Abstract—Multiple sclerosis (MS) is a severe chronic inflammatory disorder of the central nervous system (CNS) manifested by chronic inflammation, myelin loss, gliosis, varying degrees of axonal and oligodendrocyte pathology and progressive neurological dysfunction and large infiltration of a heterogeneous population of cellular and mediators of the immune system. The inflammatory lesions are manifestation of a heterogeneous population of cellular and soluble mediators of the immune system, such as T cells, B cells, macrophages and microglia, as well as a broad range of cytokines, chemokines, antibodies, complement and other toxic substances. Identification of autoantigens and autoantibodies in demyelinating diseases is essential for understanding the immunopathogenesis mechanism of MS. Recently, CSF analysis in MS has been recommended as a tool for monitoring disease activity and diagnosis. In this review we discuss the most significant autoantibodies which could be used as biomarkers for diagnosis, prognosis and treatment in MS and the hope is that biomarkers speed up the MS monitoring.

Key words—Multiple sclerosis, autoantibodies, diagnosis, biomarker

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS) manifested morphologically by inflammation, demyelination, axonal loss and gliosis. The inflammatory lesions are characterized by high infiltration of various populations of cellular and soluble mediators of the immune system, such as T cells, B cells, macrophages and microglia, as well as a broad range of cytokines, chemokines, antibodies, complement and other toxic substances.[1-3]

The cellular components involved in the neuroinflammation and neuroimmune activation in the cerebrospinal fluid (CSF) are brain microglial cells, ependymal cells, macrophages, astrocytes and mast cells. Microglial cells which constitute around 10% of the CNS are the first to respond to neuronal injury.[1,4,5] MS is considered as an autoimmune disease in which T cell reactivity to self-antigens expressed in the brain, particularly myelin antigens plays a pivotal role in MS pathogenesis. [6] In this connection, the essential contribution of B cells and autoantibodies have been demonstrated in the pathogenesis of MS, leading to interest in the use of such autoantibodies as diagnostic or prognostic biomarkers.[7] Therefore, autoantibodies profile against epitopes derived from MS brain tissue could serve as diagnostic markers and/or as basis for the identification of a subgroup of MS.[8] Autoantibodies (IgG and IgM) localized against demyelinated axons and oligodendrocytes and also antibody-antigen immune complexes were detected in foamy macrophages in active lesion areas.[9] These observations provide further evidence on the role of antibodies, complement and macrophages in plaque development, which strongly suggest that they can induce axonal injury, an important cause of disability in MS. They may provide novel therapeutic strategies to limit tissue degeneration in the disease.[10]

Identification of autoantigens in demyelinating diseases is essential for understanding the MS pathogenesis, so that immune responses against these antigens could be considered as biomarkers for diagnosis, prognosis and treatment responses. Knowledge of antigen-specific immune responses in individual patients is also a prerequisite for antigen-based therapies.[9] In the late of nineteenth century, Heinrich Irenäüs Quincke and Heinrich Georg Queckenstedt developed the methods of sampling and studying cerebrospinal fluid (CSF) .[11]CSF sampling was initially developed for therapeutic purposes in the treatment of hydrocephalus, but shortly became a very useful diagnostic test for infections and other disorders.[12]

In addition to establishing a diagnosis for MS, cerebrospinal fluid (CSF) has historically been a very attractive tissue source for neuroimmunologists. Oligoclonal bands (OCBs) are immunoglobulins (IgG, IgM, or IgA) that are generated by plasmablasts and plasma cells in the CSF or CNS compartment. In chronic infections of the CNS, the OCBs are directed against the causative agent. Therefore, identifying the molecular target of OCBs in patients with MS may provide clues to the
pathogenesis of this disorder. However, it is also conceivable that OCBs are a determinant of the disease that may not reflect the prognosis or therapeutic effects.[12] The diagnosis of MS is ultimately a clinical decision that does not require laboratory tests if neurologic dysfunction in time and space can be established. However, CSF analysis is extremely helpful in patients with an atypical clinical presentation, age of onset, or magnetic resonance imaging (MRI) findings.[12,13] It is also of paramount importance to exclude some infectious and inflammatory mimics. [12,14] For this purpose, CSF studies are undertaking routinely in some European countries.[12]

Diagnostic CSF findings in MS patients include qualitative IgG oligoclonal banding and the quantitative IgG index.[12,15] Two or more OCBs detected by separation of CSF proteins which are not demonstrable in corresponding serum but reflect a local B cell response and define the positive IgG OCBs in MS.[12,16] Despite its high sensitivity, IgG OCBs are not specific for MS. OCBs can be seen in various inflammatory and non-inflammatory disorders.

