Multiple Sclerosis

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Introduction to Multiple Sclerosis

Multiple sclerosis (MS) is a complex multifactorial polygenic disease, influenced by various factors including age, gender, hormonal, and environmental factors. Despite an unknown etiology, the (histo-) pathological hallmarks of MS lesions are well defined and include demyelination and inflammation of various brain regions. The most widely accepted hypothesis explaining MS is that autoreactive T and B cells and autoantibodies induce myelin damage, neuroinflammation, and neurodegeneration, making MS part of the group of autoimmune diseases (see also chapters "Rheumatoid arthritis" and "Diabetes mellitus"). MS affects persons of all ages, but symptoms are most likely to appear in individuals between 20 and 50 years of age. The estimated prevalence of MS is about 2.5 million people worldwide and is two to three times higher in women than in men. The diagnosis of MS requires evidence of lesions in at least two separate areas of the central nervous system (CNS), including the brain, spinal cord, and optic nerves (dissemination in space), and evidence that new lesions developed at least 1 month apart (dissemination in time). Other potential causes for CNS lesions must be excluded. Technically, diagnosis includes medical history, neurologic exam, and magnetic resonance imaging (MRI) to detect dissemination in space and time, visual-evoked potential measurement, cerebrospinal fluid analysis to detect the levels of immune system proteins and the presence of oligoclonal bands (immunoglobulin bands in gel electrophoresis analysis), and blood tests to rule out conditions causing symptoms similar to MS. The McDonald diagnostic criteria additionally require the first MS attack, which is also known as clinically isolated syndrome (CIS), to be clinical, with features typical or suggestive of MS and with objective abnormalities on neurologic examination. Such symptoms have to last for at least 24 h.

Relapsing remitting MS (RRMS) is the most common type of MS, affecting around 85 % of MS cases. RRMS means that symptoms appear (i.e., a relapse) and then fade away either partially or completely (i.e., remitting). Benign MS is usually a subset of RRMS and comprises patients who accumulate little disability over many years or even remain clinically stable. Secondary progressive MS (SPMS) is characterized by at least one relapse followed by progressive clinical worsening over time. This progressive course may develop slowly after an initial CIS, but more commonly follows a period of well-defined RRMS. Finally, primary progressive MS (PPMS) is characterized by steady worsening of neurologic functioning, without any distinct relapses or periods of remission. A PPMS patient's rate of progression may vary over time, with occasional plateaus or temporary improvements, but the progression is continuous.

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Pathophysiology of Multiple Sclerosis and Metabolic Alterations

On the histopathological level, MS lesions are characterized by oligodendrocyte loss and subsequent demyelination of axons, neuroaxonal loss, astroglia and microglia activation (i.e., gliosis), and, to a certain extent, regeneration of myelin around axons. Both white and gray matter areas of the brain are affected (Fig. 1). These pathological events are paralleled by the recruitment of peripheral inflammatory cells such as lymphocytes and monocytes. Several advanced magnetic resonance imaging (MRI) techniques have been developed that, compared with conventional MRI measures, are better able to capture the complexity of the pathological processes occurring in the CNS of MS patients. Among those, proton MR spectroscopy (^{1}H-MRS) has the unique ability to provide chemical-pathological characterization of MR-visible lesions and normal-appearing brain tissues.

^{1}H-MRS brain imaging revealed profound changes in the level of metabolites such as *N*-acetylaspartate (NAA, a derivative of aspartic acid acting as an important metabolic precursor and osmolyte in the brain), choline, creatine, myoinositol, glutamate (Glu), glutamine (Gln), macromolecules, lipids, and lactate. Reduced NAA levels indicate neuronal/axonal loss. Increased choline and creatine levels suggest gliosis and cell-membrane turnover (de- and remyelination), respectively. Furthermore, lactate, the end product of anaerobic glycolysis, is also increased in MS lesions [2, 3], along with Gln, fructose, and glucose (at early stages of MS) highlighting severe metabolic changes (Fig. 2).

