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Phenotypes and glia-immune cell interactions in animal models of multiple sclerosis

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Contents

Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
ATP	Adenosine triphosphate
APC	Adenomatous polyposis coli
APCs	Antigen representing cells
AQP4	Aquaporin 4
AVP	Arginine vasopressin
BBB	Blood brain barrier
BDNF	Brain derived neurotrophic factor
BMP 2/4	Bone morphogentic proteins2/4
CD4	Cluster of differentiation 4
CD8	Cluster of differentiation 8
CNPase	2', 3'-cyclic-nucleotide 3'-phosphodiesterase
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
COX I	Cytochrome c oxidase I
Cup	Cuprizone
DCs	Dendritic cells
EAE	Experimental allergic encephalomyelitis
eGFP	Enhanced green fluorescent protein
ER	Endoplasmic reticulum
GABA	*gamma*-Aminobutyric acid
Gal-3	Galectin-3
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GFAP	Glial fibrillary acidic protein
HLA-DR/LN3	Human leukocyte antigen – DR isotype/ Clone LN3
IFN-γ	Interferon gamma
IGF-1	Insulin-like growth factor 1
IL-1β	Interleukin-1β
IL-6	Interleukin-6
IL-12	Interleukin-12

iNOS	Inducible nitrogen oxide synthase
LFB/PAS	Luxol fast blue/Periodic acid-Schiff
MAG	Myelin-associated glycoprotein
MAO	Monoamine oxidase
MBP	Myelin basic protein
MHC	Major histocompatibility complex
MOG	Myelin oligodendrocyte glycoprotein
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NG2	Neuron-glial antigen 2
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
NRG1	Neuregulin-1
NSCs	Neural stem cells
OLIG2	Oligodendrocyte transcription factor 2
OPCs	Oligodendrocyte progenitor cells
PDGFRA	Platelet derived growth factor receptor A
PLP	Proteolipid protein
PPMS	Primary-progressive multiple sclerosis
PRMS	Progressive-relapsing multiple sclerosis
RG	Radial glial
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RRMS	Relapsing-remitting MS
S1Pr	Sphingosine 1-phosphate receptor
SPMS	Secondary-progressive MS
TGF-ß	Tumor growth factor-ß
TNF-α	Tumor necrosis factor-α
TREM2	Triggering receptor expressed on myeloid cells 2
YS	Yolk sac

1. Publication contribution

1.1. The author of this thesis contributed to the original, peer-reviewed publication “Oligodendrocyte lineage marker expression in eGFP-GFAP transgenic mice”, (Journal of Molecular Neuroscience) https:// doi.org/10.1007/s12031-020-01771-w. by i) participating in developing of the study and its design ii), writing the manuscript, iii) performing all of the animal experiments, histochemical and immunohistochemical stainings and iv) performing all of the data analyses and statistical evaluations.

1.2. The author of this thesis contributed to the original, peer-reviewed publication “Continuous cuprizone intoxication allows active experimental autoimmune encephalomyelitis induction in C57BL/6 mice”, (Histochemistry and Cell Biology) https:// doi.org/10.1007/s00418-019-01786-4. by i) participating in the animal experiments and ii) participating in data analyses and statistical evaluations.

2. Introduction

2.1. Multiple sclerosis (MS)

MS is a complex chronic immune-mediated disease of the central nervous system (CNS) characterized by brain atrophy and inflammatory demyelinated plaques located within the white and gray matter.

On the clinical level, MS can be divided into four major groups:

Relapsing-remitting MS (RRMS): This type is the most frequent, occurring in about 85% of all MS patients. During the initial RR stage of MS, symptoms appear (relapse), followed by periods of partial or complete recovery (remission).

Secondary-progressive MS (SPMS): In SPMS, symptoms continuously aggravated over time, with or without the development of relapses and remissions. Most patients (~90%) diagnosed with RRMS will develop a secondary progressive course. SPMS can be characterized by decreasing the frequency of relapse, but accelerating of neurodegeneration.

Primary-progressive MS (PPMS): This type of MS is sporadic, occurring in about 10–15% of MS patients. PPMS patients experience slowly exacerbating symptoms from the onset without distinct relapses or remissions.

Progressive-relapsing MS (PRMS): An uncommon form of MS (5%), PRMS is specified by a continuous aggravating disease state from the onset with acute relapses but no remissions, with or without recovery [1-4].

2.2. Histopathology of MS

MS is a complex, chronic, inflammatory disease of the CNS. Its pathology was originally explained by the existence of focal white matter plaques, also called lesions. There are several MS pathological basic processes, including the breakdown of the blood–brain barrier (BBB), inflammation, myelin breakdown, astrogliosis, oligodendrocyte injury, and neurodegeneration [5].

The early phase of the disease is associated with the formation of focal lesion types in the white matter associated with activated microglia and infiltrated macrophages, major histocompatibility complex (MHC) class I restricted $CD8^+$ cells, $CD4^+$ cells, and B-cells. Inflammation in the early phase of the disease triggers oxidative damage, resulting in oxidative bursts in microglia and macrophages, oligodendrocyte damage, axonal injury, and the accumulation of astrocytes which are a possible driving force for active MS lesion development [6].

