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(54) **DIAGNOSIS OF MULTIPLE SCLEROSIS AND OTHER DEMYELINATING DISEASES**

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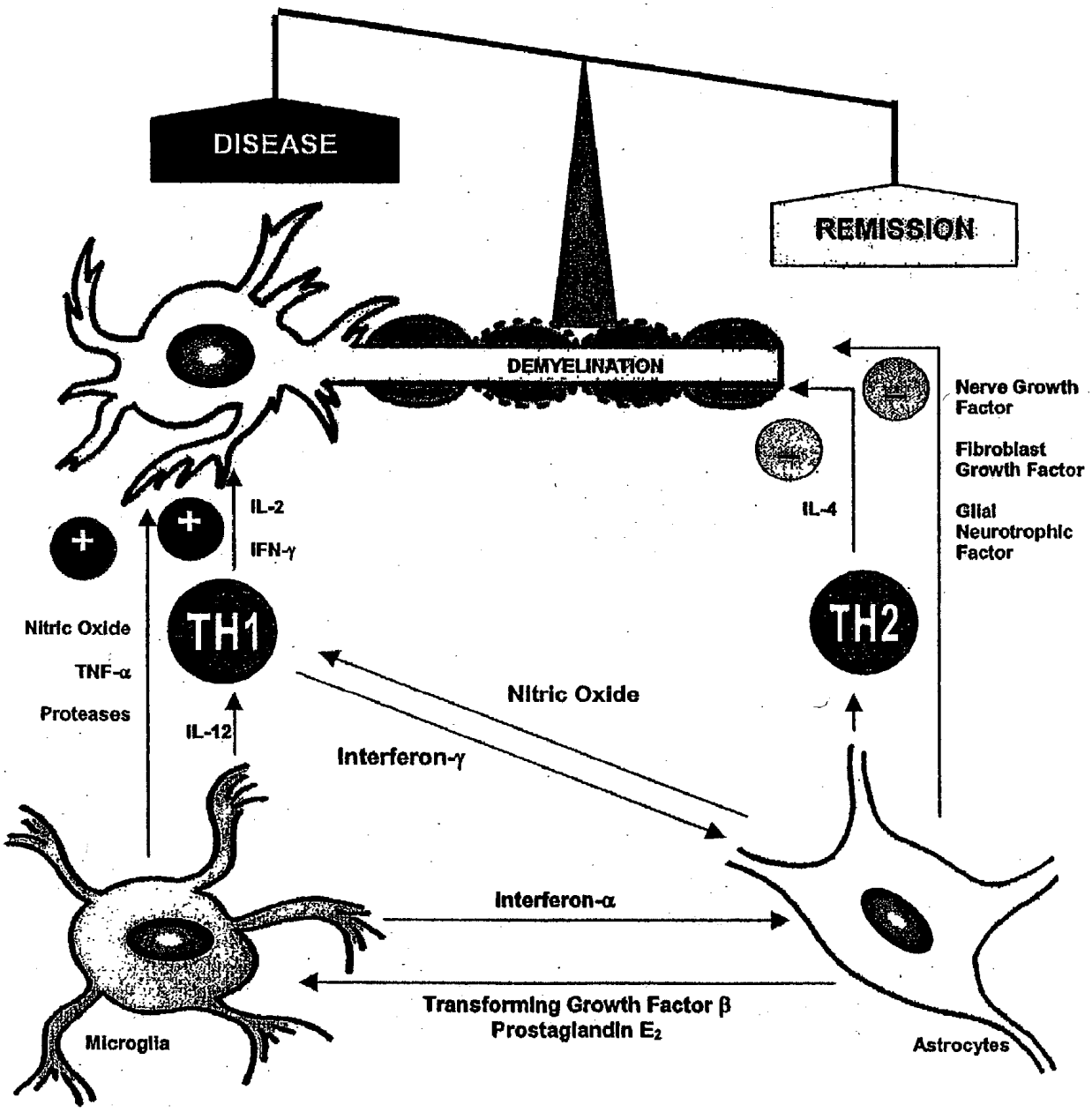
(57) **ABSTRACT**

Disclosed herein is a method of diagnosing multiple sclerosis and other demyelinating diseases or predicting a predisposition to multiple sclerosis and other demyelinating diseases. The method utilizes detection of increased amounts of memory lymphocytes reacting to MS antigens, proinflammatory cytokines, and antibodies against MS antigens.

Figure 1


DISEASE
REMISSION
DEMYELINATION
Nerve Growth Factor
Fibroblast Growth Factor
Glial Neurotrophic Factor
IL-4
IL-2
IFN-γ
Nitric Oxide
TNF-α
Proteases
TH1
TH2
IL-12
Nitric Oxide
Interferon-γ
Interferon-α
Transforming Growth Factor β
Prostaglandin E2
Microglia
Astrocytes

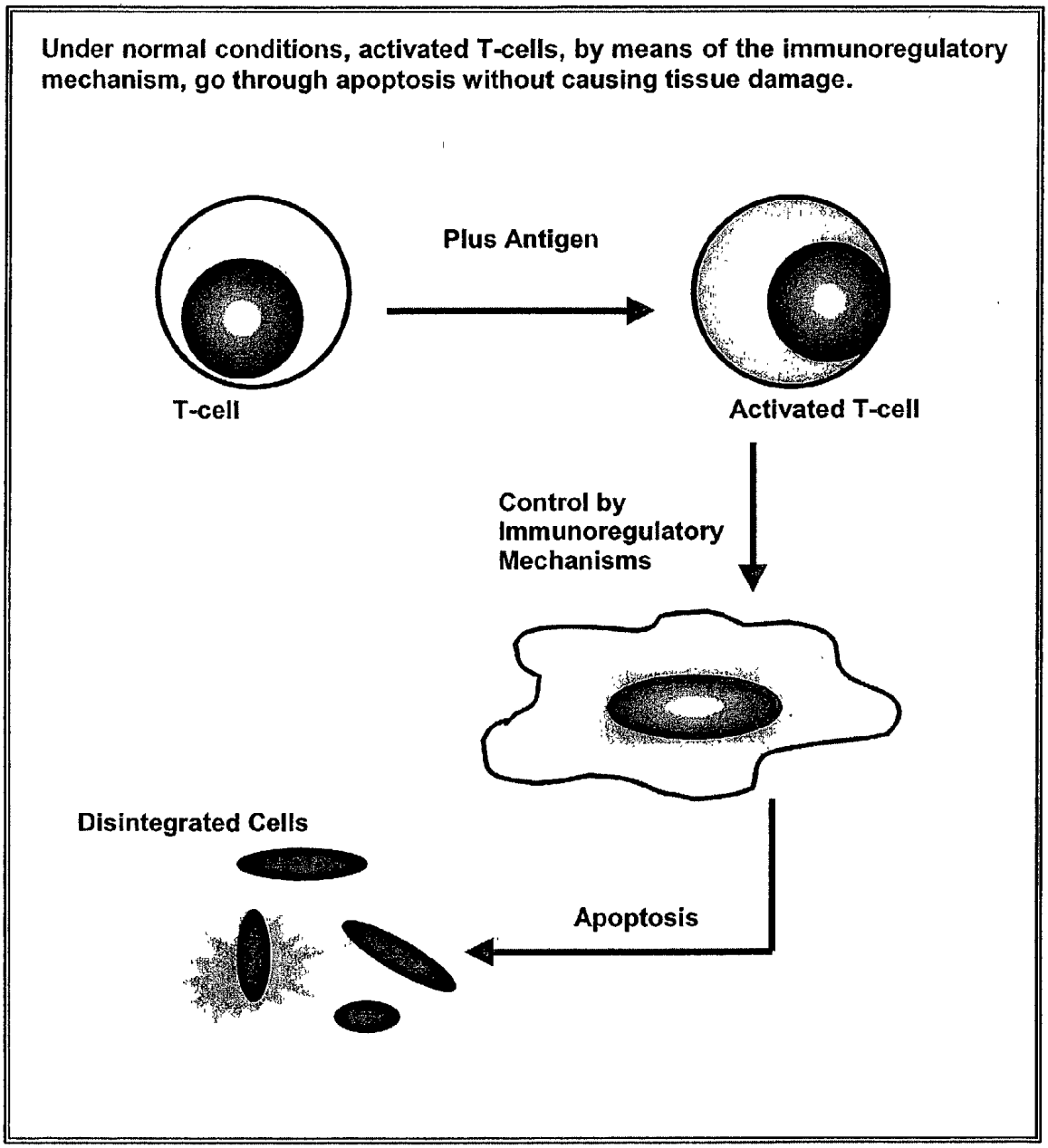
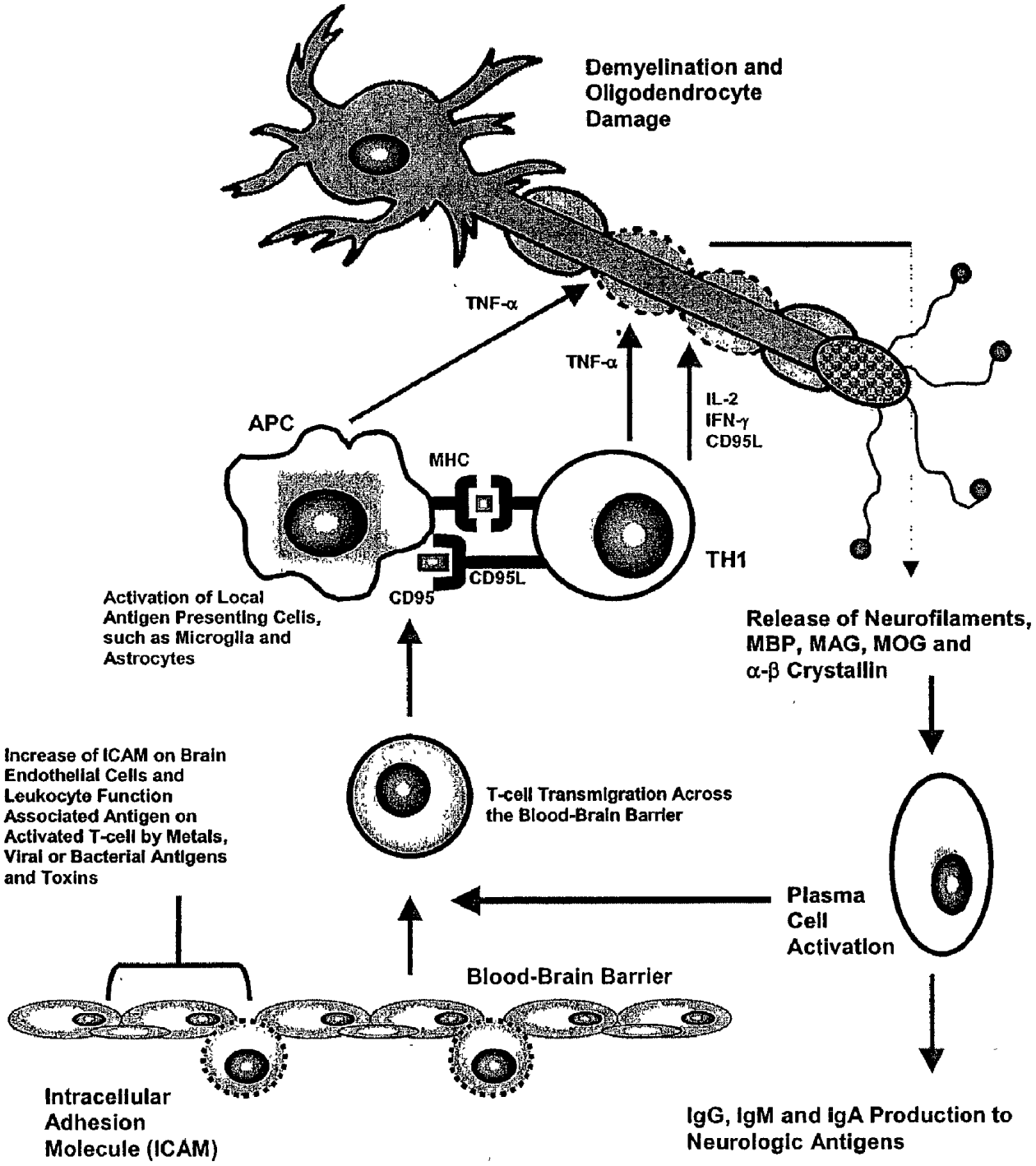


