CHAPTER 17

MULTIPLE SCLEROSIS

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Abstract: Multiple sclerosis (MS) is a chronic, complex neurological disease with a variable clinical course in which several pathophysiological mechanisms such as axonal/neuronal damage, demyelination, inflammation, gliosis, remyelination and repair, oxidative injury and excitotoxicity, alteration of the immune system as well as biochemical disturbances and disruption of blood-brain barrier are involved.[1,2] Exacerbations of MS symptoms reflect inflammatory episodes, while the neurodegenerative aspects of gliosis and axonal loss result in the progression of disability. The precise aetiology of MS is not yet known, although epidemiological data indicate that it arises from a complex interactions between genetic susceptibility and environmental factors.[3] In this chapter the brain structures and processes involved in immunopathogenesis of MS are presented. Additionaly, clinical phenotypes and biomarkers of MS are showed.

INTRODUCTION

The worldwide prevalence of multiple sclerosis (MS) is estimated at between 1.1 and 2.5 million cases. MS is the most common neurological disease in young adults (between 20-40 years) characterized by recurrent relapses and/or progression within the central nervous system (CNS).[4] MS is a complex disease, with a variable clinical course in which several pathophysiological mechanisms such as axonal/neuronal damage, demyelination, inflammation, gliosis, remyelination and repair, oxidative stress and excitotoxicity, alteration of the immune system and disruption of blood-brain barrier are involved.[2,5] The autoimmune diseases arise from a complex interactions between genetic susceptibility and environmental factors. The geographic distribution of MS is uneven. A greater frequency is observed between 40 and 60 degrees north and south latitude.[6,7]

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Geographically MS describes three frequency zones. High frequency areas have MS prevalence of 120:100,000 population, these include: Western and Northern Europe, Canada, Russia, Israel, Parts of Northern US, New Zealand and South-East Australia. Medium frequency 50:100,000 and these include southern US, most of Australia, South Africa, the southern Mediterranean basin, Russia into Siberia, the Ukraine and parts of Latin America.[5,8] Zones with a very low disease frequency of 5:100,000 population are: Asia, Sub-Saharan Africa and South America. Migrants from high to lower risk areas retain the MS risk of their birth place only if they are at least age 15 at migration.[5] Based on epidemiological considerations it is the relatively high risk of MS in the industrialised nations due to hygiene-related changes in the sequence of common infections resulting in the emergence of patterns of immune reactivity that either cause or fail to protect against the development of MS.[9-11]

In the MS pathogenesis genetic factors play very important roles. The most consistent finding in case-control genetic MS studies was the association with the major histocompatibility complex (MHC) (also called human leukocyte antigen-HLA) class II on chromosome 6p21; DR15, DQ6, Dw2 haplotype. Two genes, *HLA-DRB1* and *IL7R* (*CD127*), have been unambiguously associated with the disease susceptibility following their identification as candidates by function.[12-14]

SYMPTOMS AND THE CLINICAL PHENOTYPES OF MS

MS is variable in onset and progression. At onset of the disease the most common symptoms are impaired vision due to optic neuritis (inflammation of the optic nerve) and deficits in sensation (or over-sensation as burning or prickling).[15] The mature form of MS shows other symptoms including paresis and paralysis, ataxia, fatigue, spasticy and incontinence.[16] Cognitive impairment (difficulties with memory, concentration and other mental skills) also occurs frequently.[17] Diagnosis of MS is primarily made on clinical grounds based on the presence of multiple neurological deficits that cannot be explained by one localized CNS pathology. Symptoms must appear at more than one occasion (dissemination in time and space) for a diagnosis to be established. Radiological and laboratory tests, such as computerized tomography scan (CTS), magnetic resonance imaging (MRI), cerebrospinal fluid analyses for immunoglobulin and oligoclonal banding are helpful but only confirm clinical observations.[16] MS is classified into subtypes: Relapsing-remitting (RRMS), secondary progressive (SPMS) and primary progressive (PPMS) disorders.

Initially, more than 80%[2,4] of individuals with MS have a RRMS disease course with defined clinical exacerbations of neurologic symptoms, followed by complete or incomplete remission. RRMS is dominated by multifocal inflammation, oedema and the physiologic actions of cytokines.[2] After 10-20 years, or median age of about 39.1 years, about half of those with RRMS gradually accumulate irreversible neurological deficits in the absence of clinical relapses or new white matter lesions detected by MRI. This stage is known as SPMS which is no longer characterized by clinical attacks and remissions but by insidious progression of clinical symptoms (Fig. 1).[18-21] The remaining 20%, with progressive clinical deterioration from the onset of the disease, have PPMS. PPMS is characterized, in general, by less inflammation and earlier and more sustained axonal loss. The age at the beginnig of the PPMS is around 40 years or later than in RRMS.[22-25] Axonal injury is responsible for the transition from RRMS to progressive forms of the disease. A significantly rarer form is progressive relapsing MS, which initially presents

