Aus der Klinik für Neurologie
der Heinrich-Heine-Universität Düsseldorf
Direktor: Univ.-Prof. Dr. med. Dr. rer. nat. Sven Meuth

Analysis of the interaction between astrocytes and microglia under stimulation with HERV-W ENV

Dissertation
zur Erlangung des Grades eines Doktors der Medizin
der Medizinischen Fakultät der Heinrich-Heine-Universität
Düsseldorf

vorgelegt von

Isabel Lewen

2025

Als Inauguraldissertation gedruckt mit Genehmigung der
Medizinischen Fakultät der Heinrich-Heine-Universität Düsseldorf

Gez.:
Dekan: Prof. Dr. med. Nikolaj Klöcker
Erstgutachter: Prof. Dr. Patrick Küry
Zweitgutachterin: Prof. Dr. Christine R. Rose

Teile dieser Arbeit wurden veröffentlicht:

Gruchot J, **Lewen I**, Dietrich M, Reiche L, Sindi M, Hecker C, Herrero F, Charvet B, Weber-Stadlbauer U, Hartung HP, Albrecht P, Perron H, Meyer U, Küry P. Transgenic expression of the HERV-W envelope protein leads to polarized glial cell populations and a neurodegenerative environment. Proc Natl Acad Sci U S A. 2023 Sep 19;120(38):e2308187120. doi: 10.1073/pnas.2308187120 . Epub 2023 Sep 11. PMID: 37695891 ; PMCID: PMC10515160.

Gruchot J, Lein F, **Lewen I**, Reiche L, Weyers V, Petzsch P, Göttle P, Köhrer K, Hartung HP, Küry P, Kremer D. Siponimod Modulates the Reaction of Microglial Cells to Pro-Inflammatory Stimulation. Int J Mol Sci. 2022 Oct 31;23(21):13278. doi: 10.3390/ijms232113278 . PMID: 36362063 ; PMCID: PMC9655930.

Silva Oliveira Junior M, Schira-Heinen J, Reiche L, Han S, de Amorim VCM, **Lewen I**, Gruchot J, Göttle P, Akkermann R, Azim K, Küry P. Myelin repair is fostered by the corticosteroid medrysone specifically acting on astroglial subpopulations. EBioMedicine. 2022 Sep;83:104204. doi: 10.1016/j.ebiom.2022.104204. Epub 2022 Aug 8. PMID: 35952494; PMCID: PMC9385547.

Zusammenfassung

Die Multiple Sklerose (MS) ist die häufigste neurodegenerative Erkrankung und führt weltweit zu einem hohen Maß an Behinderung und vorzeitigem Tod. Der Pathomechanismus der MS ist Teil weltweiter Forschungsanstrengungen. Aktivierte Gliazellen konnten als relevante Faktoren der MS Pathologie benannt werden. Bisherige Therapien basieren auf einer globalen Unterdrückung des Immunsystems und können kurzzeitig zur Symptomverbesserung beitragen. Auch eine Prognoseverbesserung kann für eine gewisse Zeit erreicht werden, jedoch nur unter beachtlichen Nebenwirkungen und unter Verlust der Effektivität über Zeit. Daher gibt es großen Bedarf die Pathogenese besser zu verstehen, um neue Therapieansätze zu ermöglichen. In diesem Zug konnten humane endogene Retroviren (HERVs) mit der Pathologie der Multiplen Sklerose in Verbindung gebracht werden, wobei das Hüllprotein (ENV) von HERV-W neuroinflammatorische und neurodegenerative Prozesse antreibt.

Diese Arbeit untersucht die Auswirkungen von HERV-W ENV auf Astrozyten und Mikroglia, zwei Gliazelltypen, die für die MS-Pathologie von entscheidender Bedeutung sind, und konzentriert sich dabei auf die Immunkompetenz, die Neurotoxizität, strukturelle Veränderungen und die Expression funktioneller Proteine. Angesichts der umfangreichen Wechselwirkung zwischen diesen Zellen liegt ein besonderer Fokus auf der Auswirkung von Mikroglia auf die ENV-Aktivierung von Astrozyten. Um dies zu beurteilen, wurden Astrozyten sowohl in direkter Co-Kultur mit Mikroglia als auch in Einsatz-Systemen analysiert, bei denen nur lösliche Faktoren interagieren können.

Die Immunzytochemie zeigte, dass die HERV-W ENV-Exposition die Zellzahl sowohl bei Mikroglia als auch bei Astrozyten verringerte. Gleichzeitig wurden aktivierte morphologische Veränderungen beobachtet. Mikroglia zeigten eine erhöhte iNOS-Expression, was auf nitrosativen Stress hinweist. Astrozyten zeigten eine Verringerung der Zellfläche, was auf eine geschwächte interzelluläre Adhäsion hindeutet, was durch eine Verringerung von Strukturproteinen gestärkt wird.

In der Genexpressionsanalyse zeigte sich unter ENV-Exposition eine deutliche Hochregulierung proinflammatorischer Zytokine und eine Unterdrückung entzündungshemmender und neuroprotektiver Proteine, was eine Verschiebung hin zu einem proinflammatorischen, neurodegenerativen Phänotyp signalisiert. Unterstützt wurde dies durch die Daten der Sekretionsanalyse. Gleichzeitig zeigte sich eine Veränderung kritischer astrozytärer Funktionen wie der Regulation der Glutamathomöostase und der Zelladhäsion, was zu Neurotoxizität und einem Zusammenbruch der Blut-Hirn-Schranke beitragen kann. Das Vorhandensein von Mikroglia, auch mit Abstand, verstärkt die ENV-induzierten astrozytären Veränderungen erheblich, was darauf hindeutet, dass die Interaktionen zwischen Astrozyten und Mikroglia zum pathologischen Umfeld bei MS beitragen können. Ein direkter Kontakt war für diesen Effekt nicht notwendig, woraus man schießen kann, dass die Vermittlung über lösliche Faktoren möglich ist.

Diese Studie unterstreicht das Potenzial der gezielten Beeinflussung von HERV-W ENV und seiner Signalwege in therapeutischen Ansätzen für MS und liefert einen Beitrag zum Verständnis der Wirkungsweise des anti-HERV-W-ENV-Antikörpers Temelimab, dessen Effektivität bereits in einer Phase 2b-Studie gezeigt werden konnte. Diese Erkenntnisse erweitern unser Verständnis der zellulären Mechanismen, durch die HERV-W ENV die Neuroinflammation und Neurodegeneration bei MS verschlimmert.

Abstract

Multiple sclerosis (MS) is the most common neurodegenerative disease and leads to a high degree of disability and premature death worldwide. The pathomechanism of MS is part of a global research effort, however, activated glial cells have been identified as relevant factors in MS pathology. Current therapies are based on a general suppression of the immune system and can contribute to a short-term improvement in symptoms. An improvement in prognosis can sometimes be achieved, but only with considerable side effects and loss of effectiveness over time. There is currently a great need to obtain a better understanding of the pathogenesis in order to develop new therapeutic approaches. In this regard, human endogenous retroviruses (HERVs) have been linked to the pathology of multiple sclerosis, with the envelope protein (ENV) of HERV-W driving neuroinflammatory and neurodegenerative processes.

This work examines the effects of HERV-W ENV on astrocytes and microglia, two glial cell types critical to MS pathology, focusing on immune competence, neurotoxicity, structural changes, and the expression of functional proteins. Given the extensive interaction between astrocytes and microglia, this work aims to investigate the effects of microglia on ENV-dependent activation of astrocytes. To assess this, astrocytes were analyzed both in direct co-culture with microglia and in insert systems in which only soluble factors were able to interact.

Immunocytochemistry showed that HERV-W ENV exposure decreased the number of cells in both microglia and astrocytes, with activated morphological changes also observed. Microglia exhibited increased iNOS expression, indicating nitrosative stress. Whereas astrocytes showed a reduced cell area, indicating weakened intercellular adhesion, which was further supported by a reduction of structural proteins.

Gene expression analysis showed a clear upregulation of proinflammatory cytokines and a suppression of anti-inflammatory and neuroprotective proteins under ENV exposure, signaling a shift towards a proinflammatory, neurodegenerative phenotype, supported by secretion data. Simultaneously, an alteration of critical astrocytic functions such as regulation of glutamate homeostasis and cell adhesion were shown, which potentially contribute to neurotoxicity and a breakdown of the blood-brain barrier. The presence of microglia, even with spacing, significantly enhanced ENV-induced astrocytic changes, suggesting that interactions between astrocytes and microglia contribute to the pathologic environment in MS. Direct contact was not required for this effect, indicating that mediation via soluble factors is possible.

This study highlights the potential of targeting HERV-W ENV and its signaling pathways in therapeutic approaches for MS and contributes to our understanding of the mechanisms of the anti-HERV-W-ENV-antibody temelimab, whose effectiveness has been demonstrated in a phase 2b trial. These findings expand our understanding of the cellular mechanisms by which HERV-W ENV contributes to neuroinflammation and neurodegeneration in MS.

Abbreviations

AD ..... Alzheimer's disease
ADAM ..... Disintegrin and metalloproteinase domain-containing proteins
ALS ..... amyotrophic lateral sclerosis
ANOVA ..... analysis of variance
APOE ..... Apolipoprotein E
AQP4 ..... aquaporin 4
BBB ..... Blood brain barrier
BD ..... bipolar disorder
BDNF ..... brain-derived neurotrophic factor
BSA ..... bovine serum albumin
C3 ..... component 3
C3d ..... Complement component 3 cleaved d
Cdh2 ..... N-Cadherin
CNS ..... central nervous system
CSF ..... cerebrospinal fluid
CXCL10 ..... C-X-C motif chemokine 10
DAMPs ..... damage-associated molecular patterns
DAPI 4,6-diamidino-2-phenylindole
DMEM ..... Dulbecco's modified eagle medium
DNA ..... Desoxyribonucleic acid
EAE ..... experimental autoimmune encephalitis
EBV ..... Epstein-Barr virus
ELISA ..... Enzyme--Linked Immunosorbent Assays
ELOVL1 ..... fatty acid elongase 1
ENV ..... envelope protein
ERVs ..... human endogenous retroviruses
FCS ..... fetal calf serum
GAPDH ..... Glyceraldehyde 3-phosphate dehydrogenase
GdE ..... gadolinium-enhancing
GFAP ..... glial fibrillary acidic protein
GJA1 ..... Gap junction alpha-1 protein = connexin 43
GJB2 ..... Gap junction beta-2 protein = connexin26
GJB6 ..... Gap junction beta-6 protein = connexin 30
GLAST ..... glutamate-aspartate transporter, glutamate-aspartate transporter = SLC1A3
GWASs ..... Genome-wide association study
HHV ..... human herpesvirus
IBA1 ..... Ionized calcium binding adaptor molecule 1
IGF-1 ..... Insulin-like growth factor 1
IL1ß ..... Interleukin 1ß
IL6 ..... Interleukin 6
iNOS ..... inducible nitric oxide synthase
ITGB3 ..... Integrin ß3
ITGBA5 ..... Integrin α5
LAL ..... limulus amebocyte lysate
LCN2 ..... Lipocalin-2
MACS ..... magnetic activated cell sorting
MERTK ..... Proto-oncogene tyrosine-protein kinase MER
MG ..... microglia
MS ..... Multiple sclerosis
MSRVs ..... multiple sclerosis retroviruses

NAWM ..... normal-appearing white matter
NDS ..... normal donkey serum
NGS ..... normal goat serum
NIH ..... National Institute of Health
NMOSD ..... neuromyelitis optica spectrum disorder
NO ..... nitric oxide
NSCs ..... neural stem cells
P2RY12 ..... P2Y purinoceptor 12
PAMPs ..... pathogen-associated molecular patterns
PBS ..... phosphate buffered saline
PCR ..... polymerase chain reaction
PDL ..... Poly-D-lysine hydrobromide
PFA ..... paraformaldehyde
PPMS ..... primary progressive form of MS
qPCR ..... quantitative polymerase chain reaction
RNA ..... ribonucleic acid
RPMS ..... relapsing progressive form
RRMS ..... relapsing-remitting MS
S100a10 ..... S100 calcium-binding protein A10
S100b ..... S100 calcium-binding protein B
SCZ ..... schizophrenia
SEM ..... standard error of the mean
SERPING1 ..... Serpin Family G Member 1
SPMS ..... secondary progressive MS
STAT3 ..... Signal transducer and activator of transcription 3
TEs ..... transposable elements
TIMP1 ..... TIMP metallopeptidase inhibitor 1
TLR4 ..... Toll like receptor 4
TNFα ..... Tumor necrosis factor alpha
TREM2 ..... Triggering receptor expressed on myeloid cells 2
ZETT ..... Zentrale Einrichtung für Tierforschung und wissenschaftliche Tierschutzaufgaben

Table of contents

1 Introduction 1
1.1 CNS 1
1.1.1 Astrocytes 2
1.1.2 Microglia 3
1.2 MS 3
1.2.1 Symptoms 3
1.2.2 Epidemiology and prognosis 4
1.2.3 Etiology 5
1.2.4 Pathology 5
1.2.5 Treatment 5
1.2.6 Astrocyte and microglial crosstalk in MS 6
1.3 HERV-W ENV 8
1.3.1 History of HERV-W 8
1.3.2 HERV-W ENV and MS 8
1.4 Aim of this thesis 9
2 Material and methods 10
2.1 Material 10
2.1.1 Reagents and buffers 10
2.1.2 Media 11
2.1.3 Molecular biology kits 12
2.1.4 Antibodies and magnetic microbeads 13
2.1.5 Equipment and software 13
2.1.6 PCR primer list 15
2.2 Methods 16
2.2.1 Cell culture methods 16
2.2.1.1 Preparation of HERV-W ENV protein 16
2.2.1.2 Ethics statement 17
2.2.1.3 Preparation of primary astrocytes and microglia from P0/1 rat brains 17
2.2.1.4 Splitting of astrocyte culture 17
2.2.1.5 Purification of astrocytes via magnetic activated cell sorting 18

2.2.1.6 Purification of microglia via magnetic activated cell sorting 19
2.2.1.7 PDL coating of cell culture dishes 19
2.2.1.8 Coculturing astrocytes and microglia 20
2.2.1.9 Stimulation with recombinant HERV-W ENV protein 21
2.2.1.10 Immunocytochemistry 21
2.2.1.11 Image generation and processing 23
2.2.2 Molecular biological methods 23
2.2.2.1 Isolation of RNA 23
2.2.2.2 Reverse transcription 24
2.2.2.3 Real-time quantitative PCR 24
2.2.2.4 ELISA 25
2.2.3 Statistical Analysis 25
3 Results 26
3.1 Stimulation with recombinant HERV-W ENV reduces microglia cell count and induces a degenerative shift in microglia 26
3.2 HERV-W ENV stimulation reduces astrocyte cell count and area 27
3.3 Stimulation with recombinant HERV-W ENV decreases GLAST and increases AQP4 expression in astrocytes 29
3.4 Stimulation with recombinant HERV-W ENV increases astroglial C3d 31
3.5 Stimulation with recombinant HERV-W ENV increases LCN2 expression in astrocytes 33
3.6 Shift in gene expression and secretion in astrocytes under stimulation with HERV-W ENV 34
4 Discussion 37
4.1 Paracrine signaling is a mechanism of microglial enhancement of the ENV-effect 38
4.2 HERV-W ENV-stimulated astrocytes convert into a pro-inflammatory subpopulation 39
4.3 The influence of HERV-W ENV onto astroglia's role in neuroinflammation 42
4.4 HERV-W ENV induces neurodegenerative changes in astrocytes 43
4.5 Limitations 44
4.6 Outlook 46
4.7 Conclusion 47
5 Literaturverzeichnis 48
6 Danksagung 67

1 Introduction

1.1 CNS

The human nervous system is divided into the central nervous system (CNS) and the peripheral nervous system. The CNS comprises the brain and spinal cord, composed primarily of neurons and glial cells. Neurons are responsible for electrochemical signaling, while glial cells - initially recognized as supportive cells (Virchow 1856) - play vital roles in both structural and functional support. Glial cells are categorized into microglia and macroglia, with the latter consisting of oligodendrocytes and astrocytes. Oligodendrocytes form myelin sheaths around neurons to accelerate signal transmission, while astrocytes contribute to structural support, blood flow regulation, and immune signaling. Microglia, the brain's resident immune cells, constitute the CNS's innate immune response and are essential in neuroinflammation. Importantly, glial cells are now understood to regulate neuronal signaling and homeostasis actively.

The CNS processes efferent information from the environment and coordinates responses. The functions of the human brain are as broad as the human experience, encompassing cognitive functions, memory, perception, and motor control. As a result, neurological diseases, particularly neurodegenerative conditions, manifest through a wide array of symptoms affecting both cognitive and physical capabilities.

The CNS is protected by the blood-brain barrier (BBB), which restricts immune cell entry and modulates substance exchange between the bloodstream and brain tissue (Carson et al. 2006; Owens et al. 1998). This barrier complicates treatment by limiting drug access to the CNS. While the BBB is intact, microglia are the only immune cells in the brain. In disease states, however, the BBB can become permeable, allowing immune cells to infiltrate the brain.

Damage to the CNS, as seen in neurodegenerative diseases, has a limited capacity for repair due to an environment that is inherently unfavorable for regeneration (Nagappan et al. 2020). While some degree of regeneration is possible, the CNS often performs damage containment through the formation of glia scars (Liddelow und Barres 2016). Which, although protective, inhibits tissue repair and results in loss of functions. In contrast, there is evidence that suggests glial scars can be favorable for axonal regrowth (Liddelow und Barres 2016). Once neurons are killed by neurotoxic processes they rely on replacement by neural stem cells, but these are restricted to specific stem cell niches in limited brain regions (Lin und Iacovitti 2015). Similar for glia cells; oligodendrocytes can regenerate from oligodendrocyte progenitor cells (OPCs), which are more widely distributed, yet their regenerative capacity diminishes over time (Gruchot et al.

