DOI 10.1007/s11055-017-0431-1
*Neuroscience and Behavioral Physiology, Vol. 47, No. 5, June, 2017*

# Current Concepts of the Intrathecal Humoral Immune Response and the Diagnostic Significance of the Detection of Oligoclonal Immunoglobulins in Multiple Sclerosis

**G. S. Makshakov,[1] S. V. Lapin,[3] and E. P. Evdoshenko[1,2]**

*Translated from Zhurnal Nevrologii i Psikhiatrii imeni S. S. Korsakova, Vol. 116, No. 2, Iss. 2, Multiple Sclerosis, pp. 14–20, February, 2016.*

Multiple sclerosis (MS) is a presumptively autoimmune demyelinating diseases of the central nervous system. We present here a literature review including a list of potential antigens inducing the autoimmune response, these including myelin and nonmyelin structures. None of the antibodies to any of these antigens has sufficient specificity and sensitivity for use in routine laboratory practice. Oligoclonal immunoglobulins identified by isoelectric focusing (IEF) are currently the main immunological markers for MS. The sensitivity and specificity of the IEF method are 90% and 85%, respectively. The present article also discusses additional markers such as oligoclonal IgM and the MRZ reaction.

**Keywords:** multiple sclerosis, oligoclonal immunoglobulins, isoelectric focusing (IFE), MRZ reaction, oligoclonal immunoglobulin M.

Multiple sclerosis (MS) is a multifactorial diseases whose development depends on genetic predisposition and the actions of environmental factors [1, 2]. Autoimmune processes play a major role in the pathogenesis of this disease, producing inflammation, demyelination, degradation of axons, and restoration of damaged myelin sheaths. The processes make different contributions in each individual patient with MS, with the result that there is no single biomarker for this disease. At the same time, the main method for the laboratory diagnosis of MS, which has a good evidence base, consists of detection of oligoclonal immunoglobulins (OCI) in patients' cerebrospinal fluid (CSF) [3, 4]. Detection of OCI in the CSF when it is absent from the serum provides a highly sensitive and relatively specific method allowing differential diagnosis of MS. In most cases, detection of OCI, along with clinical and MRI data, establishes the diagnosis of MS.

**Mechanisms of Formation of OCI**

The pathogenesis of CNS damage in MS consist of a complex interaction between various cell types involved in the immune response. Although the main antigen provoking at autoimmune reaction is currently unknown, there are several theories explaining the mechanisms underlying OCI production.

Four possible pathways to intrathecal OCI production have been described [5]: 1) Ig-secreting cells undergoing differentiation in the lymphoid organs arrive in the CNS and accumulate in inflammatory foci independently of their antigen specificity; 2) in proinflammatory conditions, memory B-cells in the CNS differentiate into OCI-producing plasma cells; 3) memory cells can differentiate into long-lived plasma cells via activation of the immune response to one epitope during the process of activation of an immune response to a different epitope (bystander activation); 4) OCI-producing plasma cells may form in "tertiary" ectopic lymphoid fol-

[1] City Clinical Hospital No. 31, St. Petersburg City Center for Multiple Sclerosis and Other Autoimmune Diseases, St. Petersburg, Russia; e-mail: gleb.makshakov@gmail.com.
[2] Clinical Department of Neurology and Neurosurgery, Pavlov First St. Petersburg State Medical University, St. Petersburg, Russia.
[3] Laboratory for the Diagnosis of Autoimmune Diseases, Scientific Methodological Center for Molecular Medicine, Ministry of Health of the Russian Federation, Pavlov First St. Petersburg State Medical University, St. Petersburg, Russia.

licles, which are generally found along blood vessels in the meninges [5]. These lymphoid follicles have only been seen in patients with secondary progressive MS [6].

Most OCI are class G immunoglobulins (IgG) subclass IgG1, though they can also be IgM or IgA [7]. Although these immunoglobulins are called "oligoclonal," to emphasize their benign nature, these antibodies are in fact monoclonal paraproteins and each clone has unique properties.