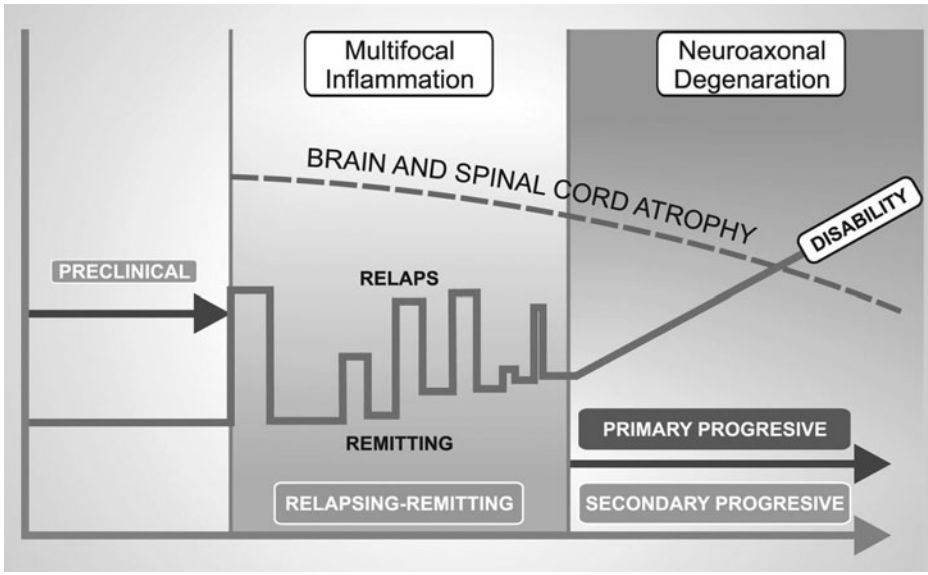

Figure 1. Pathogenical and clinical model of multiple sclerosis subtypes.

as PPMS; however, during the course of the disease these individuals develop true neurologic exacerbations.[26] Individuals with SPMS who have clinical exacerbations, followed by incomplete remission, are included in this category.[27] Axonal transection is observed in the early stage of the disease and in some cases even earlier than that (clinically isolated syndromes or CIS).[21,22] PPMS and SPMS are dominated by neuroaxonal degeneration and correlate with disability and brain and spinal cord atrophy (Fig. 1).[4,23] The most marked atrophy occurs in SPMS.[4] It is difficult to predict the clinical course of this disease.[27,28] Progression of disability seems to be increased in patients with higher number of relapses during the first and second year of the disease.[29,30]

STRUCTURES OF BRAIN INVOLVED IN IMMUNOPATHOLOGY OF MS

Blood-Brain Barier

MS is a disease of CNS where blood-brain barier (BBB) is a key structure.[31-33] The existence of BBB was first intimated by Paul Ehrlich in 1885, although the term *Bluthirnschranke* (blood-brain barrier) was first used as early as 1900 by Lewandowski (existence and cellular structure was debated well in 1960).[34,35]

The BBB is a selective physical barier that regulates transport of blood between the circulation and CNS parenchyma. This structure is composed of capillaries surrounded by perivascular macrophages and astrocytic endfeet and in this way astrocytes can directly modulate BBB function (Fig. 2). Capillaries in CNS presents tight junctions. It allows diffusion of small gaseous molecules ($NO^{\bullet}$, $O_2^{-\cdot}$ and CO_2) and smaller lipophilic compounds that easily can pass through the endothelial cell membrane across the BBB but limits the entry of large hydrophilic molecules such as proteins. Large molecules

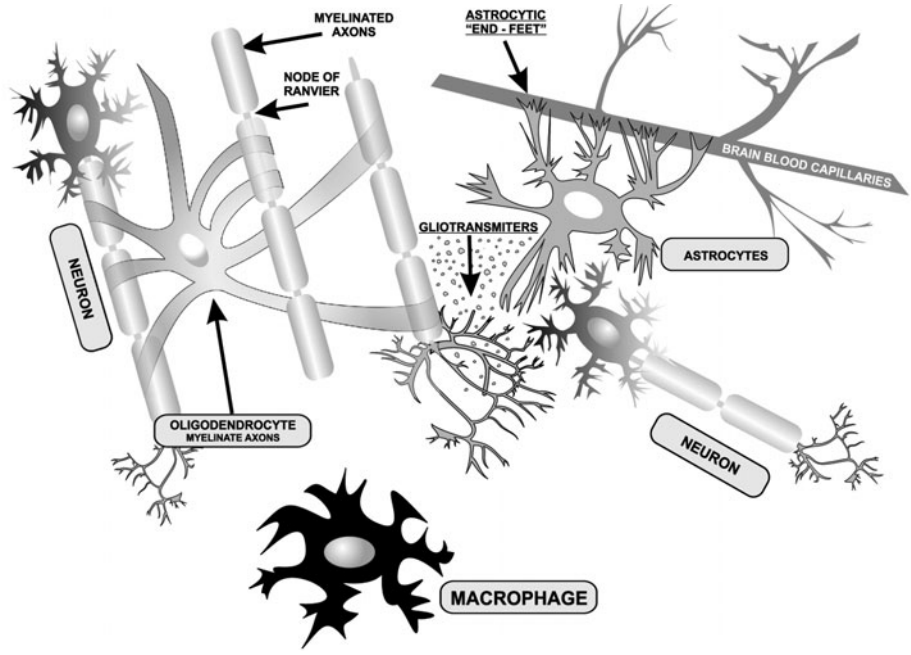

Figure 2. Brain structures involved in multiple sclerosis (MS) immunopathology.