IMMUNOPATHOGENESIS OF MS

MS, as an inflammatory demyelinating disease of the CNS is believed to have an immunopathological etiology arising from gene-environment interactions. The initiation factors of the inflammatory response remain yet unknown. However, MS is considered as a complex disease depending on genetic as well as environmental factors.[1,17] Genetic factors have a significant role in susceptibility to MS, such as genes related to interleukin-1 receptor (IL-1R), immunoglobulin Fc receptor, Apo protein E, IL-1β, immunoglobulin heavy chain, T cell receptor, tumor necrosis factor (TNF)-α, MBP, IL-2R, IL-7R and Human leukocyte antigen (HLA) genes.[18-23]

In general, MS begins with the formation of acute inflammatory lesions characterized by disruption of the blood brain barrier (BBB). Breakdown of the BBB usually lasts for about a month and then resolves, leaving a site of damage that can be investigated by conventional magnetic resonance imaging (MRI). The pathological features of MS plaques are BBB leakage, destruction of myelin sheaths, oligodendrocyte damage and cell death, axonal damage and axonal loss, glial scar formation and the presence of inflammatory infiltrates that mainly consist of lymphocytes and macrophages.[22,24,25] Despite the major advances in the current understanding of pathogenesis of MS, exact details of the inflammatory cascade of MS remain unknown. It has been demonstrated that axonal degeneration is the major feature of irreversible neurological disability in MS patients. Axonal injury initiates the disease onset and correlates with the degree of inflammation within lesions.[22,26,27] Four different patterns of pathology with resulting demyelination have been identified in MS lesions: Type I is T cell mediated where demyelination is macrophage mediated, directly or by macrophage toxins. In type II lesions, both T cells and antibodies are involved and are the most common pathology observed in MS lesions. In this pattern, demyelination occurs through specific antibodies and complement. Type III is related to distal oligodendropathy, degenerative changes which occur in distal followed by apoptosis. Type IV results in primary oligodendrocyte damage followed by secondary demyelination. The latter pattern is seen only in a small subset of primary progressive MS patients.[22,28,29]

It is known that antibodies against myelin basic protein (MBP) are present in early multiple sclerosis.[30,31] Another potential target antigen for autoreactive antibodies might be myelin oligodendrocyte glycoprotein (MOG), which is specific to the central nervous system and is located exclusively on the surface of myelin sheaths and oligodendrocytes.[32] Antibodies against MOG cause demyelination in vitro[33] and in animal models of multiple sclerosis[34,35] and have been found in active lesions in patients with multiple sclerosis.[36] A substantial subgroup of patients with multiple sclerosis mount a persistent autoantibody response to the extracellular immunoglobulin domain of MOG30 with a predominance of anti-MOG IgM antibodies.[30,31] In this review, we studied whether the presence of serum anti-MOG and anti-MBP antibodies predicts conversion to clinically defined multiple sclerosis among patients who have a clinically isolated syndrome and findings on MRI and cerebrospinal fluid analysis that are suggestive of multiple sclerosis.[37]

AUTOANTIBODIES AND DIAGNOSIS

In this connection, the most important antigens are myelin oligodendrocyte glycoprotein (MOG) and aquaporin4 (AQP4). Although T cell responses to the quantitatively major myelin proteins, myelin basic protein (MBP) and proteolipid protein (PLP), are likely to be of importance in the course of MS. The cell-mediated autoimmune responses to other myelin antigens, in particular quantitatively minor myelin antigens, such as myelin-associated glycoprotein (MAG) and the central nervous system-specific myelin oligodendrocyte glycoprotein (MOG), could also play a prevalent role in initiation or progression of the disease. The incidence of responses to MBP, PLP, and MAG did not differ greatly between MS patients and control individuals. A predominant T cell reactivity to MOG in MS suggests an important role for cell-mediated immune response to this antigen in the pathogenesis of MS.[38]