During the relapsing-remitting and progressive stages of the disease, oxidative damage induces widespread demyelination throughout the entire white and gray matter resulting in brain atrophy. Oligodendrocytes—to a great degree—and microglia/macrophages—to a lower degree—contain iron stored in ferritin. During the course of demyelination, oligodendrocytes are destroyed and iron is released from ferritin (iron storage protein) into the extracellular space [7]. Then, iron is taken up by microglia and macrophages and stored repeatedly in ferritin. In MS lesions, where free radicals are produced by oxidative bursts, iron can be released from ferritin and transformed into reactive Fe^{2+}. The produced Fe^{2+} reacts with hydrogen peroxide to generate highly reactive hydroxyl radicals and Fe^{3+} that promote oxidative damage, cellular injury, demyelination, and axonal destruction (**Fig 1**) [8, 9].

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^{\circ} + OH$$

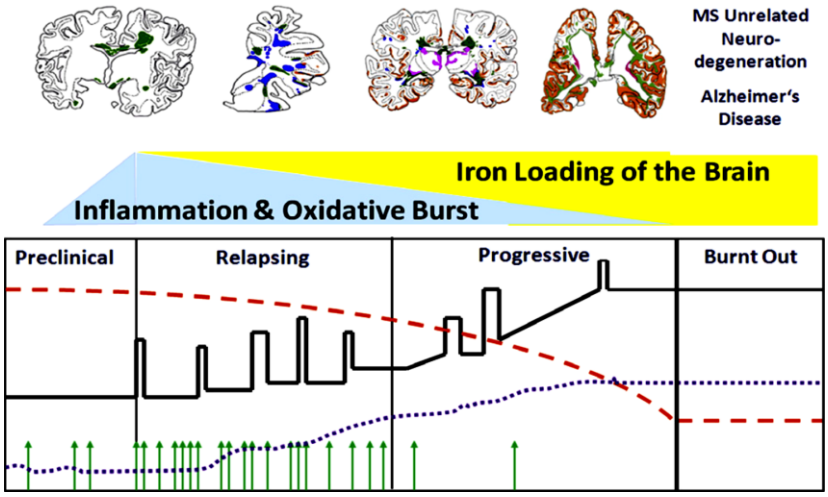

Fig 1. The pathology of MS during the disease course. The early phase of the disease is characterized by oxidative damage which is mainly driven by inflammation. In the relapsing phase, patients experience acute clinical attacks which are followed by complete or incomplete recovery (green: demyelinated and blue: remyelinated). New lesions on magnetic resonance imaging (MRI) appear (green arrows). In the progressive stage of the disease, patients experience a gradual progression of disability and massive cortical demyelination (red). Brain atrophy (blue dotted line) and reduction of brain volume (red dashed line) are augmented during this stage. During the burn out stage, brain atrophy is stable and the total brain volume is remarkably decreased. To summarize, the relapsing-remitting phase is characterized by demyelinated and remyelinated plaques. In the progressive stage, massive cortical demyelination (red) is diffused throughout the brain and continues extensively during the burnout phase [5].

In the disease course of MS, different plaque types are evident. As given below, they can be grouped into four types:

Acute active plaques: Most frequent in acute and RRMS types, acute active plaques are hypercellular demyelinated plaques massively infiltrated by macrophages/microglia that contain myelin debris. Furthermore, demyelinated plaques contain inflammatory infiltrates which are composed of lymphocytes (mostly $CD8^+$ T cells and fewer $CD4^+$ T cells), B cells, and plasma cells. Astrocytes

extensively proliferate and exhibit a hypertrophic morphology. Oligodendrocytes are destroyed in early lesions. However, oligodendroglial injury is heterogenous in acute active plaques with numerous oligodendrocytes present in some lesions, often referred to as signs of concurrent early remyelination. In these lesions, the first changes in mitochondria are mirrored by a dominant loss of immunoreactivity of the cytochrome c oxidase I (COX I) and loss of the respective complex IV activity of the mitochondrial respiratory chain. Mitochondrial injury can trigger pro-apoptotic events and lead to oligodendrocyte destruction and demyelination, additionally blocking the differentiation of oligodendrocytes progenitor cells into myelinating cells which results in remyelination failure (**Fig 2**) [5].

Chronic active plaques: The most common plaques in patients with progressive MS, they are characterized by demyelination and hypocellularity in the center and hypercellularity at the rim of the plaque. Perivascular inflammatory infiltrates are often appeared in chronic lesions but the BBB remains intact or its damages are restricted. In chronic active lesions, mitochondrial numbers and activity are increased due to the higher energy demand of demyelinated axons compared to the myelinated ones (**Fig 2**).

As plaques progress from acute active to chronic inactive, the edema resolves, inflammation decreases, and macrophages and microglia decrease in numbers. Axonal damages and loss are also apparent in chronic MS plaques [10].