can pass through the BBB via specific transporters located on CNS endothelial cells.[36] The wall thickness of brain capillaries is approximately 40% of that in other types of endothelial cell. It could be an adapation to the restrictive permeability of the BBB.[37]

Astrocyte

Astrocytes constitute approximately 90% of the human brain and support neural transmission, release neuromodulatory factors into the extracellular space and modulation of neurotransmission. Astrocytes play also a critical role in maintaining survival of neurons and other glia (Fig. 2). Communication among astrocytes follows from their syncytial arrangement and occurs via Ca^{2+} channels. Neuronal stimulation can initiate microvascular and endothelial cell responses via these glial elements.[38] Astrocytes release interleukine (IL)-6, tumor necrosis factor-α (TNF-α) and IL-1β and can tighten the BBB via tumor growth factor-β (TGF-β) secretion.[36]

Microglia

In healthy brain microglia consists mainly of macrophages and retract sampling and monitoring their microenvironment. Activated microglia, mainly by macrophages, secretes a variety of inflammatory and oxidative stress mediators including cytokines (TNF and IL-1β and IL-6) and chemokines (macrophage inflammatory protein MIP-1α, monocyte chemoattractant protein, MCP-1 and interferon (IFN) inducible protein IP-10) that promote the inflammatory state. The morphology of macrophages changes from

ramified to amoeboid due to their phagocytic role.[39] These moderately active microglia are thought to perform beneficial functions, such as scavenging neurotoxins and reactive oxygen species (ROS), removing dying cells and cellular debris and secreting trophic factors that promote neuronal survival. Persistent activation of brain-resident microglia may increase the permeability of the BBB and promote increased infiltration of peripheral macrophages, the phenotype of which is critically determined by the CNS environment.[40]

Oligodendrocyte and Axon

Oligodendrocytes myelinate axons, increase axonal stability and induce local accumulation and phosphorylation of neurofilaments within the axon. Neuronal function is further influenced by oligodendrocyte-derived soluble factors that induce sodium channel clustering, necessary for powerfull conduction along axons and maintain this clustering even in the absence of direct axon-glial contact[41,42] (Fig. 2). Neurofilaments, the major axonal cytoskeleton proteins are consist of three components that differ in molecular size: A light chain, N-L, an intermediate chain, N-M and a heavy chain, N-H. The second major component of the axonal cytoskeleton is the microtubule, which is up 100 μm in length and consists of tubulin (α and β) subunits.[41] Actin is the major component of the microfilaments. Axons contain a large volume of membranes because of their elongated shape.[42,43] Cholesterol is the main lipid in these membranes and 24S-hydroxycholesterol is a cholesterol metabolite specific to the brain. Reduced concentrations of oxysterols are associated with brain atrophy especially with cognitive impairment.[44]

IMMUNOPATHOGENESIS OF MS

MS pathogenesis is a complex autoimmunological process, is built to a large extent on results obtained from experimental autoimmune encephalomyelitis (EAE).[45] The CNS is both immune competent and actively interactive with the peripheral immune system.[46] Potentially autoaggressive T cells exist in normal immune system. Once activated, myelin-specific T cells (activation outside the CNS) (*phase 1-activation*) can cross the BBB and migrate into the CNS (*phase 2-adhesion; phase 3-connection; phase 4-penetration*) where they become locally re-activated (*phase 5-re-activation*). After that re-activated T cells proliferate and secrete pro-inflammotory cytokines which stimulate microglia, macrophages and astrocytes and recruited B cells, ultimately result in demyelinisation, damage oligodendrocytes and axons (*phase 6-myelin injury*) with concomitant neurological deficits[47] (Fig. 3).

The most possibile hypothesis how these autoreactive myelin-specific T cells become activated are molecular mimicry processes.[48] T cells recognize short (10-20 residue) linear peptides that are derived from limited proteolysis of myelin proteins. When such peptides are presented to T cells, together with MHC on the surface of antigen-presenting cell (APC), they become activated where T cells respond to environmental antigens, that resemble self-antigens, could be a potential mechanism by which these cells get activated.[49,50] The idea of molecular mimicry is that short linear peptides, derived from viral components (a proposed etiology for MS) are sufficiently similar to myelin peptide to generate autoreactive responses.[51,52] MBP-specific $CD4^+$ T cells are considered to be initiators of MS, but clonal expansion of $CD8^+$ cells has been detected in MS lesions.