ANTI-MOG ANTIBODY

Antibody against MOG is seen in patients with different diseases, such as childhood multiple sclerosis, acute demyelinating encephalomyelitis (ADEM), anti-AQP4 negative NMO, and optic neuritis, but hardly in adult MS.[9] It has been recently reported that the only antibodies recognizing conformation-dependent epitopes of MOG have a demyelinating potential in the animal model of multiple sclerosis, EAE.[39] This fact that, whether antibody to MOG can be a diagnostic marker for MS is still controversial. Recent studies reported that serum specific anti-MOG epitope antibody might be an MS specific marker. Although serum anti-MOG epitope IgG could
not differentiate MS from neuromyelitis optica (NMO), it may be a useful marker for monitoring disease activity.[40] NMO-Ab and MOG-Ab could be potential biomarkers to determine visual prognosis in patients with optic neuritis (ON).[41] Anti-MOG antibodies, measured either by ELISA or FACS were especially identified in children with demyelination. In acute disseminated encephalomyelitis (ADEM) these antibodies were highly reactive. The presence of autoantibodies was not related with EBV serostatus, age, gender or disease course. This study independently has confirmed the recent published results of seroprevalence and specificity of the assay. Due to their low sensitivity, anti-MOG antibodies can not serve as disease-specific biomarkers, but they are able to support the diagnosis of ADEM in difficult cases.[42] The high-titer of MOG-IgG antibodies are strongly detected in pediatric patients with ON, indicating that anti-MOG-specific antibodies may have a direct role in the pathogenesis of ON in this subgroup.[43]

ANTI-AQP4

Aquaporin-4 is the first specific molecule which has been defined as a target for the autoimmune response in any form of MS. It is also the first example of a water channel being the target of any autoimmune disorder.[44,45] In the past, neuromyelitis optica (NMO) was included only in the optic nerves and spinal cord. However, the discovery of highly specific anti-aquaporin-4 (AQP4) antibody for NMO enabled us to identify more diverse clinical manifestations.[46]

The pathogenesis mechanism of optic neuritis developed rapidly from the end of the 19th century to the beginning of the 20th century accompanying with progress in morpho-anatomy and physiology.[47] The process of optic neuritis is generally considered as a new phase, started by the advent of molecular immunology and genetic. Researches showed that optic neuritis associated with anti-aquaporin(AQP) 4 antibodies is refractory to steroid therapy and causes injuries to the optic nerve-optic chiasma-optic tract, resulting in a broad array of visual field abnormalities. Specifically, the disease becomes severe in individuals who possess anti-AQP4 antibodies that target astrocytes, together with anti-myelin oligodendrocyte glycoprotein (MOG), the antibodies that target myelin oligodendrocytes. Furthermore, measurement of glial fibrillary acidic protein (GFAP) levels in cerebrospinal fluid may be useful in the diagnosis of anti-AQP4 antibody positive optic neuritis.[47] Although, the anti-AQP4 antibody is specific for NMO, but is also found in limited forms. The presence of this antibody in acute transverse myelitis (ATM) has been associated with recurrence and conversion to NMO, but its influence on disability has not yet been described.[48] According to previous study, and compared with anti-AQP4 antibody-negative ones, anti-AQP4 antibody-positive patients show significantly higher frequencies of severe ON, TM, longitudinal spinal-cord segments and they are more predisposed to ON or TM relapse. Also, seropositive NMO and HR-NMO patients are more likely to develop relapses of ON or TM. Anti-AQP4 antibody may play some roles in the diagnosis and prognostic predication of demyelinating diseases in central nervous system.[49].

ANTI-MBP ANTIBODY

This autoantibody is specifically bound to disintegrating myelin around axons in the lesions of acute MS and EAE model.[50] In other words, a specific myelin protein autoantibody intervenes target membrane damage in demyelinating disease of central nervous system.[44] Furthermore, Monosymptomatic unilateral optic neuritis (ON) is a common first manifestation of multiple sclerosis (MS) in which increased numbers of autoimmune T and B cells, indicating different myelin autoantigens including myelin basic protein (MBP) and its peptides, have been implicated in a hypothetical immunopathogenesis. Using an immunospot assay to detect specific antibodies secreted by individual cells, it was analyzed the B-cell repertoire to MBP and its amino acid residues 1-20, 63-88, 110-128, and 148-165 in blood and CSF from patients with ON and MS, and from controls. The autonomy of the autoimmune B-cell response in CSF was further supported by the pronounced asynchrony of the repertoire to the four MBP peptides in CSF compared with blood in individual patients. The large number of MBP- and MBP peptide-reactive B cells in CSF in early MS, as manifested by ON, could play a major role in the immunopathogenesis and perpetuation of MS. Alternatively, they could represent myelin breakdown or restoration.[51]