2019). This intrinsic limitation in regenerative potential contributes significantly to the progressive nature of many neurodegenerative diseases.

1.1.1 Astrocytes

Astrocytes comprise roughly 30% of glial cells in the CNS, acting as support cells for neurons (Bartheld et al. 2016). They are organized into discrete territories that form a functional syncytium through gap junctions, facilitating communication and metabolic exchange with oligodendrocytes (Sofroniew und Vinters 2010; Bartheld et al. 2016; Orthmann-Murphy et al. 2008). Astrocytes are typically classified into two main types: protoplasmic astrocytes, found in gray matter, with fine, evenly distributed processes, and fibrous astrocytes, located in white matter with longer, fiber-like processes (Sofroniew und Vinters 2010).

Astrocytes are immunocompetent cells, changing into astrogliosis when triggered and able to promote further pro-inflammatory changes like cytokines and migration of macrophages and lymphocytes (Han et al. 2021). In the healthy brain, they produce neurotrophic factors (Magistretti 2006), and maintain homeostasis of glutamate, calcium, extracellular potassium, and water (Sofroniew und Vinters 2010; Ransohoff und Brown 2012). Astrocytes provide metabolic support for neurons in form of lactate and antioxidants (Anderson et al. 2004; Masutani et al. 2004); as well as neurosteroids, modulating neuronal excitability (Masutani et al. 2004).

Astrocytes are an active part of the tripartite synapses. One astrocyte hereby connects with tens of thousands of neuronal synapses (Ventura und Harris 1999), regulating synaptic transmission by controlling the release of neurotransmitters like glutamate (Bezzi und Volterra 2001; Santello und Volterra 2010). Astrocytes also prune synapses through phagocytosis. A process more prevalent in development but still important for memory and learning in the adult brain (Chung et al. 2013).

Astrocytes stand in contact with blood vessels, as part of metabolic functions, as well as immunological isolation, but they also regulate blood flow dependent on neuronal activity (Attwell et al. 2010). In the BBB the glia limitans consists of the endfeet of astrocytes, which envelope the corresponding parts of the blood vessels (Sofroniew 2015; Sofroniew und Vinters 2010), thereby forming a barrier that restricts the entry of peripheral immune cells into the CNS in the healthy brain (Brosnan und Raine 2013; Horng et al. 2017). For a long time, the brain was considered an immune-privileged organ, which has since been changed to an understanding of a heavily controlled lymphatic system (Negi und Das 2018).

1.1.2 Microglia

Microglia constitute 5–10 % of total brain cells (Li und Barres 2018). Their morphology consists of small somata with fine cellular processes in the healthy mature CNS. Microglia originate from the yolk sac and enter the brain during prenatal development (Li und Barres 2018). After closing of the BBB, they maintain their population in the CNS independent of peripheral macrophages. Microglia support brain development by mediating cell death and shaping neural structures. In the adult brain, microglia shift to roles in synaptic pruning, OPC maintenance, and myelin phagocytosis. They are crucial in disease for clearing cellular debris and facilitating tissue repair, especially in remyelination and neuroprotection (Lampron et al. 2015).

Microglia play a dual role in neuroregeneration. In neurodegenerative conditions, microglia are often activated by damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs), adopting a pro-inflammatory phenotype (Kwon und Koh 2020). This state, characterized by the release of cytokines such as TNFα, IL6, and IL1β, as well as reactive oxygen and nitrogen species, contributes to neuronal damage, synapse loss, and the exacerbation of neuroinflammation (Kutzelnigg et al. 2005; Jack et al. 2005; Lassmann 2014; Giannetti et al. 2014). However, microglia are equally critical for neuroregeneration. In an anti-inflammatory or repair-associated phenotype, microglia clear cellular debris, especially myelin, through phagocytosis, create an environment conducive to remyelination and secrete neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and insulin-like growth factor (IGF). These processes support the survival and differentiation of oligodendrocyte progenitor cells (OPCs) and promote axonal repair (Colonna und Butovsky 2017; Prowse und Hayley 2021). The balance between these opposing roles of microglia is influenced by the local CNS environment and disease context. Thus, they play a central role in the progression of many neurodegenerative diseases.

1.2 MS

1.2.1 Symptoms

Multiple sclerosis (MS) is a chronic, neurodegenerative disease affecting the CNS in humans. The disease was first described by French neurologist Jean-Martin Charcot in 1868 (JM Charcot 1868). At the beginning patients often experience a growing symptom for a few days to weeks, that remits with varying amounts of residual. Common first symptoms are optic neuritis, sensibility issues and fatigue, rarer are cranial nerve, cerebellum, or spinal involvement. Following that, focal lesions in all regions of the CNS lead to diverse symptoms including the loss of

sensitivity, paresthesia, muscle weakness and spasticity, blurred vision/blindness (optic neuritis), problems with speech and/or swallowing, etc. (Lassmann et al. 2012). Cortical signs, such as aphasia, apraxia, seizures, or extrapyramidal signs are observed more rarely (Compston 2005). The diagnosis is made primarily on the basis of the medical history, physical exam, and MRI, which was formalized as the McDonald criteria (Thompson et al. 2018): neurologic lesions, which are disseminated in both space and time. Those can be shown through a typical clinical evaluation of episodes of intermittent neurological impairment, or T2-hyperintense lesions, and gadolinium-enhancing T1 lesions on MRI or characteristic oligoclonal bands in the cerebrospinal fluid (CSF). In addition, differential diagnoses need to be excluded. With new treatment options being developed, the need for new diagnostic criteria arises. New postulations include diagnosis by dissemination in space only, via biomarkers in cerebrospinal fluid, and classification according to disease intensity.

1.2.2 Epidemiology and prognosis

MS affects approximately 2.9 million people worldwide (Wallin 2017). In Germany, the prevalence amounts to 280.000 people with a steep increase over the last 30 years, at least partially stemming from better diagnostic methods (Hemmer 2023). Strong regional differences could be described with prevalence rates particularly high in Europe, North America, and Australia (Koch-Henriksen et al. 2018). MS onset peaks between the ages of 20 and 40 affecting more women with a ratio of 2:1 (Kurtzke 2000).

The course of the disease varies so that 4 different forms are distinguished. The most common is relapsing-remitting MS (RRMS) with patients experiencing relapses of symptoms and a following remission to an asymptomatic stage. 60-70 % of those patients develop a secondary progressive MS (SPMS), where remission is no longer possible, and symptoms worsen progressively. 10 % of MS patients experience the progressive course of the disease already in the early stages, suffering from the primary progressive form of MS (PPMS) (Dutta und Trapp 2014; Hauser und Oksenberg 2006; Trapp und Nave 2008). Another 10 % of patients suffer from a relapsing progressive form (RPMS), experiencing a progressive worsening of symptoms from the beginning with added acute relapses, without remission, leading to a fast-paced accumulation of symptoms (Lublin und Reingold 1996). Despite advances in disease-modifying treatments, MS remains a progressive condition with a significant impact on life expectancy and quality of life.

1.2.3 Etiology

The etiology of MS remains a topic of research. Due to the varying prevalence, migration analyses were conducted which could show young immigrants assume the probability of disease of their new countries, suggesting environmental influences (Kurtzke 2000; Pugliatti et al. 2002; Rosati 2001). Further potential disease furthering factors include smoking, childhood obesity, low vitamin D levels, and possibly a high salt diet (Ascherio 2013; O'Gorman et al. 2012). In addition, viral infections, such as the Epstein-Barr virus (EBV) and human herpesvirus (HHV) 6, have been suggested as triggers for MS (Alvarez-Lafuente et al. 2004; Wagner et al. 2004). Lastly, there seems to be a genetic component to MS development. Genome-wide association studies (GWASs) were performed to identify risk alleles and discovered MHC locus on chromosome 6p21 as a factor in MS (Sawcer et al. 2005). This demonstrates a multifactorial genesis with various modifiable and predetermined risk factors.

1.2.4 Pathology

MS pathology is characterized by inflammation and demyelination, leading to neurodegeneration. Damage to oligodendrocytes impairs signal transmission, while immunoreactions produce a neurotoxic environment, leading to neuronal death. White matter lesions are being enclosed by astrocytes leading to the formation of glia scars. Neuronal death was seen as a result of the massive loss in oligodendroglia, but recent studies suggest that neurodegeneration can be directly mediated by inflammatory microglia and astrocytes (Muzio et al. 2021). The demyelinated areas have the potential to remyelinate due to the differentiation of resident OPCs and neural stem cells (NSCs), however, this process appears overall inefficient (Kotter et al. 2011) leading to MS progression.

1.2.5 Treatment

MS treatment follows two main approaches. During acute relapses, high-dose glucocorticoids are administered to control acute inflammation and promote remission. Long-term treatment focuses on improving prognosis, reducing disability, and enhancing quality of life. This treatment is topic of busy research. Immunomodulatory drugs ranging from Interferon, over Cladribrin/Siponimod to CD20-antibodies, are prescribed based on disease activity. In addition, symptomatic treatments are also integral, tailored to patient needs, and include physiotherapy, occupational therapy, speech therapy, psychotherapy, and antispastic therapy (Hemmer 2023).

For patients with low disease activity, the first-line treatments are the oldest drugs: Interferon-β preparations and glatiramer acetate treatment. They require frequent subcutaneous or intramuscular injections, and provide moderate efficacy, but show very rarely life-threatening adverse effects. Next in line are teriflunomide and dimethyl fumarate, they can be administered orally and show equal or better efficacy, with more potentially severe adverse effects. The most potent options—fingolimod, natalizumab, daclizumab, and alemtuzumab—are reserved for higher disease activity, due to their effectiveness but come with a greater risk of serious, possibly life-threatening side effects (Soelberg Sorensen 2017). Only laquinimod, dimethyl fumarate, and fingolimod/siponimod are known to cross the BBB (Ponath et al. 2018), with siponimod and laquinimod demonstrating positive effects on progressive MS and reducing brain atrophy. For primary progressive MS CD20-antibody, namely ocrelizumab, is solely recommended, because of its higher potential to affect neuroregeneration. However, benefits could only be shown for younger patients, indicating a great need for innovation.

1.2.6 Astrocyte and microglial crosstalk in MS

Since astrocytes and microglia heavily promote MS pathology and interact with each other, promoting inflammation and neurodegeneration, this section will illustrate the different ways in which these cell types affect each other and how this contributes to MS progression.

In a healthy state, astrocytes are anti-inflammatory, secreting low levels of cytokines like TGFβ (John et al. 2003) and IL10 (Cannella und Raine 1995). Activation, however, changes their morphology to a hypertrophic phenotype, showing an enlarged soma, and reduced process density (Brosnan und Raine 2013). Those astrocytes are present at the margins of demyelinating lesions and extend into the normal-appearing white matter (NAWM), indicating their role in lesion development (Ponath et al. 2017b; Brosnan und Raine 2013). In experimental autoimmune encephalitis (EAE), a murine MS model, astrocyte activation is detectable before immune cell infiltration, suggesting they are early players in lesion formation (Wang et al. 2005; D'Amelio et al. 1990; Pham et al. 2009). These astrocytes contain myelin, which activates NFκB signaling and chemokine secretion, further fueling immune responses (Ponath et al. 2017a; Ponath et al. 2017b).

Astrocyte activation has been categorized into a pro-inflammatory (A1) type, marked by complement component 3 (C3), and an anti-inflammatory (A2) type, which expresses neurotrophic factors (Clarke und Liddelow 2017; Liddelow und Barres 2017). However, research has shown that astrocyte phenotypes are more complex, varying according to activation mode and disease stage. In MS, an early stage was identified with pro-inflammatory cytokines and

reactive oxygen species, as well as hypertrophy and proliferation, that could coexist and shift to a later stage that was described as leaning toward an anti-inflammatory and neuroregenerative phenotype (Cordiglieri und Farina 2010).

Activated microglia also undergo distinct changes in cell shape, gene expression, and functional behavior in response to infection, trauma, or ischemia (Block et al. 2007; Colton und Wilcock 2010; Streit et al. 2005), assuming an amoeboid shape with reduced complexity of cellular processes. The microglia become motile and actively follow chemokine gradients toward lesions and infections. Blocking this microglial activation has been shown to suppress EAE (Heppner et al. 2005). The dichotomic division of microglia in M1/M2 is equally outdated. However, a more pro-inflammatory phenotype can be described to produce reactive oxygen and nitrogen species and proinflammatory cytokines, contributing to myelin destruction and neurodegeneration like in MS. The anti-inflammatory phenotype is associated with anti-inflammatory molecules and myelin debris clearance as needed in MS (Lampron et al. 2015; Orihuela et al. 2016). Recent research reveals a spectrum of mixed phenotypes influenced by local conditions (Paolicelli et al. 2022; Stratoulias et al. 2019; Wishart et al. 2023).

This complex balance between inflammatory and regenerative functions complicates the identification of therapeutic targets. As described above, microglia are essential for clearing myelin debris, which is a prerequisite for remyelination. Broadly, microglial phagocytic activity reduces damage-associated molecular patterns (DAMPs), which in turn decreases immune activity and the activation of other cells, such as astrocytes and blood-derived immune cells (Lampron et al. 2015; Orihuela et al. 2016). Astrocytes form glial scars that contain inflammation, prevent the spread of toxic agents, and stabilize damaged areas (Sofroniew und Vinters 2010). They also secret neurotrophins and growth factors that promote neurite outgrowth (Bush et al. 1999) like BDNF, whose deletion has been shown to worsen clinical outcomes in EAE (Linker et al. 2010).

Activated microglia produce reactive oxygen and nitrogen species and proinflammatory cytokines (TNFα, IL6, and IL1β, etc.) (Kutzelnigg et al. 2005; Jack et al. 2005; Lassmann 2014; Giannetti et al. 2014), which are shown to activate astrocytes. Microglial C1q complement and cytokines induce astrocytes to neurotoxicity and destruction of synapses (Liddelow et al. 2020). Myelin degradation and neurotoxic metabolites from microglial activity release DAMPs, further activating astrocytes (Srinivasan et al. 2005). Similarly, pathogen-associated molecular patterns (PAMPs) from pathogens, such as double-stranded RNA or bacterial endotoxins, also activate astrocytes (Farina et al. 2007; Jensen et al. 2013; Rothhammer und Quintana 2015a). Activated astrocytes, in turn, recruit macrophages, microglia, and lymphocytes through chemokines and cell adhesion molecules (Ponath et al. 2017a; Sørensen et al. 1999) and through ATP release,

creating a gradient sensed by microglial P2Y purinoceptor 12 (P2RY12), controlling microglial chemotaxis to injury sites. Consequently, astrocytes and microglia perpetuate mutual activation, leading to an escalating immune response.

1.3 HERV-W ENV

1.3.1 History of HERV-W

The human genome project revealed that 40-50% of the human genome consists of transposable elements (TEs) (Lander et al. 2001), including human endogenous retroviruses (HERVs) (Venter et al. 2001). ERVs were first described in the late 1960s and early 1970s (Weiss, 2006), making up 5% - 8% of the human genome (Lander et al. 2001; Stocking und Kozak 2008). There are 20 different humane ERV families (Nelson et al. 2003), and their persistence suggests some degree of symbiosis with the host. Some HERVs have been domesticated to produce proteins with crucial functions, while others may trigger immune responses, contributing to neurodegenerative diseases when activated (Küry et al. 2018). For instance, syncytin-1 and -2, derived from HERV-W and HERV-FRD, respectively, play essential roles in placental development (Xiang und Liang 2021). For many other retroviral particles activation and expression are described that lead to neurodegenerative and neuropsychiatric diseases such as MS, amyotrophic lateral sclerosis (ALS), Alzheimer's (AD) as well as schizophrenia (SCZ), and bipolar disorder (BD) (Römer 2021). Typically silenced in healthy individuals through epigenetic mechanisms (Deaton und Bird 2011; Lavie et al. 2005; Ohtani et al. 2018; Szpakowski et al. 2009).

HERV expression seems to be epigenetically silenced, so known regulators of epigenetic modifications such as UV radiation, nutritional factors, and specific drugs are proposed to be able to increase HERV expression (Gruchot et al. 2023a). This pathway does not seem to be the only reason, why HERVs are highly expressed in specific diseases. It could be shown, that HERV expression can be reactivated by inflammation. NFκB binding in proximity to HERVS is known to express regulatory functions (Manghera und Douville 2013) and is considered as a pathway for direct activation of HERVs through inflammation. Retroviral particles on the other hand can induce inflammation themselves leading indirectly to their own expression.

1.3.2 HERV-W ENV and MS

HERV-W particles were first discovered in leptomeningeal cell cultures from MS patients (Perron et al. 1989) and named multiple sclerosis retroviruses (MSRVs) (Perron und Seigneurin

1999). Subsequent studies comparing the CSF and serum of MS patients with healthy controls showed increased RNA and protein levels of the HERV-W envelope protein (ENV) in MS patients (Garson et al. 1998; Mameli et al. 2007; Mameli et al. 2009; Perron et al. 2012).