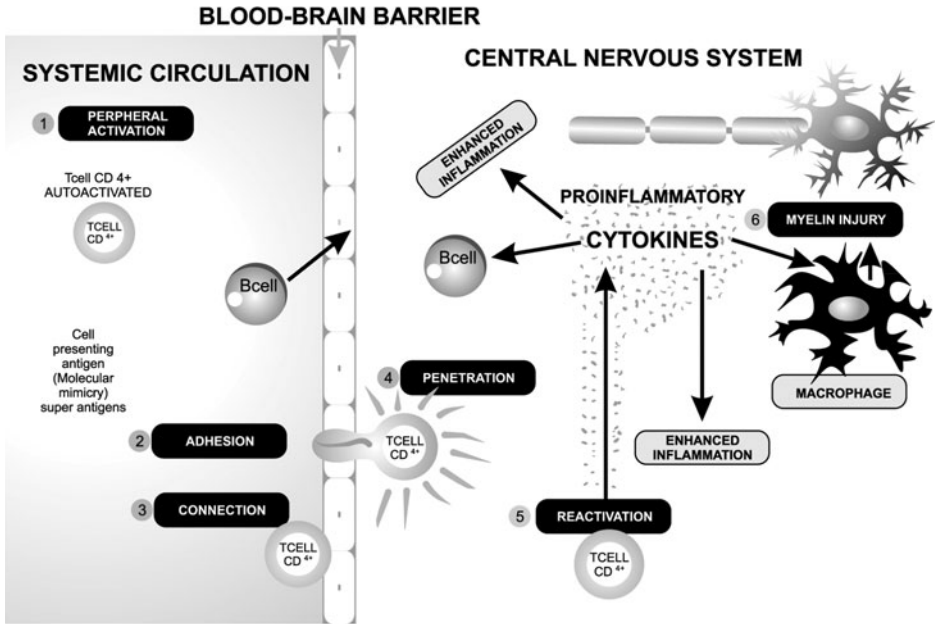

Figure 3. Pathogenesis of multiple sclerosis (MS) cascade.

$CD4^+$ T cells come in two varietes, TH1 and TH2. Recently discovered other types of T cells, so-called regulatory T cells (Treg.), suppress and control the naturally existing autoreactive immune cells. Another new subtype of T cells, TH17, play significant role as pathogenic effector T cells in EAE. Activated T cells express high levels of molecules such as very late antigen-4 (VLA-4) and leukocyte-function-associated antigen-1 (LFA-1) that facilitate their transmigration across the BBB.[53-55]

DISRUPTION OF BBB

Pathogenesis of migrating T cells across BBB consists of 4 phases: Phase 1-intravascular 'crawling' in which T cells do not 'roll' with the blood stream along the endothelium, rather actively crawl, often in a direction against the blood stream. Phase 2-diapedesis in which the crawling T cells stop and squeeze through the vessel wall. Phase 3-perivascular activation in which T cells crawl along the outside of the blood vessel. Phase 4-invasion of CNS parenchyma. During the course of its extravascular crawling the T cells encounter a perivascular antigen-presenting cell, e.g., a dendritic cell (competent APCs). If the antigen-specific receptor of the T cell recognizes 'its' antigen on the surface of dendritic cell, the T cells are re-activated and thereby become 'licensed' to leave the vessel area and penetrate into the surrounding tissues.[47] In the process of penetreting autoreactive cells into CNS are involved: Matrix metalloproteinases (MMPs), chemokines, cytokines, adhesive molecules and intergrins. Here an important step is activation of peripheral immune system that leads to BBB weakness by systemic production of inflammatory mediators.[56]

Metalloproteinases (MMPs)

MMPs are extracellular matrix remodeling neutral proteases which are important in normal development, angiogenesis, wound repair and a wide range of pathological processes.[57,58] MMP-9 can directly attack myelin components such as myelin-basic protein and facilitate T-cell migration across brain microvascular endothelial cells.[59] Tissue inhibitor of MMPs1 (TIMP-1) is present in patients with severe RRMS.[60,61]

Chemokines

Chemokines are small-molecular-weight chemotactic cytokines that attract leukocytes to sites of infection and inflammation. The expression of chemokines, like cytokines, is often triggered by inflammatory mediators, such as TNF, IFN-γ, microbial toxins, or trauma, although constitutively expressed chemokines, such as CXCL1 and CXCL12, are endogenous to neurons and astrocytes, respectively.[36,62] Chemokine receptors are present not only on inflammatory cells, but also on astrocytes, oligodendrocytes and neurons and are involved not only in chemoattraction but also neuronal development, modulation of cell adhesion, phagocytosis, T-cell differentiation, apoptosis and angiogenesis.[63] Analysis of MS tissue has revealed elevated levels of CXCL9, CXCL10, CCL2, CCL7 and CCL8 in reactive astrocytes within demyelinating lesions. Some receptors such as CXCR3 on lymphocytes correlate with tissue destruction, while others, such as CCR5 on macrophages and microglia, are associated with early remyelination.[63,64] CCR2 expression is elevated in T cells from patients with SPMS more than in those with RRMS and several studies have shown that both serum and cerebro-spinal fluid (CSF) levels of CCL2 are lower in RRMS patients. $CCR2^+$ cells migrate across a BBB model with greater efficiency than CCR2 cells, suggesting that CCL2/CCR2 may be important in the pathogenesis of MS.[65]