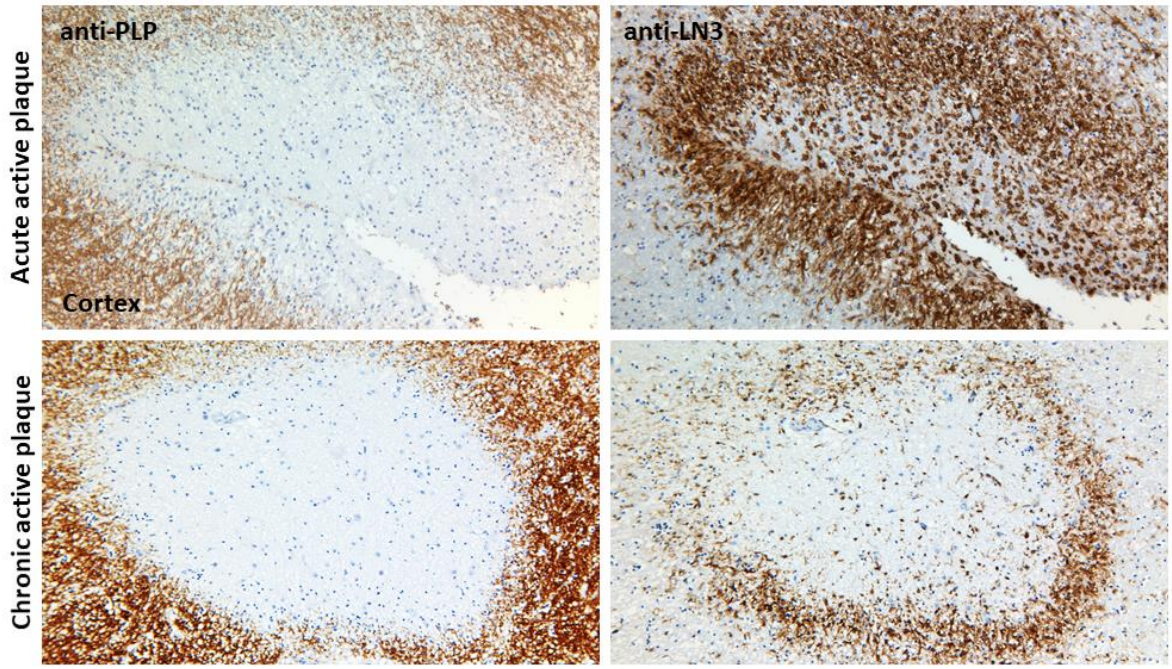

Fig 2. MS plaque types**.** The active acute plaque is characterized by active demyelination (anti-proteolipid protein (PLP)) and massively infiltrated monocytes and macrophages (anti-human leukocyte antigen–DR isotype /Clone

LN3 (HLA-DR /LN3)). The chronic active plaque is characterized by demyelination and hypocellularity in the center and hypercellularity at the rim.

Chronic inactive plaques: These are characterized by demyelination and hypocellularity at the center and the rim of the plaque. As plaques progress from the chronic active to chronic inactive stage, macrophages and microglia gradually disappear and inflammation decreases [5].

Remyelinated plaques: These are characterized by the presence of thinly myelinated axons with short internodal distances. Completely remyelinated lesions, the so-called shadow plaques, are clearly distinguishable areas with reduced myelin density and disproportionately thin myelin sheath [11].

2.3. The role of glial cells and neurons in MS

Neural stem cells (NSCs) are primary progenitor cells at different developmental stages that hold the potential to differentiate into neurons and glial cells. During early development, neuroepithelial cells proliferate and give rise to early neurons. During the embryonic period, the brain epithelium thickens and neuroepithelial cells convert into radial glial (RG) cells. RG cells divide asymmetrically and give rise to RG cells and neurons. Later in their development, the RG cells transform into astrocytes in the cortex and hippocampus. Radial glial cells also generate intermediate progenitor cells that give rise to oligodendrocytes [12, 13]. Generally, the glial cell population can be subdivided into the following five major groups in the CNS: 1) astrocytes, 2) oligodendrocytes, 3) microglia, 4) progenitor neuron-glial antigen 2 (NG2)-glial cells, and 5) ependymal cells [14]. In this section, the focus is on the role of oligodendrocytes, astrocytes, microglia, and neurons in the pathogenesis of MS.

2.3.1. Astrocytes

Astrocytes are the most abundant cells in the CNS which account for one third of the brain mass. Their function and morphology principally depend on their location, physiological situation, subtypes, and the developmental stage [15, 16]. In the gray matter, astrocytes are protoplasmic with short and large branched tertiary processes which are in close proximity to neuronal synapses. In the white matter, astrocytes are fibrous with long unbranched processes [15].