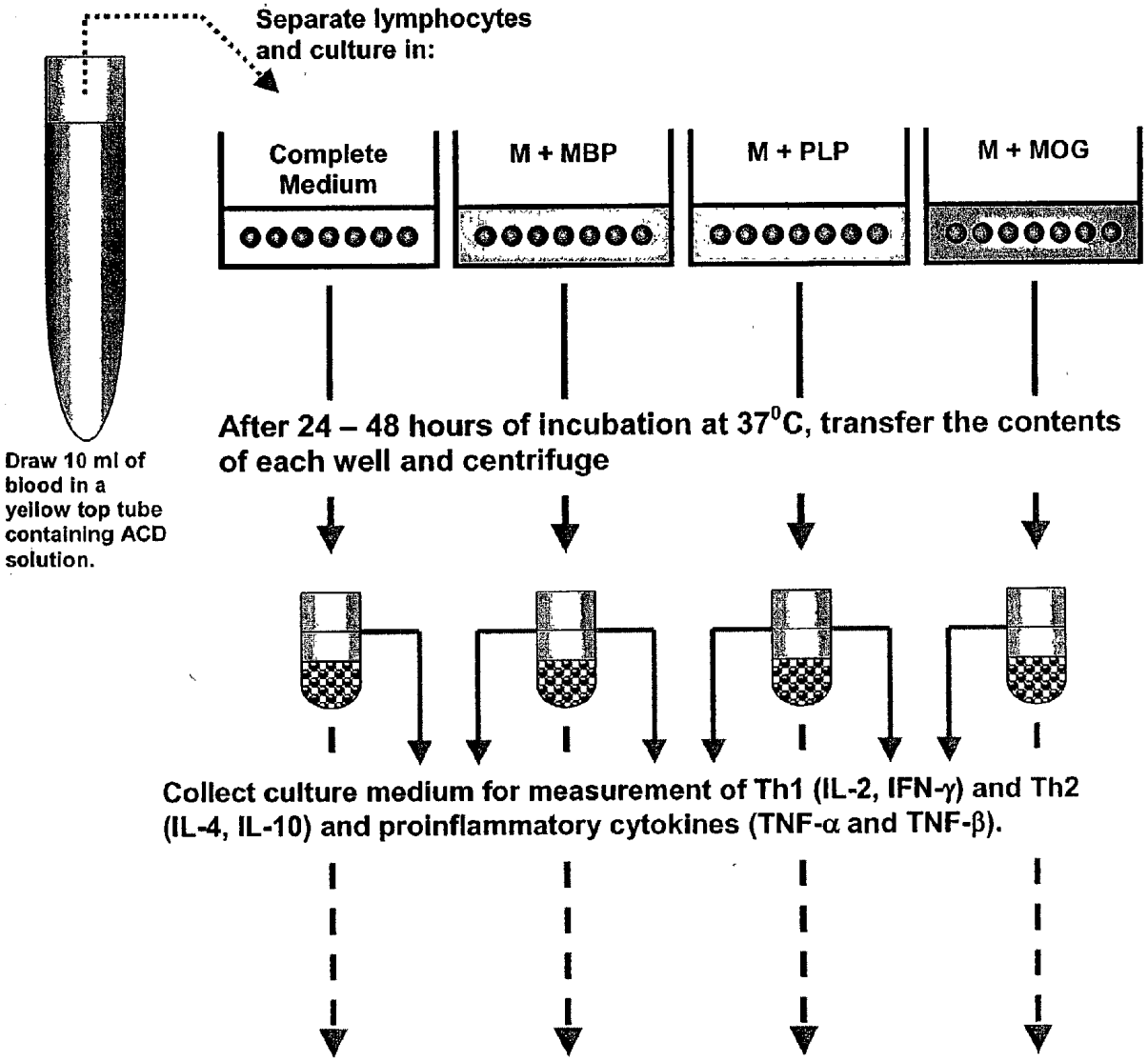
Figure 2 – Apoptosis of Activated T-Cells by Means of Immunoregulatory Mechanism, which Prevents Tissue Damage.

Figure 3 - Cellular and Humoral Immune Mechanisms in Stress, Infection and Toxic Chemical-induced Neurotoxicity, which Includes Neuronal Degeneration, Secondary Demyelination, and Reactive Astrogliosis



Under pathological conditions, pre-existing autoreactive T-cells are generated by molecular mimicry as a result of sequence homologies or matched motifs between autoantigen and viral, bacterial or parasitic proteins.

Figure 4 - Procedure for detection of myelin or other antigen-specific CD4 T-cells in patients with possible neuroimmunologic disorders.



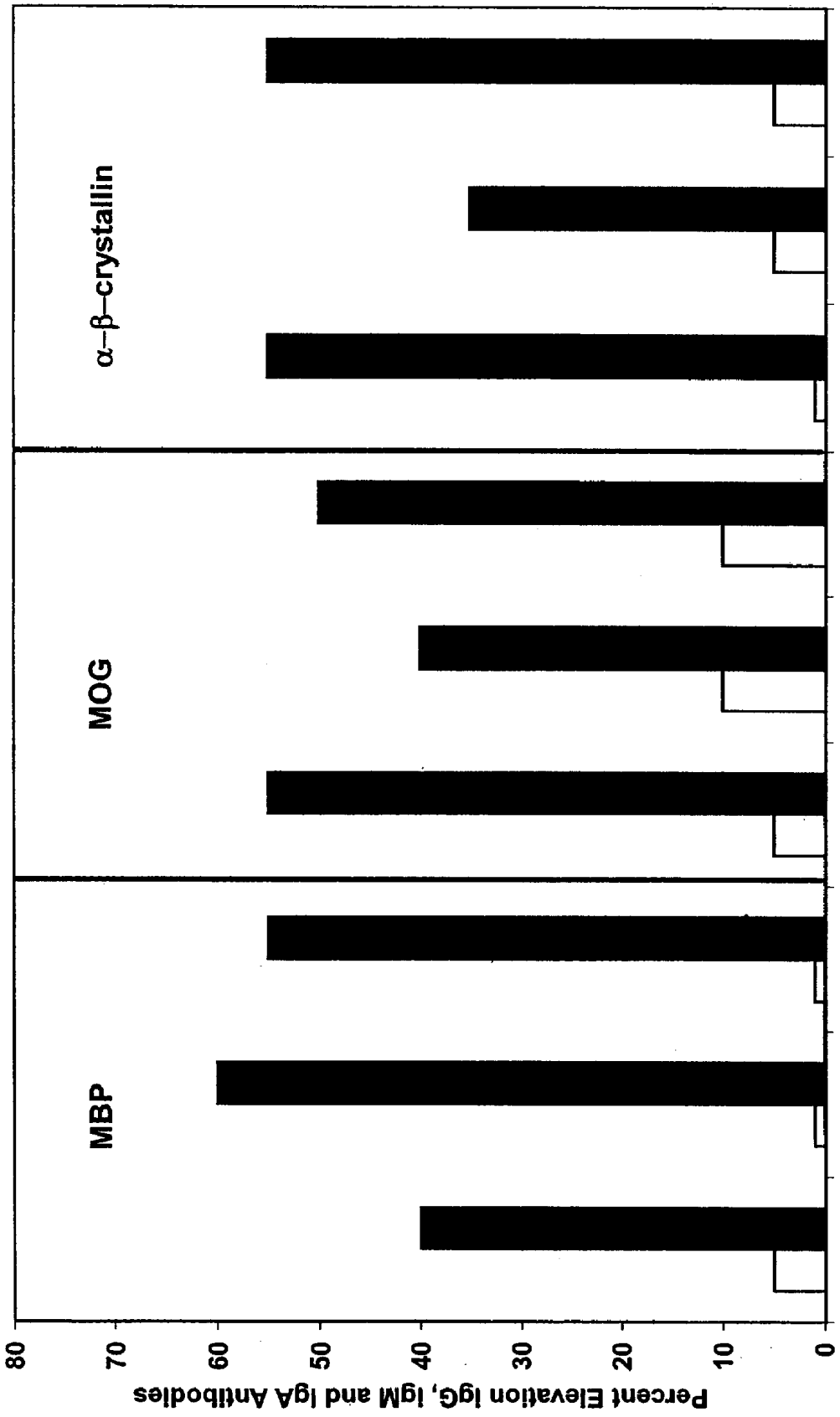
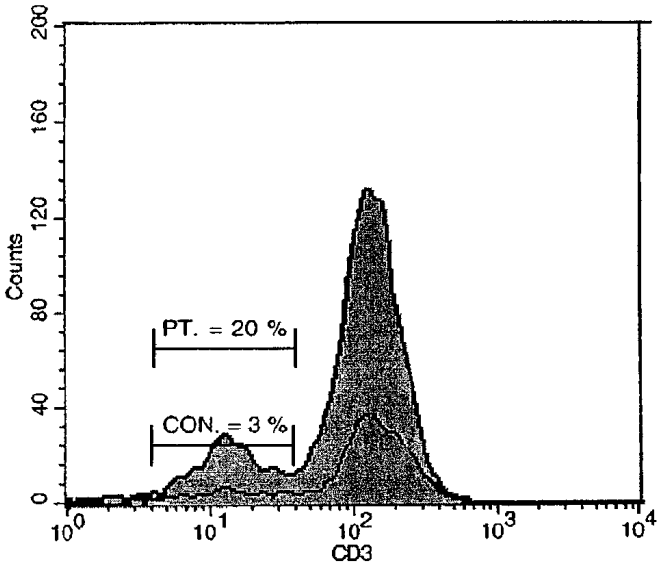
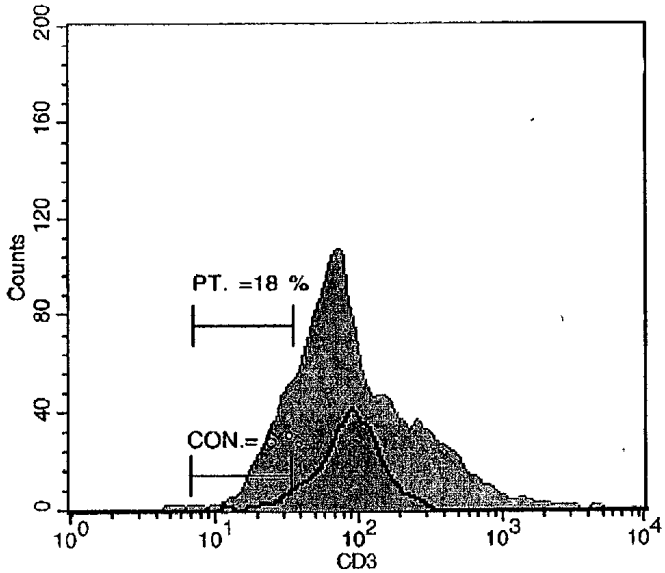


Figure 5 - Percent Elevation in IgG, IgM and IgA Antibodies Against Three Different Neurological Antigens in Controls ☐ and Patients with MS ■ at cut-off values above 2 S.D. of Mean of Controls.