Interestingly, metabolic abnormalities were also found in the "normal-appearing" white matter, hence in regions which are not yet affected by demyelination. Furthermore, studies have demonstrated a reduction in cerebral blood flow of different white [5] and gray matter regions [6] in MS patients

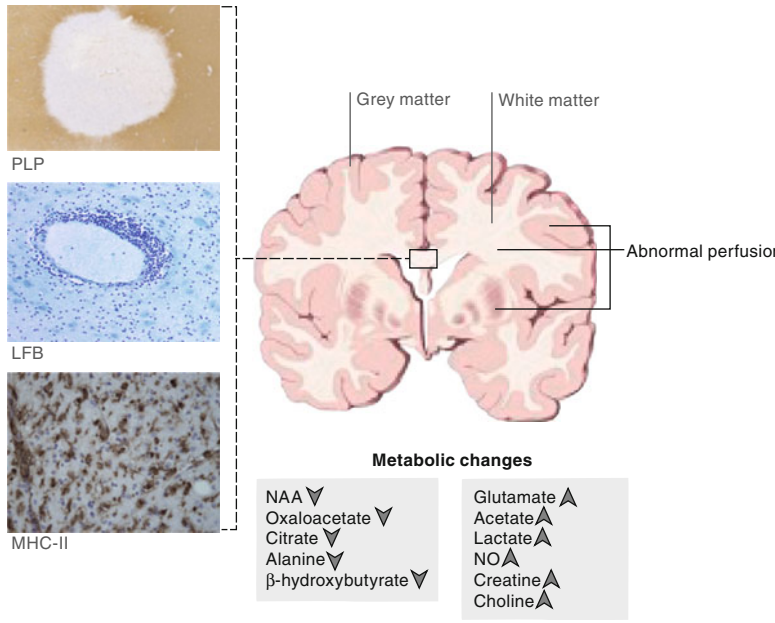

Fig. 1 Pathological and metabolic changes in the brain of an MS patient. Classical active MS lesions [1] can be found in the white and gray matter of the brain. Hallmarks of such lesions are demyelination as indicated by loss of proteolipid protein (*PLP*) staining (*top-left* image) and increased luxol fast blue (*LFB*) staining (*middle-left*), recruitment of inflammatory cells into the perivascular space, and massive accumulation of major histocompatibility complex (*MHC*) II expressing macrophages (*bottom-left* image). Likewise, abnormal perfusion is present at multiple locations in the CNS. The brain and cerebrospinal fluid demonstrate abnormal levels of several metabolites. *NAA* *N*-acetyl aspartate, *NO* nitric oxide