The antigenic stimuli initiating and maintaining autoimmune inflammation have thus far been controversial. OCI in each patient have been shown to react with multiple antigens. Because of their location in the compactly packed myelin and on the outer surfaces of myelin sheaths, myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), and myelin-associated glycoprotein (MAG) may be potential targets of OCI [1, 8, 9]. At the same time, the diagnostic and prognostic significance of anti-MBP and anti-MOG antibodies is highly controversial. Autoantibodies seen in MS patients have been found not to have high affinity for myelin basic protein [10]. Anti-MOG antibodies have been shown to recognize only conformational epitopes of MOG and not its linear sequence, which increases the probability that autoantibodies will cross-react [11, 12]. At the same time, anti-MBP antibody levels in MS patients were significantly greater than in a control group with noninflammatory CNS diseases [13]. Patients with clinically isolated syndrome (CIS) with IgG reacting with MBP and MOG were found to experience secondary exacerbations earlier than patients with CIS but without anti-MBP and anti-MOG antibodies. The relative risk of transformation into full MS in patients with anti-MBP and anti MOG antibodies was greater than that in patients lacking these antibodies [14]. Other studies have yielded data not supporting a link between anti-MOG and anti-MBP antibodies on the one hand and an increased risk of transformation into full MS on the other [15]. The list of potential antigens for autoantibodies in MS expands continually. Reactivity to both myelin and nonmyelin antigens has been demonstrated [16]. For example, the list includes the small heat shock protein αβ-crystallin, the specific neuroglial protein transaldolase, neurofascin, which is a protein located in nodes of Ranvier, neurofilament light subunits, nuclear ribonucleoprotein A1 (hn-RNP A1), and other molecules [6, 16, 17].

Antibodies can be targeted against a number of infectious agents, including Epstein–Barr virus, herpes simples virus, varicella zoster virus, and *Chlamydia pneumoniae*. Recent studies have emphasized the important role of Epstein–Barr virus in inducing MS [2, 18–20]. Despite the fact that the detailed spectrum of oligoclonal antibody specificities has yet to be characterized, the antigens of various viruses may be the targets for oligoclonal antibodies. Immunoreactivity for Epstein–Barr virus antigens such as BRRF2 and nuclear antigen-1 (EBNA-1) is significantly greater in MS patients than members of the healthy population [1]. Investigations of blood collected from MS patients before disease onset confirmed a higher titer of anti-EBNA antibodies in patients who subsequently developed MS than in the group of patients remaining healthy [21–23].

Analysis of the results of epidemiological studies has also supported the important role of Epstein–Barr virus in the pathogenesis of MS. People experiencing infectious mononucleosis or acute Epstein–Barr virus infections have an increased risk of developing MS later in life [24, 25]. It has been suggested that molecular mimicry may trigger the autoimmune reaction. The likely mechanism of induction of the autoimmune reaction is cross-reactivity between Epstein–Barr virus antigens and MBP, MOG, and transaldolase epitopes [26–28]. Furthermore, latent Epstein–Barr virus infection persisting within B cells in the CNS may have local immunomodulatory effects, stimulating interferon-α production in MS plaques [29].

Other neurotropic target viruses for OCI include measles, rubella, and varicella zoster viruses. Although immunoglobulins produced against these viruses generally account for just 2% of the total IgG content in CSF and only a small fraction of OCI, synthesis of these is very characteristic of MS and is seen in 80–100% of patients with this disease [30–35]. Detection of antibodies to this triad of viruses is known as the MRZ reaction (measles, rubella, zoster). It should be emphasized that development of MRZ antibodies is not linked with intrathecal replication of the viruses themselves, as confirmed by PCR studies of patients' CSF [30]. It has been suggested that the production of these antibodies results from secondary activation of memory B cells specific for these viruses, such that the secondary immune response reflects the individual history of illnesses or immunizations. Furthermore, higher OCI titers against rubella were seen in the CSF of patients with MS from Germany than that of patients from Cuba and this reflects epidemiological data demonstrating a lower morbidity of rubella in Cuba [36]. Virus-specific plasma cells formed from activated memory B cells are located in tertiary follicles because of the effects of the anti-inflammatory environment, which contains important trace factors such as CXCL12, BAFF, interleukin-6, and TBF-α [5]. Prolonged persistence of plasma cells in the CNS results in prolonged production of MRZ antibodies. Data from a prospective study showed that antibody titers gradually increase over time [37], which is evidence for slowly progressing recruitment of new plasma cells to the process of autoimmune inflammation. In chronic infectious diseases of the CNS, the MRZ reaction may also be positive, suggesting a link between this phenomenon and chronic inflammation [38].

