and chronic was defined as reaching the peak level of autonomic failure within 3 months. Comprehensive clinical, hematologic, biochemical, neurological,

and serologic assessments of all patients were performed at baseline. In addition, cerebrospinal fluid analysis was also conducted.

We inquired about the presence or absence of each of the following functions that are controlled by the autonomic system: syncope or orthostatic hypotension for orthostatic intolerance; sicca complex, dryness of the skin, or hypohidrosis/anhidrosis for heat intolerance; pupillary dysfunction; diarrhea or constipation for dysfunction of the gastrointestinal system; dysuria or urinary retention needing catheterization for bladder dysfunction; and sexual dysfunction. However, we were not able to assess the extent of each autonomic symptom rigorously with the composite autonomic scoring scale. Patients with known causes of autonomic failure, including multiple system atrophy, diabetes, and amyloidosis, were excluded.

Each patient went through autonomic testing, which involved the Schellong test, head-up tilt test, measurement of the coefficient of variation in R-R intervals (CV_{R-R}), noradrenaline (NA) infusion test, pupillary response to local instillation, assessment of the plasma levels of catecholamines, sweat testing, quantitative sudomotor axon reflex test (QSART), [^{123}I] metaiodobenzylguanidine (^{123}I-MIBG) myocardial scintigraphy, and cystometry. ^{123}I-MIBG, an analog of noradrenaline, is used to trace uptake and transport both in noradrenaline presynaptic sympathetic nerve terminals and in subsequent vesicular storage [19]. Postganglionic presynaptic cardiac sympathetic nerve endings can be noninvasively assessed by MIBG scintigraphy because a reduction in cardiac MIBG uptake (H/M ratio) indicates postganglionic sympathetic dysfunction. Cardiac MIBG uptake is reduced in patients with Lewy body diseases such as Parkinson's disease and dementia with Lewy bodies [20, 21]. In the standard procedure, the H/M ratio is calculated from early and delayed anterior chest planar images by drawing a region of interest including the heart (H) and the other one over the upper mediastinum (M).

17.4.2 Clinical Profile of the Anti-gAChR Antibody-Positive and Antibody-Negative Patients with AAG

The clinical characteristics of the patients are presented in Table 17.1. The age at onset and the duration of the autonomic symptoms were 48.8 ± 20.1 (mean $\pm$ standard deviation) years and 3.7 ± 6.9 years, respectively. The patterns of mode were divided into subacute and gradual groups, according to the duration to the peak of autonomic symptoms. Half of the patients with AAG had subacute onsets and half had chronic progressive presentations. Gastrointestinal tract symptoms were the most frequently observed (92.0 %). Table 17.1 compares the clinical features between patients who were positive and those who were negative for the anti-gAChR antibodies. Gradual onset was more common in the anti-gAChR antibody-positive patients than in the antibody-negative patients (62.5 % vs. 38.5 %). No significant differences in the clinical findings were noted, except

for a higher frequency of other autoimmune disease complications in patients who were positive for the anti-gAChR antibody compared to antibody-negative patients (37.5 % vs. 8.0 %, $p = 0.012$).

17.4.3 Clinical Characteristics and Autonomic Symptoms of the Patients with AAG Who Were Anti-gAChR Antibody Positive

Table 17.3 summarizes the clinical characteristics and autonomic symptoms of the patients with AAG. An antecedent event was reported in four patients shortly before the initiation of autonomic symptoms. In our study, major autonomic symptoms including orthostatic, sicca, sudomotor, papillary, gastrointestinal, and urinary symptoms were analyzed. Orthostatic hypotension for orthostatic intolerance and gastrointestinal tract symptoms were observed in 20 (83.3 %) patients and 22 (91.7 %) patients, respectively. Gastrointestinal tract symptoms were composed of various digestive system problems, such as constipation (n = 14), early satiety (n = 4), vomiting (n = 4), abdominal pain (n = 4), anorexia (n = 4), diarrhea (n = 4), ileus (n = 3), alternate stool abnormality (n = 3), taste impairment (n = 1), and achalasia (n = 3). Pupillary dysfunction was observed in 11 patients, including two patients who had Adie's tonic pupil. The initial symptoms of seropositive AAG/acute pandysautonomia (APD) in 15 patients (62.5 %) were orthostatic hypotension, involving lightheadedness, orthostatic intolerance, or syncope. The autonomic manifestations of the anti-gAChR antibody-positive patients were widespread, and they affected both sympathetic and parasympathetic functions. However, three patients (patients 6, 16, and 17) had only one symptom (disturbance of the digestive system, bladder dysfunction, and orthostatic hypotension, respectively). Patient 6 had a history of recurrent ileus and severe abdominal pain. She previously had an operation to remove a sigmoid volvulus 1 year before the onset of repeated ileus and abdominal pain. As for the other symptoms, attacks of coughing were observed in four patients (16.7 %), and six patients (patients 1, 5, 11, 12, 14, and 15) had a subjective numbness or superficial sensory disturbance in the extremities or trunk. Psychiatric symptoms were observed in three patients (patients 8, 9, and 14). Patient 8 demonstrated infantilization after the onset of autonomic symptoms and had frequent syncope with emotional strain. Patient 9 showed memory disturbances and apathy in daily living, such as domestic duties. Patient 14 also demonstrated character changes, such as the tendency to act in a childish manner. Three patients showed the following endocrine disorders: amenorrhea (patient 4), syndrome of inappropriate secretion of antidiuretic hormone (SIADH) (patient 14), and panhypopituitarism (patient 15). Nine patients presented the other following autoimmune diseases: rheumatoid arthritis (n = 2), primary biliary cirrhosis (n = 2), secondary Sjögren's syndrome (n = 2), Still's disease