In the normal CNS, astrocytes are shown to control the formation, maintenance, function, and removal of neuronal synapses through the regulated release of synaptically active molecules such as thrombospondin, glutamate, gamma-Aminobutyric acid (GABA), and purines (Adenosine triphosphate (ATP) and adenosine) [17-19]. Astrocytes form perivascular endfeet at the BBB and exert essential functions in maintaining the fluid, ion, and pH homeostasis. Additionally, they support the transmitter homeostasis of the synaptic interstitial fluid via various channels including, aquaporin 4 (AQP4) and Kir4.1 K^+, arginine vasopressin (AVP), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors [20, 21].

Astrocytes play a dual role during MS pathogenesis. They induce deleterious effects by activating the immune response and recruiting T cells [22, 23] and macrophages and microglial cells [24], inhibiting axonal regeneration [25], secreting cytotoxic factors such as nitric oxide (NO), reactive oxygen species (ROS) [26, 27], and mediating mitochondrial dysfunction [28]. On the other hand, they incite a protective role by promoting BBB integrity [29], terminating immune responses [30], protecting neurons by secreting brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF) [31], facilitating remyelination [32], regulating myelin breakdown clearance [33], as well as supporting the differentiation and proliferation of oligodendrocytes progenitors into mature, myelinating cells [34].

2.3.2. Oligodendrocytes

Oligodendrocyte progenitor cells (OPCs) are those derived from the ventral epithelium of the neural tube recognizable by marker proteins such as oligodendrocyte transcription factor 2 (OLIG2), the chondroitin sulfate proteoglycan NG2, or platelet-derived growth factor receptor A (PDGFRA). These cells have the potential to differentiate into post-mitotic, pre-myelinating oligodendrocytes (expressing the markers 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNPase), and O4), further giving rise to myelinating cells of the CNS (expressing the markers myelin basic protein (MBP), myelin-associated glycoprotein (MAG), myelin oligodendrocyte glycoprotein (MOG), and proteolipid protein (PLP)) [35]. Oligodendrocytes take up glucose, convert it to lactate and pyruvate, and then deliver these metabolites to axons to provide nutritional support to neurons [36, 37]. Moreover, oligodendrocytes maintain long-term axonal integrity and provide an insulating substance for fast and energy-efficient salutatory conduction by forming multilayered myelin sheaths [38, 39].

Oligodendrocytes are responsible for the formation of myelin in a multi-step process. In the first step, oligodendrocytes target axons with a diameter of more than 0.2µm and exclude dendrites [40]. They wrap their plasma membrane spirally around the axon, followed by the lateral growth of all layers over each other [40, 41]. In the next step, the movement of each myelin layer toward each other results in the fusion of two neighboring myelin layers, thereby forming a node of Ranvier. Sodium channels are positioned adjacent to the edge of lateral loops and the node of Ranvier which is flanked by the paranodal loops formed [42]. Finally, the compaction of myelin starts in the inner most layers where the site of the MBP local translation occurs. The MBP binds opposing inner membranes, zippers the cytoplasmic surfaces together, and precisely regulates the passing through of molecules [43].

The apoptosis of oligodendrocytes is eventually an initial event in the MS lesion formation. However, the exact mechanisms of demyelination and oligodendrocyte loss are still unknown. There are two proposed mechanisms of demyelination in MS pathogenesis. In outside-in model, the peripheral T cells migrate into the CNS and destroy, together with macrophages and B cells, the myelin and other CNS

elements together with macrophages and B cells. In inside-out model, the primary oligodendrocyte or myelin degeneration releases antigenic constitutes which, secondarily, activate autoimmune and inflammatory responses [44]. Demyelination causes an aberrant distribution of ion channels across the axonal surface and an imbalance of ion influx is associated with axonal degeneration [45]. Furthermore, the loss of oligodendrocytes and demyelination results in the disruption of axonal transport, causes neuronal homeostasis imbalance, and finally triggers axonal degeneration [46].

2.3.3. Microglia

Microglia, the brain-resident macrophages, are derived from yolk sac (YS)—primitive macrophages that migrate into the CNS during early embryogenesis and persist until adulthood [47, 48]. Macrophage and microglia activation has been classified into two different states: classic (M1) and alternative (M2). The M1 phenotype refers to a pro-inflammatory state, in which microglial cells produce pro-inflammatory mediators including interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-12 (IL-12), ROS, and inducible nitrogen oxide synthase (iNOS) [49]. The M2 phenotype refers to an anti-inflammatory state, involved in the phagocytosis function, production, and the release of trophic factors such as tumor growth factor-ß (TGF-ß) and BDNF [50-52]. However, nowadays the strict classification of M1 and M2 microglia is highly debated and it is suggested that variation in the microenvironment can affect the behavior of microglia and regulate their phenotype in a transient pattern [53, 54].

The role of microglia in MS is complex and controversial. The microglia play a crucial role in both active inflammation and remyelination. During the development of MS lesions, microglia are responsible for the phagocytosis of myelin debris [55], and it has been shown that the activation of the triggering receptor expressed on myeloid cells 2 (TREM2) expressed on microglia stimulates microglial survival and phagocytic activity [56]. Microglia also play a role in antigen presentation to T cells and the release of pro-inflammatory cytokines such as TNF-α and IL-1β in active MS lesions [57]. Furthermore, microglia that expresses insulin like growth factor-1 (IGF-1) alleviates apoptosis, and promotes the proliferation and differentiation of NSCs during neurogenesis [58].