In recent studies, the prevalence and titer of total, free, and bound cerebrospinal fluid anti-myelin basic protein antibodies as well as free/bound ratios were determined in four groups of patients with multiple sclerosis and three groups of controls. All patients with clinically active MS have increased the levels of total anti-MBP, which may be present in either free or bound forms. Patients whose disease are in remission have undetectable anti-MBP levels, and some patients with clinically stable disease with residual disability may have detectable antibody titers. Chronically progressive MS is usually associated with high levels of antibody in the bound rather than the free form, resulting in a low or normal free/bound ratio. In contrast, exacerbation of MS is characterized by relatively high levels of free anti-MBP in the cerebrospinal fluid, resulting in a high free/bound antibody ratio. The bound anti-MBP was also detected in elevated levels in 1 patient with subacute sclerosing panencephalitis and 2 of 8 patients with postinfectious encephalomyelitis. Although the elevated levels of cerebrospinal fluid anti-MBP are not specific for MS, they are strongly associated with disease activity and may be involved in the pathogenesis of demyelination in patients with MS.[52]

ANTI-PLP ANTIBODY

PLP is a component of oligodendritic glial sheaths of neuronal processes that is specifically expressed in the CNS.[53] In the pathogenesis of multiple sclerosis, autoimmune T cells reactive with proteolipid protein may play a crucial role, that is linked with HLA-DR2, w15.[54,55] In other word, equal numbers of CD4+ T cells recognizing myelin basic protein (MBP) and
proteolipid protein are found in the circulation of normal individuals and multiple sclerosis patients. [56]

THE COMPARISON BETWEEN ANTI-PLP AND ANTI-MBP IN MS

The human myelin basic protein (hMBP) and proteolipid protein (PLP) were utilized as antigens in a solid-phase radioimmunoassay to determine relative frequencies of anti-MBP and anti-PLP in CSF of optic neuritis and multiple sclerosis patients. [57] These results recommend that there are at least two immunologically distinct forms of MS, a common form which strongly associated with anti-MBP and more frequent standing out inflammatory characteristics in CSF and CNS, and an infrequent form related to anti-PLP in CSF and tissue, and less plentiful inflammation. Antigen-specific affinity chromatography was used to purify Anti-MBP from CNS tissue IgG which could react with synthetic peptides of hMBP. The anti-MBP epitope on the hMBP molecule was restricted between residues 75 and 106. The PLP epitope for anti-PLP has not yet been determined. These observations have theoretical implications for expected next specific immunotherapy of MS. [57]

On the other hand, the solid phase radioimmunoassays was utilized to purify Myelin basic protein and proteolipid protein (PLP) from non-MS human brain for detecting their specific antibodies in cerebrospinal fluid of optic neuritis and clinically definite multiple sclerosis patients. Considering the total of 370 clinically definite MS patients with active and inactive disease, 77% had increased CSF anti-MBP and 1% had increased CSF anti-PLP. [58] To study the intrathecal synthesis of anti-myelin basic protein and anti-proteolipid protein antibodies in patients in the early stages of multiple sclerosis, the cerebrospinal fluid cells that secrete anti-MBP or anti-PLP antibodies were detected in 10 of 15 and in 21 of 23 patients with acute ON, while they were detected in nine of 18 and in six of 18 patients with other neurological diseases, respectively. Patients with ON had significantly more anti-PLP-secreting cells compared with patients with other neurological diseases (P < .01). No difference was observed for anti-MBP-secreting cells. A significant correlation between the time of onset and the number of anti-PLP-secreting cells was found in patients with idiopathic ON (P < .02). These data suggest that anti-PLP antibodies are more specific finding in demyelinating disease than anti-MBP antibodies. Furthermore, they suggest that anti-PLP antibodies may arise as a consequence of the demyelinating process. [59]