Table 17.3 Clinical and autonomic characteristics at baseline of anti-gAChR Ab-positive AAG patients

Patient	Age	Sex	Onset age	Duration	Onset[a]	AE[b]	OH OI	Sicca	Coughing episodes	HI AH	Pupil abnormality[c]	GI[d]	Bladder dysfunction	Sexual dysfunction	Other clinical features	Complication: endocrine disorder, autoimmune disease	Complication: tumor
1	75	M	59	16	Gradual	–	+	+	–	+	+	+	+	+	Numbness	–	–
2[37]	60	M	60	0	Subacute	+	+	+	–	+	+	+	+	–	–	Still disease susp.	–
3	39	F	39	0	Subacute	–	+	+	–	–	–	+	–		–	ANA positive	–
4	26	F	21	5	Gradual	–	–	+	–	+	+	+	+		–	Amenorrhea	Ovarian tumor
5	68	M	53	15	Gradual	–	+	–	–	–	–	+	+	+	Numbness	–	–
6	37	F	35	2	Gradual	–	–	–	–	–	–	+	–	–	–	–	–
7	45	M	45	0	Subacute	+	+	+	–	+	+	+	+	+	–	–	–
8	60	M	60	0	Subacute	–	+	+	–	+	+	+	+	+	Mental symptom	–	–
9	79	F	77	2	Gradual	–	+	–	–	–	+	+	+		Dementia	PBC, Hashimoto dis.	–
10	78	M	78	0	Subacute	–	+	–	–	+	–	+	–	–	–	–	–
11	67	M	59	8	Gradual	–	+	+	–	–	–	+	–	–	Numbness	–	–
12	49	M	12	37	Gradual	–	+	+	+	+	+	+	+	+	Sensory disturbance	–	–
13	73	F	66	7	Gradual	–	+	+	–	+	–	+	+		–	–	Mediastinal tumor
14	16	M	16	0	Subacute	–	+	–	+	+	+	+	–	–	Numbness, character change	SIADH	–
15	56	M	55	1	Gradual	–	+	–	+	+	–	+		+	Numbness	PMR, panhypopituitarism	–
16	54	F	35	19	Gradual	–	–	–	–	–	–	–	+		–	–	–
17	84	F	84	0	Gradual	–	+	–	+	–	–	+	+		–	–	Paranasal cancer
18	37	F	37	0	Subacute	–	+	+	–	+	+	+	+		–	SLE, SS	–
19	38	F	39	0	Subacute	–	+	–	–	–	–	–	–		–	Graves' dis.	Ovarian tumor
20	68	F	66	2	Gradual	–	+	+	–	+	–	+	+		–	RA, SS	–
21	46	F	39	7	Gradual	+	+	–	–	+	–	+	+		–	RA, fibromyalgia	–
22	49	F	47	2	Gradual	–	–	+	–	–	–	+	–		–	PBC	–
23	6	F	6	0	Subacute	+	+	+	–	+	+	+	+		–	–	–
24	36	M	35	0.5	Gradual	–	+	+	–	+	+	+	+	+	–	–	–

Initial symptoms were expressed in *red*

AE antecedent event,*OH* orthostatic hypotension, *OI* orthostatic intolerance, *HI* heat intolerance, *AH* anhidrosis, *GI* gastrointestinal tract symptoms

α3 Ab = ganglionic acetylcholine receptor α3 antibody, β4 Ab = ganglionic acetylcholine receptor β4 antibody, *A.I.* antibody index, *AE* antecedent event, *OI* orthostatic intolerance, *OH* orthostatic hypotension, *HI* heat intolerance, *AH* anhidrosis, *GI* gastrointestinal tract symptoms, *Dept* department

[a]Subacute = peak of autonomic failure within 3 months; gradual = gradual onset of chronic autoimmune autonomic ganglionopathy with the peak of autonomic failure after 3 months

[b]Patient 2 = fever up; 7 = epididymitis; 21 = influenza virus type A infection; 23 = fever up and cough

[c]Patients 4 and 7 = Adie's tonic pupil. The other cases had the abnormality of papillary reflex to light bilaterally or unilaterally

[d]Patient 1 = constipation; 2 = constipation; 3 = early satiety and vomiting; 4 = constipation; 5 = constipation; 6 = constipation, ileus, and sigmoid volvulus suspected; 7 = early satiety, vomiting, alternate stool abnormality, abdominal pain, and taste impairment; 8 = constipation; 9 = constipation; 10 = constipation; 11 = anorexia and diarrhea; 12 = diarrhea and achalasia; 13 = constipation, early satiety, vomiting, and ileus; 14 = abdominal pain, anorexia, and diarrhea; 15 = diarrhea; 17 = constipation, anorexia, and achalasia; 18 = alternate stool abnormality, abdominal pain, and anorexia; 20 = constipation; 21 = constipation; 22 = early satiety, ileus, alternate stool abnormality, and abdominal pain; 23 = constipation, vomiting, and achalasia; 24 = constipation

(n = 1), Hashimoto's disease (n = 1), polymyalgia rheumatica (n = 1), systemic lupus erythematosus (n = 1), Graves' disease (n = 1), and fibromyalgia (n = 1).