2.3.4. Neurons

Neurons, specialized cells for information processing and transmission of electrochemical signaling, consist of a cell body, called soma and cellular processes called neurites. Neurites are characterized by multiple ramified dendrites that provide an extended receptive surface for the cells and increase the number of synaptic inputs. The singular axon carries messages in the form of action potentials along the length of the axon [16]. Neurons can be classified into three main groups: 1) sensory neurons transport sensory information, such as visual or auditory input, to the brain; 2) motor neurons

responsible for voluntary muscle activities and transporting information from nerve cells in the brain to the muscle; and 3) interneurons.

Axonal transport is an essential physiological function mediated via anterograde transport (from the soma towards the distal axonal site) and retrograde transport (from the distal axonal site towards the soma) [59, 60]. The disturbance of fast axonal transport along the length of the axon results in the accumulation of substances referred to as axonal spheroids [61].

One of the major causes of irreversible disability in MS patients is axonal loss, considered to be an early and persistent event in MS pathology progression [62]. Axonal damage in MS can be triggered by various mechanisms: 1) immunological attacks of cytotoxic $CD8^+$ T lymphocytes on the axon [63], 2) high intraaxonal levels of NO radicals which injure axonal mitochondria and disrupt axonal cytoarchitecture [64], and 3) demyelination.

2.3.5. Oligodendrocytes, astrocytes, and microglial crosstalk

In the healthy CNS, quiescent astrocytes support oligodendrocytes' differentiation and myelination by producing factors such as neuregulin-1 (NRG1) [65] and IGF-1 [66]. Additionally, astrocytes stimulate the oligodendrocytes' survival through a mechanism involving the interaction of α6β1 integrin on oligodendrocytes with laminin on astrocytes [67]. Microglia also drive OPCs' differentiation and enhance/support remyelination by expressing galectin-3 (Gal-3) [68].

During diverse brain insults or neurodegenerative processes, astrocytes secrete different factors such as bone morphogenetic proteins 2/4 (BMP2/4) and hyaluronan which block OPCs' maturation and impair remyelination [69, 70]. Moreover, the end-feet loss of astrocytes around the BBB is linked to its disruption and immune invasion into the CNS [71]. Microglia are also involved in the myelin damage by producing neurotoxic or neurotrophic molecules and presenting self-antigens to effector immune cells [72].

2.4. MS animal models

MS is a complex autoimmune disease with an unknown pathogenesis. There are several experimental animal models that mimic distinct aspects of MS pathology. Here, I introduce two MS animal models, namely the experimental allergic encephalomyelitis (EAE) and the cuprizone (Cup) animal model.

2.4.1. EAE

EAE is a frequently applied animal model of MS that simulates an acquired inflammatory demyelinating autoimmune disease. It can be induced by the active immunization of encephalitogenic antigens derived from CNS proteins such as PLP, MBP, and MOG or by the passive transfer of encephalitogenic

T cells [73]. The resulting EAE symptoms are scored on a scale from 0 to 5 which starts with tail limpness, followed by hind-limb paralysis and forelimb paralysis.

EAE pathogenesis is mediated by the activation of antigen representing cells (APCs) and the presentation of antigens to naive T cells. Dendritic cells (DCs) or other APCs activate $CD4^+$ T cell responses by presenting antigens via major histocompatibility complex II (MHC II) and perpetuates CNS-targeted autoimmunity [74]. The activated myelin-specific T cells enter the bloodstream in the periphery, translocate into the CNS via post-capillary venules, and cross the BBB. The BBB is composed of the endothelial cell monolayer, astrocytic glial end-feet, and two basement membranes **(Fig. 3a)**. The BBB breakdown results in the accumulation of immune cells between the two basement membranes, the formation of perivascular cuffs, and finally the infiltration of immune cells into the CNS (**Fig. 3b**) [75, 76].