We have already noted that apart from the IgG fraction, OCI also contain IgM and IgA fractions. IgM has a special role in the pathogenesis of the disease. A number of studies have shown that oligoclonal IgM synthesized by a population of $CD5^+$ B lymphocytes were linked with a more aggressive course of illness [39]. In fact, IgM species have the greatest ability to activate the C3 component of the com-

plement system, which is one of the key participants in the inflammatory response and the formation of inflammatory plaques in MS. Studies of the immunoreactivity of oligoclonal IgM have shown that a number of IgM oligoclonal fractions display high reactivity to the lipid components of myelin: phospholipids and glycolipids, particularly phosphatidylcholine. Damage to myelinated fibers due to IgM may be mediated by complement-dependent demyelination, as well as by activation of myelin phagocytosis by activated macrophages and microglial cells via an interaction with Fc receptors and complement receptors [39]. Thus, IgM species have been shown to be not only an indicator of but also a participant in immune inflammation in the CNS in MS patients.

Thus, various antibodies can trigger and maintain the autoimmune reaction, resulting in intrathecal production of antibodies by plasma cell clones, this occurring independently of antibody production in the blood. Isolated antibody synthesis in the CNS has the result that serological methods cannot be used for diagnosis of MS. Thus far, the "gold standard" for the immunological diagnosis of MS is assay of OCI in the CSF.

**Methods for Detecting Oligoclonal Antibodies**

Attempts to study γ-globulins in CSF samples by electrophoresis were first reported in the 1940s and were crowned by the detection of elevated γ-immunoglobulin synthesis in patients with neurosyphilis [40]. A similar picture was subsequently seen in the CSF of MS patients. A the end of the 1950s, studies using radioactive iodine-labeled γ-globulin showed that immunoglobulins in the CSF of MS patients do not come from the serum [41]. This provided evidence that antibodies in MS can be synthesized within the CNS. Detection of OCI in MS was linked with the use of agarose gel electrophoresis, which provides clearer separation of immunoglobulins. Studies reported by Laterre [42] emphasized the importance of comparing serum and CSF electrophoresis results, which identified a characteristic pattern of immunoglobulin synthesis, and intrathecal synthesis was subsequently demonstrated in 86.9% of patients with full MS. Electrophoresis was later replaced by isoelectric focusing (IEF) followed by immunoblotting, as developed by Keir et al. [43]. This method is currently the "gold standard" for the laboratory diagnosis of MS and is recognized by most international experts [3].

In contrast to electrophoresis, IEF involves movement of immunoglobulin molecules in a gel in an electric field in accordance with their isoelectric points, which are determined by differences in the amino acid sequences of the constant and variable regions of immunoglobulins and different variants of spatial modifications, e.g. glycosylation and sialylation. Antibodies produced by different B cell clones show significant differences in their isoelectric points [44], such that they can be separated by IEF. The separated molecules are then stained with peroxidase-labeled antibodies, producing a characteristic pattern of bands in the gel. Assessment of the result includes analysis of the clonality of the antibodies in serum and CSF. This test is qualitative, i.e., identifies the characteristic type of synthesis, the number of bands not having great clinical significance. The presence of oligoclonal antibodies can be identified when two or more clearly visualized oligoclonal bands are present. Analysis of paired serum is important for identification of the type of synthesis. There are five classical types of synthesis [4]. Type 1 is characterized by polyclonal synthesis of antibodies in serum and CSF samples, oligoclonal bands not being detected. This type of synthesis is seen in normal subjects and patients with acute inflammatory processes. In synthesis type 2, oligoclonal bands are absent from serum but present in the CSF, confirming intrathecal immunoglobulin synthesis. This type of synthesis is highly specific for MS. In type 3 synthesis, oligoclonal bands are seen in the CSF and, in smaller quantities, in serum samples, which is also evidence for intrathecal synthesis. In type 4 synthesis, there are identical quantities of oligoclonal bands in the CSF and serum. This type of synthesis implies systemic rather than intrathecal antibody production, with penetration into the CSF from the blood across a normal or damaged blood-brain barrier (BBB). Type 5 is characterized by monoclonal antibody synthesis, which is seen in multiple myeloma, CNS lymphoma, and other paraproteinemias.