Cytokines

Cytokines and their receptors are expressed in CNS cells and are important for development and function of the brain. There are proinflammatory cytokines IL-1β, IL-2, IL-6, IL-12, IL-18, TNF-α, IFN-γ and anti-inflammatory IL-4 and IL-10 (can be produced by macrophages, B cells).[36,66] IL-10, as an immunomodulator, inhibits Th1 proliferation. Some cytokines have pleiotropic activities such as IFN-β and even proinflammatory cytokines possess anti-inflammatory properties and vice versa. Both, infiltrating immune cells and resident glial cells whose activities are associated with inflammation, may also contribute to repair and regeneration through the secretion of neuroprotective factors, such as leukemia inhibitory factor (LIF), transforming growth factor-β (TGF-β) and ciliary neurotrophic factor (CNTF).

TNF-α is mostly known to exert inflammatory and neurotoxic effects and is also involved in normal development and function of the brain. TNF-α is highly expressed in the embryonic brain. IFN-γ is a major cytokine involved in demyelinating pathologies. Overexpression of IFN-γ in white matter of transgenic mice results in myelin ablation.[36,67] Evidence from a toxic demyelination model suggests that low levels of IFN-γ may also have surprisingly important role in protection against severe myelin loss. Although the beneficial effect of IFN-β in preventing deterioration of demyelinating diseases is well documented, little is known about its mechanism of action on myelin in the CNS.[68-70]

Adhesion Molecules

Two adhesion molecules, in particular ICAM-1 (intracellular adhesion molecule-1) and VCAM-1 (vascular cell adhesion molecule-1), play crucial roles in the extravacation and homing of T cells through the CNS parenchyma expecially in acute active plaques and ICAM-1 and VCAM-1 bind LFA-1 (CD11a/CD18) and VLA-4 (very late antigen-4) on activated lymphocytes, respectively. In MS tissue, ICAM-1 expression was observed in astrocytes at the edges of demyelinated lesions.[71,72] Leukocyte function-associated antigen-1 (LFA-1) controls the function and, in particular, the migration of immune cells.[73,74]

AXONAL INJURY

Axonal loss is significantly associated with disease progression and disability in MS.[1,4,8,21] Axonal transection can be achieved by inflammatory mediators released as part of the immune activation and lack of neurotrophic factors lead to the axons, by oligodendrocytes, in chronic demyelinated plaques.[17-20]

Oligodendrocytes produce protein Nogo which interacts with the Nogo receptor present on axons to inhibit neurite outgrowth. Since the identification of Nogo and several other molecules in myelin have been identified that suppress neurite, sprouting in the adult CNS including myelin-associated glycoprotein (MAG) and oligodendrocyte myelin glycoprotein (OMgp), both also stimulate Nogo receptors. Neurite sprouting is suppressed by Nogo and its related molecules. This process prevents formation of aberrant connections and regrowth and repair transected axons in demyelinated brain tissues. Recovery from an MS exacerbation is mainly the result of the re-establishment of transmission along the demyelinated axons by redistribution of sodium channels, permitting some degree of restoration of neuronal conduction.[75,76.]

REMYELINATION AND REPAIR

Remyelination and repair is observed in acute and also in chronic phase of MS and is connected with oligodendrocytes function. Endogenous remyelination occurs in white and grey matter in MS and fully remyelinated areas are often referred as “shadow plaques” by neuropathologists.[77,78]

Remyelination occurs in two major phases. The first phase consists of colonization of lesions by oligodendrocyte progenitor cells (OPCs). The second is the differentiation of OPCs into myelinating oligodendrocytes that connect demyelinated axons to generate functional myelin sheaths. Several intracellular and extracellular molecules such as brain-derived neurothrofic factor (BDNF), nerve growth factor (NGF) and insulin-like growth factor (IGF-1) have been identified that mediate these two phases of repair.[79]

GLIOSIS

Gliosis is the proliferation of astrocytes in the central nervous system in response to injury which results in scar formation. Astrocytes react to injury by hypertrophy and

up-regulation of the glial-fibrillary acidic protein. Gliosis, along with neuronal loss, is a prominent feature of MS.[79-81]

OXIDATIVE STRESS AND EXCITITOXICITY

Reactive oxygen species (ROS) can damage lipids, proteins and nucleic acids in cells causing cell death of various cell types including the CNS.[82,83] ROS, leading to oxidative stress (OS), generated in excess primarily by macrophages, have been implicated as mediators of demyelination and axonal damage in MS.[84,85] ROS cause damage to cardinal cellular components resulting in cell death by necrosis or apoptosis.[86] CNS is particularly susceptible to ROS-induced damage due to the high oxygen demands of the brain and the concentration of antioxidants may not be as high as the demand. Brain contains antioxidants enzymes, catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD) and non-enzymatic such as antioxidants glutathione, vitamins A,C,D, co-enzym Q and uric acid etc.[87,88] Enzymatic and non-enzymatic antioxidants -like vitamins, micro and macro elements can regulate progress and function of different immunological cells. Extremely fast production in CNS of several ROS including: $O_2^{-\cdot}$, $HO_2^{\bullet}$, H_2O_2, $^{\bullet}OH$ and $NO^{\bullet}$ (mainly produced by macrophages structures responsible for demyelinisation and axons disruption) takes place.[89]