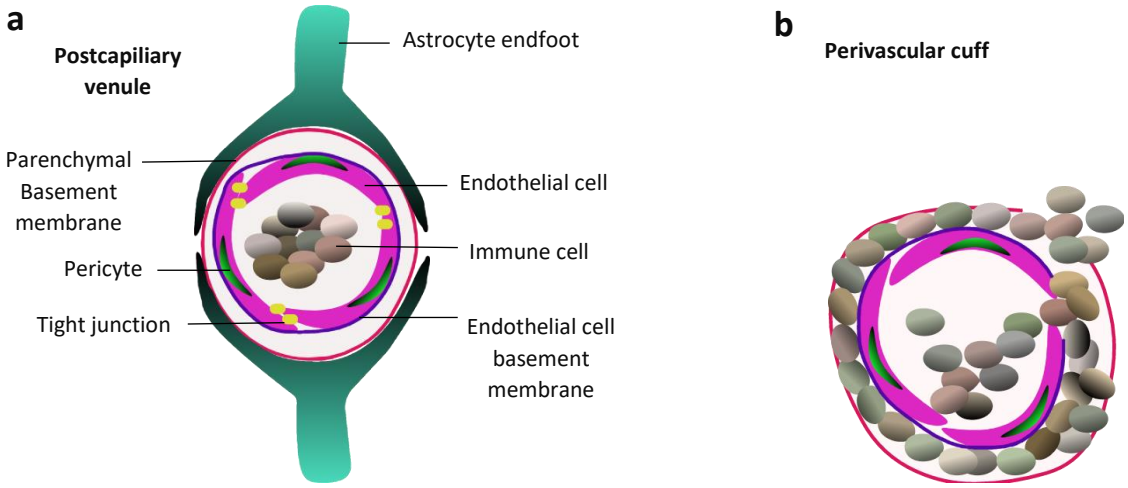

Fig 3. Schematic of the blood brain barrier and perivascular cuff formation. (a) shows astrocyte endfeet surrounding postcapillary venules and preventing immune cell egress from the venules. (b) shows the loss of astrocyte endfeet resulting in the invasion of immune cells and perivascular cuff formation.

In the CNS, T cells might be reactivated by macrophages, DCs, and B cells and release diverse cytokines (i.e., IL-7, interferon gamma (IFN-γ), TNF-α and granulocyte-macrophage colony-stimulating factor (GM-CSF)) [77-80], proteases, glutamate, and free radicals. Then, other immune cells are recruited to the site of inflammation which finally results in sometimes extensive myelin destruction, and axonal damage [81].

EAE is a useful animal model to understand the basic autoimmune mechanism of MS. Nevertheless, there are some differences between MS and EAE. For example, the autoantigen is known in the EAE, while the antigen that stimulates autoimmune reactions in MS patients has not been identified yet. The auto-immune pathogenesis is mediated by $CD4^+$ T cells in EAE, while $CD8^+$ T cells play a more vigorous role in MS pathogenesis than $CD4^+$ T cells [82, 83]. Due to these differences, some treatments which are successful in the EAE animal model cannot be translated to clinical use.

2.4.2. Cuprizone

Recent studies have shown that the loss of oligodendrocytes and neurons is the earliest hallmark of MS pathogenesis. This process is not always associated with immune cell infiltration into the CNS. To this end, the cuprizone model is appropriate to study the mechanism of oligodendrocyte apoptosis and demyelination in a non-immune situation. This animal model represents a reversible demyelination and remyelination system and partially mimics type III and IV MS lesions [84]. To induce demyelination via cuprizone, mice are intoxicated with a diet containing 0.25% cuprizone mixed into ground standard rodent chow.

It has been shown that oligodendrocyte apoptosis paralleled by the early activation of astrocytes and microglia is the first hallmark of stress and appears days after the initiation of cuprizone intoxication [85, 86]. The continuation of cuprizone intoxication for up to three weeks induces the accumulation of microglia and astrocytes as well as axonal damage specifically in the medial corpus callosum (**Fig 4**). Although during this early stage, myelin pathology cannot be clearly visualized by immunohistochemistry staining against myelin proteins, histochemistry staining methods against lipoproteins such as Luxol fast blue/Periodic acid-Schiff (LFB/PAS) can effectively reveal the demyelination after three weeks of cuprizone intoxication [87]. Cuprizone intoxication for five weeks induces acute demyelination, accompanied by massive accumulation of microglia, astrocytes, and axonal damage in the medial and lateral corpus callosum. The withdrawal of cuprizone intoxication induces robust remyelination [88].

The exact mode of action of cuprizone intoxication has not yet been fully explored. Cuprizone is a copper chelator that disturbs the function of mitochondrial enzymes containing copper as a co-factor including monoamine oxidase (MAO) and cytochrome c oxidase. Due to myelin synthesis, mature oligodendrocytes require a large amount of oxygen and ATP. To this end, high numbers of mitochondria are necessary for the regular function of oligodendrocytes. Oligodendrocytes contain little anti-oxidant enzymes; thus, free radicals such as ROS/reactive nitrogen species (RNS) cannot be efficiently detoxified and their intracellular accumulation results in the disruption of endoplasmic reticulum (ER) homeostasis. Therefore, oxidative and ER stress in concert stimulate oligodendrocyte apoptosis and myelin sheath disintegration [89, 90].