In addition, Anti-PLP IgG antibody-secreting cells were detected in blood from most MS patients, but such cells were 49-fold more frequent in CSF (mean 1 per 625 cells). PLP-reactive T and B cells were also identified in blood and CSF from control patients, but in lower number. A strong and persistent autoimmune response to PLP as well as to other myelin proteins, enriched in CSF could be pathogenetically important in MS. [60] In general, the methods of, ELISA and Western blotting are not able to detect reactivity against epitopes showed by native antigens expressed on myelin sheaths. It has been described as a cell-based assay that particularly detects serum antibodies identified against three major myelin autoantigens: MBP, PLP and MOG. The mentioned method detects antibody binding to recombinant antigens in their native conformation on MBP, PLP and MOG transfected mammalian (hamster ovary) cells. By using FACS analysis serum anti-MBP, anti-PLP and anti-MOG IgG autoantibodies were identified in MS patients and controls. Compared with healthy controls the titres of IgG autoantibodies directed against membrane-bound recombinant myelin antigens were highly raised for PLP, no quite significant for MBP and not significant for MOG. The titres of anti-MBP antibodies were low in contrast to high titre of anti-MOG antibodies in both groups suggesting a nonspecific binding. The cell-based assay detection of autoantibodies directed against recombinant myelin antigens could be a useful tool for preparing the serological markers in diagnosis and progression of MS. Indeed, it permits obtaining molecular characteristics of disease in each patient in term of an antibody response against certain myelin and non-myelin antigens. It has been shown that in RRMS patients the increased level of serum antibodies against PLP is important, so that it might be considered in search for specific immunomodulatory therapy in MS. [61] In addition, intrathecal immunoglobulin synthesis and activation of the complement cascade occurs in patients with MS. Some researches aimed the status of the relation between intrathecal immunoglobulin synthesis and complement activation by comparing total intrathecal synthesis of IgA, IgG, and IgM, the number of cells secreting anti-MBP and anti-PLP antibodies of the IgG isotype and intrathecal activation of the complement cascade in patients with possible onset symptoms of MS or clinically defined MS. Early activation of the complement cascade was correlated with intrathecal synthesis of IgM. Intrathecal IgG, IgA and IgM synthesis were also correlated weakly with the presence of cells secreting anti-MBP or anti-PLP autoantibodies. Full activation of the complement cascade had no correlation with any amount of intrathecal antibody synthesis. These findings recommend a complicated relation between different immunoglobulin isotypes and complement activation which may have similarly complex roles in the pathogenesis of MS. [62]

ANTI-HHV-6 IN MS

The viral infections are thought to play a significant role in the pathogenesis of multiple sclerosis (MS) potentially through molecular mimicry. [63] Some of the strongest associations with MS onset have been reported for human herpesviruses, particularly Epstein-Barr virus (EBV) and human herpesvirus 6 (HHV-6), so that the observed effect was directly related to anti-HHV6 IgG titers which may indicate that either HHV-6 infection or factors associated with an altered humoral immune response to HHV-6 may have an impact on MS clinical course. Therefore, anti-HHV-6 IgG titer, as an important non-autoantibody may be a useful prognostic factor in relapsing-remitting MS clinical course. [64] In a research has been shown the intrathecal production of antibody to lymphotropic herpesviruses in MS patients and the presence of human herpesvirus 1 to 7 DNAs in cerebrospinal fluid (CSF),
whereas the study did not give additional support to the possibility that HHV-6 is a common cause of MS, but the role of virus in a subset of patients cannot be excluded.[65]

It should be noted that an identical sequence was found in both myelin basic protein (MBP, residues 96–102), a candidate autoantigen for MS, and human herpesvirus-6 (HHV-6 U24, residues 4–10) that is a suspected viral agent associated with MS.[63]

**CONCLUSION**

Human myelin basic protein (hMBP) and proteolipid protein (PLP) were proposed as antigens to determine relative frequencies of anti-MBP and anti-PLP in the CSF of optic neuritis and MS patients. In addition, some studies showed that optic neuritis is associated with anti-aquaporin(AQP) 4 antibodies together with anti-myelin oligodendrocyte glycoprotein (MOG) antibodies that target myelin oligodendrocytes. The cell-based assay detection of autoantibodies directed against recombinant myelin antigens could be a useful tool for preparing the serological markers in diagnosis and progression of MS.

The correlation of B cells and autoantibodies in CSF examination has been demonstrated in MS leading to interest in the use of such autoantibodies as diagnostic or prognostic markers and as a basis for immunomodulatory therapy. Although their main role is to exclude differential diagnosis, however, the new methods are using in this field and it is expected that these biomarkers, lonely or with other alternative disease markers along with MRI, is able to help the clinician to predict disease progression in patients with various phenotypes of MS.

**References**