17.4.4 Autonomic Function Studies of the Anti-gAChR Antibody-Positive Patients with AAG

Table 17.2 shows the results of the autonomic function studies of the anti-gAChR antibody-positive patients. Some information was missing because some of the hospitals did not have adequate equipment for the autonomic function tests. Of these 24 patients, abnormalities such as orthostatic changes in systolic blood pressure and the $CV_{R\text{-}R}$ were observed at high rates (86.4 % and 75.0 %, respectively). Cardiac MIBG uptake (H/M ratio), which was measured by ^{123}I-MIBG myocardial scintigraphy in 11 anti-gAChR antibody-positive patients, was decreased in nine patients (81.8 %). Urodynamic studies were performed in five of 24 patients. Residual urine of more than 50 mL was noted in four patients (80.0 %). Sudomotor and cutaneous vasomotor functions were assessed by thermoregulatory sweat testing in three patients and a reduced ability to sweat was reported in two patients. Plasma norepinephrine values were measured in seven of 24 patients. Six had reduced supine norepinephrine below 100 pg·mL^{-1} (85.7 %). Pupillary responses to 1 % phenylephrine, 5 % tyramine, and 5 % cocaine were examined in two patients (patients 2 and 14) and 0.1–0.125 % pilocarpine in two patients (patients 4 and 7) in a totally dark room. In the former test, remarkable mydriasis was observed as a pupillary response to the local instillation of 1 % phenylephrine. In the latter test, the affected Adie's pupil reacted with miosis to low dose pilocarpine. We confirmed albuminocytologic dissociation in seven of 12 patients (58.3 %) in a cerebrospinal fluid analysis.

17.4.5 Clinical Features of AAG in Japan

AAG has two patterns of onset. In the present study, a gradual mode of onset was more common in the seropositive group than in the group that was seronegative for anti-gAChR antibodies, although Sandroni et al. have reported that subacute onset was more common in the seropositive group [22]. There was no difference between the demographic features of the seropositive patients in gradual onset and subacute AAG in seropositive patients (Table 17.4) and no relationship between antibody status and the temporal profile. Subacute AAG was often preceded by an illness that was presumed to be a viral or bacterial infection. Patients with chronic AAG with mild or restricted autonomic failure usually present with low antibody levels, whereas high levels of antibodies are associated with severe acute/subacute AAG subtypes. However, the highest A.I., which was found in patient 7, was associated

Table 17.4 Demographic features of patients with subacute and gradual AAG

	Subacute patients with AAG/APD	Gradual patients with AAG/APD	
	Anti-gAChR Ab positive	Anti-gAChR Ab positive	P value
Number of patients	9	15	
Age (year)	42.1 ± 22.3	57.8 ± 17.3	0.066
Age at onset (year)	42.1 ± 22.3	49.5 ± 20.1	0.417
Sex (female, %)	4 (44.4)	9 (60.0)	
Antecedent event (%)	3 (33.3)	1 (6.7)	0.106
Orthostatic hypotension and/or orthostatic intolerance (%)	9 (100.0)	11 (73.3)	0.062
Sicca complex (%)	6 (66.7)	8 (53.3)	0.553
Coughing episodes (%)	1 (11.7)	3 (20.0)	0.612
Heat intolerance and/or anhidrosis (%)	7 (77.8)	8 (53.3)	0.256
Pupil abnormality (%)	6 (66.7)	5 (33.3)	0.129
Gastrointestinal tract symptoms (%)	8 (88.9)	14 (93.3)	0.756
Bladder dysfunction (%)	5 (55.6)	11 (73.3)	0.401
Sexual dysfunction[a] (%)	2 (40.0)	5 (71.4)	0.432
Anti-gAChRα3 Ab (A.I., LIPS)	2.057 ± 1.906	2.071 ± 0.968	0.404
Anti-gAChRβ4 Ab (A.I., LIPS)	0.653 ± 0.319	0.910 ± 0.473	0.165

[a]We reviewed the 26 male patients only
[b]Numbness, mental symptom, dementia, character change, and back pain
[c]Amenorrhea, eating disorder, SIADH (syndrome of inappropriate secretion of antidiuretic hormone), and panhypopituitarism
[d]Still disease, *PBC* primary biliary cirrhosis, Hashimoto's disease, *PMR* polymyalgia rheumatica, *SLE* systemic lupus erythematosus, SS Sjögren's syndrome, Graves' disease, *RA* rheumatoid arthritis, fibromyalgia, and other autoantibodies positive
[d]Ovarian tumor, pancreas cancer, mediastinal tumor, and paranasal cancer

with chronic AAG and severe autonomic dysfunction. These findings suggested that it is necessary to compile information about patients with AAG more precisely. The main findings of the clinical and autonomic characteristics were that patients with AAG were associated with a high rate of orthostatic hypotension for orthostatic intolerance and upper and lower gastrointestinal tract symptoms in both the seropositive and seronegative group. There was no significant difference in the occurrence of autonomic symptoms between the seropositive and seronegative group. It remains possible that unknown autoantibodies and other causative agents exist in the seronegative group. Clinically, it is difficult to distinguish the chronic form of seronegative AAG from other degenerative disorders of the autonomic nervous system (e.g., pure autonomic failure). Of note, three patients had achalasia in the seropositive group. The pathogenesis of achalasia remains unclear, but anti-gAChR antibodies might contribute to it. All of the anti-gAChR antibody-positive patients, except for patients 6, 16, and 19, presented pandysautonomia. Patient 6 had been diagnosed with a chronic intestinal pseudo-obstruction (CIPO) before

being tested with the autoantibodies. Our data were consistent with prior reports that showed that patients with limited autonomic symptoms demonstrated a relatively lower A.I. Sandroni et al. have previously reviewed other autonomic neuropathies that are associated with occasional positivity to gAChR antibodies. These include postural orthostatic tachycardia, CIPO, chronic idiopathic anhidrosis, and distal small fiber neuropathy [23]. All of these were listed as possible clinical phenotypes of anti-gAChR autoimmunity, although they show low antibody levels. The results of our study indicated a similar tendency and suggested a possible correlation between antibody levels and the phenotype of the dysautonomia. In the present study, however, we were not able to perform a correlational analysis between the A.I. levels and the clinical severity of the autonomic symptoms because we did not perform a quantitative assessment for autonomic dysfunction.