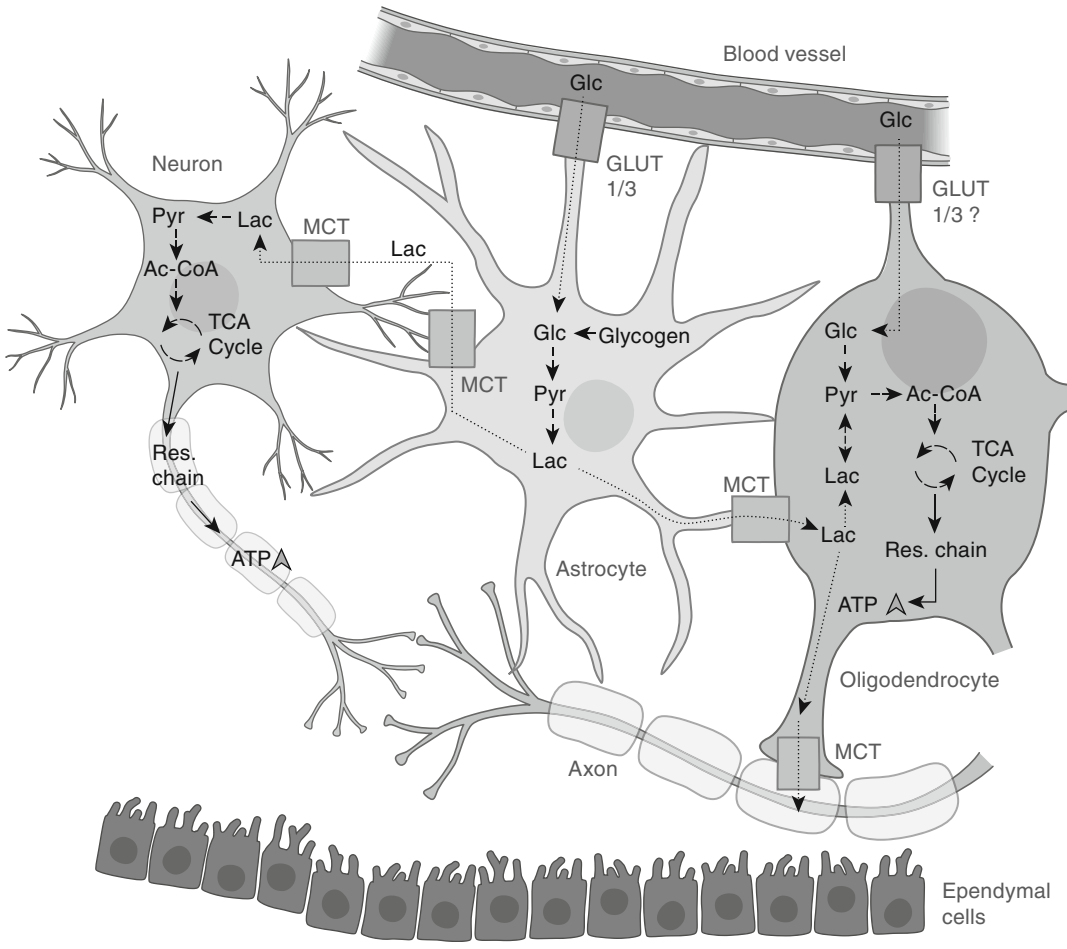

Fig. 2 Movement of metabolites. The brain is mainly fueled by glucose (*Glc*), transported via the blood and taken up by glucose transporters (*GLUT*) 1 and 3 (expressed in endothelial and brain cells, respectively). Alternatively, astrocytes are the main reservoir of glycogen in the brain. Therefore, they might serve as supporters for neurons and oligodendrocytes during energy deprivation periods. According to the lactate (*Lac*) shuttle hypothesis for energy transfer between cells, a heavily glycolytic (i.e., non-oxidative) cell (e.g., an astrocyte) produces large amounts of pyruvate (*Pyr*) and subsequently lactate, which is then transported out of the cell along its concentration gradient through specific monocarboxylate transporters (*MCTs*). The extracellular lactate is taken up via distinct MCTs. Intracellularly, lactate is recycled to pyruvate and transformed into acetyl-CoA (*Ac-CoA*), which is metabolized in the tricarboxylic acid cycle (*TCA*, also called citric acid cycle) to finally produce ATP via oxidative phosphorylation in the respiratory chain of the mitochondria. This interdependence is hypothesized to occur mainly between astrocytes (*center*) and neurons (*left*). Recently, oligodendrocytes have been included in the hypothesis as critical intermediaries for lactate transport to neurons [4]. Oligodendrocytes are also able to take up glucose directly from the endothelial cells of the vessels, probably via GLUT 1 and 3 as well

(Fig. 1). The globally decreased blood perfusion in MS is thought to result from diffuse perivascular inflammation leading to microvascular damage, thrombosis, and fibrin deposition. Furthermore, hypoperfusion might result from widespread astrocyte dysfunction, which contributes to the regulation of vascular tone in the CNS [7].

Metabolic alterations in MS patients can also be detected by ^{1}H-MRS in biofluids, most importantly cerebrospinal fluid (CSF), instead of imaging the brain. This enables quantification of a much larger range of biochemical compounds. In this context, it is important to note that many MS lesions are located in the periventricular white

matter of the brain as well as in superficial areas of the spinal cord, which are close to the CSF space. Since MS lesions are not routinely biopsied, CSF analysis remains an important tool to discern MS pathology. CSF is a clear, colorless fluid surrounding the brain and the spinal cord to protect them from injury and is predominantly produced by the choroid plexus, a dense network of blood vessels located in each of the four ventricles. CSF contains trace proteins, electrolytes, and nutrients that are needed for the metabolism and normal function of the brain. Remarkably, it also serves to remove waste products from the CNS parenchyma and is thus a vital source of information on physiological and pathological processes occurring within the brain parenchyma.