Nitric Oxide

Nitric oxide (NO) is a free radical gas that at physiological concentrations is essential for many cellular processes such as neurotransmission, differentiation and signal transduction.[87] These physiological processes are regulated by NO produced by nNO synthetase (nNOS) at steady state concentrations from ~50 nM to ~500 nM. Neurons produce NO at concentration of 33 nM during normal physiological functions (low flux NO). Activated microglial and astrocytes can produce NO at steady state concentrations as high as 1 μM (high flux NO). NO, released during CNS pathology, can react with $O_2^{-\cdot}$ and form reactive nitrogen species (RNS), such as peroxynitrite ($ONOO^-$) which cause damage to a variety of macromolecules including proteins. $ONOO^-$ is formed in a reaction that is limited only by the diffusion rates of the molecules.[90,91] Peroxynitrite has been associated with damage to neurons and is a pathogenic factor in MS. NO produced by glial cells within MS lesions also has been shown to directly reduce axonal conduction. $ONOO^-$ can mediate a variety of destructive interactions including oxidation, lipid peroxidation, DNA strand breaks and nitration of amino acids, mainly tyrosine residues in proteins. Uric acid (UA) is a natural scavenger of $ONOO^-$.[91] Peroxynitrite-dependent nitration of tyrosine residues, forming 3-nitrotyrosine (3NY), disrupts protein structure and function, thereby interrupting or altering cell signaling. Nitrotyrosine is found in the CNS of patients with MS and is considered a footprint for peroxynitrite mediated damage in the cell. In MS, progression and severity is tightly associated with levels of reactive nitrogen species (RNS) such as NO and peroxynitrite in the cerebrospinal fluid (CSF) and blood serum.[92]

Endogenous Antioxidants in Prevention of MS

Several enzymes, including SOD, GPx, glutatione reductase and catalase are endogenous antioxidants that possess specific free radical scavenging properties and

reduce ROS levels in brain. Three types of SODs exist in brain cell. CuZn-SOD is a cytosolic enzyme that requires both copper and zinc ions as cofactors. Mn-SOD is a mitochondrial enzyme with requirements for Mn^{2+}. A copper-containing SOD is present in the extracellular space. The CuZn-SOD has been used extensively to reduce brain injury induced by ischemia. The short half-life of CuZn-SOD (6 minutes) in circulating blood and its failure to pass through the BBB make it difficult to use this enzyme in clinical therapy.[93] The glutathione levels in a phase of remission and at an exacerbation, both in RRMS as well as SPMS are low. It is accompanied by the very low activity of erythrocyte glutathione reductase, the only enzyme which provides restoration of oxidized glutathione. The compensatory activation of serum catalase was observed only in exacerbation of RRMS.[87,94] Polysaturated fatty acids, the major components of neuron membranes are higly susceptible to ROS attack and results in lipid peroxidation products. In MS lipid peroxidation is incresed.[95]

In MS low molecular weight antioxidants such as immunoregulatory vitamins C, D, E, UA, glutation and co-enzym Q also play important roles.[87]

Uric Acid

The antioxidant, UA, is a product of purine metabolizm. Serum levels of UA are relatively high, averaging around 4-5 mg/dl in women and 5-6 mg/dl in men.[96] Clinical studies show that the mean serum UA concentration is lower in the MS group and the mean serum UA level from the patients with active MS is significanthly lower than in inactive MS patients. Serum UA levels significantly increased during immunomodulatory treatment. Lower serum UA level in MS may represent a primary, by significant loss of protection against nitric oxide and peroxinitrite and the development of CNS inflammation and tissue damage since UA is an scavenger of peroxynitrite.[97,98]

Vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)$_2$D)

Hypovitaminosis D is currently one of the most studied environmental risk factors for MS. This vitamin could play an immunomodulatory role in the CNS.[99] The biologically active metabolite of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)$_2$D) is able to skew the T-cell compartment into a more anti-inflammatory and regulated state, with inhibition of Th1 and Th17 cells and promotion of Th2 and Treg cells.[100,101] Studies on EAE suggest that treatment with vitamin D prevented and even cured some of the MS patients. Near the equator, where vitamin D synthesis due to sunlight is relatively higher, MS incidence is low. Furthermore, high sun exposure and a good vitamin D status in childhood and adolescence, reflected by the high serum values of 25-hydroxyvitamin D (25(OH)D), have been associated with a decreased risk for developing MS.[102,103]