**Use of IEF in Medical Practice**

In most clinical studies of MS, the sensitivity of IEF for detection of OCI is greater than 90%, while levels of specificity reported by different authors are over 86% [4, 45]. Despite the fact that to date the role of OCI is not entirely clear, detection in the CSF has a high correlation with the presence of MS (70–95%) and in many cases helps to establish the correct diagnosis. However, oligoclonal bands are not seen in some MS patients. In addition, detection of type 2 OCI synthesis is not absolutely specific for MS. OCI in the CSF can be detected in 60% of cases of neuroborreliosis and neurosarcoidosis and 11–50% of patients with herpes, tuberculous, and HIV encephalitis, and also in rheumatic diseases (for example, systemic lupus erythematosus) and systemic forms of vasculitis (for example, Sjögren's syndrome). In establishing the diagnosis, the physician must therefore include data from the clinical picture and results from other investigations. This view is reflected in the criteria of Poser and McDonald (2001, 2005), where one of the supplementary methods is analysis of CSF for OCI [46]. A positive test for OCI softens the MRI criterion of spatial dissemination. According to the 2010 McDonald criteria, this analysis is recommended only for the diagnosis of the primary progressive form of MS [47].

Analysis of OCI by IEF is a widely used and well studied method for the laboratory diagnosis of MS, and it also has prognostic significance [48]. The role of oligoclonal antibodies as an indicator predicting the risk that CIS will develop into MS has been studied. Data from a prospective study of patients with CIS showed that when OCI are detected in the CSF, the risk of this progression increases

significantly [48]. An additional factor increasing this risk is detection of foci of demyelination on MRI scans, which is characteristic of MS; the risk is significantly greater when both factors are present than when only one is positive or when both are negative [49]. OCI detected by IEF constitute a prognostic factor for earlier disability: thus, the times at which EDSS scores of 4 and 6 are reached in OCI-positive patients are earlier than those in OCI-negative patients [50].

An interesting feature of OCI is that once detected by IEF, these antibodies do not disappear as the disease progresses, even when immunosuppressive therapy is used. Methods based on direct actions on B cells, such as treatment with rituximab (Mabthera), also have no effect. Controversial data have been reported on the conversion of patients from OCI-positive to OCI-negative after natalizumab (Tysabri) treatment [51], though this was refuted by data reported by other investigators [52]. Autologous stem cell transplantation (ASCT) also had no effect on intrathecal OCI synthesis [53]. Despite pre-transplantation chemotherapy, OCI synthesis beyond the BBB persists in the same way as on use of other biopreparations. This fact does not downplay the efficacy of ASCT treatment [54], though it does suggest that if islets of tertiary lymphoid tissue beyond the BBB persist, then the effect of ASCT may be incomplete. A potential method with direct influences on the cross-barrier OCI synthesis consists of intrathecal administration of biopreparations with effects on immune cells. These methods are currently under study [38].

The MRZ reaction, based on assay of titers of oligoclonal antibodies against three neurotropic viruses, has not achieved wide use in the diagnosis of MS. At the same time, measurement of MRZ antibodies may provide a more specific tool for the diagnosis of MS than detection of oligoclonal bands [55]. A positive MRZ reaction is obtained when there are elevated indexes of antibody activity against two or more viruses. Data have been reported showing that the MRZ reaction is encountered statistically significantly more often among patients with CIS which transforms into full MS by two years. The combination of positive tests for OCI and a positive MRZ reaction with two or more foci on MRI scans has greater diagnostic significance than positive results from a combination of two markers [55]. The fact that a positive MRZ reaction can be obtained in the absence of OCI in 1/3 of patients with recurrent-remitting MS and 2/3 of OCI-negative patients with secondary and primary progressive forms of MS [56] is interesting. Use of the MRZ reaction for the diagnosis of complex cases among OCI-negative patients may simplify the final diagnosis of MS. Detection of OCI fractions against measles virus is significantly linked with a larger number of foci of demyelination in the T2 regime [57].

IgM OCI constitute a prognostic marker for disease and are also seen when the course of MS is more aggressive. The probability that CIS will convert to full MS when there is intrathecal synthesis of IgM antibodies during the first year of illness is 90% [58]. In five-year observations, exacerbations of MS were found more frequently among patients with intrathecal synthesis of oligoclonal IgM [58]. Detection of IgM OCI specific for the lipid structures of myelin was associated with a short time interval between the first and second exacerbations and with a greater degree of disability on the EDSS. Interferon-1β treatment had a marked therapeutic effect on patients with IgM OCI. This group retained a relatively high frequency of exacerbations, which was significantly different from that in a group of patients negative for IgM OCI against lipid structures [39]. These data provide evidence of a link between IgM OCI and more severe disease, a greater exacerbation frequency, and a higher degree of disability.