In animal models of MS, the concentration of several metabolites was altered in the CSF. As an example, significantly lower levels of arginine were observed during the early stage of experimental autoimmune encephalomyelitis (EAE), which is one of the most commonly used MS animal models. Arginine is the main substrate for nitric oxide (NO) synthesis, and its reduction likely results from increased activity of inducible NO synthase (iNOS) in activated immune cells and microglia. This NO can react with the free radical superoxide O_2^-, arising from oxidative phosphorylation in the mitochondria to create peroxynitrite anion, a very reactive oxidizing agent, capable of inducing cell death through multiple pathways. Thus, lowered levels of arginine might be an indirect indicator of cell stress and death.

Furthermore, levels of alanine and branched-chain amino acids (leucine, isoleucine, and valine) are decreased in early EAE and likely MS. These amino acids are utilized as a source of pyruvate for energy metabolism; thus, decreased levels indicate increased cell turnover. As the onset of EAE is associated with maximum infiltration of the CNS by blood monocytes and T cells, the observed decrease may suggest these metabolites are utilized for energy metabolism by invading cells [8].

Human MS patients additionally show increased levels of lactate in the CSF correlating with disease activity in the brains of patients with CIS [9]. Increased levels of lactate likely arise from increased anaerobic glycolysis, an attempt to compensate for reduced oxidative phosphorylation caused by perturbed microcirculation and hypoxia-like injury during MS plaque formation. Continued perturbation of mitochondrial function results in cellular energy deficit and loss of mitochondrial transmembrane potential ultimately leading to apoptotic cell death. Thus, the increase in lactate in active plaques and CSF can be interpreted as a result of inflammation, local ischemia, and mitochondrial dysfunction.

Similarly, the concentration of β-hydroxybutyrate (a ketone body and substrate for gluconeogenesis) is increased in the CSF of CIS patients corresponding to the presence of inflammatory lesions [9]. Recent evidence suggests that astrocytes are in principle capable of gluconeogenesis and glucose release. Thus, the increased levels of β-hydroxybutyrate may reflect a perturbation of astrocytic gluconeogenesis potentially due to the presence of inflammatory plaques or due to reduced cerebral blood flow as a result of a perturbation of microcirculation.

Another important metabolic factor playing a role in MS pathogenesis is nitric oxide (NO). NO products are significantly raised in the CSF of MS patients [10]. NO competitively inhibits the binding of oxygen to mitochondrial respiratory complex, and, thus, its increase perturbs ATP synthesis [11]. This condition, in which oxygen is in principle available but cells are unable to use it, mimics hypoxia and is called histotoxic or metabolic hypoxia. Again, subsequent mitochondrial respiratory dysfunction may cause cell death and, in consequence, demyelination and neurodegeneration.

Elevation of Glu levels in CSF, a known CNS neurotoxic trigger, compounded by low levels of oxaloacetate (an inhibitor of neuronal cell death, Fig. 1) may contribute to axonal loss and represents an area for further study [12].

Treatment of Multiple Sclerosis

Despite tremendous scientific efforts, to date, MS has no cure. Treatment usually focuses on strategies to treat acute MS attacks, to lower attack frequency, and to reduce progression (i.e., progression of clinical disability).

Corticosteroids are mainly used to reduce the inflammation that spikes during a relapse. These drugs inhibit lymphocyte proliferation, synthesis of pro-inflammatory cytokines, and expression of cell surface molecules required for immune function (see chapter "Overview" under part "Immune system"). Furthermore, it is believed that corticosteroids stabilize the blood-brain barrier (BBB), for example, by decreasing the expression of angiopoietin-1 and vascular endothelial growth factor A (VEGFA), both well known to regulate the permeability of the BBB (see chapters "Overview" under part "Brain" and "Stroke"). Alternatively, plasmapheresis (meaning removal of blood components) might be applied to help combat severe symptoms of relapses in patients who are not responding to corticosteroids.