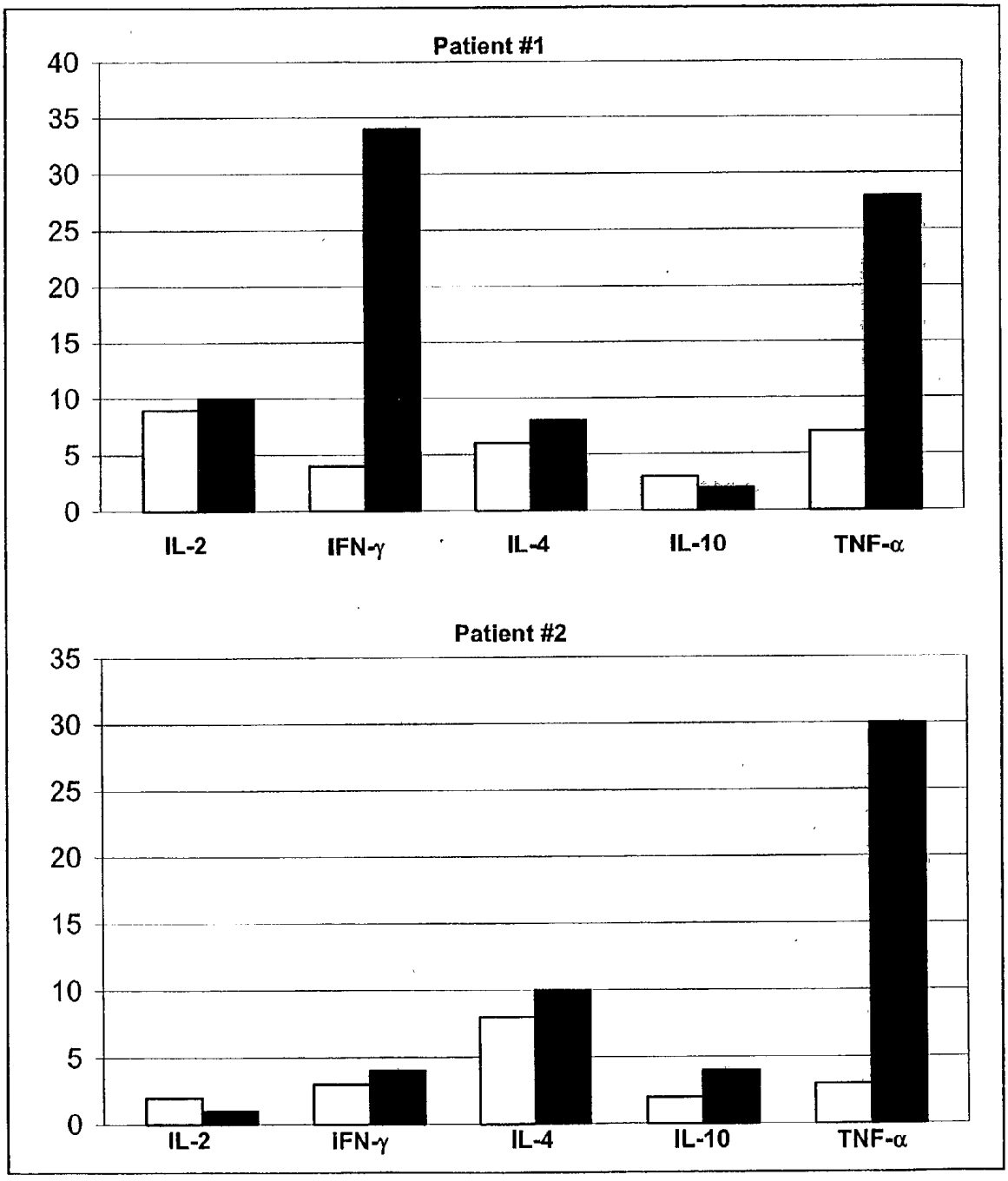
Figure 6 - In Vitro Stimulation Study of Myelin-specific Lymphocytes in Controls and Patients with Possible Multiple Sclerosis





Different histograms of % activated antigen-specific CD4 T-cells in the blood of control subjects and patients with possible multiple sclerosis

Figure 7 - Measurement of Th-1, Th-2 and proinflammatory cytokine production in blood samples of two different controls ☐ and two patients ■ with possible MS. Note that in patient #1, IFN-γ, as well as TNF-α are elevated; while in patient #2, only TNF-α is increased.



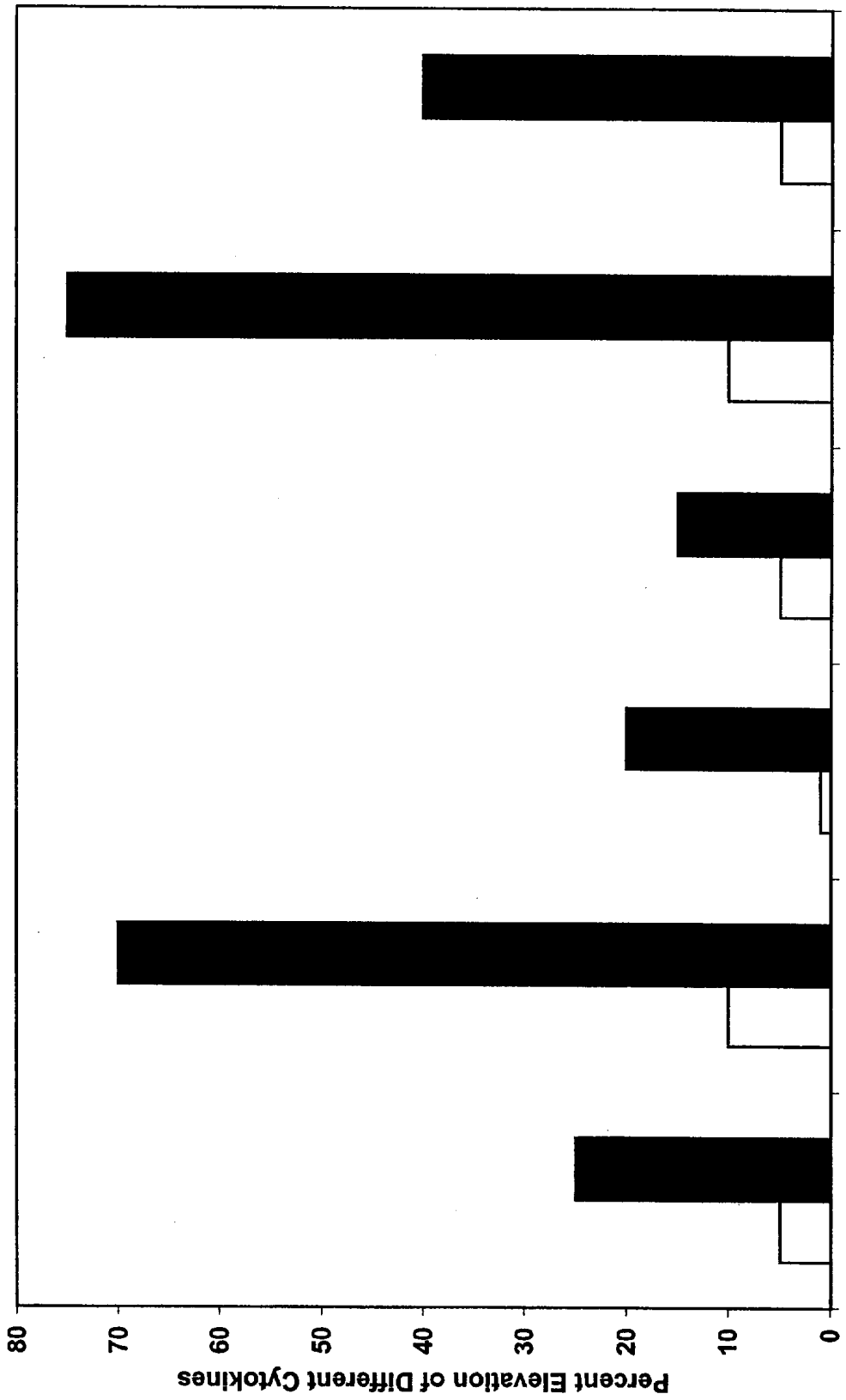


Figure 8 - Percent Elevation of Different Cytokines Produced by MBP-Reactive T-cells in Controls and Patients with MS at cut-off values above 2 S.D. of Mean of Controls.

DIAGNOSIS OF MULTIPLE SCLEROSIS AND OTHER DEMYELINATING DISEASES

BACKGROUND OF THE INVENTION

[0001] 1. Field of the Invention

[0002] The invention relates to a method of diagnosing multiple sclerosis and other demyelinating diseases.

[0003] 2. Description of the Related Art

[0004] Autoimmune neurologic disorders occur when immunologic tolerance to myelin and other neurologic antigens of the Schwann cell, the axon and the motor or ganglioside neuron are lost. The resulting demyelinating diseases share the pathologic features of destruction of myelin, accompanied by an inflammatory infiltration in the brain, spinal cord, or the optic nerve. Based on the location of the lesions, the occurrence of relapses, and the nature of events, it is possible to separate the clinical neurologic syndromes of multiple sclerosis, acute disseminated encephalomyelitis, acute transverse myelitis, and optic neuritis (1,2).

[0005] The most common demyelinating disease is multiple sclerosis. Multiple sclerosis (MS) is a disease of the myelin central nervous system (CNS) that is clinically characterized by episodes of neurologic dysfunction separated by time and space.

[0006] Currently, there is no specific diagnostic test for Multiple Sclerosis ("MS"). The diagnosis is made based on clinical grounds, which may vary from clinician to clinician. Supportive evidence to clinical grounds can come from the MRI of the brain, cerebrospinal fluid studies, and evoked response (1,5).

[0007] MRI is usually the procedure of choice for corroboration of a clinical diagnosis of MS, particularly when gadolinium enhancement is used. High signal intensity lesions on T-2 weighted images, particularly in the periventricular areas, support a diagnosis of MS. MRI is not specific for MS, since several diseases of the white matter such as ischemic, infectious, metabolic and neoplastic present similar pictures.

[0008] Cerebrospinal fluid examination is an additional supportive technique for the diagnosis of MS. CSF total protein is usually normal but CSF IgG levels may be increased and the ratio of CSF IgG to CSF albumin is often elevated. The presence of discrete IgG oligoclonal band by immunofixation electrophoreses is more characteristic but not specific for MS. This oligoclonal band may be found in many conditions including: subacute sclerosing panencephalitis, neurosyphilis, Lyme Disease, HTLV-1 associated myelopathy, Sjögren Syndrome, sacoidosis, meningeal carcinomatosis and HIV infection.

[0009] The third technique for support in the diagnosis of MS is evoked response, which includes: pattern-sensitive visual-evoked potential, the brainstem auditory-evoked potential (5).

[0010] Overall, the combination of MRI, the CSF examination and evoked responses support a clinical diagnosis of MS in a majority of cases. However, all three determinants (MRI, CSF examination and evoked response) are not always positive in the same patient. For example, abnormal MRI alone or abnormal MRI with normal CSF and abnormal evoked response can challenge many clinicians over the diagnosis of MS. Hence, there is no definitive test available to diagnose multiple sclerosis.

[0011] Therefore, there is a need for additional markers to aid in the diagnosis of MS. These biomarkers become very useful when the immunological mechanisms behind the development of neurological dysfunction associated with MS are understood.

SUMMARY OF THE INVENTION

[0012] The preferred embodiment provides a method for diagnosing the likelihood and severity of a demyelinating disease in a patient, comprising the steps of: a) determining a level of antibodies against a neuron-specific antigen in a sample from the patient; b) comparing the level of antibodies determined in step a) with a normal level of the antibodies, wherein (i) normal level of antibodies for neuron-specific antigen indicate optimal conditions; (ii) lower than normal level of antibodies for neuron-specific antigen indicate absence of the demyelinating disease; and (iii) higher than normal level of antibodies for neuron-specific antigen indicate a likelihood of the demyelinating disease.

[0013] Another preferred embodiment provides a method for diagnosing the likelihood and severity of a demyelinating disease in a patient, comprising the steps of: a) isolating peripheral blood mononuclear cells (PBMCs) from the patient; b) incubating PBMCs with a neuronal antigen or peptide; c) measuring a concentration of cytokines resulting from step b); and d) comparing the concentration of cytokines determined from step c) with a normal level of cytokines, wherein (i) normal level of cytokines for the neuronal antigen or peptide indicate optimal conditions; (ii) lower than normal level of cytokines for the neuronal antigen or peptide indicate absence of the demyelinating disease; and (iii) higher than normal level of cytokines after challenge with the neuronal antigen or peptide indicate a likelihood of the demyelinating disease.