**Conclusions**

In conclusion, OCI are a product of the immune response to a number of protein and nonprotein CNS antigens. Data have been obtained supporting the involvement of OCI in the pathogenesis of the disease. Detection of IgG OCI is currently regarded as the main diagnostic and prognostic biomarker for MS for the immunological laboratory diagnosis of MS. IEF used for detection of OCI has high sensitivity and specificity, though the pathological type of synthesis detected by this method can be encountered in other chronic inflammatory diseases involving the CNS. Testing of CSF for IgG OCI is therefore regarded as a supplementary method for the diagnosis of MS, reflecting the 2010 revision of the McDonald criteria [47]. Other laboratory markers, such as the MRZ reaction and detection of IgM OCI, have limited use in clinical practice. The development of new techniques for evaluating local and systemic immune reactions involving B cells in patients with CIS and MS, as well as other inflammatory diseases, should throw new light on the role of OCI and open a pathway to more specific methods for the diagnosis of MS.

This study was carried out at the First St. Petersburg State Medical University, Russian Ministry of Health, with financial support from the Russian Scientific Foundation.

**REFERENCES**

1. C. F. Lucchinetti and R. Hohlfeld, *Multiple Sclerosis 3*, in: *Blue Books of Neurology Series*, Saunders (2010), Vol. 35, No. 6, pp. 122–124.
2. A. N. Boiko, N. F. Smirnova, S. N. Zolotova, and E. I. Gusev, "Epidemiology and etiology of multiple sclerosis," *Consilium Medicum*., **10**, No. 7, 5–8 (2008).
3. M. Freedman, E. Thompson, F. Deisenhammer, et al., "Recommended standard of cerebrospinal fluid analysis in the diagnosis of multiple sclerosis," *Arch Neurol*., **62**, No. 6 (2005), doi:10.1001/archneur.62.6.865.
4. S. V. Lapin and E. P. Evdoshenko, "Laboratory methods for the diagnosis of multiple sclerosis and other neurological diseases," in: *Handbook of Main Clinical Diagnostic Laboratories*, No. 1, 22–32 (2011).
5. E. Meinl, M. Krumbholz, and R. B. Hohlfeld, "B lineage cells in the inflammatory central nervous system environment: Migration, main-