It has further been shown that the expression of HERV-W ENV triggers an immune response (Perron et al. 2001; Rolland et al. 2006), it activates TLR4, induces the production and secretion of pro-inflammatory cytokines, and activates human monocytes (Rolland et al. 2006). The expression could be linked to myeloid cells and astrocytes in humans (Kremer et al. 2013; van Horssen et al. 2016). In vitro studies could show an influence onto TLR4- expressing OPCs at the border of MS lesions reducing their differentiation capacity and in turn reducing myelin expression and therefore reducing repair activities. This was proposed to be mediated by nitrosative stress (Kremer et al. 2013). Similarly, microglia were shown to increase pro-inflammatory cytokine and chemokine production as well as nitric oxide levels after exposure to HERV-W ENV protein. As described above, these microglia were shown to act in an axon-damaging way (Kremer et al., 2019). Blockage of TLR4 or HERV-W ENV leads to diminished effects of ENV in models of disease, proving their role in mediating those effects (Göttle et al. 2019; Göttle et al. 2021; Kremer et al. 2015). These findings could be transferred to MS in humans with the anti-HERV-W ENV antibody, temelimab, which has demonstrated safety and efficacy in MS treatment in humans through phase 2b trials. Latest studies showed fewer new T1-hypointense lesions, reductions in brain atrophy, and higher myelin integrity under treatment with temelimab in comparison to placebo (Hartung et al. 2022).

1.4 Aim of this thesis

As highlighted, HERV-W ENV is strongly linked to MS. While the impact on oligodendrocytes and microglia is relatively well-studied, the role of astrocytes remains less understood, despite their significant involvement in MS pathogenesis. Previous work by our group showed a neurodegenerative switch of microglia after stimulation with HERV-W ENV and even showed them to be in proximity to axons, suggesting a neurotoxic role. Given the intricate crosstalk between astrocytes and microglia, this study aims to investigate astrocytic responses to HERV-W ENV, both in isolation and in the presence of microglia. Understanding these expression and secretion patterns may offer valuable insights for diagnosis and treatment. Portions of these results have been published in Gruchot, Lewen et al. “Transgenic expression of the HERV-W envelope protein leads to polarized glial cell populations and a neurodegenerative environment” 2023.

2 Material and methods

2.1 Material

2.1.1 Reagents and buffers

Name	Supplier
4,6-diamidino-2-phenylindole (DAPI)	Roche, Basel, Switzerland
Bovine serum albumin (BSA) Fraction V 7.5%	Thermo Fisher Scientific, Waltham, USA
Chloroform	Merck, Darmstadt, Germany
Citifluor mounting medium	Citifluor, London, UK
HERV-W ENV	Protein'eXpert, Grenoble, France
Ethanol ≥ 96%	Merck, Darmstadt, Germany
RedDot	Biotium; Fremont, USA
Isoflouran	Piramal-Healthcare, Mumbai India
L-accutase	Thermo Fisher, Waltham, USA
LiChrosolv® water	Merck, Darmstadt, Germany
NGF	R&D Systems, Minneapolis, USA
Normal goat serum (NGS)	Sigma-Aldrich, St. Louis, USA
Normal donkey serum (NDS)	Sigma-Aldrich, St. Louis, USA
Paraformaldehyde (PFA)	Merck, Darmstadt, Germany
Poly-D-lysine hydrobromide (PDL)	Sigma-Aldrich, St. Louis, USA
RNase-free water	Qiagen, Hilden, Germany
Shandon immu-mount	Thermo Fisher Scientific, Waltham, USA
TRI-reagent	Sigma-Aldrich, St. Louis, USA
Triton X-100	Sigma-Aldrich, St. Louis, USA
Trypan blue, 0.4%	Sigma-Aldrich, St. Louis, USA
β-mercaptoethanol	Sigma-Aldrich, St. Louis, USA

Buffer	Merck, Darmstadt, Germany
Tris-HCl 20 mM pH 7.5	
SDS 1.5%	
DTT 10mM	
NaCl 150mM	
PB buffer	
Bovine serum albumin 0,5 %	Thermo Fisher Scientific, Waltham, USA
In Dulbecco´s phosphate buffered saline (PBS)	Sigma-Aldrich, St. Louis, USA
RLT-buffer	Qiagen, Hilden, Germany

2.1.2 Media

Solution A	
MEM	Thermo Fisher Scientific, Waltham, USA
10% 30 U/ml papain	Worthington / Cell Systems, Lakewood, USA
20% 0.24 mg/ml L-cysteine	Sigma-Aldrich-Aldrich, St. Louis, USA
2% 40 µg/ml DNase	Worthington / Cell Systems, Lakewood, USA
Solution B: trypsin inhibitor solution	
1 mg/ml trypsin inhibitor	Sigma-Aldrich-Aldrich, St. Louis, USA
5 mg/mL albumin BSA-V	Thermo Fisher Scientific, Waltham, USA
40 µg/ml DNase I	Cell Systems, Lakewood, USA
in 1 ml Leibovitz's medium L-15	Sigma-Aldrich-Aldrich, St. Louis, USA
Solution C	
Dulbecco's modified eagle medium (DMEM) low glucose	Thermo Fisher Scientific, Waltham, USA
10% fetal calf serum (FCS)	Capricorn Scientific, Palo Alto, USA

Cell culture media

Dulbecco's modified eagle medium (DMEM) low glucose	Thermo Fisher Scientific, Waltham, USA
10% fetal calf serum (FCS)	Capricorn Scientific, Palo Alto, USA
2 mM L-glutamine 50 U/mL	Thermo Fisher Scientific, Waltham, USA
50 U/mL penicillin/streptomycin	Thermo Fisher Scientific, Waltham, USA

Trypsin

Leibovitz's medium L-15	Sigma-Aldrich-Aldrich, St. Louis, USA

Inhibitor medium

0.1 mg/mL trypsin inhibitor	Cell Systems, Lakewood, USA
5 mg/mL albumin BSA-V	Sigma-Aldrich-Aldrich, St. Louis, USA
0.4 µg/mL DNase I	Thermo Fisher Scientific, Waltham, USA

2.1.3 Molecular biology kits

High-Capacity cDNA Reverse Transcription Kit	Thermo Fisher Scientific, Waltham, USA
Neural Tissue Dissociation Kit (P)	Miltenyi Biotec, Bergisch Gladbach, Germany
PureLink Genomic DNA Purification Kit	Thermo Fisher, Waltham, USA
Red HS Taq Master Mix	Biozyme, Hessisch Oldendorf, Germany
RNeasy Mini Kit	Qiagen, Hilden, Germany
SYBR Green Master Mix	Thermo Fisher Scientific, Waltham, USA

2.1.4 Antibodies and magnetic microbeads

anti-CD11b magnetic microbeads rat	Miltenyi Biotec, Bergisch Gladbach, Germany
anti-ACSA1-biotin microbeads rat	Miltenyi Biotec, Bergisch Gladbach, Germany
anti-biotin microbeads	Miltenyi Biotec, Bergisch Gladbach, Germany
goat anti mouse Alexa Flour 594	Thermo Fisher Scientific, Waltham, USA
goat anti mouse Alexa Flour 405	Thermo Fisher Scientific, Waltham, USA
goat anti mouse Alexa Flour 488	Thermo Fisher Scientific, Waltham, USA
goat anti rat Alexa Flour 594	Thermo Fisher Scientific, Waltham, USA
goat anti rabbit Alexa Flour 488	Thermo Fisher Scientific, Waltham, USA
goat anti rabbit Alexa Flour 594	Thermo Fisher Scientific, Waltham, USA
donkey anti goat Alexa Flour 594	Thermo Fisher Scientific, Waltham, USA
donkey anti rabbit Alexa Flour 488	Thermo Fisher Scientific, Waltham, USA
donkey anti mouse Alexa Flour 647	Thermo Fisher Scientific, Waltham, USA

2.1.5 Equipment and software

7900HT Fast Real-Time PCR System	Thermo Fisher Scientific, Applied Biosystems, Foster City, USA
96 Well Fast Thermal Cycler	Fritz Gössner GmbH, Hamburg, Germany
Autoclave GLA30	JENOPTIK AG, Jena, Deutschland
AxioCam HR c	Zeiss, Oberkochen, Germany
Axioplan 2 Fluorescence microscope	Zeiss, Oberkochen, Germany
Axiovision 4.2 software	Thermo Scientific, Waltham, USA
BBD 6220 CO2 incubator	Greiner, Kremsmünster, Österreich
Cell culture inserts	Swiss Academic Software GmbH, Wädenswil, Schweiz
Citavi 6.18	Miltenyi Biotec, Bergisch Gladbach, Germany
Columns	Bio-Rad, München, Germany
Electrophoresis device and power supplier	Eppendorf, Wesseling-Berzdorf, Germany
Eppendorf Centrifuge 5804	
Thermo Fisher Scientific, Waltham, USA	

Excella E24 incubator	USA Scientific, Orlando, USA
GraphPad PRISM software 8.01	GraphPad Software, Inc, La Jolla, USA
Herasafe HSP 12 sterile bench	Heraeus, Hanau, Germany
ImageJ software	National Institute of Health, Rockville, USA
Megafuge 3.0R	Thermo Scientific, Waltham, USA
Microsoft 365 MSO	Microsoft Corporation, Redmond, USA
NanoDrop ND 1000	PeqLab, Erlangen, Germany
Primer Express 3.0.1	Thermo Fisher Scientific, Waltham, USA
Tecan i-control	Tecan, Männedorf, Switzerland
Thermomix	Biotech international, Worcester, UK
Thoma counting chamber (Depth: 0.100 mm Area: 0.0025 mm²)	Optik Labor, Görlitz, Germany
Veriti thermocycler	Applied Biosystems, Foster City, USA
Water bath	GFL, Burgwedel, Germany
Zeiss LSM 510 microscope	Zeiss, Oberkochen, Germany
Zen black software	Zeiss, Oberkochen, Germany
Zen blue software	Zeiss, Oberkochen, Germany

2.1.6 PCR primer list

Name	Forward sequence	Reverse sequence
rAdam	CAG AGT GGT GCA ATG GAA CCT	TAG ACC CCA TCC TCC ACA TAC C
rApoe	GCA GGC GGA GAT CTT CCA	TGT CTT CCA CTA TTG GCT CGA A
rAqp4	CAT GGC CAG CAG TGA GGT TT	CAT CGC CAA GTC CGT CTT CT
rBdnf	GGT ATC AAA AGG CCA ACT GA	GCA GCC TTC CTT GGT GTA AC
rC3d	GGT CTG CGG AAG TGT TGT GA	GGC GCT GGC AGC TGT ACT
rCdh2	CAA GCA CAG CGG ACG TCT A	CAA GTT GAT CGG AGG GAT GAC
rCxcl10	GGG ATC CCT CTC GCA AGA A	CTC AGC GTC TGT TCA TGG AAG T
rElovl1	CCT GAA GCA CTT GG ATG GT	CAA TGA CCT TG AAA GCA TGA A
rGapdh	GAA CGG GAA GCT CAC TGG C	GCA TGT CAG ATC CAC AAC GG
rGja1	CCC ACT GAG CCC ATC AAA A	CAG CCA TTG AAG TAG GCG TAT TT
rGjb2	AGT GTA ACG CCT GGC CTT GT	TTC TGT GGG CCT GGA AAT G
rGjb6	TGC CCT GGG TGC TGA AA	TGA AGC AGT CCA CGA GAT TGG
rGlast	TCA CCG TCA GCG CTG TCA	ACG GTC GGA GGG CAA ATC
rIgf1	AGA CGG GCA TTG TGG ATG A	ACA TCT CCA GCC TCC TCA GAT C
rIl1b	GAA ACA GCA ATG GTC GGG AC	AAG ACA CGG GTT CCA TGG TG
rIl6	GTT GTG CAA TGG CAA TTC TGA	TCT GAC AGT GCA TCA TCG CTG
rNos2	GAC GAG ACG GAT AGG CAG AG	GTG GGG TTG TTG CTG AAC TT
rItgb3	CCT CTC AGA TGC GCA AGC TT	TCC ACA AAG GCC CCA AAG
rItgba5	TCT TCC GAT TGA GGA TCT CTT CA	TGA ATG CCC CAG GTG ATG T
rLcn2	GGG CAG GTG GTT CGT TGT C	AGC GGC TTT GTC TTT CTT TCT G
rMertk	TCT GAC AGA GAC CGC AGT CTT C	TGG ACA CCG TCA GTC CTT TG
rS100a10	GCC ATC CCA AAT GGA GCA T	CCC CTG CAA ACC TGT GAA AT
rS100b	GAG CAG GAA GTG GTG GAC AAA	CAC TCC CCA TCC CCA TCT T
rSerping1	GAC AGC CTG CCC TCT GAC A	GCA CTC AAG TAG ACG GCA TTG A
rSlc1a3	TCA CCG TCA GCG CTG TCA	ACG GTC GGA GGG CAA ATC
rStat3	CCG GCC CTT AGT CAT CAA GA	TTG ACC AGC AAC CTG ACT TTT G
rTimp1	CGC AGC GAG GAG TTT CTC AT	GGC AGT GAT GTG CAA ATT TCC
rTrem2	CCA AGG AGC CAA TCA GGA AA	GGC CAG GAG GAG AAG AAT GG

2.2 Methods

2.2.1 Cell culture methods

2.2.1.1 Preparation of HERV-W ENV protein

The recombinant full-length HERV-W envelope protein was produced by Proteine'Xpert, Grenoble (France) in BL21 Escherichia coli. The HERV-W ENV protein consists of 548 amino acids and has a molecular weight of 61,44 kDa. The protein sequence is shown in Figure 1.

MALPYHTFLFTVLLPPFALTAPPPCCCTTSSSPYQEFLWRTRLPGNIDAPSYRSLSKG
NSTFTAHTHMPRNCYNSATLCMHANTHYWTGKMINPSCPGGLGATVCWTYFTHTS
MSDGGGIQGQAREKQVKEAISQLTRGHSTPSPYKGLVLSKLHETLRTHTRLVSLFNTTL
TRLHEVSAQNPTNCWMCLPLHFRPYISIPVPEQWNNFSTEINTTSVLVGPLVSNLEITH
TSNLTCVKFSNTIDTTSSQCIRWVTPPTRIVCLPSGIFFVCGTSAYHCLNGSSESMCFLSF
LVPPMTIYTEQDLYNHVVPKPHNKRVPILPFVIRAGVLGRLGTGIGSITTSTQFYYKLSQ
EINGDMEQVTDSLVTLQDQLNSLAAVVLQNRRALDLLTAKRGGTCLFLGEERCYYVN
QSRIVTEKVKEIRDRIQCRAEELQNTERWGLLSQWMPWTLPFLGPLAAIIFLLLFGPCIF
NFLVKFVSSRIEAVKLQIVLQMEPQMQSMTKIYRGPLDRPARLCSDVNDIEVTPPEEIS
TAQPLLHSNSVGSSHHHHHH

Fig. 1 ***Protein sequence of HERV-W ENV.*** *Written in single-letter amino acid code*

Our partners from GeNeuro SA, Plan-les-Ouates (Switzerland) evaluated sterility and purity of HERV-W ENV by measuring endotoxin levels using the limulus amebocyte lysate (LAL) test and found them to be below the detection limit of <5EU/ml.

The HERV-W ENV was diluted in buffer to a concentration of 1 mg/ml. In the following the HERV-W ENV will be referred to as "ENV" and the dilution buffer, which was used as a control will be referred to as "buffer". The stock solution of ENV and buffer were stored in aliquots at -80°C until further use.

2.2.1.2 Ethics statement

All animal procedures were approved by the Animal Research Institute of the Heinrich-Heine-University Düsseldorf: ZETT (Zentrale Einrichtung für Tierforschung und wissenschaftliche Tierschutzaufgaben) under the license O69/11 and were performed in compliance with regional experimental guidelines.

2.2.1.3 Preparation of primary astrocytes and microglia from P0/1 rat brains

The generation of astrocytes and microglia from early postnatal rat cortices was performed by laboratory technicians of our research group following a protocol based on McCarthy and De Vellis (McCarthy und Vellis 1980). For steps of preparation and experiments see Fig. 2. The animals were bred at the ZETT and kept under defined and sterile conditions. The P0/1 Wistar rats were anesthetized with isoflurane, disinfected with ethanol and decapitated. The brain was separated from the surrounding tissue and the meninges were removed from the cortex. The cortical lobes were separated and cut into small pieces. The cortex tissue of 6 rats was placed in cell culture medium and centrifuged for 1 min at 2000 rpm. The supernatant was exchanged to 1 ml of solution A to digest the tissue and incubated for 45 min at 37°C and 5 % CO2. After mixing with 1 ml of solution B to stop the digesting process it was incubated for 4 min at 37°C and 5 % CO2. The supernatant was discarded and replaced again by 1 ml of solution B to further digest the tissue. It was titrated with Pasteur pipettes to dissociate mechanically. Then 10 ml solution C was added and centrifuged for 5 min at 1200 rpm. The supernatant was discarded. The dissociated rat cortices were resuspended in 15 ml cell culture medium and cultured in 3 T-75 flasks precoated and filled with 15 ml cell culture medium. The flasks were incubated at 37°C and 10 % CO2 and 98 % humidity with medium changes twice a week.

2.2.1.4 Splitting of astrocyte culture

The primary astrocytes were split 1:1 or 1:2 depending on the cell density. The cell culture flasks with astrocyte and microglia coculture were placed at the orbital shaker for 2 h at 37°C and 180 rpm to detach microglia from the adhering astrocytes. The medium containing the microglia was further cultivated. To the flasks containing the astrocytes 10 ml fresh cell culture medium was added and they were placed at the orbital shaker for another 24 h at 37°C and 180 rpm to further purify astrocytes. The medium was collected again to cultivate microglia. The flasks were washed 3 times with PBS. 5 ml trypsin was added to each flask and incubated for 5-10 min at 37°C with a closed lid to detach the cell layer. The flasks were tapped against an edge to mechanically

detach the cells from the bottom of the flask. Adding 10 ml cell culture medium stopped the digesting process of the trypsin and resuspended the cells. The cell suspension was collected and centrifuged for 5 min at 1200 rpm at 4°C. Supernatant was discarded and astrocytes were resuspended in 1 ml cell culture medium. The astrocytes were cultured in prepared T-75 flasks filled with 20 ml cell culture medium and incubated at 37°C and 10 % CO2 and 98 % humidity for two days.