The patients with AAG in Japan were younger and more male predominant than those in Western countries [9]. In the present study, Japanese patients with AAG also had a lower age at onset. The seronegative and seropositive groups did not differ significantly in age and gender, although the seropositive group showed a female predominance. Of the 29 Japanese patients previously reported, 10 (34.5 %) had coughing episodes and 12 (41.4 %) had psychiatric symptoms. The present study found several anti-AChR antibody-positive patients with coughs (patients 12, 14, 15, and 17) and psychiatric symptoms (patients 8, 9, and 14), but these patients had a low frequency when compared with the findings of previous reports. Six AAG anti-gAChR antibody-positive patients (patients 1, 5, 11, 12, 14, and 15) complained of subjective numbness as an extra autonomic symptom, and the numbness might have been caused by extensive disturbance of the sympathetic and parasympathetic nervous system. However, this important symptom may distinguish AAG from other disorders such as acute autonomic sensory neuropathy (AASN), Guillain-Barré syndrome (GBS), and chronic inflammatory demyelinating polyneuropathy (CIDP). A nerve conduction study could be performed to assess this, and a nerve biopsy may be necessary in the patient with AAG.

Additionally, attention should be paid to the endocrine disorders that are complications of AAG, because there were five patients (patients 4, 9, 14, 15, and 19) with endocrine disorders in the study. Three of the five patients who were seropositive for the anti-gAChR antibodies presented with amenorrhea, SIADH, and panhypopituitarism. Several Japanese neurologists have already reported cases of acute pandysautonomia or acute autonomic and sensory neuropathy with amenorrhea and/or SIADH [24–26]. They have suggested that patients with autonomic neuropathy might have both peripheral and central nervous system manifestations.

nAChRs are associated with cholinergic neurotransmission, modulation of dopamine function, inflammation, and activity of the hypothalamic-pituitary-adrenal axis [27, 28]. We presumed that nAChRs are involved in a variety of neurological systems that are implicated in the pathophysiology of central nervous system involvement including endocrine disorders and psychiatric symptoms.

Ganglionic AChR antibodies have the potential to impair autonomic ganglionic synaptic transmission [1, 29, 30]. Because both sympathetic and parasympathetic ganglia utilize nicotinic cholinergic synapses, antibodies that interfere with

ganglionic transmission could cause pandysautonomia. Our clinical and serological observations suggest that pandysautonomia may be mediated by autoantibodies that interfere with autonomic ganglionic transmission. The patients who were identified in the present study had a failure of both the sympathetic (orthostatic hypotension and anhidrosis) and parasympathetic (gut dysmotility, sicca complex, and pupil abnormalities) functions. The autonomic function tests that were performed in this study were incomplete and inadequate, but most of the results (H/M ratio in ^{123}I-MIBG myocardial scintigraphy and pupillary response to local instillation) demonstrated postganglionic parasympathetic involvement. Kimpinski et al. characterized the unique sudomotor dysfunction in AAG as widespread, predominantly postganglionic, and a result of lesions at both the ganglia and distal axon [31]. Manganelli et al. have also demonstrated in a sudomotor function study and skin biopsy findings that there is postganglionic autonomic damage in patients with AAG [32]. They attributed this damage to prolonged and severe impairment of synaptic transmission. These reports coincide with our deduction that the anatomic pattern of autonomic dysfunction was predominantly postganglionic.

Another important observation was that the impairment of the autonomic function might be partially reversible in AAG. Patients 1 and 4 (the illustrative cases) improved in response to immunotherapy (plasmapheresis, intravenous methylprednisolone (IVMP), intravenous immunoglobulin (IVIg), and immunosuppressant drugs) with symptomatic therapy. We interpreted this improvement correlated with the decrease in the levels of anti-gAChR antibodies in each case. Some patients with seropositive AAG responded to treatment with IVMP, plasmapheresis, or IVIg, and most of these required combined or subsequent treatments to maintain the improvement [33–37]. The more severely affected patients who did not respond to IVMP or PP monotherapy benefited from combined therapy with other first-line therapy and immunosuppressant agents, such as prednisolone, azathioprine, and rituximab [38]. They also required prolonged immunotherapy for sustained clinical improvement. These results suggest that antibody production may be ongoing and not self-limited. Combined treatment with any of the immunosuppressant agents reduced antibody production to levels that were adequate for clinical benefit in our patients. In patient 4, menstruation restarted after a series of immunotherapy, suggesting an autoimmune mechanism in this process. Thus, immunotherapy can also be efficacious for the treatment of the endocrinological abnormalities of patients with AAG.

17.5 Future Endeavors

17.5.1 The Animal Model of Experimental AAG

An animal model of this disorder can be induced in rabbits by immunization with ganglionic AChR subunit proteins. Rabbits with experimental autoimmune

autonomic ganglionopathy (EAAG) manifest symptoms of autonomic failure similar to those seen in AAG patients and show a deficit in synaptic transmission in autonomic ganglia. Furthermore, autonomic deficits can also be transferred to mice by passive transfer of IgG from AAG patients via serum [7, 39]. We have identified a subunit-specific antibody for the gAChR, the anti-AChRβ4 antibody for the first time. Another subunit-specific antibody for gAChR, anti-AChRα3 antibody, causes several autonomic dysfunctions in an experimental AAG model [7, 39, 40]. However, it is unclear whether the anti-AChRβ4 antibody is involved in the pathogenesis of AAG. Additional experiments and investigations using the animal model are necessary to clarify the role of AChR β4 antibodies in the pathogenesis of AAG.