[0014] Another preferred embodiment provides a method for diagnosing the likelihood and severity of a demyelinating disease in a patient, comprising the steps of: a) isolating peripheral blood mononuclear cells (PBMCs) from the patient; b) incubating PBMCs with neuronal antigen or peptide; c) determining an amount of neuronal antigen- or peptide-specific activated T-cells or neuronal-specific memory lymphocytes resulting from step b); d) obtaining a stimulation index from step c); and e) comparing the stimulation index from step d) with a normal stimulation index, wherein (i) normal stimulation index indicates optimal conditions; (ii) lower than normal stimulation index indicates absence of the demyelinating disease; and (iii) higher than normal stimulation index indicates a likelihood of a demyelinating disease.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] **FIG. 1** is a diagram that shows the regulation of Th1/Th 2 responses by the balance or imbalance between microglia and astrocytes in demyelinating processes.

[0016] **FIG. 2** is a diagram that shows apoptosis of activated T-cells by means of immunoregulatory mechanism, which prevents tissue damage.

[0017] **FIG. 3** is a diagram that shows cellular and humoral immune mechanisms in stress, infection and toxic chemical-induced neurotoxicity, which includes neuronal degeneration, secondary demyelination, and reactive astrogliosis.

[0018] **FIG. 4** is a diagram that shows a procedure for detection of myelin and other antigen-specific CD4 T-cells in patients with possible neuroimmunologic disorders.

[0019] **FIG. 5** is a graph that shows percent elevation in IgG, IgM, and IgA antibodies against three different neurological antigens in controls and patients with multiple sclerosis at cut-off values above about 2 standard deviations of the mean of the controls.

[0020] **FIG. 6** is a graph showing an in vitro stimulation study of myelin-specific lymphocytes in controls and patients with possible multiple sclerosis.

[0021] **FIG. 7** is a graph showing measurement of Th-1, Th-2, and proinflammatory cytokine production in blood samples of two different controls and two patients with possible multiple sclerosis.

[0022] **FIG. 8** is a graph showing percent elevation of different cytokines produced by MBP-reactive T-cells in controls and patients with multiple sclerosis at cut-off values above 2 standard deviations of the mean of the controls.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0023] The hallmark of the MS lesion is a plaque, an area of demyelination sharply demarcated from the usual white matter shown in MRI scans. The histological appearance of the plaques varies in different stages of the disease. In active lesions, the blood-brain barrier is damaged, thereby permitting extravasation of serum proteins into the extracellular space. Inflammatory cells can be seen in perivascular cuffs and throughout the white matter. Activated monocytes-derived macrophages and activated lymphocytes predominate. CD4 T-cells, especially T-helper-1 (but not CD8 cells) accumulate around postcapillary venules at the edge of the plaque and are also scattered in the white matter (3-5). In active lesions, up-regulation of adhesion molecules and markers of lymphocyte and monocyte activation, such as IL2-R and CD26 have also been observed. Demyelination in active lesions is not accompanied by destruction of oligodendrocytes. In contrast, in the chronic phase of the disease, the lesions are characterized by the loss of oligodendrocytes and hence, the presence of myelin oligodendrocytes glycoprotein (MOG) antibodies in the blood. T-cells bearing the γ-6 T-cell receptor are found in MS lesions and may be involved in the selective destruction of oligodendrocytes. The γ-8 T-cells are reacting with heat shock proteins (HSP65), such as α-β crystallin, which may be found in oligodendrocytes under stressful conditions. This particular reaction of γ-6 T-cells with oligodendrocytes results in selective cellular destruction, the release of α-β crystallin into circulation, the presentation of macrophages and T-cells, and the production of specific antibodies against myelin oligodendrocyte glycoprotein (MOG) and α-β crystallin (6-14).

[0024] The activated helper T-cells that are CD45RA (phenotype associated with memory or activated T-cells) accumulate in the brain and spinal cord of MS sufferers. These findings imply that activated T-cells, activated monocytes/macrophages and their cytokines have a special role in the pathogenesis of the disease (15-20). Activated T-helper cells release interleukin-2, interferon-γ and lymphotoxins, while monocytes release tumor necrosis factor-α (TNF-α). The monocytes are primed by T-cell-derived interferon-γ to release TNF-α. TNF-α and lymphotoxins have been reported to be injurious to myelin and oligodendrocytes. Indeed, it can be said that lymphotoxins or TNF-β can cause apoptosis of cultured oligodendrocytes (20-26). Thus, the liberation of toxic cytokines by monocytes and T-helper-1 cells, coupled with macrophage activation with release of free radicals, may ultimately culminate in the destruction of myelin in MS.

[0025] The Role of Th1/Th2 Cytokines, Microglia and Astrocytes in Regulating Immune Responses and the Development of Neuropathologies

[0026] T-helper-1 (Th1) and Th2 cells can be redefined as polarized forms of immune responses that not only represent a useful model for understanding the pathogenesis of several diseases, but also one that can provide the basis for the development of immunotherapeutic strategies. Mechanisms that regulate the balance of Th1 and Th2 cells, such as cytokines, are of great interest because they can determine the outcome of the disease. For example, interleukin-12 (1L-12) promotes the development of Th1 cells, whereas 1L-4 leads to the expansion of Th2 cells. In CNS inflammation, it has been shown that there might be a balance between microglia and astrocytes in regulating local immune reactions, including Th1/Th2 responses (21-24). This positive and negative regulation of Th1/Th2 by the microglia and astrocytes is shown in **FIG. 1**.

[0027] As shown in the **FIG. 1**, microglia produces IL-12, which primarily promotes the development of Th1 cells. Astrocytes cannot produce IL-12 and induce mainly Th2-cell responses, thereby representing important homeostatic mechanisms during recovery from Th1-mediated inflammation (21, 22,27-30).

[0028] The capacity of microglia and astrocytes to stimulate Th1 and Th2 cells depends on their surface molecules, such as MHC class II, B7 and CD40. MHC class II-positive microglia directly induce encephalitogenic myelin basic protein (MBP)-reactive $CD4^+$ T-cells to produce interferon-γ (IFN-γ) and TNF-α in vivo. After treatment with IFN-γ and/or bacterial antigens (LPS), microglia express CD40, which contributes to Th1 activation (31-33).

[0029] Th1 cells can stimulate microglia to produce prostaglandin E_2 (PGE_2), which provides a negative feedback mechanism for downregulation of Th1-cell responses within the CNS. During antigen presentation within the CNS, IFN-γ secreted by activated microglia and Th1 cells can induce astrocytes to secrete PGE_2 and contribute to the downregulation of microglia and Th1-cell responses (34,35).

[0030] Lymphocyte Reaction to Myelin and Other Neurologic Antigens

[0031] The major question, then, is "What triggers the influx of activated T-cells and monocytes into the CNS?" Considerations include a failure of immunoregulation between astrocytes and microglia that permits T-cells specific for myelin antigens to be induced and to enter the CNS (13). One way of examining this question is to study an

experimental animal model that resembles the human disease MS. EAE, an animal disease induced by immunization with spinal cord homogenate or myelin proteins or by the adoptive transfer of T-cells reactive to myelin antigens, shares many features with MS. The disease declares itself as an ascending paralysis, characterized by weakness of the tail, which is followed by paralysis of the hind limbs and the fore limbs (19-21). This adoptive transfer of EAE to healthy animals with sensitized lymphocytes from sick animals clearly indicates that neurologic, antigen-specific T-lymphocytes can actually induce disease. In fact, many investigations have shown that if myelin-specific CD4 Th1 type (which produces IL-2, IFN-γ, LT and TNF-α) is adoptively transferred to the naïve animal, EAE will be induced. Thus, the myelin antigen-specific CD4 T-cells are central to the initiation of demyelinating diseases (19,24,26).

[0032] Kinetic studies have shown that after the transfer of CD4, Th1 cells reactive to MBP are the first cells to infiltrate the central nervous system and are detected within four to five days after the transfer. As the lesion evolves, the MBP-specific CD4 Th1 cells constitute only between 1%-3% of the infiltrating cells, thereby indicating recruitment of other mononuclear cells. Activated lymphocyte to other myelin components, such as proteolipid protein (PLP), is equally important in the pathogenesis of demyelinating diseases (15-20).

[0033] In addition to Th1, Th2 and proinflammatory cytokines abnormalities and myelin antigen-specific CD4 T-cell evaluation, a number of other immune regulation abnormalities have been reported to occur in the blood and spinal fluid of MS patients. An increase in IgG and the occurrence of oligoclonal bands representing restricted populations of antibodies in the spinal fluid is a consistent finding. While the antigens with which the oligoclonal band antibodies react are not known, recent evidence has clearly identified antigens such as myelin basic protein, myelin oligodendrocyte glycoprotein and α-β crystallin against which the autoimmune response in MS is directed.

[0034] With immunogold-labeled peptides of myelin antigens and high-resolution microscopy, techniques that can detect antigen-specific antibodies in situ, scientists have identified autoantibodies specific for the central nervous system myelin antigen myclin/oligodendrocyte glycoprotein. These autoantibodies were specifically bound to disintegrating myelin around axons in lesions of acute multiple sclerosis and the marmoset model of allergic encephalomyelitis. These findings represent direct evidence that autoantibodies against a specific myelin protein mediate target membrane damage in central nervous system demyelinating disease (18-20).

[0035] In the complete collection of proteins extracted from MS-affected myelin, the dominant human antigen for CD4+T-cells appears to be α-β crystallin, a small heat shock protein. Enhanced levels of α-β crystallin are present in the cytosol of oligodendrocytes and astrocytes in MS lesions, where it is up-regulated at the earliest stages of lesional formation. After myelin phagocytosis in MS lesions, α-β crystallin becomes available to T-cells, suggesting the important role of this autoantigen in the pathogenesis of MS. The presentation of these antigens by T-cells to B-cells results in autoantibody production. It can therefore be said that IgG, IgM and IgA antibodies against myelin basic protein, myelin associated glycoprotein, myelin oligodendrocyte glycoprotein, proteolipid protein, phosphodiesterase, transaldolase, glutamate receptor, S-100 protein, small heat shock protein, such as α-β-crystallin, and other antigens, can aid in the diagnosis of MS and other demyelinating diseases.

[0036] Immunological Mechanisms of Injury in Multiple Sclerosis

[0037] Based on a review of the literature and results presented here, we propose that the following chain of events may lead to MS.

[0038] As a result of molecular mimicry and sequence homology between autoantigens and bacterial, viral or parasitic antigens, autoantibodies and autoreactive T-cells are generated in the blood. Under normal conditions, these autoreactive T-cells go through programmed cell death without causing any tissue damage, as shown in **FIG. 2**. However, for cross-reactive circulating T-cells and antibodies to become pathogenic, they can cross the blood-brain barrier.

[0039] Environmental factors such as stress, infections and toxic chemicals or their metabolites can disrupt the blood-brain barrier.

[0040] Viral particles, bacterial toxins, superantigens and reactive metabolites facilitate the movement and entrance of autoreactive T-cells and cross-reactive antibodies from the systemic circulation into the central nervous system.

[0041] In the central nervous system, the infectious agents antigens and toxic reactive metabolites up-regulate the expression of endothelial adhesion molecules, which further facilitates the entry of T-cells into the central nervous system.

[0042] Proteases, such as matrix metalloproteinases and others may further enhance the migration of autoreactive immune cells into the central nervous system by degrading extracellular-matrix macromolecules.

[0043] Through communication with macrophages, activated T-cells release significant amounts of proinflammatory cytokines, such as interferon-γ, tumor necrosis factor alpha and tumor necrosis factor beta.

[0044] Proinflammatory cytokines may directly damage the myelin sheath or up-regulate the expression of cell-surface molecules on neighboring lymphocytes and antigen-presenting cells.