2.2.1.5 Purification of astrocytes via magnetic activated cell sorting

The purification of astrocytes was achieved by magnetic activated cell sorting (MACS) modified based on the protocol from Miltenyi Biotec. Since this is a rather time-consuming procedure, it is important to work efficiently and keep the solutions at 2-8°C. Two days after splitting the astrocytes the flasks were placed at the orbital shaker for 24 h at 37°C and 180 rpm to minimize microglial contamination. The medium was discarded and the cell layer was washed with 10 ml PBS. 5 ml trypsin was added and incubated for 5 min to detach the cell layer. The reaction was stopped by adding 10 ml cell culture medium and the cells of two flasks were collected in a flacon. The cell suspension was purified via magnetic activated cell sorting according to modified protocol. Briefly, the cell suspension was centrifuged at 1200 rpm for 5 min at 4°C. The supernatant was discarded and the cells were resuspended in 160 µl PB buffer (kept on ice). 40 µl anti-GLAST (ACSA-1)-biotin microbeads were added and incubated for 10 min at 4°C labeling the astrocyte-specific GLAST-protein with biotin. The cells were washed with 2 ml PB buffer and centrifugated at 1200 rpm for 5 min at 4°C. After discarding the supernatant, the cells were resuspended in 160 µl PB buffer, mixed with 40 µl anti-biotin micro-beads, and incubated for 15 min at 4°C to add a magnetic body to the labeled astrocytes. The cells were again washed with 2 ml PB buffer, centrifuged at 1200 rpm for 5 min at 4°C and the supernatant was discarded. The cells were resuspended in 2 ml PB buffer. The cell suspension was added to a prepared column in a magnetic field, beforehand rinsed with 3 ml PB buffer. After the cell suspension went into the column so that the magnetically labeled astrocytes bound to it, the column was washed 3 times with 3 ml PB buffer to rinse out all non-labeled cells. Then the column was taken out of the magnetic field and the astrocytes were flushed out into a collection tube with 5 ml PB buffer whilst pushing the plunger into the column. The purified cell suspension was centrifuged at 1200 rpm for 5 min at 4°C, the supernatant was discarded and the astrocytes were resuspended in cell culture medium.

2.2.1.6 Purification of microglia via magnetic activated cell sorting

Similar to the astrocyte purification, primary microglia were isolated by MACS-based protocols, modified from Miltenyi Biotec. To separate microglia from primary astrocyte and oligodendrocyte cultures the flasks were put on the orbital shaker for 2 h and got a medium change before they were put on the shaker for another 24 h. The medium from the 2 h shake was collected, centrifugated at 1200 rpm for 5 min and the supernatant was exchanged for fresh cell culture medium. The cell suspension was incubated in a 10 cm petri dish at 37°C and 10 % CO2 and 98 % humidity until the next day. The medium from the 24 h shake was collected in 10 cm petri dishes and incubated for at least two hours before collecting the medium and washing the dishes with 5 ml PBS. 3 ml L-accutase were added to the cell layer and incubated for 5 min at 37°C and 10 % CO2 to detach the cells from the dish. After mechanical detachment, 4 ml cell culture medium was added and microglial cells were collected in a falcon tube. The cell suspension was purified via magnetic activated cell sorting according to modified protocol. Briefly the cell suspension was centrifuged at 1200 rpm for 5 min at 4°C. The supernatant was exchanged for 80 µl PB buffer and 20 µl CD11b/c microbeads were added to magnetically label microglia. The suspension was incubated for 15 min at 7°C and then washed with 2 ml PB buffer and centrifuged at 1200 rpm for 5 min at 4°C. The supernatant was discarded and the cells were resuspended in 500 µl PB buffer. The cell suspension was added to a prepared column in a magnetic field, beforehand rinsed with 500 µl PB buffer. After the cell suspension went into the column and the magnetically labelled microglia have bound to it, the column was washed 3 times with 500 µl PB buffer to rinse out all non-labelled cells. Then the column was taken out of the magnetic field and the microglia were flushed out into a collection tube with 1 ml PB buffer by pushing the plunger into the column. The purified cell suspension was centrifuged at 1200 rpm for 5 min at 4°C, the supernatant was discarded and the microglia were resuspended in cell culture medium.

2.2.1.7 PDL coating of cell culture dishes

For easy ICC, PDL coated glass coverslips were used. Briefly, 13 mm sterile glass cover slips were placed in a 24-well plate. PDL was filtered and diluted to the concentration of 0,1 mg/ml with PBS. 500 µl of this PDL solution were added to each well onto the coverslip and incubated overnight at 4°C. Before cultivation of the astrocytes the cover slips were washed three times with PBS, to eliminate residual PDL. After adding 500 µl medium and ensuring that the coverslips are attached to the bottom of the well the plate was incubated at 37°C and 10 % CO2 for equilibration.

2.2.1.8 Coculturing astrocytes and microglia

For reliable results a confluent astrocyte cell layer was obligatory. Preliminary experiments identified 30.000 astrocytes per well as expedient and this number was chosen as a standard. For determination of the number of astrocytes per microliter 10 µl of the purified cell suspension was mixed with 10 µl trypan blue solution. 10 µl of this mixture was added to each side of a Thomas counting chamber. 30.000 astrocytes per well were added into a precoated 24-well-plate prepared with 500 µl medium. Through gentle motions the cells were distributed evenly to assist growing into a confluent cell layer. The cells were incubated at 37°C and 10 % CO2.

The primary astrocytes were cultivated for three days and given a medium change on day three if not confluent by then. The microglia were counted in the same way using trypan blue and a Thomas counting chamber. On day four 100.000 microglia were added to the astrocytes directly or in a cell culture insert prepared with an additional 200 µl medium. One third of the astrocytes were cultivated alone as a control group. For visualization of different conditions see fig. 2.

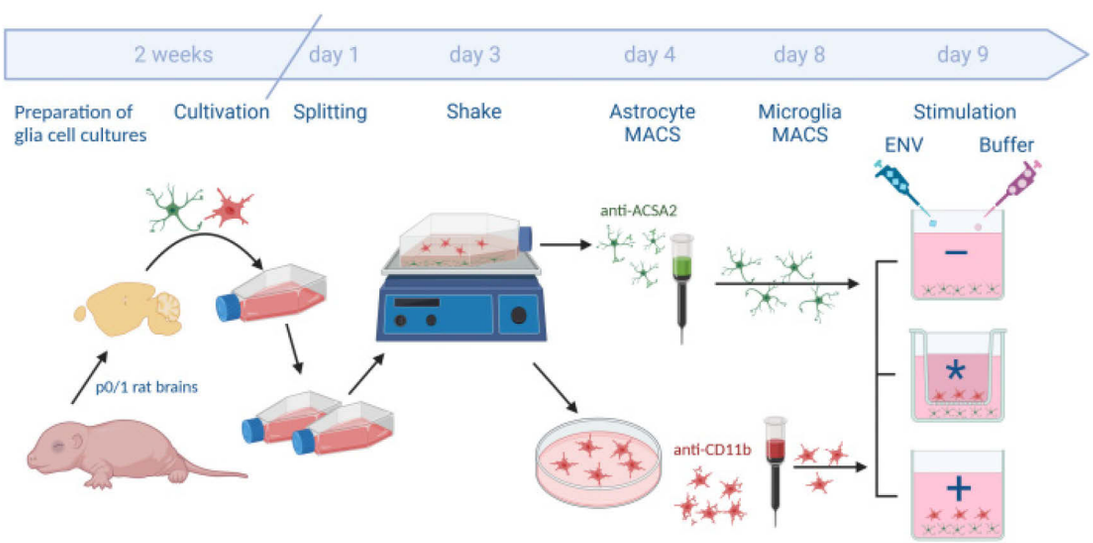

Fig. 2 ***Illustrated timeline*** *of experimental procedure. Isolation of glia cells from p0/1 rat brains. Cultivation, splitting and shake of mixed cultures. Magnetic activated cell sorting (MACS) of astrocytes, further cultivation and following isolation of microglia via MACS. Planting of astrocytes for all conditions; "-": without microglia; "*": microglia in transwells; "+": with microglia. Stimulation with ENV or buffer. Image generated using biorender.com*

2.2.1.9 Stimulation with recombinant HERV-W ENV protein

24 h after adding the microglia the cocultures were stimulated with ENV. Therefore, the stock solution of ENV was diluted in medium to a concentration of 1 µg/ml. The buffer solution was treated in the same way. The medium in the wells was changed to 500 µl ENV/buffer-medium. The medium in the transwell culture insert was changed to 200 µl ENV/buffer-medium respectively.

2.2.1.10 Immunocytochemistry

Analyzing timepoints were set to 1 and 3 d after stimulation. For immunocytochemistry the cocultures were fixed with paraformaldehyde (PFA). Therefore, the medium was aspirated and the cells were fixed with 4% PFA in PBS, 500 µl was added per well and 200 µl per transwell culture insert. The fixation was incubated for 15 min at room temperature. The cover slips were carefully washed three times with PBS and the plate was sealed and stored at 4°C.

For immunocytochemistry the coverslips were placed in a coloring chamber and kept wet at all times. For detail and concentrations of the blocking and staining solutions see table 1. Volumes of 80 µl were used per cover slip. First the cells were permeabilized and blocked with Triton X-100 and serum in PBS for 40 min at room temperature to avoid unspecific staining. The primary antibodies were incubated for 24 h at 4°C. After washing the coverslips 3 times with PBS the secondary antibodies were incubated for 2 h at room temperature in light-protected conditions. For the secondary antibodies appropriate IgG conjugated to Alexa Fluor594, Alexa Fluor488, Alexa Fluor405, or Alexa Fluor647 were used. 4´,6-diamidin-2-phenylindol (DAPI; 1:100) or RedDot (1:300) were used to visualize the nuclei. After washing 3 times with PBS the coverslips were mounted.

antigens	Triton X-100 concentration		serum	primary antibodies	supplier	dilution	nucleus staining	secondary antibodies 1:500 in
	for blocking	for primary antibody solution	for blocking and primary antibody solution	(RRID AB_)				
GFAP - IBA1	0,5%	0,1%	10% NDS	gt IBA1 (2224402)	Abcam, Cambridge, UK	1:500	DAPI	PBS
				rb GFAP (10013382)	Dako Agilent, Santa Clara, USA	1:1000		
				rb IBA1 (839504)	WAKO Pure Chemical Corporation, Osaka Japan	1:500	RedDot	PBS
				ms GFAP (94844)	Merck Millipore, Darmstadt, Germany	1:1000		
				gt SOX9 (2194160)	R and D Systems, Minneapolis, USA	1:750		
AQP4 - GLAST	0,5%	0,1%	2% NGS	rb AQP4 (258270)	Sigma-Aldrich, Darmstadt, Germany	1:1000	DAPI	PBS
				ms GLAST (10829302)	Miltenyi Biotec, Bergisch Gladbach, Germany	1:250		
C3d - GFAP	1%	1%	10% NGS	rb C3d (578478)	Agilent, Santa Clara, USA	1:500	DAPI	10% NGS, 1% Triton X-100
				ck GFAP (304558)	Abcam, Cambridge, UK	1:1000		
LCN2 - GFAP	0,5%	0,1%	10% NDS	gt LCN2 (355022)	R and D Systems, Minneapolis, USA	1:100	DAPI	PBS
				ck GFAP (304558)	Abcam, Cambridge, UK	1:1000		
				free-floating				
IBA1 - iNOS	0,5%	0,1%	10% NDS	rb iNOS (10688716)	Abcam, Cambridge, UK	1:500	DAPI	PBS
				gt IBA1 (2224402)	Abcam, Cambridge, UK	1:500		

Table 1 ***Staining procedures.*** *Contents and concentrations.*

For the staining of the membranes of the cell culture inserts these membranes were cut out of the inserts as close to the frame as possible. The membranes were placed inside a 48-well plate and blocked free-floating in 200 µl Triton X-100 and NDS (for details see table 2) for 40 min at room temperature. The primary antibodies were incubated for 24 h at 4°C and the membranes were carefully washed 3 times. The secondary antibodies were incubated for 2 h at room temperature in light-protected conditions and after washing 3 times the membranes were mounted between a glass slide and a coverslip.

2.2.1.11 Image generation and processing

For documentation of the immunocytochemic images were taken in Z-scheme using the Axioplan 2 fluorescence microscope with Axiovision 4.2 software and the Zeiss LSM 510 microscope with Zen black/blue software. 7 images per coverslip were taken with a 20x objective lens. Two coverslips per condition for each individual experiment were evaluated. For further analyses, Fiji (Schindelin et al. 2012) based on ImageJ (National Institute of Health (NIH) USA) was used to define positive cells, count them, and analyze their intensity, area, and area fraction.

2.2.2 Molecular biological methods

2.2.2.1 Isolation of RNA

To isolate ribonucleic acids from the astrocytes and cocultures the cells were lysed. β-mercapto-ethanol was diluted 1:100 in RLT-buffer. The medium was exchanged with 350 µl β-mercapto-ethanol dilution and the cells were further lysed mechanically. Cell lysates were immediately frozen on dry ice and stored at -80°C or directly processed.

For isolation of RNA, the RNeasy Mini Kit from Qiagen was used to the manufacturer's protocol: For homogenization, the lysate was placed in Qia-shreeder-column and centrifuged at 14.000 rpm for 1 min. The eluate was mixed with 350 µl 70 %-ethanol and added to the RNeasy-column. The column was centrifuged at 10.000 rpm for 1 min to bind the RNA to the column and centrifuged again at 10.000 rpm for 1 min with 350 µl RW1-buffer to get rid of debris. The eluate was discharged and 75 µl RNAfree DNAse buffer (1:8 DNAse in RDD) was incubated for 15 min at room temperature to cleave the DNA and then centrifuged with 350 µl RW1-buffer at 10.000 rpm for 1 min to wash the DNA-fragments out. The column was washed twice with 500 µl RPE-buffer and centrifuged each time at 10.000 rpm for 1 min. To remove the remaining ethanol the column was centrifuged at 14.000 rpm for 1 min dry with a new collection tube. Finally, RNA was washed out with 21 µl RNAse free ddH2O after 2 min incubation at 10.000 rpm for 1 min. RNA was stored at -30°C until further processing.

2.2.2.2 Reverse transcription

In order to perform real-time quantitative polymerase chain reaction (qPCR) analysis RNA was reverse transcribed with the High-Capacity cDNA Reverse Transcription Kit with RNase inhibitor according to manufacturer's protocol: RNA concentration was measured with the nano drop and diluted to 19,8 µl RNA solution containing 250 ng RNA. A master- mix (see Table 2) was prepared and 10,2 µl was added to the RNA solution. The reverse transcription was then performed in a thermocycler in four steps: first 10 min at 25°C to mix the components, then 2 h at 37°C to complement the RNA strands with DNA for double-stranded RNA-DNA strands, these strands were detached at 85°C for 5 seconds and stored at 4°C until further processing.

Reagent	Volume in µl
10x RT Buffer	3
10x RT Random Primers	3
25x dNTP Mix (100 mM)	1,2
Reverse Transcriptase 50 U/µl	1,5
RNAse Inhibitor 20 U/µl	1,5
Total volume	10,2

Table 2 ***Reverse transcription master mix****. Components and their respective volumes per sample in µl.*

2.2.2.3 Real-time quantitative PCR

The determination of relative DNA concentrations was performed using the SYBR Green procedure and master mix and the 7900 HT Fast Real-Time PCR System. The RNA was diluted to a volume of 100 µl and 4 µl per well was placed in a 96-well q-RT PCR plate. A master mix was produced according to the supplier protocols (see Table 3). 16 µl of the master mix were added to each well.

Reagent	Volume in µl
oligonucleotide forward primer (5 µM)	1,2
oligonucleotide reverse primer (5 µM)	1,2
LiChrosolv H2O	3,6
SYBR Green master mix	10
Total volume	16

Table 3 ***qPCR master mix****. Components of the qPCR master mix and their respective volumes per sample.*

PCR was performed using an amplification protocol involving an activation phase at 50°C for 2 min followed by a denaturation phase at 95°C for 10 min and 40 cycles of one minute at 60°C for amplification and 15 seconds at 95°C for separation. The relative amount of amplificated

double strands was measured in each circle through the incorporation of SYBR Green. GAPDH proved to be the most accurate and stable normalization gene and was therefore used as reference gene. Relative gene expression levels were determined according to the ΔΔCt method. All samples were analyzed in duplicates.

Specific primers were designed using the Primer Express software and tested for specificity with amplicon melt curve analysis.

2.2.2.4 ELISA

The determination of secreted molecules was performed using enzyme-linked immunosorbent assays (ELISA). To assess astrocyte secretion of TNFα, CXCL10, and IL-6, the following colorimetric sandwich ELISA kits were used: rat TNF alpha ELISA Kit (ab100785, Abcam), rat CXCL10 ELISA Kit (ab270896, Abcam) and rat IL-6 ELISA kit (ab234570, Abcam). Culture media were collected at 1 and 3d time points. The medium was centrifuged at 1000 × g for 5 min at 4 °C, and stored at −80 °C until further processing. Prior to use, all reagents were thawed and adjusted to room temperature. Culture media were measured in duplets according to the supplier's protocol.