17.5.2 Antibodies to Other Subunits of Nicotinic AChR

Nicotinic acetylcholine receptors (nAChRs) are a family of ligand-gated cation channels found throughout the central and peripheral nervous system. Baker et al. previously described CNS involvement in AAG [41]. They found antibodies that specifically bind neuronal α5 and α7 nAChRs. The α7 nAChR is a homopentameric ligand-gated ion channel predominantly expressed in the cerebral cortex and hippocampus. Anti-sense knockdown of α7 nAChRs impairs learning and reduced expression of α7 nAChRs has been observed in the temporal cortex of Alzheimer's disease patients, suggesting that targeted autoimmunity against these receptors may impair neurocognitive function. Interestingly, the α4 nAChRs are more widely depleted in Alzheimer's brains than the α7 subtype. Gibbons et al. investigated the relationship between orthostatic hypotension, antibody titers, and cognitive impairment in patients with AAG [42]. In the current study, several patients with AAG demonstrated CNS involvement (memory disturbance, apathy, childish behavior, emotional instability, etc.) (Table 17.3). Watson et al. found antibodies that specifically blocked the function of α7 nAChRs in patients with Rasmussen encephalitis [43]. Therefore, it may be necessary to establish a LIPS assay for the detection of autoantibodies to these nAChRs.

It is also notable that six patients have suffered from numbness and sensory disturbance in Table 17.3. Sometimes it is difficult to differentiate AAG from other neuropathies including autonomic dysfunction (e.g., AASN, GBS). Koike et al. reported testing for anti-gAChR antibodies in six patients with AASN, all of whom were negative. They demonstrated significant differences in the frequency of an antecedent event, age, and progression in comparison between AASN and seropositive AAG [44]. We are performing the LIPS test for the anti-AChR antibodies in many cases of AASN, GBS, CIDP, and other neuropathies. In nociceptive neurons from the thoracic and lumbar dorsal root ganglia (DRG), other nAChR subunits were found. Therefore, we are planning to test autoantibodies for other subunits of the nAChRs in the CNS and the DRG. Diligent clinical observation is required to clarify the role of novel autoantibodies to nAChRs,

because the lack of overt symptoms in patients screened for autoantibodies does not disprove their possible pathogenicity.

17.5.3 Future Plans

Moreover, several research groups have reported that patients with other neuroimmunological disorders (myasthenia gravis, Lambert-Eaton myasthenic syndrome, GBS, and CIDP) have autoantibodies to gAChR and autonomic symptoms [45–47]. We need to verify the pathogenicity of these autoantibodies and the correlation between antibody levels and disease severity.

The seropositive group in our study had a significant overrepresentation of autoimmune diseases. Autonomic dysfunctions have been reported in association with Sjögren's syndrome [48, 49], systemic sclerosis [50], systemic lupus erythematosus [51], rheumatoid arthritis [52, 53], and mixed connective-tissue disease [54]. Although AAG and other autoimmune diseases can coexist due to the same background of autoimmunity, few reports have referred to anti-gAChR antibodies in these autoimmune diseases. In a previous report, AAG and Sjögren's syndrome were found to coexist in two patients [55]. The frequency of positive anti-gAChR antibody status in large populations or among patients with autonomic neuropathy in Sjögren's syndrome has not been determined. We believe it is important to clarify the clinical and immunological characteristics of the coexistence of AAG and the other autoimmune diseases.

A prospective, multicenter clinical observation is necessary to confirm the relationships between the antibody levels, symptoms, and results of the autonomic functions tests.

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Chapter 18
Novel Treatment

Katsuichi Miyamoto

Abstract Novel therapeutic drug for neuroimmune diseases has appeared in multiple sclerosis (MS) and neuromyelitis optica (NMO). Fingolimod is the first oral drug as disease-modifying drug (DMD) for MS. It has higher efficacy than IFNβ; however its side effects should be noted. Natalizumab is the first molecular target drug for MS. Although the effect of preventing the recurrence is high, since the serious side effects such as progressive multifocal leukoencephalopathy have been reported, it should be used by considering risk and benefits. Dimethyl fumarate is an oral drug that has been used as an anti-psoriatic agent in Germany. It has begun treatment for MS. Alemtuzumab is a molecular targeted drug against CD52; it has been applied to the treatment of chronic lymphocytic leukemia. Alemtuzumab is one of the drugs having the strongest effects as the DMD of MS; it has many side effects such as thyroid dysfunction. The new treatment attempts using molecular targeted agents have begun for NNO. Tocilizumab and eculizumab are both molecular targeted drugs and are used for other diseases. These agents have begun to apply for refractory NMO in some countries. Since there is no data for long-term use, it is necessary to pay attention to the adverse events.

Keywords Fingolimod • Natalizumab • Alemtuzumab • Dimethyl fumarate • Tocilizumab • Eculizumab

18.1 Multiple Sclerosis

In addition to IFNβ as the standard disease-modifying drug (DMD) for multiple sclerosis (MS), fingolimod and natalizumab have appeared. Furthermore new drugs such as alemtuzumab and dimethyl fumarate have been marketed (Table 18.1).