[0045] Putative MS antigens, myelin basic protein, myelin proteolipid protein, myelin oligodendrocyte glycoprotein, myelin associated glycoprotein, α-β-crystallin phosphodiestrases and S-100 protein and other antigens are presented by macrophages with the help of MHC Class II, T-cell receptor and costimulatory molecules CD28-CTLA-4 to T-helper cells, which trigger enhanced immune response against one or all of MS antigens.

[0046] If this antigen presentation results in activation of T-helper cells and the production of proinflammatory cytokines, such as interferon-γ and TNF-α, it can trigger a cascade of events resulting in a proliferation of proinflammatory CD4 and T-helper-1 cells and ultimately cause further damage or injury to the myelin and oligodendrocytes.

[0047] Injury to the myelin and oligodendrocytes results in the proliferation of a significant amount of antigens into the circulation, which begins a vicious cycle of antibody (IgG, IgM, IgA) production against the MS antigens.

[0048] The binding of neuron-specific antibodies to myelin and oligodendrocytes and the formation of antigen-antibody complex with the involvement of complement cascades will induce antibody-dependent, cell-mediated cytotoxicity, apoptosis or death of neurons, which are observed as white spots in the MRI of the brain. A summary of these cellular and humoral immune mechanisms resulting in tissue damage is shown in **FIG. 3**.

[0049] This injury to the myelin membrane or the neurons results in axons that are no longer able to transit action potentials efficiently within the central nervous system. Blocking of the action potential results in the production of neurologic symptoms, which are detected by evoked responses (5).

[0050] Based on these immunological mechanisms, behind the injury to the neurons, it is possible to culture lymphocytes from patients with questionable MS and neurological antigens, and replicate a majority of these steps in a tissue culture environment. Only lymphocytes of MS patients, which possess prior memory of exposure to MS antigens in vivo, will be stimulated when they are exposed to MS antigens in the test tube. This will result in the production of a significant amount of proinflammatory cytokines, such as interferon-γ, TNF-α, TNF-β or all three cytokines.

[0051] Due to repeated injury to the neurons by cytokines, activated helper cells, macrophages, complement and proteases, neuron-specific antigens are released in the circulation. The release of these brain antigens and an initiation of immune response against them results in (IgG, IgM, IgA) antibodies in the blood of MS patients against one or all of the following MS antigens: myelin basic protein, myelin associated glycoprotein, myelin oligodendrocyte glycoprotein, proteolipid protein, phosphodiesterase, gangliosides, transaldolase, glutamate receptor, S-100 protein, glial fibrillary acidic protein, and small heat shock protein, such as α-β-crystallin.

[0052] The detection of a high percentage of lymphocytes reacting to MS antigen(s) and the production of a significant amount of proinflammatory cytokines in culture along with high levels of IgG, IgM or IgA antibodies against the neurologic antigen(s) will significantly enhance the sensitivity of MS detection.

[0053] The inventor has developed a laboratory test for diagnosing multiple sclerosis and other demyelinating diseases or predicting a predisposition to multiple sclerosis and other demyelinating diseases. The test utilizes detection of increased amounts of memory lymphocytes reacting to MS antigens, proinflammatory cytokines, and antibodies against MS antigens.

[0054] The preferred embodiment provides a method for diagnosing the likelihood and severity of a demyelinating disease in a patient, comprising the steps of: a) determining a level of antibodies against a neuron-specific antigen in a sample from the patient; b) comparing the level of antibodies determined in step a) with a normal level of the antibodies, wherein (i) normal level of antibodies for neuron-specific antigen indicate optimal conditions; (ii) lower than normal level of antibodies for neuron-specific antigen indicate absence of the demyelinating disease; and (iii) higher than normal level of antibodies for neuron-specific antigen indicate a likelihood of the demyelinating disease.

[0055] Another preferred embodiment provides a method for diagnosing the likelihood and severity of a demyelinating disease in a patient, comprising the steps of: a) isolating peripheral blood mononuclear cells (PBMCs) from the patient; b) incubating PBMCs with a neuronal antigen or peptide; c) measuring a concentration of cytokines resulting from step b); and d) comparing the concentration of cytokines determined from step c) with a normal level of cytokines, wherein (i) normal level of cytokines for the neuronal antigen or peptide indicate optimal conditions; (ii) lower than normal level of cytokines for the neuronal antigen or peptide indicate absence of the demyelinating disease; and (iii) higher than normal level of cytokines for the neuronal antigen or peptide indicate a likelihood of the demyelinating disease.

[0056] Another preferred embodiment provides a method for diagnosing the likelihood and severity of a demyclinating disease in a patient, comprising the steps of: a) isolating peripheral blood mononuclear cells (PBMCs) from the patient; b) incubating PBMCs with neuronal antigen or peptide; c) determining an amount of neuronal antigen- or peptide-specific activated T-cells or neuronal-specific memory lymphocytes resulting from step b); d) obtaining a stimulation index from step c); and e) comparing the stimulation index from step d) with a normal stimulation index, wherein (i) normal stimulation index indicates optimal conditions; (ii) lower than normal stimulation index indicates absence of the demyelinating disease; and (iii) higher than normal stimulation index indicates a likelihood of a demyelinating disease.

[0057] The laboratory tests are summarized in the following parts A-C, shown in Table 1.

TABLE 1

Part A: Test for memory lymphocytes reacting to MS antigens

1. Myelin lymphocyte immune function assay to myelin basic protein (MBP)
2. Myelin lymphocyte immune function assay to myelin basic protein peptides
3. Myelin lymphocyte immune function assay to myelin oligodendrocyte glycoprotein (MOG)
4. Myelin lymphocyte immune function assay to myelin oligodendrocyte glycoprotein peptides
5. Myelin lymphocyte immune function assay to myelin associated glycoprotein (MAG)
6. Myelin lymphocyte immune function assay to myelin associated glycoprotein peptides
7. Myelin lymphocyte immune function assay to proteolipid protein (PLP)
8. Myelin lymphocyte immune function assay to proteolipid protein peptides
9. Myelin lymphocyte immune function assay to small heat shock protein small heat shock protein, such as α-β-crystallin
10. Myelin lymphocyte immune function assay to transaldolase
11. Myelin lymphocyte immune function assay to transaldolase peptides
12. Myelin lymphocyte immune function assay to glial fibrillary acidic proteins (GFAP)
13. Myelin lymphocyte immune function assay to S-100 proteins
14. Myelin lymphocyte immune function assay to cross-reactive peptides from dietary proteins and infectious agents

TABLE 1-continued

15. Myelin lymphocyte immune function assay to glutamate receptor
16. Myelin lymphocyte immune function assay to phosphodiesterase

Part B: Test for proinflammatory cytokines

1. Production of interleukin-2 or T-helper-1 cytokine
2. Production of interferon-γ or T-helper-1 cytokine after stimulation of lymphocytes with neuron-specific antigens
3. Production of tumor necrosis factor alpha or proinflammatory cytokines after stimulation of lymphocytes with neuron-specific antigens
4. Production of tumor necrosis factor beta or lymphotoxin (proinflammatory cytokine) after stimulation of lymphocytes with neuron-specific antigens
5. Production of interleukin-12

Part C: Test for antibodies against MS antigens

1. Elevation of IgG, IgM, or IgA antibodies against myelin basic protein (MBP)
2. Elevation of IgG, IgM, or IgA antibodies against myelin basic protein peptides
3. Elevation of IgG, IgM, or IgA antibodies against myelin oligodendrocyte glycoprotein (MOG)
4. Elevation of IgG, IgM, or IgA antibodies against myelin oligodendrocyte glycoprotein peptides
5. Elevation of IgG, IgM, or IgA antibodies against myelin associated glycoprotein (MAG)
6. Elevation of IgG, IgM, or IgA antibodies against myelin associated glycoprotein peptides
7. Elevation of IgG, IgM, or IgA antibodies against proteolipid protein (PLP)
8. Elevation of IgG, IgM, or IgA antibodies against proteolipid protein peptides
9. Elevation of IgG, IgM, or IgA antibodies against small heat shock protein small heat shock protein, such as α-β-crystallin
10. Elevation of IgG, IgM, or IgA antibodies against transaldolase
11. Elevation of IgG, IgM, or IgA antibodies against transaldolase peptides
12. Elevation of IgG, IgM, or IgA antibodies against glial fibrillary acidic proteins (GFAP)
13. Elevation of IgG, IgM, or IgA antibodies against S-100 proteins
14. Elevation of IgG, IgM, or IgA antibodies against cross-reactive peptides from dietary proteins and infectious agents
15. Elevation of IgG, IgM, or IgA antibodies against glutamate receptor
16. Elevation of IgG, IgM, or IgA antibodies against phosphodiesterase

[0058] A normal baseline for the tests is obtained by averaging the results for activated T-cells or memory lymphocytes reacting to MS antigens, proinflammatory cytokines, or antibodies against MS antigens for individuals without symptoms relating to multiple sclerosis or other demyelinating diseases. Hence, if an individual exhibits a measurement for activated T-cells or memory lymphocytes reacting to MS antigens, proinflamrnatory cytokines, or antibodies against MS antigens above the baseline, the above-normal measurement indicates a presence or predisposition to multiple sclerosis and other demyelinating diseases. Preferably, a patient will show above normal measurements for activated T-cells or memory lymphocytes reacting to MS antigens, proinflammatory cytokines, or antibodies against MS antigens; more preferably, a patient will show measurements above about two standard deviations for activated T-cells or memory lymphocytes reacting to MS antigens, proinflammatory cytokines, or antibodies against MS antigens.

[0059] Presence or predisposition of multiple sclerosis results in significant levels of activated T-cells or memory lymphocytes reacting to MS antigens, proinflammatory cytokines, or antibodies against MS antigens. The antibodies can be present as IgG, IgM, or IgA.

[0060] The test methods of detection of increased amounts of activated T-cells or memory lymphocytes reacting to MS antigens, proinflammatory cytokines, and antibodies against MS antigens can be used to predict a predisposition to multiple sclerosis and other demyelinating diseases. Any test result showing above-normal measurements for activated T-cells or memory lymphocytes reacting to MS antigens, proinflammatory cytokines, or antibodies against MS antigens without symptoms or a clinical diagnosis shows a predisposition to multiple sclerosis or other demyelinating disease.

[0061] To test for antibodies to neuronal antigens, an immunoassay can be used immunoassays include, but are not limited to, ELISA test, RIA test, latex agglutination, beads assay, and proteomic assays. A preferable immunoassay is the ELISA test. Other immunoassays can be used and the choice of immunoassay can be determined by one of ordinary skill in the art.

[0062] To test for amount of lymphokines, a method can be selected from, but not limited to, the following: bioassay, immunoassay, flow cytometry, and RIA. Other methods can be used and the choice of method can be determined by one of ordinary skill in the art.

[0063] To test for amount of neuronal antigen- or peptide-specific activated T-cells or neuronal-specific memory lymphocytes, a method can be selected from, but not limited to, the following: flow cytometry and thymidine incorporation. Other methods can be used and the choice of method can be determined by one of ordinary skill in the art.

[0064] Furthermore, a combination of clinical test results with the tests for markers, such as activated T-cells or memory lymphocytes reacting to MS antigens, proinflammatory cytokines, and antibodies against MS antigens, can diagnose multiple sclerosis and other demyelinating diseases. Clinical test results can come from MRI, evoked response, and cerebrospinal fluid. For example, a combination of abnormal MRI and evoked response (even with normal cerebrospinal fluid) with activated T-cells or memory lymphocytes reacting to MS antigens and production of proinflammatory cytokines plus antibodies against MS antigens will support the clinical diagnosis of MS in more than 95% of patients, as shown in Table 2. Table 2 shows some possible combinations of test results using clinical data along with testing of markers, such as activated T-cells or memory lymphocytes reacting to MS antigens, proinflammatory cytokines, or antibodies against MS antigens.