2.2.3 Statistical Analysis

Data are shown as mean values ± standard error of the mean (mean ± SEM). Statistical analyses were conducted using Graph-Pad Prism. Normality was confirmed by Shapiro-Wilk test. Significance was assessed using Student's unpaired t-test. Multiple comparisons were assessed by 2-way ANOVA followed by Sidak's post hoc test. The experimental groups were considered significantly different at *P < 0.05, **P < 0.01, ***P < 0.001.

3 Results

3.1 Stimulation with recombinant HERV-W ENV reduces microglia cell count and induces a degenerative shift in microglia

Recent studies have shown that HERV-W ENV exerts a pro-inflammatory, neurotoxic effect on microglia, most likely through TLR4 signaling (Kremer et al. 2019). Given the close functional interaction between microglia and astrocytes, both of which express TLR4, this study hypothesized that ENV might also activate astrocytes. To look into that, astrocytes were cocultured with microglia and stimulated with ENV. Control groups with buffer and without microglia (-MG) in all combinations were analyzed. To differentiate between different activation mechanisms, microglia were introduced to astrocytes either in direct co-culture (+MG) or separated by transwell culture dishes (*MG), allowing only soluble factors to interact.

Prior findings demonstrated that co-cultured microglia undergo a degenerative shift under ENV via nitrosative stress among others (Kremer et al. 2019). To assess whether similar changes occur in the transwell setup, transwell membranes were examined and stained for Ionized calcium-binding adaptor molecule 1 (IBA1) as a microglial marker and inducible nitric oxide synthase (iNOS) as a nitrosative stress marker (Fig. 3A) (Gruchot, Lewen et al.2023b). All cells on the membrane stained positive for IBA1, identifying them as microglia (Fig. 3B). The number of microglia seemed to be less under treatment with ENV. In the coculture (+MG) at both time points, the number of IBA1-positive microglia has been lower under treatment with ENV than with buffer. The reduction was statistically significant for the 3-day timepoint (Fig. 3D) (Gruchot, Lewen et al.2023b). The number of microglia in the transwell and in coculture were hereby comparable. ENV treatment led to a statistically significant increase in iNOS-positive microglia in the transwell setup (Fig. 3C), indicating pro-inflammatory activation (Gruchot, Lewen et al.2023b), as before described for cocultured microglia (Kremer et al. 2019).

Activated microglia phenotypically change into an amoeboid form, with fewer processes and a broader cell body (Kaur et al. 2001). So, the size of microglia in coculture was analyzed. The area per cell was bigger under treatment with ENV (Fig. 3A,E), which showed consistency with a more stretched-out, amoeboid form. The number of microglia in cocultures grew from the 1-day to the 3-day timepoint, as did the area per microglia. This should be considered as an additional factor for differences in astrocytes between the time points.

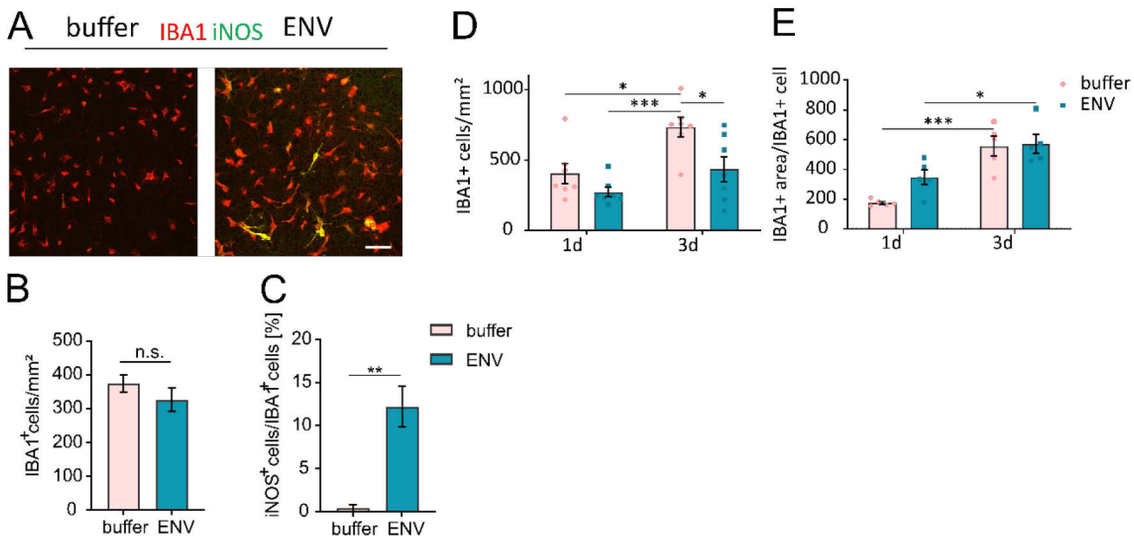

Fig. 3 ***Reduction of microglia cell count and increase of microglial iNOS and cell area under ENV stimulation****. **A** Representative immunocytochemical images of IBA1-positive microglia cells grown in transwells expressing iNOS treated with ENV and buffer after 24 h. **B** Quantification of IBA1-positive microglia in transwells under stimulation with ENV and buffer after 24 h. **C** Ratio of iNOS-positive microglia under stimulation with ENV and buffer after 24 h. **D** Quantification of IBA1+ microglia in coculture under stimulation with ENV and buffer after 24 h and 72 h. **E** Area per IBA1-positive microglia under stimulation with ENV and Buffer after 24 h and 72 h. IBA1 (Ionized calcium-binding adaptor molecule 1), iNOS (inducible nitric oxide synthase). Data represented as mean +/-SEM. Significance of microglia analyses (B and C) was assessed by Student's unpaired t-test. All other quantifications were analyzed via 2-way ANOVA followed by Sidak's post hoc test. Data was considered statistically significant (95% CI) at *P < 0.05, **P < 0.01, ***P < 0.001. (Scale bar: 50 µm. 20x objective)*

3.2 HERV-W ENV stimulation reduces astrocyte cell count and area

To ensure accurate astrocyte analysis, microglial contamination in monocultures and transwell conditions was evaluated using glial fibrillary acidic protein (GFAP) as an astrocyte marker (Liddelow und Barres 2017) and IBA1 as a microglial marker (Fig. 4A). The astrocyte purity was over 98% across all conditions (Fig. 4B) (Gruchot, Lewen et al.2023b).

Before analyzing the changes in astrocyte function and secretion, the influence of different conditions on astrocyte cell count and size was measured. The astrocytes were quantified by staining for GFAP. ENV exposure decreased astrocyte cell counts, statistically significant in co-culture after 3 days (Fig. 4E,F) (Gruchot, Lewen et al.2023b). This reduction was stronger in the presence of microglia and there was no shown effect of microglia on astrocytes under buffer treatment, suggesting that ENV-activated microglia amplify the effects of ENV on astrocytes, both directly and indirectly.

Morphological changes of astrocytes were examined by quantifying the area per astrocyte. For this, the GFAP-positive area was divided by the number of astrocytes. There appears to be a trend at the 3-day timepoint for a decrease of astrocyte area under treatment with ENV in the monoculture and the transwells (Fig. 4C,D), pointing towards weakened intercellular adhesion, supported by the loss of structural proteins shown below. This decrease in astrocyte area cannot be seen in coculture.

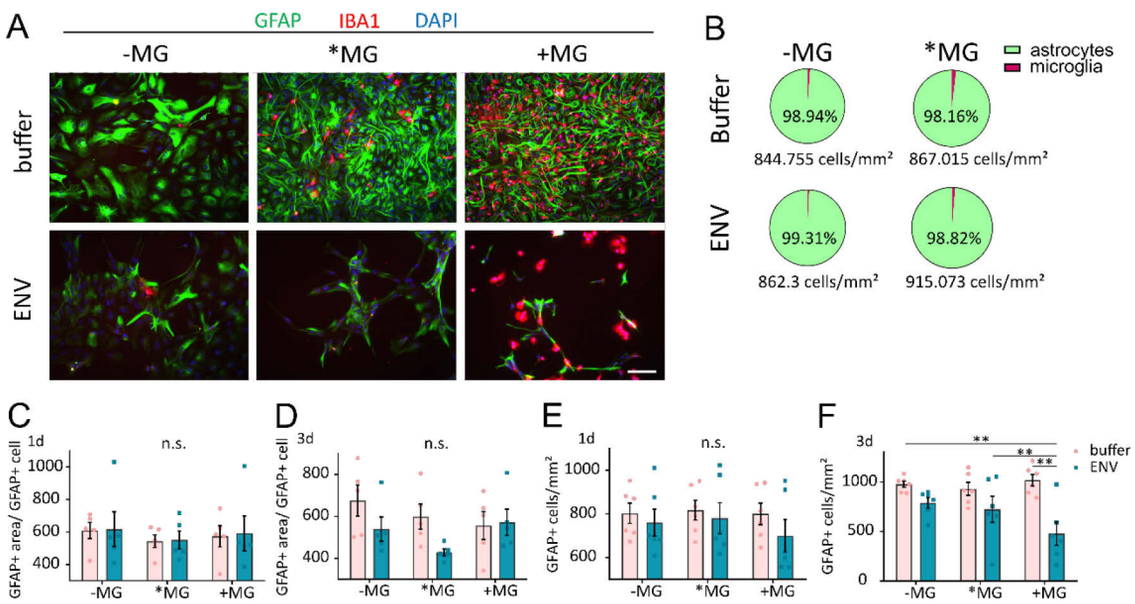

Fig. 4 ***Astrocyte purity and reduction of astrocyte cell count and area under stimulation with ENV. A*** *Representative immunocytochemical images of GFAP-positive astrocytes and IBA1-positive microglia cells treated with ENV and buffer after 72 h.* ***B*** *Microglial contamination and astrocyte cell count of astrocyte cultures without microglia and with microglia in transwells.* ***C,D*** *Area per GFAP-positive astrocyte in all six conditions after 24 h (C) and 72 h (D).* ***E,F*** *Quantification of GFAP+ astrocytes in all six conditions after 24 h (E) and 72 h (F). IBA1 (Ionized calcium-binding adaptor molecule 1), glial fibrillary acidic protein (GFAP). - MG: without microglia; + MG: with microglia; * MG: microglia in transwells. Data represented as mean +/- SEM. Significance was analyzed via 2-way ANOVA followed by Sidak's post hoc test. Data was considered statistically significant (95% CI) at *P < 0.05, **P < 0.01. (Scale bar: 100 µm. x20 objective)*

3.3 Stimulation with recombinant HERV-W ENV decreases GLAST and increases AQP4 expression in astrocytes

Astrocytic functions were assessed by examining the expression of aquaporin 4 (AQP4) and glutamate-aspartate transporter (GLAST), both of which are disrupted in MS pathology (Liddelow et al. 2017; Mandolesi et al. 2015). In acute MS lesions synaptic changes such as elevated glutamate concentrations could be shown, caused by a reduced capacity of glutamate transporters (Srinivasan et al. 2005), suggesting a reduction of GLAST.

GLAST showed a trend towards reduction by ENV in this work. In immunocytochemistry every astrocyte was positive for GLAST, but in varying intensity. Its gene expression, measured by PCR, as well as its intensity in immunocytochemistry appeared to be noticeably reduced by ENV, but without statistical significance (Fig. 5A,B,C). Because of the experimental design, gene expression levels could only be analyzed for astrocytes without direct contact with microglia, otherwise overlapping expression levels of both cell types would have been measured. The total area fraction, as in the fraction of GLAST-positive area to total area, was statistically significantly reduced under treatment with ENV, when microglia were present (Fig. 5C), pointing towards synaptic modulation.

AQP4 is a water channel located in astrocytic endfeet directly regulating water and potassium homeostasis and functions as an adhesion molecule that is involved in cell migration and neuroexcitation, synaptic plasticity, and memory function. It has been shown increased in inflammatory lesions modulating astrocyte-to-microglia communication and promoting inflammation by inducing among others LCN2 and complement factors (Ikeshima-Kataoka 2016).

In this work, AQP4 was increased by ENV. The gene expression of AQP4 was increased under treatment with ENV, statistically significant without microglia at the 1-day time point (Fig. 5E,H). The number of AQP4-positive cells in immunocytochemistry was quantified. Both conditions with microglia were comparable so the focus was laid on the transwell condition. The relative number of AQP4-positive cells to GLAST-positive cells was increased under treatment with ENV, statistically significant in the presence of microglia after 1 day (Fig. 5F,I). The intensity of AQP4 seemed to be increased under ENV in the presence of microglia at the 3-day time point (Fig. 5G). The fraction of AQP4-positive area to GLAST-positive area was increased under ENV in presence of microglia at both time points, statistically significant in the coculture (Fig. 5G). This pattern indicates that ENV increases AQP4 expression, with microglia potentially enhancing this effect at the protein level.

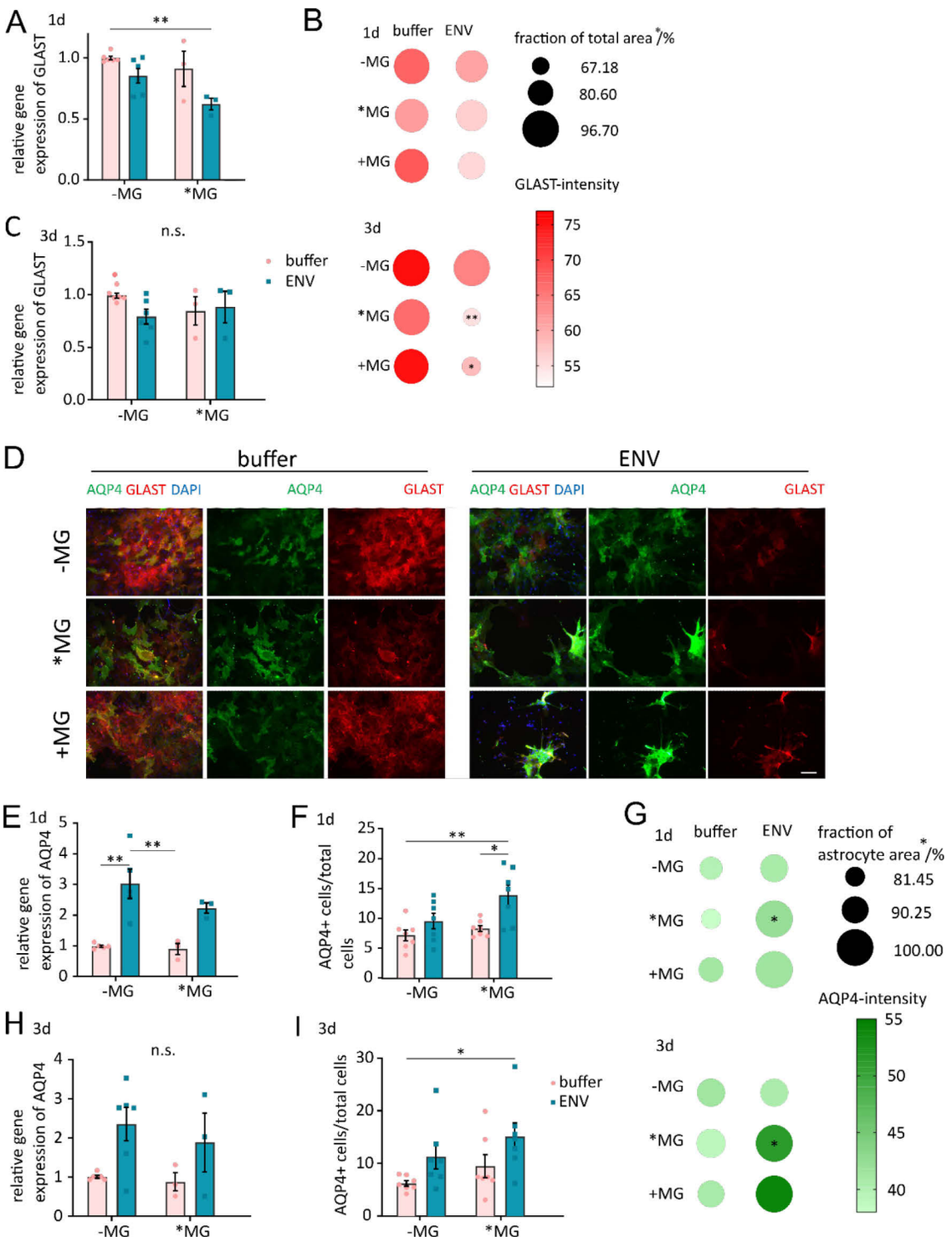

Fig. 5 ***Decrease of GLAST and increase of AQP4 expression under stimulation with HERV-W ENV. A,C*** *Relative gene expression levels of GLAST in astrocytes in absence and presence of microglia under stimulation with ENV and buffer after 24 h (A) and 72 h (C).* ***B*** *Representation of GLAST-Intensity and GLAST-positive area.* ***D*** *Representative immunocytochemical images of astrocytes expressing GLAST and AQP4 in all six conditions after 72 h. Channels splitted.* ***E,H*** *Relative gene expression levels of AQP4 in astrocytes in absence and presence of microglia under stimulation with ENV and buffer after 24 h (E) and 72 h (H).* ***G*** *Representation of ApP4-Intensity and fraction of ApP4-positive area of astrocyte area.* ***F,I*** *Quantification of strong AQP4-positive astrocytes in all six conditions after 24 h (F) and 72 h (I). GAPDH was used as reference gene. Aquaporin 4 (AQP4), glutamate-aspartate transporter (GLAST). - MG: without microglia; + MG: with microglia; * MG: microglia in transwells. Data represented as mean +/-SEM. Significance was analyzed via*

*2-way ANOVA followed by Sidak's post hoc test. Data was considered statistically significant (95% CI) at *P < 0.05, **P < 0.01. (Scale bar: 100 µm. 20x objective)*

3.4 Stimulation with recombinant HERV-W ENV increases astroglial C3d

In order to analyze the effect of HERV-W ENV onto inflammatory astrocytes complement fragment C3d was used. C3d has been shown to be statistically significantly expressed in astrocytes of MS patients as well as in MS models such as EAE (Tassoni et al. 2019; Michailidou et al. 2017) and as part of the innate immune system leads to astrogliosis (Escartin et al. 2021; Pekna und Pekny 2021).