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Table 18.1 Novel treatment

Applied disease	Drug name	Action mechanism	Administration
MS	Fingolimod	Sphingosine 1-phosphate receptor modulator	0.5 mg orally once daily
MS	Natalizumab	mAb against the cell adhesion molecule α4-integrin	300 mg infused intravenously, every 4 weeks
MS	Dimethyl fumarate	Inhibit immune cells and molecules	Starting: 120 mg orally twice daily, for 7 days
			Maintenance: 240 mg orally twice daily
MS	Alemtuzumab	Anti-CD52 mAb	Intravenous infusion:
			1'st: 12 mg/day on 5 consecutive days
			12 months after
			2'nd: 12 mg/day on 3 consecutive days
NMO	Tocilizumab	Anti-IL6 receptor mAb	8 mg/kg infused intravenously, every 4 weeks
NMO	Eculizumab	Anti-C5 mAb	Intravenous infusion:
			Induction: 900 mg weekly, 4 times
			Maintenance: 1200 mg every 2 weeks

MS multiple sclerosis, *NMO* neuromyelitis optica, *mAb* monoclonal antibody

18.1.1 Fingolimod

Fingolimod (Gilenya®/Imusera®) is an oral medication used for treating relapsing-remitting MS (RRMS). It is used for reducing the frequency of relapses and for delaying the occurrence of physical disability in patients with RRMS. The US Food and Drug Administration (FDA) approved fingolimod in September 2010.

Fingolimod may work by reducing the number of circulating lymphocytes, leading to reduced migration of white blood cells into the central nervous system. Fingolimod is phosphorylated to form fingolimod phosphate, which resembles naturally occurring sphingosine 1-phosphate (S1P), an extracellular lipid mediator whose major effects are mediated by cognate G protein-coupled receptors. There are at least five S1P receptor subtypes. These receptors are expressed on a wide range of cells that are involved in many biological processes relevant to MS [1].

S1P1 plays a key role in the immune system, regulating lymphocyte egress from lymphoid tissues into the circulation. Fingolimod phosphate initially activates lymphocyte S1P1 via high affinity receptor binding yet subsequently induces S1P1 downregulation that prevents lymphocyte egress from lymphoid tissues, thereby reducing autoaggressive lymphocyte infiltration into the central nervous system (CNS). S1P receptors are also expressed by many CNS cell types and have

been shown to influence cell proliferation, morphology, and migration. Fingolimod crosses the blood–brain barrier and may therefore have direct CNS effects, distinguishing it from immunologically targeted MS therapies [2].

In large multinational trials in adult patients with RRMS, oral fingolimod 0.5 mg/day was more effective (annualized relapse rates, T2 lesion volume) than oral placebo (FREEDOMS) [3] and recommended dosages of intramuscular IFNβ-1a (TRANSFORMS) [4] in reducing the annualized relapse rate and was also generally more effective at slowing progression of neurological disability and at reducing the burden and activity of disease.

Fingolimod was generally well tolerated in these trials, with most adverse events being manageable and of mild to moderate severity; there were two deaths from opportunistic infections, albeit these occurred with fingolimod 1.25 mg/day (higher than the recommended dosage). Limited long-term data indicated that no new safety concerns had arisen after 5 years of fingolimod treatment [5].

Incidentally, phase III study (INFORMS) in primary progressive multiple sclerosis (PPMS) did not show a significant difference between fingolimod and placebo on a combination of disability measures [6].

The recommended dose is 0.5 mg orally once daily. Doses higher than 0.5 mg cause more adverse reactions without providing additional benefit [5]. The following adverse reactions have been reported: bradyarrhythmia and atrioventricular blocks, infections, macular edema, posterior reversible encephalopathy syndrome, respiratory effects, liver injury [7]. The first dose of fingolimod should be administered in a setting in which resources to appropriately manage symptomatic bradycardia are available. In order to assess patient response to the first dose of fingolimod, observe all patients for 6 h for signs and symptoms of bradycardia with hourly pulse and blood pressure measurement. Obtain in all patients an electrocardiogram prior to dosing and at the end of the observation period. Because patients may be more likely to get infections when taking fingolimod, some vaccines should be avoided during treatment with fingolimod and for 2 months after discontinuation. Serious brain infections by varicella zoster virus or progressive multifocal leukoencephalopathy (PML) have been reported [8]. If the number of lymphocytes decreased continuously, pause of medication should be considered.

Further clinical experience is required to fully determine the long-term safety profile of fingolimod, particularly with regard to any potentially serious or life-threatening adverse events.

18.1.2 Natalizumab

Natalizumab (TYSABRI®) is a humanized monoclonal antibody against the cell adhesion molecule α4-integrin. It is used in the treatment of MS and Crohn's disease. For RRMS, natalizumab can slow the worsening of symptoms and can decrease the number of relapses.

α4-Integrin is required for white blood cells to move into organs, and action mechanism of natalizumab is believed to be the prevention of immune cells from crossing blood vessel walls to reach affected organs [9]. The symptom-causing lesions of MS are believed to be caused when inflammatory cells such as lymphocytes pass through the blood–brain barrier through interaction with receptors on the endothelial cells. Natalizumab appears to reduce the transmission of immune cells into the CNS by interfering with the α4β1-integrin receptor molecules on the surfaces of cells. The effect appears to occur in endothelial cells expressing the VCAM-1 gene and in parenchymal cells expressing the osteopontin gene. In animals used to model MS and test therapies, repeated administration of natalizumab reduced migration of leukocytes into the brain's parenchyma and also reduced lesioning, though it is uncertain if this is clinically significant for humans [10].

Natalizumab is administered by intravenous infusion every 28 days. It has proven effective in treating the symptoms of both diseases, preventing relapse, vision loss, and cognitive decline and significantly improving quality of life in people with MS.