BLOOD PLATELETS IN MS ETIOLOGY

In the etiology of MS blood platelets play an important role.[104,105] The platelets possess an unexpectedly large variety of receptors and release many different compounds from specific granules. Their classical role is hemostasis and thrombosis, also they participate in inflammation, immunity and tissue repair. Platelets are the first cells at sites of vascular injury, suggesting that they may be central players in neurodegenerative

diseases. Like erythrocytes, they are anucleated cells but unlike erythrocytes they do possess mitochondria.[106] Platelet activation, caused by diffrent stimuli (adhesion, aggregation, secrection), is also accompanied by the release of numerous substances from specialized granules. Released platelet factor 4 (PF4; CXCL4) is a cytokine secreted in abundance from platelets upon activation. Its measurement has been taken as an index of platelet activation, also in MS. Platelets are the main source of CD40L which delivers costimulatory signals to antigen-presenting cells (APC's). Platelets can induce maturation and activation of dendritic cells (DC), probably involving else than CD40L. The role of CD40L in MS is well described in reviews[104,105] and is a target of new therapies. The discovery of Toll-like receptors (TLRs) on platelets in 2004 was another completely unexpected development. Other immune functions of platelets had been noted earlier, including generation of killer-like ROS, phagocytic activity, secretable antimicrobials (thrombocidins), interactions with leukocytes and endothelial cells by direct contact or secretory signaling. The platelets and leukocytes co-operation would facilitate disruption of endothelial junctions of BBB.[105]

The involvement of platelets in MS was first studied by Putnam in 1935,[107] who described venule thrombosis in CNS demyelination. Epidemiological studies have found principal source of CD40L (CD154). The platelet activating factor (PAF), secreted by the prevalence of immune thrombocytopenic purpura (ITP)-like thrombocytopenia in MS patients is about 25-fold higher than in the general population. The adhesion molecule, PECAM-1, may also be important in this regard. It was reported that levels of serum soluble PECAM-1 (sPECAM-1) are significantly elevated in patients with active lesions.[108] In platelets from MS the exposure of P-selectin on platelet surface has been observed; it indicates that platelets are partly activated.[106]

BIOMARKERS OF MS

It is very difficult to assign a biomarker as a surrogate for a clinical outcome in MS characterized by complex pathophysiology. An individual biomarker reflects only one of many pathogenic processes.[2-4] In diseases including MS, based on processes such as inflammation, immunopathology, oxidative stress and markers of immunological activation are signifcantly sensitive on infectious processes, menstrual cycles and may also be affected by age and sex.[3] In MS biomarkers of demyelination, axonal damage, oxidative stress, gliosis and remyelination can be extremely valuable since, based on experience with MRI markers of axonal damage,[109] they may correlate better with the development of long-term disability, may have higher prognostic value and may signifcantly enhance our understanding of the mechanism of action of employed therapies. Availiable specimens for the markers are: Urine, blood, cerebrospinal fluid (CSF) and tears.

Presently classification of biomarkers of MS, based on published studies examining pathophysiological mechanisms, has been divided into the following seven categories: Those: (i) reflecting alteration of the immune system (ii) of axonal/neuronal damage (iii) of blood-brain barrier disruption (iv) of demyelination (v) oxidative stress and excitotoxicity (vi) of gliosis (vii) of remyelination and repair (see Table 1).[2]

Biomarkers reflecting pathophysiological processes can be used for MS diagnostics and identification of disease phenotypes, prediction of disease course and onset, treatment selection and effectivity, leading to novel therapeutics.[110,111]

TREATMENT

The treatment of MS involves "acute relapse treatment" with corticosteroids and symptom management with appropriate agents and disease modification with disease-modifying drugs (DMD). DMD include β-interferon (1b,1a) and Glatatimer acetate (GA). Interferons have anti-inflammatory effect on CNS primarily by preventing autoaggressive T-cell migration through the blood-brain barrier (BBB), GA has no such limiting effect on penetration of the BBB.[22] Instead, GA appears to function via the infiltration of GA-specific T helper 2 cells which produce "bystander suppression" of the autoreactive process.[112]

The main advantage of DMD for relapsing remittance MS treatment is their established good safety profiles. Treatment of the relapsed MS with corticosteroids improves the rate of recovery mainly due to its anti-inflammmatory effect. [8,47] These drugs modify the immune response that occurs in MS through various immunomodulatory or immunosuppressive effects.[113,114]

A new generation of therapies including newly developed monoclonal antibodies such as Natalizumab and Cladribine seems to be more effective than DMD. Natalizumab is a therapeutic humanised monoclonal antibody against the adhesion molecule very late activation (VLA)-4 or integrin alpha-4beta-1. It blocks the interaction of this lymphocyte bound receptor with its endothelial ligands: Vascular cell adhesion molecule (VCAM)-1 on brain endothelium and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) on vascular endothelial cells in the gut. It has been found very effective in preventing relapses of MS through its inhibition of transendothelial migration of lymphocytes across the blood-brain-barrier (BBB) and is now widely used as the presently most effective registered therapeutic agent in severe MS.[115] Cladribine, also known as 2-chlorodeoxyadenosine, is a synthetic adenosine deaminase-resistant purine nucleoside analog that preferentially depletes lymphocyte subpopulations. This sustained effect on lymphocytes is advantageous for patients with MS.[116]

Currently these therapies are rare because they have rare but serious complications such as the development of progressive multifocal leukoencephalopathy (PML) and high cost of treatment.[8] Furthermore, DMD and monoclonal antibodies can decrease the frequency of relapses and can slow down the accumulation of irreversible disability, only if employed at the early stage of onset of the disease.