This work showed a trend toward stimulation of C3d expression by both ENV and microglia separately. Again, gene expression as well as protein expression were analyzed. While the relative gene expression of C3d was increased upon ENV stimulation, statistically significant without microglia at the 3-day time point (Fig. 6A,D), the relative number of C3d-positive cells in astrocytes as a trend was increased by ENV as well as by microglia. A statistically significant increase was measured under treatment with ENV in presence and absence of microglia, but also under treatment with buffer in presence of microglia (Fig. 6B,E) (Gruchot, Lewen et al.2023b). C3d intensity seems to be weakest without microglia upon treatment with buffer and higher stimulated with ENV and/or microglia. The fraction of the C3d-positive area to GFAP-positive area was increased under ENV at both time points, statistically significant without microglia at both time points and in the coculture at the 1-day time point (Fig. 6C).

In conclusion, there is an induction of C3d under ENV, stronger in presence of microglia. In contrast to AQP4 there also is an induction of C3d under microglia and buffer especially at protein levels.

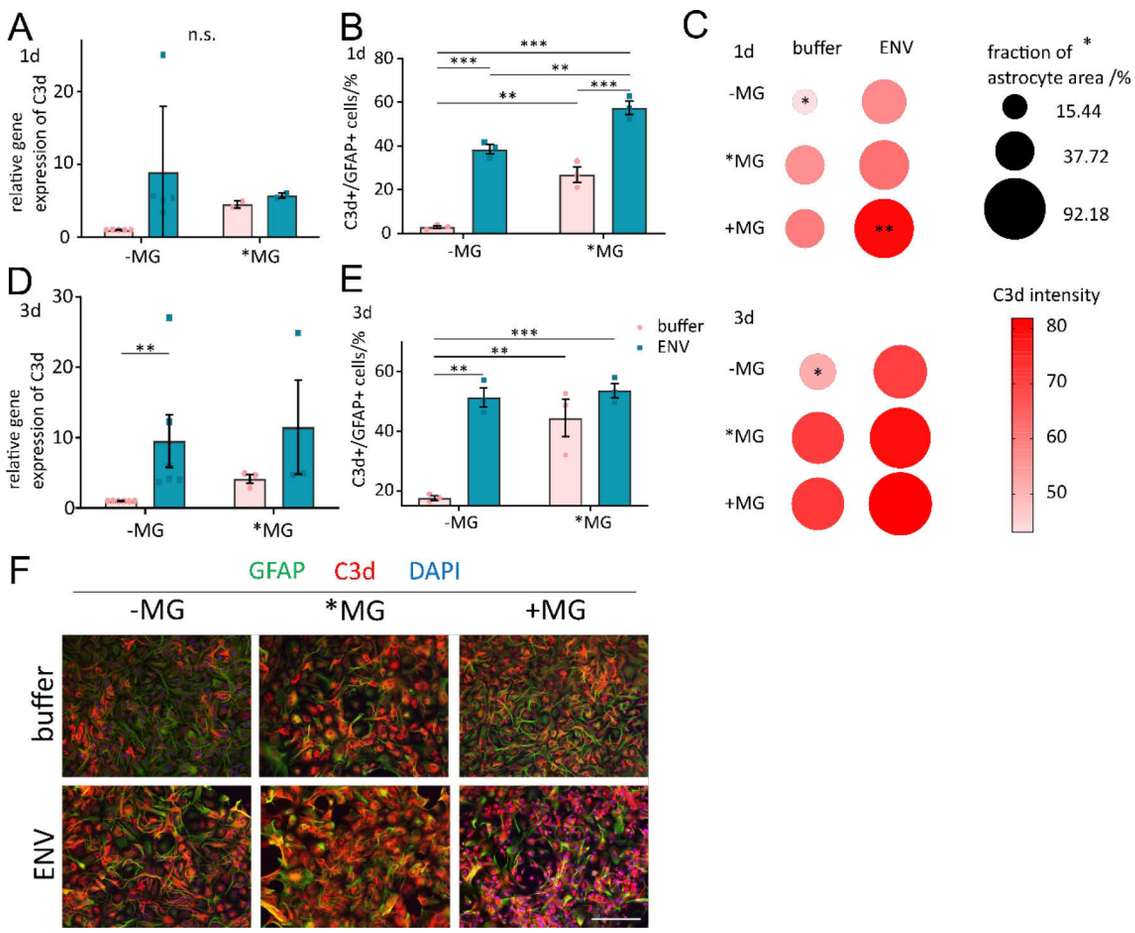

Fig. 6 ***Increase of C3d expression under stimulation with HERV-W ENV.*** ***A,D*** *Relative gene expression of C3d in astrocytes with and without microglia in transwells under stimulation with ENV and buffer after 24 h (A) and 72 h (D).* ***B,E*** *Ratio of C3d- and GFAP-double positive astrocytes.* ***C*** *Representation of C3d-Intensity and fraction of C3d-positive area of astrocyte area.* ***F*** *Representative immunocytochemical images of astrocytes expressing GFAP, C3d, and DAPI under all six conditions after 72 h. GAPDH was used as reference gene. Complement fragment C3d, glial fibrillary acidic protein (GFAP). - MG: without microglia; + MG: with microglia; * MG: microglia in transwells. Data represented as mean +/-SEM. Significance was analyzed via 2-way ANOVA followed by Sidak's post hoc test. Data was considered statistically significant (95% CI) at *P < 0.05, **P < 0.01, ***P < 0.001. (Scale bar: 200 µm. x20 objective)*

3.5 Stimulation with recombinant HERV-W ENV increases LCN2 expression in astrocytes

Lipocalin-2 (LCN2) is shown to be increased in astrocytes of MS patients (Al Nimer et al. 2016; Khalil et al. 2016). Furthermore, it was shown to be secreted where it induces neurotoxicity in different in vivo models. (Bi et al. 2013). LCN2 was therefore used here in order to analyze neurotoxic-associated astrocytes.

Here a general trend for an increase of LNC2 expression was shown. Relative gene expression was induced by ENV as well as in the presence of microglia, but due to wide variation across a limited number of trials, no statistically significant changes could be shown (Fig. 7A). The relative number of LCN2-positive cells in all GFAP-positive astrocytes was statistically significantly increased under treatment with ENV in all conditions, which was to be amplified in the presence of microglia (Fig. 7B) (Gruchot, Lewen et al.2023b). Area fraction and intensity of LCN2 could be interpreted as increased under ENV in the 1-day time point, but no statistical significance could be shown (Fig. 7C). This is in accordance with AQP4 enhancement, which can be an upstream regulator of LCN2.

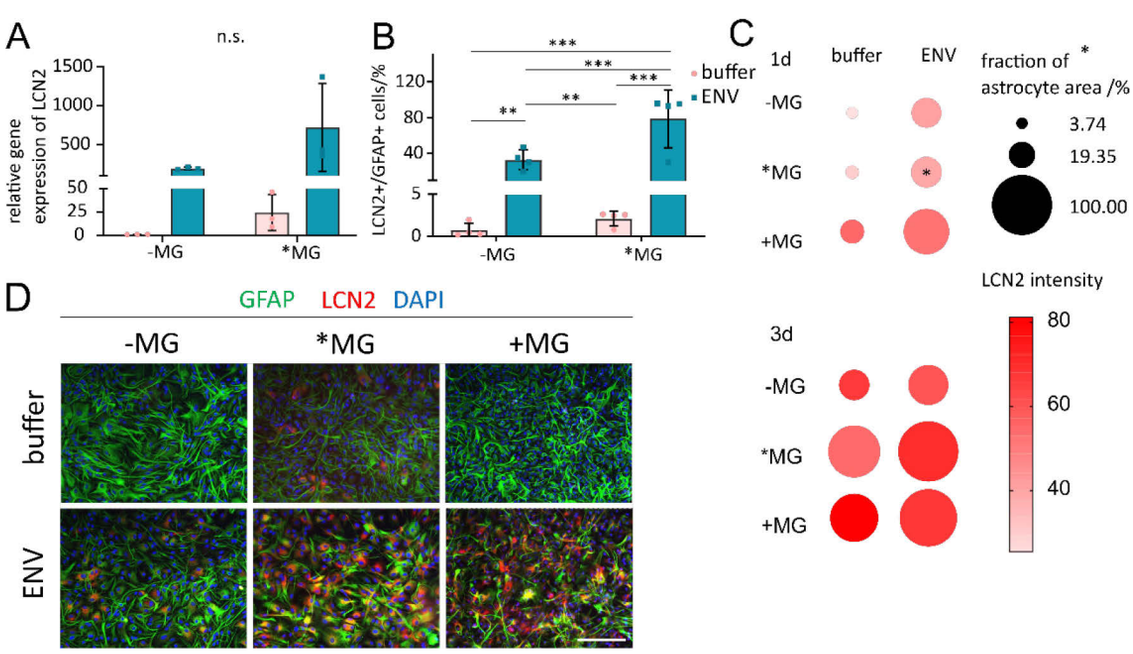

Fig. 7 ***Increase of LCN2 expression under HERV-W ENV stimulation. A*** *Relative gene expression of LCN2 in astrocytes with and without transwells under stimulation with buffer and ENV after 24h.* ***B*** *Ratio of LCN2- and GFAP-double positive astrocytes with and without transwells under stimulation with buffer and ENV after 24h.* ***C*** *Representation of C3d-Intensity and fraction of C3d-positive area of astrocyte area.* ***D*** *Representative immunocytochemical images of astrocytes expressing GFAP, LCN2, and DAPI under all six conditions after 72h. GAPDH was used as reference gene. Lipocalin-2 (LCN2), glial fibrillary acidic protein (GFAP). - MG: without microglia; + MG: with microglia; * MG: microglia in transwells. Data represented as mean +/-SEM. Significance was analyzed via 2-way ANOVA followed by Sidak's post hoc test. Data was*

*considered statistically significant (95% CI) at *P < 0.05, **P < 0.01, ***P < 0.001. (Scale bar: 200 µm. x20 objective)*

3.6 Shift in gene expression and secretion in astrocytes under stimulation with HERV-W ENV

It has previously been shown that astrocytes contribute to MS pathology through glial scar formation, loss of neurotrophic functions, and increased nitric oxide and cytokine production. In this light, different astrocytic factors were analyzed for relative gene expression standardized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH), which could be established as a reliable reference gene in our group (Küry et al. 2002). The observed changes at the 3-day time point proved to be less meaningful than the 1-day time point so the focus was set on 24 hours.

For pro-inflammatory proteins, such as IL6 and TNFα, a trend toward an increase in expression was shown. A statistically significant induction of gene expression could be shown under treatment with ENV in presence of microglia and slight induction under ENV without microglia and no change under treatment with buffer and microglia (Fig. 8A,B).

To show that gene expression makes a difference in glial secretion, specific proteins were analyzed in the cell media of the monoculture and the transwell condition using ELISAs. For TNFα and IL6 secretion there is a clear trend towards an induction by ENV, increased by microglia. The secretion of TNFα was induced by ENV in astrocytes and microglia, with the highest secretion in coculture with ENV. A statistically significant change was seen under treatment with ENV and microglia compared to all other conditions (Fig. 8D) (Gruchot, Lewen et al.2023b). IL6 secretion was significantly induced by ENV as well as by microglia with buffer, displaying a statistically significant increase under addition of microglia to ENV (Fig. 8E) (Gruchot, Lewen et al.2023b). A mixed trend was observed for CXCL10 secretion. The transwell condition showed a higher secretion of CXCL10 compared to the monoculture. Interestingly, CXCL10 secretion was reduced by ENV in both astrocytes and microglia, with overall similar levels between buffer-only and ENV plus microglia conditions (Fig. 8C) (Gruchot, Lewen et al.2023b). The analysis of secreted proteins could indicate, that HERV-W ENV creates a specific secretion pattern in astrocytes and microglia. The microglia condition is hereby a combination of astrocytic and microglial secretion so statements about astroglial secretion under the influence of microglia are difficult to make.

While HERV-W ENV could be shown to induce the expression and secretion of pro-inflammatory cytokines, anti-inflammatory cytokines were decreased. As indicated by the heatmap, anti-inflammatory proteins like IGF1, as well as neuroprotective proteins like S100a10 (Fig. 8A,B) were

statistically significantly decreased under treatment with ENV in presence and absence of microglia.

Interestingly, the neurotrophic factor BDNF showed a mixed trend. BDNF gene expression was increased when astrocytes were stimulated with HERV-W ENV alone, while it was downregulated when astrocytes were exposed to both microglia and ENV protein, compared to the condition using HERV-W ENV alone. This indicated that the induction of BDNF through ENV is neutralized in the presence of microglia.

TREM2, associated with synaptic pruning (Krasemann et al. 2017; Rosciszewski et al. 2018) was initially downregulated by ENV regardless of microglial presence but statistically significantly increased after 72 hours under ENV in combination with microglia (Fig. A,B).

The structural function of astrocytes is an important factor in lesion formation and scaring in MS. The loss of structure and communication proteins like connexins and N-cadherin has been shown to be negatively involved in the remyelination process by favoring local inflammation and could therefore be associated with the spread of chronic MS lesions (Basu und Das Sarma 2018; Li et al. 2020; Kanemaru et al. 2013). A trend towards a reduction in gene expression was observed for the gap junction proteins. Relative gene expression of GJB2, GJA1 (not statistically significant for 3 day), GJB6 under treatment with ENV in presence and absence of microglia were statistically significantly reduced, indicating interesting parallels to MS pathology (Fig. 8A,B) (Gruchot, Lewen et al.2023b).

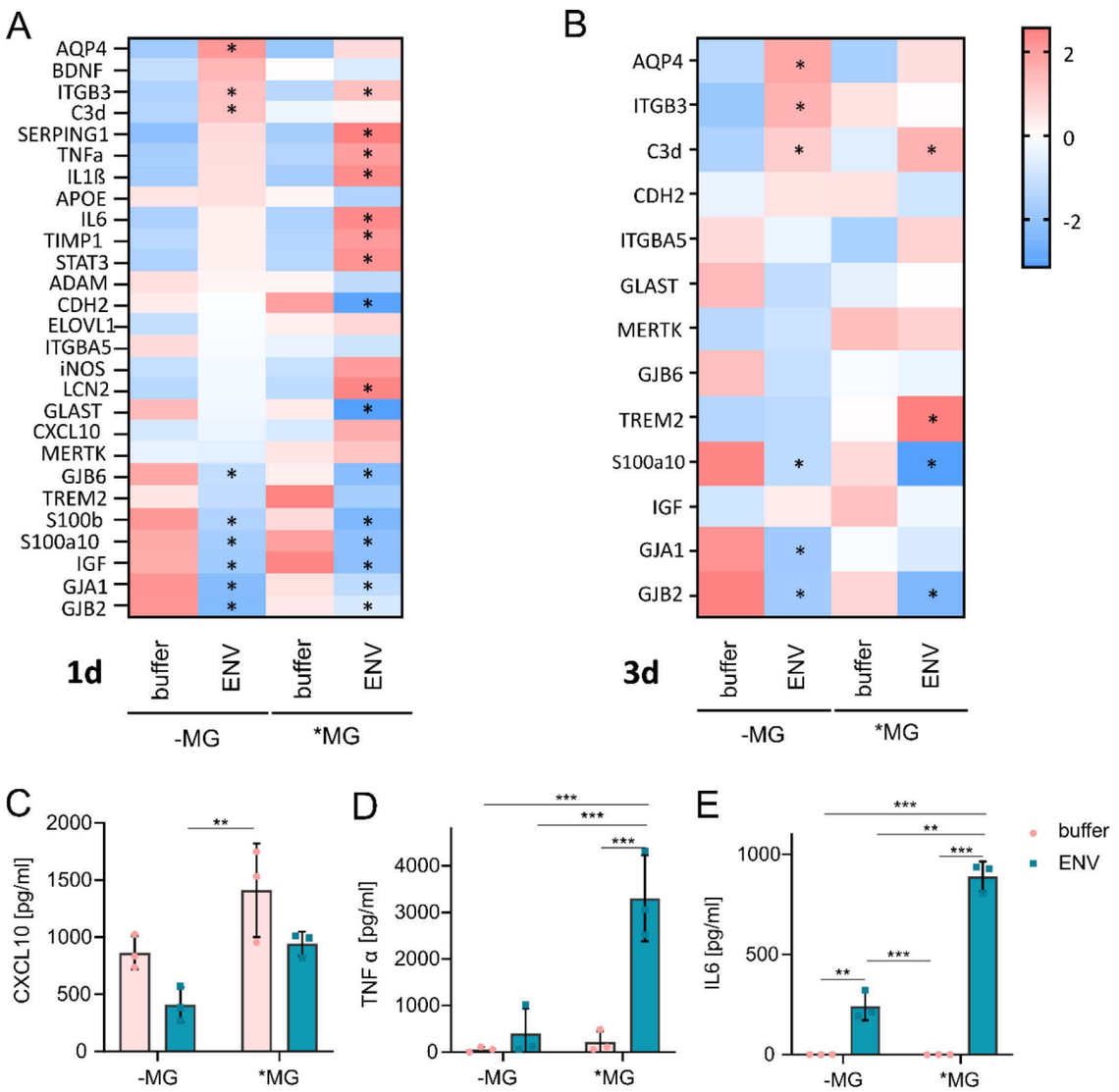

Fig. 8 ***Gene expression and protein secretion. A,B*** *relative gene expression in astrocytes in absence and presence of microglia treated with buffer and ENV after 24 h (A) and 72 h (B). Data are presented as z-scores.* ***C,D,E*** *Quantification of protein secretion by sandwich ELISA using media collected in absence and presence of microglia and in response to buffer or ENV after 24 h, identifying secreted Cxclc10 (C), TNFα (D) and IL6 (E). GAPDH was used as reference gene. - MG: without microglia; * MG: microglia in transwells. Data represented as mean +/-SEM. Gene expression was analyzed via 2-way ANOVA followed by original FDR method of Benjamini and Hochberg. Secretion data was analyzed via 2-way ANOVA followed by Sidak's post hoc test. Data was considered statistically significant (95% CI) at *P < 0.05, **P < 0.01, ***P < 0.001.*

4 Discussion

Multiple sclerosis is the most common neurodegenerative disease, marked by a range of motor and cognitive symptoms that impose significant burdens on patients as well as the health care system. Previous medications have focused primarily on immunodepressive mechanisms, leading to heavy side effects, which significantly reduced the quality of life for most patients and declining efficacy over time, necessitating medication changes. Furthermore, there is currently no medication that is capable of preventing degeneration or fostering regeneration. Consequently, global research is increasingly focused on uncovering the underlying mechanisms of MS to develop new therapeutic approaches.