tenance, local antibody production, and therapeutic modulation," *Ann. Neurol.*, **59**, No. 6, 880–892 (2006), doi:10.1002/ana.20890.
6. B. Serafini, B. Rosicarelli, R. Magliozzi, et al., "Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis," *Brain Pathology*, **14**, No. 2, 164–174 (2004), doi:10.1111/j.1750-3639.2004.tb00049.x.
7. G. Owens, J. Bennett, D. Gilden, and M. Burgoon, "The B cell response in multiple sclerosis," *Neurological Research*, **28**, No. 3, 236–244 (2006), doi:10.1179/016164106x98099.
8. V. P. Chekhonin, A. V. Semenova, O. I. Turina, and T. B. Dmitrieva, "Myelin-oligodendrocyte complex: structure, function, and role in the pathogenesis of demyelinating diseases," *Biomed. Khim.*, **49**, No. 5, 411–423 (2003).
9. T. V. Demina, M. V. Davydovskaya, N. V. Khachanova, et al., "Multiple sclerosis: pathogenesis, diagnosis, differential diagnosis, and treatment," *Nevrol. Revmatol.*, Suppl. to *Consilium Medicum*, No. 1, 84–90, (2008).
10. G. Owens, J. Bennett, H. Lassmann, et al., "Antibodies produced by clonally expanded plasma cells in multiple sclerosis cerebrospinal fluid," *Ann. Neurol.*, **65**, No. 6, 639–649 (2009), doi: 10.1002/ana.21641.
11. T. Menge, P. Lalive, H. von Budingen, and C. Genain, "Conformational epitopes of myelin oligodendrocyte glycoprotein are targets of potentially pathogenic antibody responses in multiple sclerosis," *J. Neuroinflamm.*, **8**, No. 1, 161 (2011). doi:10.1186/ 1742-2094-8-161.
12. T. Menge, H. von Budingen, P. Lalive, and C. Genain, "Relevant antibody subsets against MOG recognize conformational epitopes exclusively exposed in solid-phase ELISA," *Eur. J. Immunol.*, **37**, No. 11, 3229–3239 (2007), doi:10.1002/eji.200737249.
13. E. Klawiter, L. Piccio, J. Lyons, et al., "Elevated intrathecal myelin oligodendrocyte glycoprotein antibodies in multiple sclerosis," *Arch Neurol.*, **67**, No. 9 (2010), doi:10.1001/archneurol.2010.197.
14. V. Tomassini, L. De Giglio, M. Reindl, et al., "Anti-myelin antibodies predict the clinical outcome after a first episode suggestive of MS," *Mult. Scler.*, **13**, No. 9, 1086–1094 (2007), doi:10.1177/135245 8507077622.
15. J. Kuhle, C. Pohl, M. Mehling, et al., "Lack of association between antimyelin antibodies and progression to multiple sclerosis," *NEJM*, **356**, No. 4, 371–378 (2007), doi:10.1056/nejmoa063602.
16. C. M. Levin, "Autoantibodies to non-myelin antigens as contributors to the pathogenesis of multiple sclerosis," *J Clin. Cell. Immunol.*, **4** (2013), doi:10.4172/2155-9899.1000148.
17. S. Ousman, B. Tomooka, J. Van Noort, et al., "Protective and therapeutic role for alphaB-crystallin in autoimmune demyelination," *Clin. Immunol.*, **123**, 139 (2007), doi:10.1016/j.clim.2007.03.034.
18. G. Owens, D. Gilden, M. Burgoon, et al., "Viruses and multiple sclerosis," *The Neuroscientist*, **17**, No. 6, 659–676 (2011), doi:10.1177/ 1073858411386615.
19. R. Farrell, D. Antony, G. Wall, et al., "Humoral immune response to EBV in multiple sclerosis is associated with disease activity on MRI," *Neurology*, **73**, No. 1, 32–38 (2009), doi:10.1212/wnl.0b013 e3181aa29fe.
20. G. Fust, "The role of the Epstein–Barr virus in the pathogenesis of some autoimmune disorders – similarities and differences," *Eur. J. Microbiol. Immunol.*, **1**, No. 4, 267–278 (2011), doi:10.1556/eujmi. 1.2011.4.2.
21. P. Sundstrom, P. Juto, G. Wadell, et al., "An altered immune response to Epstein–Barr virus in multiple sclerosis: A prospective study," *Neurology*, **62**, No. 12, 2277–2282 (2004), doi:10.1212/01. wnl.0000130496.51156.d7.
22. L. Levin, "Temporal relationship between elevation of Epstein–Barr virus antibody titers and initial onset of neurological symptoms in multiple sclerosis," *JAMA*, **293**, No. 20, 2496 (2005), doi:10.1001/ jama.293.20.2496.
23. G. DeLorenze, K. Munger, E. Lennette, et al., "Epstein–Barr virus and multiple sclerosis," *Arch Neurol.*, **63**, No. 6, 839 (2006), doi:10.1001/archneur.63.6.noc50328.