TABLE 2

Combination of MRI, Evoked Response with Memory Lymphocytes, Proinflammatory Cytokines and Neuron-Specific Antibodies for the Diagnosis of Multiple Sclerosis

MRI	Evoked Response	Cerebrospinal Fluid	Memory Lymphocytes	Proinflammatory Cytokines	Specific Antibodies	Diagnosis
Zero Positive				Not Tested		Normal
One or Two Positive				Not Tested		Possible MS
All three Positive				Not Tested		Possible MS
Zero Positive				Zero Positive		Normal
One or Two Positive				Zero Positive		Possible MS

TABLE 2-continued

Combination of MRI, Evoked Response with Memory Lymphocytes, Proinflammatory Cytokines and Neuron-Specific Antibodies for the Diagnosis of Multiple Sclerosis

MRI	Evoked Response	Cerebro-spinal Fluid	Memory Lympho-cytes	Proinflam-matory Cytokines	Specific Anti-bodies	Diag-nosis
	All three Positive			Zero Positive		Possible MS
	Zero Positive			One Positive		Neuro-immune
	One Positive			One Positive		Possible MS
	Two Positive			One Positive		MS
	Three Positive			One Positive		MS
	Zero Positive			Two Positive		Neuro-immune
	One Positive			Two Positive		Early MS
	Two Positive			Two Positive		MS
	Three Positive			Two Positive		Definite MS
	Zero Positive			Three Positive		Neuro-immune or very early MS
	One Positive			Three Positive		Early MS
	Two Positive			Three Positive		Definite MS
	Three Positive			Three Positive		Definite MS

[0065] The disclosure below is of specific examples setting forth preferred methods for the preferred embodiments. These examples are not intended to limit the scope, but rather to exemplify preferred embodiments.

EXAMPLE 1

[0066] Materials and Methods

[0067] Blood samples from twenty subjects (8 males and 12 females) 32-48 years of age with abnormal MRI and evoked potential and diagnosis of possible MS were sent by different clinicians to our laboratory for neuroimmunological examination. For comparison, blood samples from 40 healthy, age- and sex-matched controls were included in this study.

[0068] Myelin basic protein (MBP), myelin associated glycoprotein (MAG), proteolipid protein (PLP), transaldolase, α-β-crystallin, and S-100 proteins were purchased from SIGMA (St. Louis, Mo.). Glial Fibrillary Acidic Protein (GFAP) was purchased from Boeringer Mannheim.