The pathology of MS is characterized by lesions of demyelination, axon death and inflammation, often attributed to oligodendrocytes. However, recent research highlights the critical involvement of astrocytes and microglia. Activated microglia are known to foster a pro-inflammatory environment that accelerates neuronal death, while astrocytes contribute to lesion formation and compromise the integrity of the blood-brain barrier (BBB). Importantly, these two types of glial cells interact dynamically: astrocytes produce ATP gradients that attract microglia to injury sites, and microglial cytokines, in turn, activate astrocytes, creating a feedback loop that amplifies neuroinflammation.

HERV-W ENV has been implicated in both the onset and progression of MS. ENV activation triggers inflammatory responses in microglia via Toll-like receptor 4 (TLR4), resulting in reduced phagocytosis, increased expression of pro-inflammatory cytokines, and increased levels of nitrosative stress in microglia. Immunocytochemistry studies in this work have confirmed that ENV-stimulated microglia exhibit increased nitrosative stress through inducible nitric oxide synthase (iNOS) expression, which, at excessive levels, contributes to neuronal dysfunction and death (Butterfield et al. 2006; Smith und Lassmann 2002). To establish the harmful concentration range, future studies should compare nitric oxide (NO) levels through ENV with those in MS lesions.

Studies show that microglia activated by ENV often reside near myelinated axons, suggesting a direct link between ENV and axonal damage (Kremer et al. 2019). Those ENV-positive microglia in lesion proximity were described with enlarged morphologies and increased cell numbers upon ENV stimulation, as they were described here in coculture as well as in the transwell culture insert. This connection has been substantiated by human studies, which revealed that microglia-related lesions are more common in progressive MS than in relapsing-remitting MS (RRMS) (Matthews 2019). The persistence of microglial inflammation in primary progressive MS (PPMS), which lacks BBB breakdown and blood-derived immune cell infiltration typical in relapsing forms,

suggests that microglial activation could be a significant driver of disease progression especially in PPMS.

Notably, recent studies have identified therapies like siponimod that may modulate microglial phenotypes, potentially promoting a subpopulation that supports remyelination rather than degeneration (Gruchot et al. 2022)

4.1 Paracrine signaling is a mechanism of microglial enhancement of the ENV-effect

An important part of this work was to identify differences in the effect of HERV-W ENV on astrocytes whether microglial cells are present or not. This is of great interest since multiple recent publication have identified strong interaction between microglial and astroglial cells in many neurodegenerative diseases but especially in MS (Charabati et al. 2023; Sanmarco et al. 2021; Healy et al. 2022). In order to differentiate between direct (cell-cell contact) and indirect (paracrine signals) effects of microglia onto astroglial cells, so called cell culture inserts were used that allow diffusion of secreted proteins.

To this end, it was important to prove the viability of microglial cells in cell culture inserts. ENV-positive microglia in lesion proximity were described with activated morphologies and increased cell numbers (Kremer et al. 2019), as they were described here in coculture as well as in the transwell inserts. Another key factor of HERV-W-mediated microglial activation was shown to be nitrosative stress indicated by an increased expression of iNOS (Kremer et al., 2019). In order to prove that microglial cells in cell culture inserts can be activated by HERV-W ENV immunocytochemistry for iNOS was performed, displaying increased levels of iNOS expression. While nitrosative stress can also be beneficial in low concentrations, levels, as they are detected in MS, lead to neuronal dysfunction and death (Butterfield et al. 2006; Smith und Lassmann 2002). Taken together this indicates, that microglial cells do show proper functions within the transwell culture dishes.

This work revealed that ENV's effects on astrocytes were consistently amplified in the presence of microglia. Paracrine signaling alone was sufficient to enhance ENV's impact, though direct contact yielded stronger responses, suggesting either additional signaling mechanisms or a concentration effect in localized environments. Previous studies showed ENV-induced microglial changes—such as increased cytokine secretion—supporting the hypothesis of paracrine enhancement (Kremer et al. 2019). Some of the described effects occurred in presence of

microglia without the interference of ENV, most notably C3d, but also LCN2 and connexins, showing an intricate combination of reciprocal effects.

4.2 HERV-W ENV-stimulated astrocytes convert into a pro-inflammatory subpopulation

Astrocytes also play a significant role in MS, raising the question of ENV's impact on these cells. The protein syncytin-1, encoded by another HERV-W gene, is known to increase the release of redox reactants by astrocytes, thereby stressing oligodendrocytes (Antony et al. 2007) and activating CRP via TLR3 (Wang et al. 2018). Syncytin-1 differs biochemically from ENV, suggesting that comparable effects are unlikely (Charvet et al. 2021). This study therefore investigated ENV's influence on astrocytes to enrich our understanding of MS pathology, particularly regarding the role of HERV-W ENV. It could be shown a loss of astrocytes under ENV and revealed that astrocytes exposed to ENV exhibit neurodegenerative characteristics. Recent advances in transcriptome analysis have enabled a more nuanced view beyond the traditional A1/A2 classification of astrocytes, identifying astroglial signatures associated with specific diseases and functions. This work identified distinct gene and protein expression patterns in astrocytes affected by ENV, contributing to a deeper understanding of how these cells influence MS pathology and revealing potential biomarkers for improved diagnosis and treatment. This work could further show how microglia promoted changes astrocytes undergo while stimulated with ENV, furthering the understanding of crosstalk between glia cells in MS and opening research to find new targets for regulation of immune response. As described before, astrocytes play a significant role in disease formation of MS. In this context, they can be involved in glia scar formation, contribute to BBB- leakage, and are described to contribute to neuroinflammation and degeneration(Farina et al. 2007; Jensen et al. 2013; Rothhammer und Quintana 2015b; Lander et al. 2001; Choi et al. 2014; Lee und Benveniste 1999). This study's findings align with these results. However, the role of cytokines in neurodegeneration is critical yet dual (Kany et al. 2019): they support injury response, tissue clearance, and infection control, yet in chronic inflammatory diseases like MS, these mechanisms contribute to ongoing tissue damage and degeneration (Murakami und Hirano 2012). Reactive astrocytes embody this dual role, as shown in experimental autoimmune encephalomyelitis (EAE) models, where in acute EAE astrocyte depletion exacerbates neuroinflammation (Toft-Hansen et al. 2011), while in chronic EAE, astrocyte absence reduces inflammation (Mayo et al. 2014). This dual role of immune activation in injury and repair highlights the need for targeted therapies. Non-specific immunosuppressants

fail to address the root cause of neuroinflammation in MS; therefore, therapies that target specific immune pathways may offer a more precise and less damaging approach.

Under this pretense, this work analyzed the effects of the ENV protein in presence and absence of microglia onto astroglial cells in order to shed light on the beneficial or detrimental roles of ENV-mediated inflammation. ENV exposure led to a rise in pro-inflammatory proteins and a decrease in anti-inflammatory molecules in astrocytes, including IGF1 and neuroprotective proteins like S100a10, which are essential for astrocyte survival, proliferation, and differentiation (King et al. 2020; Hou et al. 2022)

Furthermore, ENV induced the transcript transcoding for the neurotrophic protein BDNF, which supports remyelination and has neuroprotective effects, as shown in MS and EAE models (Stadelmann et al. 2002; Lee et al. 2012; Fulmer et al. 2014; Rosciszewski et al. 2018). However, this effect appeared to be negated in the presence of microglia, suggesting that microglial interaction with astrocytes under ENV stimulation may reduce BDNF availability, thus impairing neuronal support and promoting degeneration and remyelination failure - central aspects of MS pathology.

For pro-inflammatory proteins, an overall induction of gene expression could be shown under treatment with ENV. Many of the proteins examined can be shown as increased in MS tissue and animal models such as EAE, proving a direct connection to MS pathology, such as proteins of the complement pathway like C3. C3 or its cleaved form C3d are described to characterize neurotoxic populations of astrocytes and are even handled as a treatment option (Schartz und Tenner 2020). Expression and cleavage of C3 have been shown in many neurodegenerative diseases including MS (Watkins et al. 2016). In MS lesions C3-positive astrocytes were associated with activated microglia (Qian 2023), which seemed to induce them via C1q, IL1β, and TNF. Chemokines, which are shown to be secreted under stimulation with ENV (Kremer et al. 2019), leading to the hypothesis that ENV and microglia induce C3d in astrocytes. In the experiments described above a neurotoxic shift and elevated C3d expression have been shown under ENV as well as microglia. That leaves the question of whether the effects of ENV protein and microglia are added, or if microglia specifically enhance the effect of ENV, as they seem to do for other proteins like AQP4. An induction of C3d could be shown without ENV through microglia, especially on the protein level and an effect of microglial secretion is described in former studies, so an added effect seems to be probable. Notably, corticosteroids like medrysone have been shown to reduce C3d-positive astrocytes in MS models (Silva Oliveira Junior et al. 2022), offering potential therapeutic insights.

Another important ENV-induced protein, lipocalin-2 (LCN2), has been identified as a biomarker in MS, detectable in patient serum and cerebrospinal fluid (Al Nimer et al. 2016; Berard et al. 2012; Kalinin et al. 2022; Khalil et al. 2016), further supported by studies demonstrating the neurotoxicity of astrocytic LCN2 secretion in animal models (Bi et al. 2013). And while C3d expression can be mediated via microglial C1qa (Schartz und Tenner 2020), LCN2 cannot be expressed through C1qa signaling alone (Liddelow et al. 2017). However, it was also shown that LPS can induce LCN2 expression in astrocytes (Liddelow et al. 2017). An interesting notion, since the LPS signaling pathway includes TLR4, which is also described to be a main mediator of HERV-W ENV signaling (Bohannon et al. 2013; Rolland et al. 2006), suggesting ENV as a potential activator of LCN2 expression, which could be corroborated in this work.

While this work was able to provide information that indicates an increased secretion of TNFα and Il6 as well as a reduced secretion of CXCL10, HERV-W ENV-mediated LCN2 secretion remains to be shown.

Parallel to the here described in vitro experiments, additional analyses were performed on different experimental mouse models by colleagues with compatible results (Gruchot, Lewen et al.2023b). In cuprizone-mediated demyelination as well as experimental autoimmune encephalomyelitis, transgenic ENV-expressing animals displayed an increase in C3d as well as LCN2 positive astrocytes and therefore providing a strong proof of principle. This astrocyte profile, associated with neurodegeneration and remyelination impairment (Bi et al. 2013; Al Nimer et al. 2016; Liddelow und Barres 2017) demonstrates the neurotoxic role of ENV in astrocyte response in MS. Based on this, a transcriptome analysis was performed, which confirmed these results by showing an inflammatory phenotype and an increase in LPS and iNOS in astrocytes (Gruchot et al. 2024)

AQP4 regulates the water permeability of the brain. It is highly expressed in the endfeet of astrocytes and has been shown to function also as an adhesion molecule and a neuroimmunological inducer, involved in all astrocyte functions (Ikeshima-Kataoka 2016). It has been shown to be increased in inflammatory lesions modulating astrocyte-to-microglia communication and promoting inflammation by inducing among others LCN2 and complement factors (Ikeshima-Kataoka 2016). And indeed, also in this work an increased expression of AQP4 could be shown to be mediated by HERV-W ENV protein, particularly if microglia are present. And while a connection between HERV-W and AQP4 has not yet been made AQP4 and antibodies against it play a significant role in the pathogenesis of neuromyelitis optica spectrum disorder (NMOSD), another chronic demyelinating disease. Whereby HERV-W and antibodies against it could replicate the same effect but are not observed in patients with NMOSD, pointing towards

different mechanisms of degeneration and inflammation in NMOSD and MS (Arru et al. 2020; Katoozi et al. 2017).

Taking together this work showed an astrocyte subpopulation with a shift to pro-inflammatory proteins, that are shown to be detrimental for neuroregeneration. It could be shown, that this shift is congruent to observations made in MS and models of MS, showing a connection that astrocytes draw between ENV and MS.

4.3 The influence of HERV-W ENV onto astroglia's role in neuroinflammation

While neuroinflammation is recognized as a significant contributor to the degenerative and anti-regenerative processes in multiple sclerosis (MS), myelin debris is also a critical factor. Studies have shown that myelin debris can inhibit regeneration and act as a secondary trigger for neuroinflammation (Grajchen et al. 2020; Berglund et al. 2020; Li et al. 2023). Phagocytosis, primarily performed by microglia but also by astrocytes, is essential for clearing myelin debris from MS lesions, facilitating remyelination and recovery (Sen et al. 2022; Li et al. 2023). A key protein involved in this process is MERTK, a receptor expressed in astrocytes that regulates phagocytosis in response to neuronal activity (Chung et al. 2013). In this study, MERTK expression in astrocytes was modestly affected by HERV-W ENV alone but was notably increased in the presence of microglia, suggesting a potential phagocytic role of astrocytes during inflammation.

Another protein crucial for phagocytosis, TREM2, plays a role in mediating synapse elimination in both developing and adult brains (Krasemann et al. 2017; Rosciszewski et al. 2018). ENV-stimulated astrocytes displayed increased TREM2 expression, which has been linked to a neurodegenerative phenotype in microglia via APOE induction (Krasemann et al., 2017). APOE is among the most highly expressed astrocyte genes (Cahoy et al. 2008) and as stated above supports phagocytosis of apoptotic cells and debris in macrophages and astrocytes (Koistinaho et al. 2004; Grainger et al. 2004). In this study, APOE was decreased in astrocytes under ENV treatment, hinting at a potentially protective role. Important to notice is, that TREM2 as an upstream regulator was reduced after one day of ENV exposure, it increased statistically significantly after three days, indicating that future studies should closely examine the phagocytic function of astrocytes under prolonged ENV stimulation.

Additionally, TREM2 plays a role in cholesterol transfer between astrocytes and microglia (Nugent et al. 2020), indicating a potential disruption of metabolic support under ENV. For instance, the induction of ELOVL1, a protein involved in fatty acid elongation critical for synaptic function, was also observed following ENV treatment, suggesting broader metabolic implications (Guttenplan et al. 2021).

Phagocytic pathway activation in astrocytes, as proposed by increased protein markers, supports a potential role in MS-related phagocytosis. Hereby, phagocytosis assays may provide direct evidence of astrocytic phagocytosis, as gene expression analysis alone yields mixed results. However, the functional consequences of this activation remain unclear; literature suggests that phagocytic pathways might also contribute to inflammatory and metabolic disturbances, complicating the interpretation of astrocytic phagocytosis in neurodegeneration.

4.4 HERV-W ENV induces neurodegenerative changes in astrocytes

Excitotoxicity is seen as another key mechanism of neurodegeneration as it can damage and stress neurons and has also been reported in MS (Ghirotto et al. 2022; Kuzmina et al. 2019; Akyuz et al. 2023). To this end, the neurotransmitter glutamate is a regulator of excitotoxicity. One of the main functions of astrocytes is the maintenance of glutamate homeostasis, which is primarily performed by the glutamate transporter GLAST. To this end, the GLAST expression upon exposure to HERV-W ENV was of great interest. Here, GLAST expression statistically significantly decreased following ENV exposure, both with and without microglia, indicating that glutamate homeostasis will very likely be impaired due to HERV-W. Impaired glutamate uptake in MS lesions is a known contributor to excitotoxicity, leading to excessive synaptic glutamate and resulting in neuron degeneration (Pitt et al. 2000; Werner et al. 2001). Excess extracellular glutamate activates NMDA receptors (NMDARs), increasing intracellular calcium and free radicals, ultimately causing neuronal death (Kaul et al., 2001). Thus, the observed GLAST reduction aligns with these findings and may explain how ENV contributes to excitotoxicity in MS.