24. E. Thacker, F. Mirzaei, and A. Ascherio, "Infectious mononucleosis and risk for multiple sclerosis: A meta-analysis," *Ann. Neurol.*, **59**, No. 3, 499–503 (2006), doi:10.1002/ana.20820.
25. T. Nielsen, K. Rostgaard, N. Nielsen, et al., "Multiple sclerosis after infectious mononucleosis," *Arch Neurol.*, **64**, No. 1, 72 (2007), doi:10.1001/archneur.64.1.72.
26. M. Esposito et al., "Human transaldolase and crossreactive viral epitopes identified by autoantibodies of multiple sclerosis patients," *J Immunol.*, **163**, No. 7, 4027–4032 (1999).
27. J. Lunemann, T. Kamradt, R. Martin, and C. Munz, "Epstein–Barr virus: environmental trigger of multiple sclerosis?" *J. Virol.*, **81**, No. 13, 6777–6784 (2007), doi:10.1128/jvi.00153-07.
28. H. Lang, H. Jacobsen, S. Ikemizu, et al., "A functional and structural basis for TCR cross-reactivity in multiple sclerosis *nature," Immunology*, **3**, No. 10, 940–943 (2002), doi:10.1038/ni835.
29. U. Meier, G. Giovannoni, J. Tzartos, and G. Khan, "Translational mini-review series on B cell subsets in disease. B cells in multiple sclerosis: drivers of disease pathogenesis and Trojan horse for Epstein–Barr virus entry to the central nervous system?" *Clin. Exp. Immunol.*, **167**, No. 1, 1–6 (2011), doi:10.1111/j.1365-2249.2011.04446.x.
30. H. Reiber, S. Ungefehr, and C. Jacobi, "The intrathecal, polyspecific and oligoclonal immune response in multiple sclerosis," *Mult. Scler.*, **4**, No. 3, 111–117 (1998), doi:10.1177/135245859800400304.
31. K. Felgenhauer and H. Reiber, "The diagnostic significance of antibody specificity indices in multiple sclerosis and herpes virus induced diseases of the nervous system," *Clin Invest.*, **70**, No. 1, 28–37 (1992), doi:10.1007/bf00422934.
32. C. Sindic, P. Monteyne, and E. Laterre, "The intrathecal synthesis of virus-specific oligoclonal IgG in multiple sclerosis," *J. Neuroimmunol.*, **54**, No. 1–2, 75–80 (1994), doi:10.1016/0165-5728(94)90233-x.
33. H. Frederiksen and C. Sindic, "Intrathecal synthesis of virus-specific oligoclonal IgG, and of free kappa and free lambda oligoclonal bands in acute monosymptomatic optic neuritis. Comparison with brain MRI," *Mult. Scler.*, **4**, No. 1, 22–26 (1998), doi:10.1191/ 135245898 678909213.
34. S. Jarius, P. Eichhorn, C. Jacobi, et al., "The intrathecal, polyspecific antiviral immune response: Specific for MS or a general marker of CNS autoimmunity?" *J. Neurol. Sci.*, **280**, No. 1–2, 98–100 (2009), doi:10.1016/j.jns.2008.08.002.
35. S. Jarius, D. Franciotta, R. Bergamaschi, et al., "Polyspecific, antiviral immune response distinguishes multiple sclerosis and neuromyelitis optica," *J. Neurol. Neurosurg. Psychiatry*, **79**, No. 10, 1134–1136 (2008), doi:10.1136/jnnp.2007.133330.
36. M. Robinson-Agramonte, H. Reiber, J. Cabrera-Gomez, and R. Galvizu, "Intrathecal polyspecific immune response to neurotropic viruses in multiple sclerosis: a comparative report from Cuban patients," *Acta Neurol. Scand.*, **115**, No. 5, 312–318 (2007), doi:10.1111/j.1600-0404. 2006.00755.x.
37. I. Brecht, B. Weissbrich, J. Braun, et al., "Intrathecal, polyspecific antiviral immune response in oligoclonal band negative multiple sclerosis," *PLoS ONE*, **7**, No. 7, e40431 (2012), doi:10.1371/journal. pone.0040431.
38. M. Bonnan, "Does disease-irrelevant intrathecal synthesis in multiple sclerosis make sense in the light of tertiary lymphoid organs?" *Front. Neurol.*, **5** (2014), doi:10.3389/fneur.2014.00027.
39. L. Villar, M. Sadaba, E. Roldan, et al., "Intrathecal synthesis of oligoclonal IgM against myelin lipids predicts an aggressive disease course in MS," *J. Clin. Invest.*, **115**, No. 1, 187–194 (2005), doi:10. 1172/jci200522833.
40. E. Kabat, D. Moore, and H. Landow, "An electrophoretic study of the protein components in cerebrospinal fluid and their relationship to the serum proteins," *J. Clin. Invest.*, **21**, No. 5, 571–577 (1942), doi:10.1172/jci101335.
41. E. Frick and L. Scheid-Seydel, "Untersuchungen mit J131-markiertem Globulin zur Frage der Abstammung der Liquoreiweissk," *Klin. Wochenschr.*, **36**, No. 18, 857–863 (1958), doi:10.1007/bf01485232.