[0069] The following peptides were purchased from Research Genetics (Huntsville, Ala.):

```
Human MBP Peptides:
  87–106  VVHFFKNLVTPRTPPPSQGK        (SEQ ID NO:1)

  83–89   ENPVVHFFKNIVTPRTP           (SEQ ID NO:2)
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   1–11   ASQKRPSQRSK                 (SEQ ID NO:3)

 200–211  ANMQRQAVPTL                 (SEQ ID NO:4)
Other peptides from 1–250 AA

Proteolipid Protein Peptides
  40–60   TGTEKLIETYFSKNYQDYEYL       (SEQ ID NO:5)

  89–106  GFYTTGAVRQIIFGDYKTT         (SEQ ID NO:6)

 103–120  YKTTICGKGLSATVTGGQ          (SEQ ID NO:7)

 125–143  SRGQHQAHSLERVCHCLGK         (SEQ ID NO:8)

 139–154  HCLGKWLGHPDKFVGI            (SEQ ID NO:9)
Other peptides from 1–250 AA

Transaldolase Peptides
  11–25   MESALDQLKQFTTVV             (SEQ ID NO:10)

  21–35   ETTVVADTGDFHAID             (SEQ ID NO:11)

  31–45   FHAIDEYKPQDATTN             (SEQ ID NO:12)

  71–85   KLGGSQEDQIKNAID             (SEQ ID NO:13)

  81–95   KNAIDKLFVLFGAEI             (SEQ ID NO:14)

 261–275  GELLQDNAKLVPVLS             (SEQ ID NO:15)

 271–285  VPVLSAKAAQASDLE             (SEQ ID NO:16)

 311–325  GIRKFAADAVKLERM             (SEQ ID NO:17)
Other peptides from 1–337 AA

Myelin Oligodendrocyte Glycoprotein Peptides
   1–20   GQFRVIGPRHPIRALVGDEV        (SEQ ID NO:18)

  61–80   QAPEYRGRTELLKDAIGEGK        (SEQ ID NO:19)

 101–120  RDHSYQEEAAMELKVEDPFY        (SEQ ID NO:20)

 145–160  VFLCLQYRLRGKLRAE            (SEQ ID NO:21)
Other peptides from 1–218 AA

Myelin Associated Glycoprotein Peptides
  37–60   REIVDRKYSICKSGCFYQKKEEDW    (SEQ ID NO:22)
Other peptides from 1–81 AA
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EXAMPLE 2

[0070] Enzyme-Linked Immunosorbent Assay (ELISA) Procedure

[0071] Enzyme-linked immunosorbent assay (ELISA) was used for testing antibodies against different neuron-specific antigens in the sera of patients with possible MS and control subjects. Antigens or peptides were dissolved in methanol at a concentration of 1.0 mg/ml, then diluted 1:100 in 0.1 M carbonate-bicarbonate buffer, pH 9.5, and 50 μl were added to each well of a polystyrene flat-bottom ELISA plate. Plates were incubated overnight at 4° C. and then washed three times with 20 mm tris-buffered saline (TBS) containing 0.05% Tween 20, pH 7.4. The nonspecific binding of immunoglobulins was prevented by adding a mixture of 1.5% bovine serum albumin (BSA) and 1.5% gelatin in TBS, and then incubating for 2 h at room temperature, and then overnight at 4° C. Plates were washed as in the above, and then serum samples diluted 1:100 in 1% BSA-TBS were added to duplicate wells and incubated for 2 h at room temperature. Sera from patients with multiple sclerosis, polyneuropathies and other neurological disorders with known high titers of IgG, IgM and IgA against different

neurological antigens were used to rule out non-specific antibody activities of inter- and intra-assay variability. Plates were washed, and then peroxidase-conjugated goat anti-human IgG, IgM or IgA antiserum (KPI, Gaithersburg, Md.) diluted 1:400 in 1% BSA-TBS was added to each well; the plate was incubated for an additional 2 h at room temperature. After washing five times with TBS-Tween buffer, the enzyme reaction was started by adding 100 μl of o-phenylene diamine in citrate-phosphate buffer, pH 5.0 and hydrogen peroxide diluted 1:10,000. After 45 min, the reaction was stopped with 50 μl of 2 N H_2SO_4. The optical density (O.D.) was read at 492 nm by means of a microtiter reader. Several control wells containing all reagents, but human serum, were used for detecting nonspecific binding.

EXAMPLE 3

[0072] Detection of Neurologic Antibodies

[0073] Using ELISA assays, sera from 20 healthy subjects and 20 patients with possible MS were analyzed for the presence of IgG, IgM, and IgA antibodies against three neuron-specific antigens. The ELISA results expressed as mean O.D. at 492 nm are summarized in Table 3. The O.D. for IgG antibody values obtained with 1:100 dilution of healthy control sera ranged from 0.03 to 0.78, varying among subjects and antigens. The mean±standard deviation (S.D.) of these O.D. values, as shown in Table 3, ranged from 0.15±0.06 to 0.19±0.16. The corresponding IgG O.D. values from MS patients sera ranged from 0.06 to 2.27 and with the mean±S.D. of IgG values, which ranged from 0.58±0.49 to 0.75±0.73. For all three antigens, the differences between mean±S.D. of control sera and MS patients sera were highly significant ($p<0.001$). At a cutoff value of 2 S.D. above the mean of control values, levels of IgG antibody against these antigens were calculated in control and patients sera and found that while 0-5% of control sera had IgG values higher than 2 S.D. of controls, the MS group showed elevated IgG values from 40 to 55% ($p<0.001$) (**FIG. 5**).

[0074] Levels of IgM antineuron-specific antigens in sera of healthy controls and patients with MS are shown in Table 3. These serum IgM antibodies against all three different tested antigens were significantly higher in patients than in controls. The mean±S.D. for controls ranged from 0.14±0.04 to 0.17±0.10 O.D. and for patients ranged from 0.35±0.29 to 0.47±0.39 O.D. ($p<0.001$). When the 2 S.D. mean of controls was used as a cut-off point, 0 to 10% of controls versus 35 to 60% of MS patients sera showed elevated IgM antibody levels ($p<0.001$) (**FIG. 5**). Likewise, IgA antibody levels against these neurological antigens were examined in both groups. Individual and mean±S.D. data depicted in Table 3 showed significant differences between control and patients groups. The mean±S.D. for IgA antibody levels in controls ranged from 0.12±0.06 to 0.17±0.12 and in patients, from 0.44±0.46 to 0.48±0.42 ($p<0.001$). Percent elevated serum IgA anti-neuronal autoantibodies at the O.D. value of greater than 2 S.D. of mean controls, was significantly higher in MS patients than in controls. The percent positive for IgA antibodies in controls ranged from 0 to 10% and in patients 50-55% ($p<0.001$) (**FIG. 5**).

TABLE 3

Measurement of Antibodies against Neuron-Specific Antigens in Controls and Patients with Multiple Sclerosis Expressed by ELISA Optical Densities.

	Myelin Basic Protein					
Specimen	IgG		IgM		IgA	
#	C	P	C	P	C	P
1	0.15	0.87	0.21	0.32	0.11	0.94
2	0.11	0.23	0.15	0.37	0.24	0.19
3	0.24	0.17	0.18	0.19	0.23	0.20
4	0.05	1.53	0.18	0.99	0.08	1.23
5	0.17	0.06	0.13	0.27	0.18	0.31
6	0.03	1.28	0.21	0.80	0.15	0.57
7	0.09	0.20	0.14	0.21	0.08	0.36
8	0.17	1.95	0.08	0.61	0.05	0.58
9	0.36	0.12	0.17	0.21	0.09	0.23
10	0.20	0.35	0.12	1.85	0.07	1.24
11	0.12	0.27	0.16	0.34	0.09	0.21
12	0.15	0.13	0.17	0.24	0.16	0.88
13	0.28	0.89	0.12	0.59	0.19	0.42
14	0.78	0.25	0.07	0.32	0.15	0.06
15	0.04	1.98	0.12	0.63	0.02	0.18
16	0.15	0.27	0.09	0.19	0.11	0.37
17	0.18	0.26	0.18	0.34	0.15	0.20
18	0.03	0.15	0.09	0.06	0.14	0.25
19	0.32	2.27	0.05	0.26	0.08	0.87
20	0.24	1.81	0.15	0.35	0.20	0.24
Mean	0.19	0.75	0.14	0.46	0.13	0.47
±	±	±	±	±	±	±
S.D.	0.16	0.73	0.04	0.40	0.06	0.36
	Myelin Oligodendrocytes					
Specimen	IgG		IgM		IgA	
#	C	P	C	P	C	P
1	0.22	0.36	0.18	0.15	0.12	0.54
2	0.16	1.72	0.23	0.95	0.25	0.17
3	0.15	0.16	0.29	0.24	0.18	0.15
4	0.23	0.34	0.16	0.41	0.09	0.89
5	0.17	1.76	0.09	0.35	0.16	0.98
6	0.20	0.13	0.19	1.62	0.53	0.27
7	0.46	0.18	0.15	0.22	0.14	0.32
8	0.14	0.26	0.12	0.31	0.11	0.38
9	0.15	0.12	0.18	0.34	0.19	0.27
10	0.11	0.23	0.05	1.41	0.14	1.89
11	0.16	1.52	0.12	0.31	0.12	0.91
12	0.07	0.75	0.11	0.64	0.06	0.36
13	0.05	0.92	0.18	0.61	0.21	0.83
14	0.13	0.22	0.15	0.19	0.44	0.13
15	0.28	1.34	0.49	0.21	0.15	0.36
16	0.03	0.22	0.24	0.17	0.13	0.41
17	0.07	0.09	0.13	0.28	0.08	0.27
18	0.16	0.35	0.14	0.33	0.05	0.15
19	0.05	1.61	0.35	0.45	0.09	0.25
20	0.09	0.98	0.02	0.24	0.14	0.20
Mean	0.15	0.66	0.17	0.47	0.17	0.48
±	±	±	±	±	±	±
S.D.	0.09	0.60	0.10	0.39	0.12	0.42
	α-β-Crystallin					
Specimen	IgG		IgM		IgA	
#	C	P	C	P	C	P
1	0.26	0.29	0.17	0.38	0.14	0.53
2	0.18	0.12	0.26	0.22	0.31	0.22
3	0.14	0.23	0.13	0.07	0.12	0.15
4	0.10	1.35	0.04	0.13	0.17	0.87
5	0.11	0.38	0.15	0.26	0.19	0.32
6	0.13	0.24	0.11	0.32	0.17	0.09

TABLE 3-continued

Measurement of Antibodies against Neuron-Specific Antigens in Controls and Patients with Multiple Sclerosis Expressed by ELISA Optical Densities.

7	0.18	0.51	0.19	0.98	0.02	1.31
8	0.21	0.27	0.56	0.20	0.14	0.11
9	0.12	0.29	0.08	0.17	0.04	0.36
10	0.09	1.15	0.18	0.24	0.06	0.15
11	0.05	0.36	0.21	0.35	0.15	0.86
12	0.14	0.28	0.08	0.24	0.13	0.18
13	0.23	1.34	0.16	0.69	0.11	0.27
14	0.18	0.27	0.12	0.14	0.08	0.22
15	0.11	0.98	0.18	0.26	0.09	0.33
16	0.08	0.36	0.16	0.25	0.17	0.45
17	0.29	1.87	0.14	1.24	0.02	1.94
18	0.11	0.09	0.12	0.34	0.07	0.24
19	0.18	0.89	0.03	0.33	0.14	0.28
20	0.10	0.47	0.24	0.26	0.11	0.09
Mean	0.15	0.58	0.16	0.35	0.12	0.44
±	±	±		±	±	±
S.D.	0.06	0.49	0.11	0.29	0.06	0.46

EXAMPLE 4

[0075] Assay Variation of IgG, IgM, IgA

[0076] Coefficients of interassay variation were calculated by running five samples eight times in one assay. Coefficients of interassay variation were determined by measuring the same samples in six consecutive assays. This replicate testing established the validity of the ELISA assays, determined the appropriate dilution with minimal background and detected serum IgG, IgM and IgA against different antigens. Two sera from healthy controls, two nonspecific sera from MS patients and two sera from autistic children were used to construct standard control curves. These sera were diluted 1:25, 1:50, 1:100, 1:200 and 1:400. At dilutions of 1:50-1:200, the standard curve for MS sera was linear and antibodies from healthy controls were not detected against the three tested antigens. Coefficients of intra-assay variations for IgG, IgM, and IgA against the three antigens were less than 8%. Coefficients of interassay variations were less than 10%.

EXAMPLE 5

[0077] Lymphocyte Proliferation Assay and Cytokine Production

[0078] Peripheral blood mononuclear cells (PBMCs) were isolated from blood drawn in ACD yellow top tubes by Ficoll Density Centrifugation (SIGMA, St. Louis, Mo.). PBMCs were incubated at a cell density of 1×10^6/ml in complete RPMI alone or in complete RPMI (CRPMI) containing different neuronal antigens or peptides, at a final concentration of 10 μg/ml. After 48 hours incubation at 37° C., the contents of each well was transferred to a separate tube and centrifuged at 1,500 g. The cells were labeled with CD25+CD69 monoclonal antibodies and % antigen-specific CD3 activated T-cells were measured by flow cytometry (Becton Dickinson FacScan). The stimulation index was calculated by dividing the reactive well containing cells+antigen by controls containing only cells in complete medium. Supernatant was removed and used for measurement of TH_1 (IL-2, IFN-γ), TH_2 (IL-4, IL-10) and proinflammatory cytokines (TNF-α and TNF-β). Cytokine concentrations were measured in picograms per ml of cell culture supernatants by ELISA, using kits manufactured by Biosourse International (Camarillo, Calif.). A summary of this procedure for the measurement of neuronal antigen-activated lymphocyte and cytokine production is shown in **FIG. 4**.

EXAMPLE 6

[0079] Detection of Neurological Antigen-Specific Reactive T-Cells

[0080] MBP, MOG and α-β-crystallin reactive T-cells were tested in a proliferation assay. Histogram of two controls with 3% and 6% and two patients with 20% and 18% of MBP-reactive T-cells are shown in **FIG. 6**. The percentage of reactive T-cells of controls and patients cultured in medium alone or medium+MBP, medium+MOG and medium+α-β-crystallin are shown in Table 4. Comparison of individual values of controls and patients with MS, showed significant differences in their lymphocyte reactivity without antigenic stimulation. The mean±S.D. of this spontaneous T-cell reactivity in controls was 4.2±2.2 and for patients, 8.6±3.4 ($p<0.05$).

[0081] The percentage of MBP, reactive T-cells of controls ranged from 1-12% with mean±S.D. of 5.0±2.4; MOG was 2-9% with mean±S.D. of 4.9+2.1; and α-β-crystallin was 1-8% at 4.2±1.8. The corresponding values in MS patients ranged from 4-35% with mean±S.D. of 18.4±9.8 for MBP; MOG was 6-27% with mean±S.D. of 15.1±6.4; and α-β-crystallin was 5-21% at 10.7±4.5. The differences between lymphocyte reactivity to all tested neurological antigens in controls and MS patients were highly significant ($P<0.001$). The pattern of lymphocyte reactivity varied from antigen to antigen in different patients (Table 4). Some reacted to none of the antigens, ore reacted only to MBP or to a combination of MBP+MOG, MBP+α-β-crystallin or to MBP+MOG+α-β-crystallin.

TABLE 4

Percent Memory Lymphocyte Immune Stimulation Assay in Medium Alone (M) or M + MBP, M + MOG and M + α-β-crystallin in Controls (C) and Patients (P) with Multiple Sclerosis, Performed by Culture and Flow Cytometry

Specimen	Medium (M)		M + MBP		M + MOG		M + α-β-crystallin	
#	C	P	C	P	C	P	C	P
1	2.0	9.0	3.0	20.0	5.0	14.0	6.0	18
2	5.0	11.0	6.0	18.0	4.0	17.0	2.0	9.0
3	3.0	12.0	4.0	27.0	6.0	21.0	5.0	15.0
4	5.0	13.0	7.0	25.0	2.0	16.0	4.0	11.0
5	2.0	5.0	4.0	8.0	3.0	6.0	2.0	5.0
6	1.0	6.0	3.0	7.0	4.0	8.0	1.0	7.0
7	3.0	15.0	5.0	35.0	2.0	27.0	6.0	14.0
8	6.0	10.0	12.0	28.0	8.0	14.0	4.0	12.0
9	4.0	5.0	8.0	6.0	7.0	9.0	8.0	10.0
10	7.0	6.0	5.0	15.0	9.0	18.0	6.0	11.0
11	1.0	8.0	4.0	21.0	3.0	17.0	5.0	16.0
12	3.0	4.0	2.0	13.0	5.0	15.0	4.0	12.0
13	5.0	12.0	6.0	29.0	8.0	23.0	2.0	14.0
14	2.0	7.0	5.0	17.0	6.0	11.0	3.0	8.0
15	6.0	9.0	3.0	16.0	4.0	10.0	5.0	6.0
16	5.0	4.0	4.0	10.0	5.0	9.0	3.0	7.0
17	3.0	11.0	1.0	33.0	2.0	24.0	4.0	21.0
18	4.0	5.0	6.0	5.0	7.0	9.0	5.0	8.0
19	9.0	14.0	8.0	30.0	6.0	26.0	7.0	6.0

TABLE 4-continued

Percent Memory Lymphocyte Immune Stimulation Assay in Medium Alone (M) or M + MBP, M + MOG and M + α-β-crystallin in Controls (C) and Patients (P) with Multiple Sclerosis, Performed by Culture and Flow Cytometry

Specimen	Medium (M)		M + MBP		M + MOG		M + α-β-crystallin	
#	C	P	C	P	C	P	C	P
20	8.0	6.0	5.0	4.0	3.0	8.0	2.0	5.0
Mean	4.2	8.6	5.0	18.4	4.9	15.1	4.2	10.7
±	±	±	±	±	±	±	±	±
S.D.	2.2	3.4	2.4	9.8	2.1	6.4	1.8	4.5

C = control
P = patient

EXAMPLE 7

[0082] Cytokine Production

[0083] Cytokine production of cell culture supernatants from MBP-reactive T-cells were determined by ELISA and expressed by picogrars/ml. This pattern of cytokine production in supernatants of two controls and two MS patients is illustrated in **FIG. 6** and 20 controls and 20 MS patients in Table 5. As shown in **FIG. 7**, patient 1 produced significant levels of TNF-α and IFN-γ while patient 2 produced high levels of TNF-α but not IFN-γ. Furthermore, analysis of cytokine levels in all 20 controls and patients, showed TNF-α first, with mean±S.D. of 24.7±15.0, then IFN-γ with mean±S.D. of 20±16.6 and TNF-levels with mean±S.D. of 13.8±10.4. Production of these cytokines by activated T-cells was significantly above the background levels produced by controls lymphocytes ($p<0.001$). For IL-2, 1L-4 and IL-10, the differences between controls and patients were not significant (Table 5). The percent of elevated cytokine production by different MS patients and controls at 2 S.D. above the mean values of controls, were analyzed and found to be significantly higher in MS patients than in controls. The percent of elevation for TNF-β, TNF-α, and IFN-γ production in controls ranged from 5-10%, and in patients was at 40%, 70% and 75%, respectively **(FIG. 7)**.