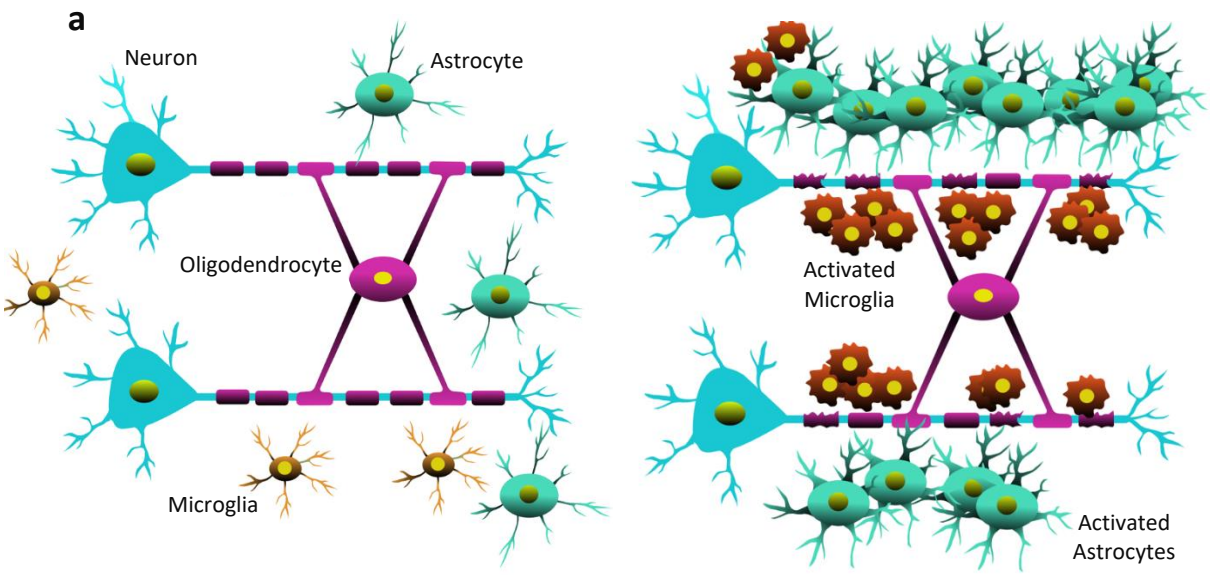

Fig 4. Schematic of glia cells activity in (a) a normal and (b) a cuprizone intoxicated brain. (a) shows resting astrocytes and microglia supporting myelinating oligodendrocytes. (b) shows that cuprizone intoxication triggers the accumulation and activation of astrocytes and microglia which results in demyelination.

There are similarities between the cuprizone animal model and MS. Different aspects of progressive MS pathology are mimicked in the cuprizone animal model. For example, axonal damage and apoptosis of oligodendrocytes are the hallmarks of MS active lesions and cuprizone-induced white matter lesions. Additionally, the cuprizone animal model is an appropriate model to investigate the mechanism of demyelination and remyelination and examine the effect of myelin protective agents [91].

2.5. Hypothesis

- Oligodendroglial and astrocytes' markers are co-expressed during a cellular stress state.

- The Cup-EAE animal model is an appropriate tool for studying the mechanisms involved during inside-out MS lesion development.

2.6. Aims of the Studies

- **To investigate whether the enhanced green fluorescent protein-glial fibrillary acidic protein (eGFP-GFAP) transgenic mice are an appropriate tool to study astrocyte pathophysiology in the cuprizone model**

  Oligodendrocytes, the myelinating cells of the CNS, orchestrate several key cellular functions in the brain and spinal cord. They can be visualized by different markers including anti-CC1, anti-OLIG2, and anti-NG2. In this study, we sought to investigate whether eGFP-GFAP$^+$ cells, which label astrocytes, co-express oligodendroglial markers' proteins during an experimental stress state (i.e., cuprizone-induced demyelination).

- **Introduction of a new MS animal model (Cup-EAE)**

  In a classical MS pathogenesis model**,** oligodendrocyte and myelin degeneration are viewed as a direct consequence of an auto-immune mediated, inflammatory attack. In contrast, the loss of oligodendrocytes eventually is the earliest hallmark during MS lesion development and, sometimes, is associated with immune cell infiltration into the CNS. It is assumed that at least in some lesions, the primary pathological event is oligodendrocyte damage which is, secondarily, followed by peripheral immune recruitment into the CNS parenchyma. An appropriate animal model to study this series of cellular events is necessary. To this end, we aimed to introduce a combinatory Cup-EAE MS model that allows to study the direct interplay of immune-mediated and metabolic oligodendrocyte injury.

3. Cumulative papers

3.1. Oligodendrocyte Lineage Marker Expression in eGFP-GFAP Transgenic Mice

Journal of Molecular Neuroscience. 2020 Dec 21. https://doi.org/10.1007/s12031-020-01771-w.

Newshan Behrangi, Peter Lorenz, and Markus Kipp.

3.2. Continuous cuprizone intoxication allows active experimental autoimmune encephalomyelitis induction in C57BL/6 mice

Histochemistry and Cell Biology. 2019 Aug; 152(2):119-131. https://doi.org/10.1007/s00418-019-01786-4.

Vladislav Yakimov, Felix Schweiger, Jiangshan Zhan, **Newshan Behrangi**, Anja Horn, Christoph Schmitz, Tanja Hochstrasser, and Markus Kipp.

4. Summary

MS is a complex chronic immune-mediated disease of the central nervous system that is associated with the development of large demyelinated plaques, oligodendrocyte destruction, and axonal degeneration. Underlying mechanisms of demyelination and neurodegeneration in MS are still poorly understood.