42. E. C. Laterre, "Agar gel electrophoresis of cerebrospinal fluid proteins. Semiology of the γ-zone," *Acta Neurol. Psych. Belg.*, **66**, 289–304 (1966).
43. G. Keir, R. Luxton, and E. Thompson, "Isoelectric focusing of cerebrospinal fluid immunoglobulin G: An annotated update," *Ann. Clin. Biochem.*, **27**, No. 5, 436–443 (1990), doi:10.1177/00045632902700504.
44. T. Holmoy, "The discovery of oligoclonal bands: a 50-year anniversary," *Eur.Neurol.*, **62**, No. 5, 311–315 (2009), doi:10.1159/000235944.
45. A. Bourahoui, J. de Seze, R. Guttierez, et al., "CSF isoelectrofocusing in a large cohort of MS and other neurological diseases," *Eur. J. Neurol.*, **11**, No. 8, 525–529 (2004), doi:10.1111/j.1468-1331.2004.00822.x.
46. C. Polman, S. Reingold, G. Edan, et al., "Diagnostic criteria for multiple sclerosis: 2005 revisions to the 'McDonald Criteria,' *Ann. Neurol.*, **58**, No. 6, 840–846 (2005), doi:10.1002/ana.20703.
47. C. Polman, S. Reingold, B. Banwell, et al., "Diagnostic criteria for multiple sclerosis: 2010 Revisions to the McDonald criteria," *Ann. Neurol.*, **69**, No. 2, 292–302 (2011), doi:10.1002/ana.22366.
48. M. Tintore, A. Rovira, J. Rio, et al., "Do oligoclonal bands add information to MRI in first attacks of multiple sclerosis?" *Neurology*, **70**, No. 13, Part 2, 1079–1083 (2007), doi:10.1212/01.wnl.0000280576.73609.c6.
49. J. Rojas, L. Patrucco, and E. Cristiano, "Erratum to: Oligoclonal bands and MRI in clinically isolated syndromes: predicting conversion time to multiple sclerosis, " *J. Neurol.*, **257**, No. 7, 1231–1231 (2010), doi:10.1007/s00415-010-5561-0.
50. F. Joseph, C. Hirst, T. Pickersgill, et al., "CSF oligoclonal band status informs prognosis in multiple sclerosis: a case control study of 100 patients," *J. Neurol. Neurosurg. Psych.*, **80**, No. 3, 292–296 (2009), doi:10.1136/jnnp.2008.150896.
51. F. von Glehn, A. Farias, A. de Oliveira, et al., "Disappearance of cerebrospinal fluid oligoclonal bands after natalizumab treatment of multiple sclerosis patients," *Mult. Scler.*, **18**, No. 7, 1038–1041 (2011), doi:10.1177/1352458511428465.
52. J. Stark, D. Koffler, and S. Sadiq, "Persistence of cerebrospinal fluid oligoclonal bands after natalizumab treatment in multiple sclerosis patients (P05.187)," *Neurology*, **80**, No. 7, Supplement, P05.187 (2013).
53. G. Mancardi and R. Saccardi, "Autologous haematopoietic stem-cell transplantation in multiple sclerosis," *The Lancet Neurology*, **7**, No. 7, 626–636 (2008), doi:10.1016/s1474-4422(08)70138-8.
54. C. Rogojan and J. Frederiksen, "Hematopoietic stem cell transplantation in multiple sclerosis," *Acta Neurol. Scand.*, **120**, No. 6, 371–382 (2009), doi:10.1111/j.1600-0404.2009.01168.x.
55. J. Brettschneider, H. Tumani, U. Kiechle, et al., "IgG antibodies against measles, rubella, and varicella zoster virus predict conversion to multiple sclerosis in clinically isolated syndrome," *PLoS ONE.*, **4**, No. 11, 7638 (2009), doi:10.1371/journal.pone.0007638.
56. I. Brecht, B. Weissbrich, J. Braun, et al., "Intrathecal, polyspecific antiviral immune response in oligoclonal band negative multiple sclerosis," *PLoS ONE.*, **7**, No. 7, 40431 (2012), doi:10.1371/journal.pone.0040431.
57. B. Rosche, S. Laurent, S. Conradi, et al., "Measles IgG antibody index correlates with T2 lesion load on MRI in patients with early multiple sclerosis," *PLoS ONE.*, **7**, No. 1, 28094 (2012), doi:10.1371/journal.pone.0028094.
58. L. Villar, J. Masjuan, P. Gonzalez-Porque, et al., "Intrathecal IgM synthesis predicts the onset of new relapses and a worse disease course in MS," *Neurology*, **59**, No. 4, 555–559 (2002), doi:10.1212/wnl.59.4.555.