TABLE 5

Measurement of T-helper-1/T-helper-2 and Proinflammatory Cytokines after 48 Hours Culture of Human Lymphocytes with Myelin-Basic Protein, Myelin-Oligodendrocytes and α-B-Crystallin Expressed by picogram/ml.

Specimen	Interleukin-2		Interferon-γ		Interleuken-4	
#	C	P	C	P	C	P
1	9.0	10.0	4.0	34.0	6.0	8.0
2	2.0	1.0	1.0	4.0	3.0	10.0
3	5.0	11.0	4.0	8.0	4.0	7.0
4	3.0	6.0	3.0	28.0	8.0	10.0
5	10.0	16.0	3.0	51.0	3.0	7.0
6	1.0	9.0	7.0	11.0	6.0	4.0
7	6.0	8.0	2.0	27.0	9.0	14.0
8	5.0	2.0	1.0	9.0	5.0	3.0
9	7.0	5.0	2.0	36.0	8.0	6.0
10	3.0	1.0	3.0	12.0	4.0	9.0
11	6.0	8.0	10.0	9.0	3.0	7.0
12	2.0	5.0	8.0	19.0	5 0	4.0
13	3.0	7.0	3.0	6.0	3.0	2.0

TABLE 5-continued

Measurement of T-helper-1/T-helper-2 and Proinflammatory Cytokines after 48 Hours Culture of Human Lymphocytes with Myelin-Basic Protein, Myelin-Oligodendrocytes and α-B-Crystallin Expressed by picogram/ml.

14	8.0	10.0	1.0	44.0	9.0	12.0
15	5.0	9.0	6.0	2.0	3.0	15.0
16	1.0	14.0	3.0	4.0	2.0	7.0
17	12.0	18.0	7.0	31.0	6.0	4.0
18	2.0	8.0	2.0	16.0	5.0	9.0
19	7.0	6.0	3.0	6.0	7.0	13.0
20	4.0	15.0	11.0	58.0	8.0	10.0
Mean	5.0	8.4	4.0	20.7	5.6	8.0
±	±	±	±	±	±	±
S.D.	2.8	4.2	3.0	16.6	2.2	3.8

C = control
P = patient

Specimen	Interleukin-10		TNF-α		TNF-β	
#	C	P	C	P	C	P
1	3.0	2.0	7.0	28.0	3.0	8.0
2	2.0	4.0	3.0	30.0	7.0	5.0
3	1.0	1.0	3.0	3.9	5.0	18.0
4	2.0	4.0	3.0	26.0	9.0	41.0
5	3.0	1.0	9.0	7.0	8.0	23.0
6	5.0	7.0	6.0	26.0	4.0	17.0
7	4.0	2.0	12.0	41.0	2.0	14.0
8	5.0	1.0	4.0	16.0	6.0	12.0
9	3.0	6.0	3.0	47.0	16.0	27.0
10	8.0	4.0	5.0	33.0	8.0	15.0
11	7.0	16.0	2.0	7.0	7.0	3.0
12	6.0	4.0	8.0	22.0	5.0	14.0
13	10.0	8.0	3.0	6.0	4.0	29.0
14	3.0	4.0	9.0	38.0	11.0	5.0
15	2.0	5.0	4.0	29.0	6.0	3.0
16	1.0	3.0	1.0	12.0	7.0	10.0
17	6.0	4.0	16.0	33.0	5.0	21.0
18	2.0	13.0	4.0	15.0	14.0	6.0
19	4.0	9.0	2.0	5.0	8.0	3.0
20	6.0	5.8	7.0	38.0	7.0	2.0
Mean	4.1	5.2	5.5	24.7	7.1	13.8
±	±	±	±	±	±	±
S.D.	2.4	3.9	3.7	15.0	3.4	10.4

C = control
P = patient

[0084] In this analysis, IFN-γ, TNF-α, and TNF-β were considered to be produced by TH_1 cells, IL-4 by TH_2 cells, and IL-10 by both subsets, except at lower levels in which case they are produced by TH_1 cells. TH_0 cells produce both IL-4 and IFN-Y. Compared with unaffected individuals, the MBP-reactive T-cells in MS patients exhibited TH_1 cytokine profiles (Table 5 and **FIG. 7**).

[0085] Many modifications and variations of the embodiments described herein may be made without departing from the scope, as is apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only.

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[0126]

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What is claimed is:

1. A method for diagnosing the likelihood and severity of a demyelinating disease in a patient, comprising the steps of:

a) determining a level of antibodies against a neuron-specific antigen in a sample from the patient;

b) comparing the level of antibodies determined in step a) with a normal level of the antibodies, wherein

(i) normal level of antibodies for neuron-specific antigen indicate optimal conditions;

(ii) lower than normal level of antibodies for neuron-specific antigen indicate absence of the demyelinating disease; and

(iii) higher than normal level of antibodies for neuron-specific antigen indicate a likelihood of the demyelinating disease.

2. The method according to claim 1, wherein the demyelinating disease is multiple sclerosis.

3. The method according to claim 1, wherein the normal, level of antibodies is calculated by taking a mean of levels of antibodies in individuals without symptoms relating the demyelinating disease.

4. The method according to claim 1, wherein the higher than normal level of antibodies is higher than about two standard deviations of normal level of antibodies of a control group.

5. The method according to claim 1, wherein the neuron-specific antigen is selected from the group consisting of myelin basic protein, myelin basic protein peptide, myelin oligodendrocyte glycoprotein, myelin oligodendrocyte glycoprotein peptide, myelin associated glycoprotein, myelin associated glycoprotein peptide, proteolipid protein, proteolipid protein peptide, small heat shock protein, transaldolase, transaldolase peptide, glial fibrillary protein, S-100 protein, cross-reactive peptide from dietary protein, cross-reactive peptide from infectious agent, glutamate receptor, and phosphodiesterase.

6. The method according to claim 5, wherein the myelin basic protein peptide contains a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4.

7. The method according to claim 5, wherein the proteolipid protein peptide contains a sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9.

8. The method according to claim 5, wherein the transaldolase peptide contains a sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

9. The method according to claim 5, wherein the myelin oligodendrocyte glycoprotein peptide contains a sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.

10. The method according to claim 5, wherein the myelin associated glycoprotein peptide contains SEQ ID NO:22.

11. The method according to claim 1, wherein determining the level of antibodies in any or all of steps a) and b) is accomplished using an immunoassay.

12. The method according to claim 11, wherein the immunoassay is an ELISA test.

13. The method according to claim 1, wherein the antibodies are selected from the group consisting of IgG, IgA, and IgM.

14. A method for diagnosing the likelihood and severity of a demyelinating disease in a patient, comprising the steps of:

a) isolating peripheral blood mononuclear cells (PBMCs) from the patient;

b) incubating PBMCs with a neuronal antigen or peptide;

c) measuring a concentration of cytokines resulting from step b); and

d) comparing the concentration of cytokines determined from step c) with a normal level of cytokines, wherein

(i) normal level of cytokines for the neuronal antigen or peptide indicate optimal conditions;

(ii) lower than normal level of cytokines for the neuronal antigen or peptide indicate absence of the demyelinating disease; and

(iii) higher than normal level of cytokines for the neuronal antigen or peptide indicate a likelihood of the demyelinating disease.

15. The method according to claim 14, wherein the demyelinating disease is multiple sclerosis.

16. The method according to claim 14, wherein the normal level of cytokines is calculated by taking a mean of levels of cytokines in individuals without symptoms relating to the demyelinating disease.

17. The method according to claim 14, wherein the higher than normal level of cytokines is higher than about two standard deviations of normal level of cytokines of a control group.

18. The method according to claim 14, wherein cytokine is selected from the group consisting of T-helper-1 cytokine and proinflammatory cytokine, interleukin-2, interferon-γ, tumor necrosis factor alpha, tumor necrosis factor beta, and interleukin-12.

19. The method according to claim 18, wherein the T helper-1 cytokine, interferon-γ, tumor necrosis factor alpha, proinflammatory cytokine, tumor necrosis factor beta, or lymphotoxin are produced after stimulation of lymphocytes with neuron-specific antigens.

20. The method according to claim 14, wherein determining the level of cytokines is accomplished using a method selected from the group consisting of bioassay, immunoassay, flow cytometry, and RIA.

21. The method according to claim 20, wherein the immunoassay is an ELISA test.

22. A method for diagnosing the likelihood and severity of a demyelinating disease in a patient, comprising the steps of:

a) isolating peripheral blood mononuclear cells (PBMCs) from the patient;

b) incubating PBMCs with neuronal antigen or peptide;

c) determining an amount of neuronal antigen- or peptide-specific activated T-cells or neuronal-specific memory lymphocytes resulting from step b);

d) obtaining a stimulation index from step c); and

e) comparing the stimulation index from step d) with a normal stimulation index, wherein

(i) normal stimulation index indicates optimal conditions;

(ii) lower than normal stimulation index indicates absence of the demyelinating disease; and

(iii) higher than normal stimulation index indicates a likelihood of a demyelinating disease.

23. The method according to claim 22, wherein the demyelinating disease is multiple sclerosis.

24. The method according to claim 22, wherein the normal stimulation index is calculated by taking a mean of stimulation indices in individuals without symptoms relating to the demyelinating disease.

25. The method according to claim 22, wherein the higher than normal stimulation index is higher than about two standard deviations of normal stimulation index of a control group.

26. The method according to claim 22, wherein the neuron-specific antigen is selected from the group consisting of myelin basic protein, myelin basic protein peptide, myelin oligodendrocyte glycoprotein, myelin oligodendrocyte glycoprotein peptide, myelin associated glycoprotein, myelin associated glycoprotein peptide, proteolipid protein, proteolipid protein peptide, small heat shock protein, transaldolase, transaldolase peptide, glial fibrillary protein, S-100 protein, cross-reactive peptide from dietary protein, cross-reactive peptide from infectious agent, glutamate receptor, and phosphodiesterase.

27. The method according to claim 26, wherein the myclin basic protein peptide contains a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4.

28. The method according to claim 26, wherein the proteolipid protein peptide contains a sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9.

29. The method according to claim 26, wherein the transaldolase peptide contains a sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17.

30. The method according to claim 26, wherein the myelin oligodendrocyte glycoprotein peptide contains a sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.

31. The method according to claim 26, wherein the myelin associated glycoprotein peptide contains SEQ ID NO:22.

32. The method according to claim 22, wherein determining the stimulation index is obtained by a method of flow cytometry or thymidine incorporation.

33. The method according to claim 32, wherein the stimulation index is determined by antigen-specific CD3 activated T-cells.

34. A method for diagnosing the likelihood and severity of a demyelinating disease in a patient, comprising the steps of:

a) determining a level of antibodies against a neuron-specific antigen in a sample from the patient;

b) comparing the level of antibodies determined in step a) with a normal level of the antibodies, wherein

(i) normal level of antibodies for neuron-specific antigen indicate optimal conditions;

(ii) lower than normal level of antibodies for neuron-specific antigen indicate absence of the demyelinating disease; and

(iii) higher than normal level of antibodies for neuron-specific antigen indicate a likelihood of the demyelinating disease;

further comprising performing all of the steps of the method of claim 14.

35. A method for diagnosing the likelihood and severity of a demyelinating disease in a patient, comprising the steps of:

a) determining a level of antibodies against a neuron-specific antigen in a sample from the patient;

b) comparing the level of antibodies determined in step a) with a normal level of the antibodies, wherein

(i) normal level of antibodies for neuron-specific antigen indicate optimal conditions;

(ii) lower than normal level of antibodies for neuron-specific antigen indicate absence of the demyelinating disease; and

(iv) higher than normal level of antibodies for neuron-specific antigen indicate a likelihood of the demyelinating disease;

further comprising performing all of the steps of the method of claim 22.

36. A method for diagnosing the likelihood and severity of a demyelinating disease in a patient, comprising the steps of:

a) isolating peripheral blood mononuclear cells (PBMCs) from the patient;

b) incubating PBMCs with a neuronal antigen or peptide;

c) measuring a concentration of cytokines resulting from step b); and

d) comparing the concentration of cytokines determined from step c) with a normal level of cytokines, wherein

(i) normal level of cytokines for the neuronal antigen or peptide indicate optimal conditions;

(ii) lower than normal level of cytokines for the neuronal antigen or peptide indicate absence of the demyelinating disease; and

(iii) higher than normal level of cytokines for the neuronal antigen or peptide indicate a likelihood of the demyelinating disease;

further comprising performing all of the steps of the method of claim 22.

37. A method for diagnosing the likelihood and severity of a demyelinating disease in a patient, comprising the steps of:

a) determining a level of antibodies against a neuron-specific antigen in a sample from the patient;

b) comparing the level of antibodies determined in step a) with a normal level of the antibodies, wherein

(i) normal level of antibodies for neuron-specific antigen indicate optimal conditions;

(ii) lower than normal level of antibodies for neuron-specific antigen indicate absence of the demyelinating disease; and

(v) higher than normal level of antibodies for neuron-specific antigen indicate a likelihood of the demyclinating disease;

further comprising the method comprising the steps of:

c) isolating peripheral blood mononuclear cells (PBMCs) from the patient;

d) incubating PBMCs with a neuronal antigen or peptide;

e) measuring a concentration of cytokines resulting from step d); and

f) comparing the concentration of cytokines determined from step e) with a normal level of cytokines, wherein

(i) normal level of cytokines for the neuronal antigen or peptide indicate optimal conditions;

(ii) lower than normal level of cytokines for the neuronal antigen or peptide indicate absence of the demyelinating disease; and

(iii) higher than normal level of cytokines for the neuronal antigen or peptide indicate a likelihood of the demyelinating disease;

further comprising performing all of the steps of the method of claim 22.

* * * * *

专利名称(译)	诊断多发性硬化症和其他脱髓鞘疾病		
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摘要(译)

本文公开了诊断多发性硬化和其他脱髓鞘疾病或预测多发性硬化和其他脱髓鞘疾病的倾向的方法。该方法利用检测增加量的记忆淋巴细胞，其与MS抗原，促炎细胞因子和针对MS抗原的抗体反应。

Figure 1