In many studies, anti-CC1 antibodies, presumably recognizing the protein adenomatous polyposis coli (APC) antigen, are used to label mature, myelinating oligodendrocytes. However, anti-CC1 antibodies could as well recognize other cell populations, particularly astrocytes, under pathological conditions. To examine this hypothesis, we used the cuprizone animal model, which is an appropriate model to study the mechanism of the apoptosis of oligodendrocytes and demyelination. We applied transgenic mice in which astrocytes are labeled by an eGFP under the control of the human GFAP promoter. Furthermore, we investigated the co-localization of oligodendrocyte markers, including anti-OLIG2, anti-CC1, anti-NG2, and the astrocyte marker anti-GFAP in the control and five weeks curprizone intoxicated eGFP-GFAP mice. Results of this study suggest that not all $CC1^{+}$ cells are mature oligodendrocytes, and a continuum might exist between activated astrocytes and oligodendrocytes in cuprizone intoxicated mice.

In the context of elucidating underlying mechanism of MS lesion development, some results suggest that inflammatory lesion development starts with a degenerative process within the brain, most likely oligodendrocyte stress, or even degeneration. Therefore, appropriate animal models to study the interplay of inflammation and metabolic injury are necessary. To this end, we introduced a combinatory Cup-EAE animal model, in which lymphocyte recruitment into the forebrain occurs as a consequence of simultaneous cuprizone intoxication and active EAE induction. This model recapitulates important histopathological characteristics of type III MS lesions. In summary, we provide a protocol that allows to study the direct interplay of immune-mediated and metabolic oligodendrocyte injury, and its consequences for the cerebral white and gray matter.

5. Zusammenfassung

Bei der Multiplen Sklerose handelt es sich um eine immunvermittelte chronische Erkrankung des Zentralnervensystems, die mit der Zerstörung von Oligodendrozyten, großen demyelinisierten Plaques und axonaler Schädigung einhergeht. Der genaue Mechanismus der Demyelinisierung und Neurodegeneration bei MS ist noch unklar.

In vielen Studien werden anti-CC1 Antikörper, die das adenomatous polyposis coli (APC) Protein binden, verwendet um reife und myelinisierende Oligodendrozyten darzustellen. Studien weißen allerdings darauf hin, dass Antikörper gegen CC1 unter pathologischen Bedingungen auch andere Zell-Populationen, insbesondere Astrozyten binden können. Um diese Hypothese zu testen, habe ich das Cuprizone-Tiermodell verwendet, welches ein geeignetes Modell zur Untersuchung der Apoptose von Oligodendrozyten und der Demyelinisierung darstellt. Im Rahmen meiner Experimente habe ich transgene Mäuse verwendet, in deren Astrozyten eGFP unter Kontrolle des Promotors für das humane GFAP exprimiert wird. Zusätzlich führte ich Immunofloureszenz-Dopplemarkierung mit anti-CC1, anti-OLIG2 und anti-NG2 in den transgenen Tieren durch. Die Ergebnisse meiner Untersuchungen zeigen eine deutlichen Co-Lokalisation von eGFP und verschiedenen Oligodendrozyten Markerproteinen. In dieser Arbeit konnte ich somit zeigen, dass nicht alle $CC1^{+}$-Zellen reife Oligodendrozyten sind, sondern dass vermutlich auch aktivierten Astrozyten CC1 exprimieren können.

Zur Entwicklung von MS Läsionen gibt es einige Hinweise, dass die Bildung inflammatorischer Läsionen mit degenerativen Prozessen im Gehirn beginnen, insbesondere Stress oder Degeneration von Oligodendrozyten. Zu genaueren Untersuchung dieses Wechselspiels zwischen Entzündung und Stoffwechselschädigung sind geeignete Tiermodelle notwendig. Hierfür haben wir ein kombiniertes Cup-EAE-Tiermodell etabliert, bei dem durch die gleichzeitige Behandlung mit Cuprizone und der Induktion einer aktiven EAE eine Rekrutierung von Lymphozyten ins Vorderhirn induziert wird. Dieses Modell spiegelt wichtige histopathologische Eigenschaften von Typ-III MS Läsionen wider. Zusammenfassend lässt sich feststellen, dass wir ein geeignetes Protokoll erarbeiten konnten, mit dem es möglich ist, das Wechselspiel der immunvermittelten und der metabolischen Oligodendrozytenschädigung und deren Auswirkung auf die die weiße und graue Substanz des Gehirns zu untersuchen.

6. Perspective

Siponimod (Mayzent® and Novartis) is a sphingosine 1-phosphate receptor (S1Pr) modulator that can selectively bind to S1Pr1 and S1Pr5. Siponimod has shown therapeutic effects and has been approved for the treatment of progressive MS. Future work remains to fully understand the protective effect of siponimod in the cuprizone and Cup-EAE animal model. In this project, we investigate the mechanism of action of siponimod and its molecular signaling pathways.

7. References

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