Since MS is a disease with wide range of symptoms-like fatigue, paraesthesias, muscular weakness and spasticity, double vision, optic neuritis, ataxia, bladder control problems, dysphagia, dysarthria and cognitive dysfunction, the rehabilitation is very important to improve the qualiy of life.[117]

CONCLUSION

MS is a relentless lifelong neurodegenerative disease that, in the early phase, results in severe disabilities including impaired vision due to optic neuritis (inflammation of the optic nerve) and deficits in sensation (or over-sensation as burning or prickling).[15] In the mature phase of MS appears other symptoms including paresis and paralysis, ataxia, fatigue, spasticy and incontinence. Cognitive impairment (difficulties with memory, concentration and other mental skills) also occurs frequently in complex immunopathogenesis. Despite the

Table 1. Classification of biomarkers of MS

I. Biomarkers Reflecting Alteration of the Immune System	
a. Cytokines and their receptors	(IL)-1β, IL-2, IL-6, IL-12 (p40), interferon (IFN)-γ, (TNF)-α, IL-10, TGF-β, IL-4, IL-12 (p70)/IL-23
b. Chemokines and their receptors	CCR5, CXCR3, CXCL10, CCR2/CCL2, *CXCR3/CXCL10 – marker activated T cells
c. Complement-related biomarkers	C3, C4, activated neo-C9, regulators of complement activation (CD35, CD59) Activated neo-C9
d. Adhesion molecules	E-selectin, L-selectin, ICAM-1, VCAM-1, CD31, surface expression of LFA-1 and VLA-4
e. Biomarkers reflective of antigen-processing and presentation	CD40/CD40L, CD80, CD86, heat shock proteins (hsp), *CD40/CD40L (differentiate between RR and SP-MS)
f. Cell-cycle and apoptosis-related biomarkers	Fas (CD95) and Fas-L, FLIP, Bcl-2, *TRAIL (reflective clinical response to INF β therapy in MS)
g. Antibodies	CSF IgG index, κ-light chains, oligoclonal bands, *Anti-myelinoligodendrocyte glycoprotein (anti-MOG Ab), anti-myelin basic protein (anti-MBP) (possibile marker prediction of definite MS after first clinical symptom (CIDS).
h. Biomarkers reflective immune-mediated neuroprotection	BDNF expresion
II. Biomarkers of Axonal/ Neuronal Damage	Cytosceletal proteins (action, tubulin and neurofilaments), tau protein, *24S-Hydroxycholesterol (marker of axonal loss-MSPP), amyloid precursor protein (APP) (marker acute amonal injury), N-acetylaspartic acid (marker of disability and axonal volume), 14-3-3 protein—predictor clinically definite MS
III. Biomarkers of Blood-Brain Barrier (BBB) Disruption	Matrix metalloproteinases (MMPs) MMP-9 and their inhibitors (TIMP), platelet activating factor, thrombomodulin
IV. Biomarkers of Demyelination	MBP and MBP-like material, proteolytic enzymes, endogenous pentapeptide QYNAD, gliotoxin with Na-channel bloking properties
V. Biomarkers of Oxidative Stress and Excitotoxicity	*Nitric oxide derivatives CSF (PPMS; brain atrophy) *Isoprostanes F2-isoprostanes (markers of lipid peroxidation) *Uric acid (strong natural peroxynitrate scavenger)
VI. Biomarkers of Gliosis	Glial fibrillary acid protein (GFAP), S-100 protein,
VII. Biomarkers of Remyelination and Repair	NCAM (neural cell adhesion molecule), CNTF (Ciliary neurotrophic factor), MAP-2 + -13 (microtubuleassociated protein-2 exon 13), CPK-BB (creatine phosphatase BB), PAM (peptidylglycine α-amidating monooxygenase)

*candidate biomarker

fact a wealth of information is available describing the structure function and composition of brain and brain-associated proteins and enzymes, exact reason and mechanism for the induction of MS is still unknown.

Earlier studies on the autopsied brains showed demylination of the myeline layer appearing in the form of plaques, like the plaques form by viral infection. It was thus hypothesised that MS may be caused by some unknown class of virus, although no virus could be isolated from the disease specimens. Later the viral theory of MS was discarded and detailed molecular analysis of normal brains and brains suffered from MS pin-pointed that MS is an autoimmune disease with complex immunopathogenesis.

Further investigations are required both, to understand the root cause of MS together with the cellular immunology of neurodegeneration and also to investigate which gene(s) may be involved, mutation in which leads to MS. Such studies will help to understend not only the disease mechanisms but provide real therapeutic benefits for diseases where we can often do nothing more than palliate the irreversible loss of neurological function.

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