In phase III clinical trials, natalizumab safety and efficacy in RRMS (AFFIRM) and safety and efficacy of natalizumab in combination with IFNβ-1a in patients with RRMS (SENTINEL), natalizumab use significantly improved clinical and magnetic resonance imaging outcomes over 2 years in patients with relapsing MS [11]. Natalizumab therapy significantly improved the relapse rate and accumulation of brain lesions in patients with RRMS [12, 13]. Natalizumab was effective both as monotherapy and in combination with IFNβ-1a in patients with RRMS [14].

In 2004, the FDA approved natalizumab after years of testing. It was subsequently withdrawn from the market by its manufacturer after it was linked with three cases of PML when administered in combination with IFNβ-1a, another immunosuppressive drug often used in the treatment of MS.

After a review of safety information and no further deaths, the drug was returned to the US market in 2006 under a special prescription program. Twenty-four cases of PML had been reported since its reintroduction by October 2009, showing a sharp rise in the number of fatalities and prompting a review of the chemical for human use by the European Medicines Agency [15]. In the European Union, it has been approved for human use only for the treatment of MS and only then as a monotherapy.

Natalizumab increases the risk of PML that usually leads to death or severe disability, because there is no known treatment, prevention, or cure for PML. The risk of getting PML may be higher if a patient is also being treated with other medicines that can weaken immune system, including other treatments for MS. Even if a patient uses natalizumab alone to treat MS, he/she can still get PML. The risk of getting PML is higher if a patient has received natalizumab for a long time, especially longer than 2 years.

JC virus (JCV) is a common virus that is harmless in most people but can cause PML in people who have weakened immune system [16]. Most people who are

Table 18.2 Estimated US incidence of PML stratified by risk factor

Anti-JCV antibody negative	Natalizumab exposure	Anti-JCV antibody positive	
		No prior immunosuppressant use	Prior immunosuppressant use
0.1/1000	1–24 months	0.7/1000	1.8/1000
	25–48 months	5.3/1000	11.2/1000
	49–72 months	6.1/1000	Insufficient data

Data from Biogen idec at Mar. 2013

exposed to JCV in childhood do not know it or do not have any symptoms. The risk of getting PML is greatest if a patient have these three risk factors (Table 18.2).

Natalizumab may increase the risk of getting an infection and may cause liver damage and allergic reactions (e.g., hives, itching, trouble breathing, chest pain, dizziness, wheezing, chills, rash, nausea, flushing of skin, low blood pressure). Common side effects include headache, urinary tract infection, lung infection, pain in arms and legs, vaginitis, stomach pain, feeling tired, joint pain, depression, diarrhea, rash, and nausea.

18.1.3 Dimethyl Fumarate

Dimethyl fumarate (Tecfidera®/BG-12®) is an oral DMD for RRMS. Studies have shown that dimethyl fumarate reduces the number of relapses by around one half and slows down progression. Dimethyl fumarate is taken as a capsule, twice daily. A chemically related compound, called Fumaderm® (dimethyl fumarate and fumaric acid esters), has been used at higher doses for decades in Germany to treat psoriasis. Action mechanism of dimethyl fumarate is not fully understood, but it is thought to inhibit immune cells and molecules and may have antioxidant properties that could be protective against damage to the brain and spinal cord. Laboratory studies suggest that dimethyl fumarate reduces the inflammation caused when the immune system attacks myelin, resulting in less damage to myelin [17].

Two main studies have provided the evidence to support approval of dimethyl fumarate for MS. Both studies found dimethyl fumarate effective at reducing the number of relapses to approximately half of that seen in those taking placebo. In one study (DEFINE) the progression of disability was reduced for people taking dimethyl fumarate; this effect was not seen in the second study (COMFIRM).

In the DEFINE study, 2-year study compared dimethyl fumarate taken either two or three times daily and placebo in more than 1200 participants with RRMS. Compared to placebo, the drug reduced the number of relapses in 1 year by 53 % for the twice daily dosing and 48 % for the three times a day dosing. Dimethyl fumarate twice daily reduced the risk of disability progression by 38 %, while dimethyl fumarate three times per day reduced this risk by 34 % [18].

CONFIRM study, 2-year study with 1232 participants, was similar to DEFINE, but with an additional group who took glatiramer acetate for comparison. Dimethyl fumarate reduced the number of relapses in 1 year by 44 % for the twice daily dose and by 51 % for the three times daily dose, compared to placebo. In contrast, glatiramer acetate reduced the number of relapses by 29 % compared to placebo [19]. The reduction in disability progression observed in the DEFINE study was not seen in the CONFIRM study.

The most common side effects include flushing and feeling hot, gastrointestinal upset, diarrhea, nausea, abdominal pain, and headache. Seriously adverse reactions have been reported such as anaphylaxis and angioedema, PML, and lymphopenia. Long-term observation is necessary in order to confirm whether new side effects appear.

18.1.4 Alemtuzumab

Alemtuzumab, an anti-CD52 monoclonal antibody, was licensed in 2001 for the treatment of chronic lymphocytic leukemia (CLL) [20]. Alemtuzumab is seen as having potential in other therapeutic areas, including MS. Then it is used in the treatment of MS, for which phase III clinical trials started in 2007. Hopeful results were reported in 2011 from phase III trial against IFNβ-1a. A combination trial with glatiramer acetate is being considered and is expected to work synergistically [21, 22]. On November 2014, alemtuzumab was finally approved by the FDA. The European Commission approved alemtuzumab for the treatment of RRMS patients in September 2013. Alemtuzumab is now used in the treatment of CLL, cutaneous T-cell lymphoma, and T-cell lymphoma under the trade names Campath®, MabCampath®, and Campath-1H® and in the treatment of MS as Lemtrada®.