Disease-modifying drugs are prescribed with the aim to reduce relapse frequency. Currently, this group includes β-interferons (Avonex, Betaseron, Extavia, and Rebif), fingolimod (Gilenya), glatiramer acetate (Copaxone), mitoxantrone (Novantrone), natalizumab (Tysabri), teriflunomide (Aubagio), and dimethyl fumarate (Tecfidera or BG-12).

β-interferons balance the expression of pro- and anti-inflammatory agents in the brain and reduce the number of inflammatory cells that cross the BBB.

Fingolimod, which is in vivo converted to its active form fingolimod phosphate, suppresses lymphocyte egress from lymphoid tissues into the circulation.

Glatiramer acetate is a mixture of random polymers of four amino acids, which mimics the antigenic properties of the myelin basic protein, a component of the myelin sheath of nerves with which it competes for presentation to T cells.

Mitoxantrone is a type II topoisomerase inhibitor; it disrupts DNA synthesis and DNA repair in both healthy cells and cancer cells. Hence, it suppresses the proliferation of T cells, B cells, and macrophages (see chapter "Overview" under part "Immune system"), impairs antigen presentation, and decreases the secretion of pro-inflammatory cytokines.

Natalizumab is a humanized monoclonal antibody against the cell adhesion molecule integrin α4 and reduces the ability of inflammatory immune cells to pass through the BBB. Teriflunomide belongs to a class of drugs called pyrimidine synthesis inhibitors. Its ability to inhibit the mitochondrial enzyme dihydroorotate dehydrogenase, which is relevant for the de novo synthesis of pyrimidine, is believed to exert the most important therapeutic effect. By inhibiting dihydroorotate dehydrogenase and diminishing DNA synthesis, teriflunomide has a cytostatic effect on proliferating B and T cells.

Dimethyl fumarate is a lipophilic, highly mobile molecule in human tissue. As a electrophilic compound, dimethyl fumarate is rapidly attacked by the detoxifying agent glutathione (GSH). GSH depletion and subsequent induction of the anti-inflammatory stress protein HO-1 is thought to be one of the mechanisms responsible for the immunomodulatory actions of dimethyl fumarate. Other postulated mechanisms of action include direct cytoprotective effects through upregulation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and subsequent induction of an antioxidant response.

Influence of Treatment on Metabolism

There is some evidence that the metabolic changes in MS can be ameliorated by the administration of disease-modifying drugs. For example, CIS patients who received glatiramer acetate or interferon beta 1b showed improvement in brain neuroaxonal integrity, as indicated by an increased NAA/creatine ratio [13, 14]. However, our knowledge about the efficacy of disease-modifying drugs to modulate metabolic changes in MS is in its infancy and warrants further studies. One has to point out that currently available immune-directed therapies have been shown to decelerate the inflammatory process in MS and, thus, restore acute clinical deficits, which occur during a relapse. However, such therapy is less effective in preventing the progression of the disease and neurodegeneration, which appear to be at least in part independent from inflammatory attacks.

Perspectives

Clearly, several metabolic pathways are disturbed in the CNS of MS patients (see above). However, we are far from understanding how these alterations impact cellular physiology and whether a manipulation of these pathways might result in new therapeutic options in the future. Recent findings suggest that neurons, astrocytes, and oligodendrocytes form a so- called tri-cellular compartmentation of brain metabolism [15], meaning that complex metabolic pathways are scattered over different cell types and the metabolites are then transported to their target cells. This is, for example, well described for NAA metabolism and catabolism. Aspartate needed for NAA production can only be synthesized de novo in astrocytes and is transported to neurons in the form of glutamine. In neurons, NAA is then assembled and released. Subsequently, NAA is hydrolyzed to acetate and aspartate by aspartoacylase, which is predominantly located in oligodendroglia. Following this theory, oligodendrocytes and astrocytes are pivotal for neuronal survival and functioning by providing energy metabolites to axons and/or neuronal cell bodies. A better understanding of these cellular interactions will help to generate new treatment strategies and diagnostic tools in the future.

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