CD52 presents on the surface of mature lymphocytes, but not on the stem cells from which these lymphocytes are derived. After treatment with alemtuzumab, these CD52-bearing lymphocytes are targeted for destruction.

In the CAMMS223 phase II trial, involving 334 patients, the proportion of clinically disease-free patients was significantly higher in alemtuzumab-treated patients than in the IFNβ-1a (Rebif®) group over 3 years: 86 vs 63 % at year 1, 81 vs 48 % at year 2, and 71 vs 39 % at year 3 ($p < 0.0001$). Clinically disease-free was defined as the absence of both relapses and sustained accumulation of disability during the assessment period [23].

The 4-year follow-up data of phase II trials showed a significant 72 % reduction in relapse rate. The data showed sustained accumulation of disability by 73 % 3 years after the patients received their last dose of alemtuzumab. Five-year follow-up data from the phase II clinical trials showed that alemtuzumab outperformed IFNβ-1a in many respects. The majority (65 %) of MS patients who were administered alemtuzumab continued to be free of clinically active disease. Just 27 % of the patients administered IFNβ-1a was found to be free of clinically active disease. The study also showed that 72 % of alemtuzumab-treated patients

were relapse-free, compared to 41 % in the IFNβ-1a administered patient group. About 87 % of the patients administered alemtuzumab were free of sustained accumulation of disability, compared to 62 % for the IFNβ-1a administered patient group [24].

Thyroid dysfunction was more common with alemtuzumab than with IFNβ-1a. During a median follow-up of 57.3 months, 34 % of alemtuzumab and 6.5 % of IFNβ-1a patients had thyroid dysfunction ($P < 0.001$). With alemtuzumab, Graves' hyperthyroidism occurred in 22 %, hypothyroidism in 7 %, and subacute thyroiditis in 4 % [25].

18.2 Neuromyelitis Optica

Neuromyelitis optica (NMO) is a relatively rare autoimmune disease that predominantly affects the spinal cord and optic nerve. Anti-aquaporin-4 antibody (AQP4-Ab), which is a disease marker of NMO, has an important role in causing the destruction of astrocytes that express AQP4. Although prednisolone is the first choice for prevention of the recurrence, if the effect is insufficient, combination therapy with immunosuppressive drugs is done [26]. Still for intractable cases, molecular target drugs such as tocilizumab and eculizumab have been attempted.

18.2.1 Tocilizumab

Tocilizumab (Actemra®/RoActemra®) is a humanized anti-interleukin-6 receptor (IL-6R) monoclonal antibody and has been approved in more than 100 countries for use in the treatment of rheumatoid arthritis. Recently plasmablasts are a subpopulation of B cells, increased in the peripheral blood of patients with NMO, and are a major source of anti-AQP4-Ab among peripheral blood B cells [27]. Exogenous IL-6 promotes the survival of plasmablasts and their production of anti-AQP4-Ab in vitro. Given the increased levels of IL-6 in the serum and CSF during relapses of NMO, blocking IL-6R pathways reduce the disease activity of NMO by inactivating the effector functions of plasmablasts [28].

Seven patients with NMO were recruited on the basis of their limited responsiveness to their current treatment. They were given a monthly injection of tocilizumab (8 mg/kg) with their current therapy for a year. Six females and one male with NMO were enrolled. After a year of tocilizumab treatment, the annualized relapse rate decreased from 2.9 to 0.4 ($p = 0.005$). The expanded disability status scale (EDSS), neuropathic pain, and general fatigue also declined significantly. Pain management is a difficult problem in patients with NMO. The intractable pain reduced gradually after the patients started tocilizumab treatment. After 6 or 12 months of therapy, three of the six patients with pain were completely free of pain [29]. These results suggested a role of IL-6 in NMO pain and the possible

merits of the use of tocilizumab in clinical practice as a pain reliever. IL-6 trans-signaling via the soluble IL-6R could be pivotal in causing pain in NMO, although alternative possibilities cannot be excluded.

Reactions to tocilizumab infusions, including fever and chills, can occur, but these are rare. The most concerning potential side effect with regular therapy is the risk of infection. Tocilizumab has been associated with increased cholesterol levels, some liver enzymes, or a decrease in the white blood cells and/or platelets.

18.2.2 Eculizumab

Eculizumab (Soliris®) is a recombinant humanized monoclonal antibody that specifically binds to a C5 terminal complement, and it inhibits the cleavage reaction to C5a and C5b from C5 through complement activation [30]. Eculizumab has been already clinically applied for paroxysmal nocturnal hemoglobinuria. For neurological disorders, clinical study has been started on NMO and myasthenia gravis that the complement-mediated has been elucidated.

Two US centers recruited 14 NMO patients into an open-label clinical trial of eculizumab. After 12 months of eculizumab treatment, 12 patients were relapse-free; two had had possible attacks. The median number of attacks per year fell from three before treatment (range two to four) to zero (zero to one) during treatment ($p < 0.0001$). Median score on EDSS improved from 4.3 (range 1.0–8.0) before treatment to 3.5 (0–8.0) during treatment ($p = 0.0078$) [31].

One patient had meningococcal sepsis and sterile meningitis about 2 months after the first eculizumab infusion, but resumed treatment after full recovery. No other drug-related serious adverse events occurred. Eculizumab has been approved by the Orphan Drug Designation by the FDA in 2013. Infectious disease is the side effect of eculizumab most attention was focused on. Especially death caused by meningococcal infections has been reported in the USA. As long as the risk of delayed treatment does not exceed the risk of meningococcal infection onset, meningococcal vaccination is recommended before the treatment with eculizumab [32].

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