Astrocytes contribute structurally to cellular networks, scar formation, and the blood-brain barrier (BBB) maintenance, with adhesion molecules playing crucial roles in these processes. Given the statistically significant astrocyte loss observed under ENV combined with microglia, this study explored whether ENV affects astrocyte adhesion molecules, potentially leading to cellular detachment and death. Since a detachment of astroglial cells could be observed in a previous study (Gruchot et al., 2023; data not shown), the hypothesis was that astroglial cells upon HERV-W ENV stimulation display a decreased presence of certain adhesion molecules that are important for the intracellular networks and ultimately lead to astroglial detachment in vitro. In this regard here provided results support this theory as a decrease in the expression of structure proteins like connexins (gap junction proteins) and cadherin could be observed. However, other mechanisms, such as cell cycle arrest that leads to regulated apoptosis or destruction of the cell

integrity, meaning necrosis integrity disruption, may also contribute to astrocyte reduction, warranting further investigation.

Interestingly, however, connexin loss is also associated with inflammatory processes. The loss of Cx43/Cx47 has hereby been directly associated with the spread of chronic MS lesions (Basu und Das Sarma 2018). Cx43 (GJA1) is also negatively involved in the remyelination process by favoring local inflammation so that astroglial Cx43 is promoted as a therapeutic target for chronic EAE and MS (Takase et al. 2024). Furthermore, calcium-dependent N-cadherin up-regulation in astrocytes has been shown to be essential for reactive astrogliosis and neuroprotection. In this work, N-Cadherin (Cdh2) was shown to be statistically significantly decreased under treatment with ENV, an effect that seemed microglia-dependent. Since calcium signaling is an important mechanism for the release of gliotransmitters and can result in a toxic environment, future experiments could focus on how ionic signaling, and especially calcium are influenced by HERV-W ENV. The described decrease of connexins and N-cadherin under treatment with ENV not only explains the structural failure observed under ENV but also shows strong parallels to MS pathology such as the loosening of the BBB and excitotoxicity.

Overall, this work observed reduced astrocyte area under ENV, suggesting weakened intercellular adhesion. In cocultures, astrocyte reduction was not apparent, possibly due to microglial presence altering spatial distribution. This work revealed changes in the structure and binding proteins that were confirmed by transcriptome analysis (Gruchot et al. 2024), opening up speculations about the integrity of the blood-brain barrier. A loosening of the cellular bonds would allow for the brain to be infiltrated by peripheral immune cells, as can be observed in acute MS flares. BBB function is still not fully understood, but Bock and colleagues postulate astroglial connexins as an important regulator of BBB integrity, among others via inflammation (Bock et al. 2017). It could be shown, that ENV has a pro-inflammatory effect on endothelial cells, that is mediated via TLR4 and increases adhesion in lymphocytes, helping them cross the BBB (Duperray et al. 2015). Furthermore, reactive astrocytes can actively promote the infiltration of leukocytes into MS lesions by releasing diverse chemokines such as CXCL10, indicating that future experiments should quantify the immune cell infiltration upon HERV-WENV exposure.

4.5 Limitations

Considering the described experiments there are certain limitations, that should not go unnamed. The used ENV protein is a bacterial product. Even though bacterial contamination could not be found, a limited amount of bacterial byproducts such as LPS would cause a significant

reaction in rat cells, that would be confused with the effect of ENV. Therefore, an ENV product of the same origin as the analyzed cells would be required to rule out this possibility with certainty. This has been successfully done for OPC experiments, which reinforces the statements made above (Kremer et al. 2013). However, the goal of these experiments was to take a look at the microglial astroglial axis upon HERV-W stimulation. The published validation in vivo (Gruchot, Lewen et al.2023b) confirms most of the here presented effects. Another uncertainty is the comparability of the dosage of the ENV protein to human endogenous production in MS. Should the concentration of ENV in the described experiments be orders of magnitude away from MS lesions statements should be taken with caution. Therefore, an idea of the quantity of ENV in the human brain and in lesions specifically would be helpful. However, it must be made clear that an attempt is being made here to imitate a disease within a few days that very probably takes many years before the first symptoms appear. Therefore, it might be very challenging to use ENV dosage according to brain levels.

A general limitation of cell culture is the transfer to complex living organs and organisms such as the brain and the animal or human. Findings from this work have been replicated in mouse models. So, a microglial activation could be shown in transgenic ENV-producing mice under cuprizone treatment, as well as astrocyte activation. The increase in LCN2 and C3d could be shown in EAE and for transgenic ENV-producing mice under cuprizone treatment on a genetic as well as protein level(Gruchot, Lewen et al.2023b), indicating that important observations could already be transferred to the in vivo situation and even fit clinical observations in humans (Michailidou et al. 2017; Al-Temaimi et al. 2017). Of note, in the last years, a lot of efforts could produce an antibody to HERV-W ENV, called temelimab. This antibody has been tested in ENV-induced EAE as an animal model of MS and has proven its efficacy (Curtin et al. 2015). It has been shown as safe and effective in treating MS up to phase 2b trials. The primary endpoint, reduction of acute gadolinium-enhancing (GdE) lesion formation, for anti-inflammatory action, has not been met. And while this has been the conventional primary endpoint in trial design, secondary endpoints have been implemented to better represent the effects on the prevention of progression and the ability to regenerate. Latest studies showed an improvement of neurodegeneration in these secondary endpoints such as reduced brain atrophy, number and size of T1 hypointense lesions, and better myelin integrity, measured with magnetization transfer ratio (Hartung et al. 2022). Further to this, the diagnostic criteria for MS are changing due to better control of disease activity and disability. Dissemination in time is no longer required, dissemination in space has relaxed criteria, and kappa-free light chains (KFLC) are accepted as a tool for diagnosis comparable to oligoclonal bands (Montalban 2024). Further secondary endpoints are therefore feasible. In the context of this work, GFAP in serum should be mentioned,

which was confirmed as a marker for disease activity (Abdelhak et al. 2018, 2018). The approval of temelimab as a treatment for MS remains to be shown.

4.6 Outlook

Thinking further upon the described experiments there are multiple questions still open. The described results are shown for only two time points. Expanding the timeline could provide insight into more immediate, or long-term changes in astrocytes under ENV, granting a better understanding of astroglial development.

It could be shown that microglia change the effect exerted by HERV-W ENV protein on astrocytes through cytokines. To get a better understanding of the mechanisms between microglia and astrocytes, the medium of stimulated microglia should be the point of focus. Secretome assays can be used to define the secreting pattern, measuring concentrations for reactive oxygen and nitrogen species and ions like calcium or potassium would give an understanding of the cell environment and a microglia medium transfer onto astrocytes could show the specific changes achieved by microglial secretion. This setup also allows for testing of neutralizing antibodies or drugs (Guttenplan und Liddelow 2019). In this light, an exchange of secreting and reacting cells could be interesting to define astrocytic manipulation of microglia. A human secretome could also be used for diagnostic purposes like CSF analysis, making early diagnosis and intervention possible.

The evaluation of the shift in gene expression could be expanded to define the specific subpopulation of astrocytes induced by ENV and compared to those of human astrocytes in MS. Therefore, transcriptome analyses are valuable and can make statements regarding astrocyte functions and pathological processes of neurodegeneration. To evaluate the transcriptome of specific glia cell subtypes under HERV-W ENV snRNA-seq can be used. Recent single-cell RNAseq experiments could show the high complexity of microglial and astroglial immune polarization, where further experiments could enable comparison between different modes of activation and reaction (Escartin et al. 2021; Paolicelli et al. 2022). Again, potentially being able to reveal new biomarkers for detecting early forms of MS.

Other experiments should look at further immune cells such as lymphocytes and blood-born macrophages, which are present in later stages of MS when the blood-brain barrier is not fully intact anymore. ENV can influence the T helper cell differentiation as well as the activation of T cells (Perron et al. 2012; Rolland et al. 2006). However, a direct effect of ENV on lymphoid cells has not been shown in clinical trials (Hartung et al. 2022). Thinking further, interactions between

astrocytes and other glia cells as well as neurons should be topic of research, eventually aiming to understand the complex interaction inside the human brain.

While this work could show the effects of ENV and some transmitting cytokines the question of reception and signaling pathways remains unanswered for astrocytes. And while TLR4 is proposed as the major receptor in microglia, some propose different modes of activation like ASCT1, or (MCT)-1 (Antony et al. 2007; Blanco-Melo et al. 2017). Experiments using e.g. TLR4-KO mice, different knock-down/knock-outs, or the application of specific blocking reagents should be performed to prove specific signaling pathways and therefore points of treatment.

Further experiments should expand their view to other neurodegenerative diseases or even neuropsychiatric disorders that are shown to be linked to HERV-W ENV or other HERVs, as many pathways are homologous between them. Since HERVs can be found in many neurodegenerative diseases such as MS, Amyotrophic Lateral Sclerosis (ALS) but also Alzheimer's disease (AD) (Römer 2021) it remains to be answered if there might be a common mechanism of neurodegeneration connecting their etiology and possible treatment.

4.7 Conclusion

This dissertation explores the impact of the HERV-W envelope protein (ENV) on astrocytes and microglia within the context of multiple sclerosis (MS), revealing its role in promoting a pro-inflammatory and neurotoxic astrocytic phenotype. HERV-W ENV exposure was shown to impair key astrocytic functions, including glutamate clearance, potentially exacerbating neuroinflammation and excitotoxicity. Additionally, HERV-W ENV exposure led to structural disruptions in astrocytes, reducing adhesion molecule expression and potentially compromising the blood-brain barrier (BBB), which could permit immune cell infiltration in MS. A critical finding is the role of microglia in amplifying HERV-W ENV's effects on astrocytes via cytokine-mediated paracrine signaling, which enhances inflammatory responses and leads to additional astrocytic dysfunction.

Future research should focus on defining specific HERV-W ENV signaling pathways in astrocytes, possibly involving receptors like TLR4, to identify actionable therapeutic targets. Extending these findings to other neurodegenerative conditions linked to HERVs, such as Alzheimer's disease and ALS, could further reveal the broader mechanisms of retrovirus-driven neurodegeneration.

In summary, this work establishes HERV-W ENV as a significant factor in MS pathology through its detrimental effects on astrocyte function and glial interactions, providing insights that may guide targeted therapies for MS and related neurodegenerative diseases.

5 Literaturverzeichnis

Abdelhak, A.; Huss, A.; Kassubek, J.; Tumani, H.; Otto, M. (2018): Serum GFAP as a biomarker for disease severity in multiple sclerosis. In: *Scientific reports* 8 (1), S. 14798. DOI: 10.1038/s41598-018-33158-8.

Al Nimer, Faiez; Elliott, Christina; Bergman, Joakim; Khademi, Mohsen; Dring, Ann M.; Aeinehband, Shahin et al. (2016): Lipocalin-2 is increased in progressive multiple sclerosis and inhibits remyelination. In: *Neurology(R) neuroimmunology & neuroinflammation* 3 (1), e191. DOI: 10.1212/NXI.0000000000000191.

Al-Temaimi, Rabeah; AbuBaker, Jehad; Al-Khairi, Irina; Alroughani, Raed (2017): Remyelination modulators in multiple sclerosis patients. In: *Experimental and molecular pathology* 103 (3), S. 237–241. DOI: 10.1016/j.yexmp.2017.11.004.

Alvarez-Lafuente, Roberto; las Heras, Virginia de; Bartolomé, Manuel; Picazo, Juan José; Arroyo, Rafael (2004): Relapsing-remitting multiple sclerosis and human herpesvirus 6 active infection. In: *Archives of neurology* 61 (10), S. 1523–1527. DOI: 10.1001/archneur.61.10.1523.

Antony, Joseph M.; Ellestad, Kristofor K.; Hammond, Robert; Imaizumi, Kazunori; Mallet, François; Warren, Kenneth G.; Power, Christopher (2007): The human endogenous retrovirus envelope glycoprotein, syncytin-1, regulates neuroinflammation and its receptor expression in multiple sclerosis: a role for endoplasmic reticulum chaperones in astrocytes. In: *Journal of immunology (Baltimore, Md. : 1950)* 179 (2), S. 1210–1224. DOI: 10.4049/jimmunol.179.2.1210.

Arru, Giannina; Sechi, Elia; Mariotto, Sara; Zarbo, Ignazio R.; Ferrari, Sergio; Gajofatto, Alberto et al. (2020): Antibody response against HERV-W in patients with MOG-IgG associated disorders, multiple sclerosis and NMOSD. In: *Journal of Neuroimmunology* 338, S. 577110. DOI: 10.1016/j.jneuroim.2019.577110.

Ascherio, Alberto (2013): Environmental factors in multiple sclerosis. In: *Expert Review of Neurotherapeutics* 13 (12 Suppl), S. 3–9. DOI: 10.1586/14737175.2013.865866.

Attwell, David; Buchan, Alastair M.; Charpak, Serge; Lauritzen, Martin; MacVicar, Brian A.; Newman, Eric A. (2010): Glial and neuronal control of brain blood flow. In: *Nature* 468 (7321), S. 232–243. DOI: 10.1038/nature09613.

Bartheld, Christopher S. von; Bahney, Jami; Herculano-Houzel, Suzana (2016): The search for true numbers of neurons and glial cells in the human brain: A review of 150 years of cell counting. In: *Journal of Comparative Neurology* 524 (18), S. 3865–3895. DOI: 10.1002/cne.24040.

Basu, Rahul; Das Sarma, Jayasri (2018): Connexin 43/47 channels are important for astrocyte/oligodendrocyte cross-talk in myelination and demyelination. In: *J Biosci* 43 (5), S. 1055–1068. DOI: 10.1007/s12038-018-9811-0.

Berard, Jennifer L.; Zarruk, Juan G.; Arbour, Nathalie; Prat, Alexandre; Yong, V. Wee; Jacques, Francois H. et al. (2012): Lipocalin 2 is a novel immune mediator of experimental autoimmune encephalomyelitis pathogenesis and is modulated in multiple sclerosis. In: *Glia* 60 (7), S. 1145–1159. DOI: 10.1002/glia.22342.

Berglund, Rasmus; Guerreiro-Cacais, Andre Ortlieb; Adzemovic, Milena Z.; Zeitelhofer, Manuel; Lund, Harald; Ewing, Ewoud et al. (2020): Microglial autophagy-associated phagocytosis is essential for recovery from neuroinflammation. In: *Science immunology* 5 (52). DOI: 10.1126/sciimmunol.abb5077.

Bezzi, P.; Volterra, A. (2001): A neuron-glia signalling network in the active brain. In: *Current Opinion in Neurobiology* 11 (3), S. 387–394. DOI: 10.1016/S0959-4388(00)00223-3.

Bi, Fangfang; Huang, Cao; Tong, Jianbin; Qiu, Guang; Huang, Bo; Wu, Qinxue et al. (2013): Reactive astrocytes secrete lcn2 to promote neuron death. In: *Proceedings of the National Academy of Sciences* 110 (10), S. 4069–4074. DOI: 10.1073/pnas.1218497110.

Blanco-Melo, Daniel; Gifford, Robert J.; Bieniasz, Paul D. (2017): Co-option of an endogenous retrovirus envelope for host defense in hominid ancestors. In: *eLife* 6. DOI: 10.7554/eLife.22519.

Block, Michelle L.; Zecca, Luigi; Hong, Jau-Shyong (2007): Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. In: *Nature reviews. Neuroscience* 8 (1), S. 57–69. DOI: 10.1038/nrn2038.

Bock, Marijke de; Leybaert, Luc; Giaume, Christian (2017): Connexin Channels at the Glio-Vascular Interface: Gatekeepers of the Brain. In: *Neurochemical research* 42 (9), S. 2519–2536. DOI: 10.1007/s11064-017-2313-x.

Bohannon, Julia K.; Hernandez, Antonio; Enkhbaatar, Perenlei; Adams, William L.; Sherwood, Edward R. (2013): The immunobiology of toll-like receptor 4 agonists: from endotoxin tolerance to immunoadjuvants. In: *Shock (Augusta, Ga.)* 40 (6), S. 451–462. DOI: 10.1097/SHK.0000000000000042.

Brosnan, Celia F.; Raine, Cedric S. (2013): The astrocyte in multiple sclerosis revisited. In: *Glia* 61 (4), S. 453–465. DOI: 10.1002/glia.22443.

Bush, T. G.; Puvanachandra, N.; Horner, C. H.; Polito, A.; Ostenfeld, T.; Svendsen, C. N. et al. (1999): Leukocyte infiltration, neuronal degeneration, and neurite outgrowth after ablation of

scar-forming, reactive astrocytes in adult transgenic mice. In: *Neuron* 23 (2), S. 297–308. DOI: 10.1016/s0896-6273(00)80781-3.

Butterfield, D. Allan; Abdul, Hafiz Mohmmad; Newman, Shelley; Reed, Tanea (2006): Redox proteomics in some age-related neurodegenerative disorders or models thereof. In: *NeuroRx : the journal of the American Society for Experimental NeuroTherapeutics* 3 (3), S. 344–357. DOI: 10.1016/j.nurx.2006.05.003.

Cahoy, John D.; Emery, Ben; Kaushal, Amit; Foo, Lynette C.; Zamanian, Jennifer L.; Christopherson, Karen S. et al. (2008): A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. In: *The Journal of neuroscience : the official journal of the Society for Neuroscience* 28 (1), S. 264–278. DOI: 10.1523/JNEUROSCI.4178-07.2008.

Cannella, B.; Raine, C. S. (1995): The adhesion molecule and cytokine profile of multiple sclerosis lesions. In: *Annals of Neurology* 37 (4), S. 424–435. DOI: 10.1002/ana.410370404.

Carson, Monica J.; Doose, Jonathan M.; Melchior, Benoit; Schmid, Christoph D.; Ploix, Corinne C. (2006): CNS immune privilege: hiding in plain sight. In: *Immunological reviews* 213, S. 48–65. DOI: 10.1111/j.1600-065X.2006.00441.x.

Charabati, Marc; Wheeler, Michael A.; Weiner, Howard L.; Quintana, Francisco J. (2023): Multiple sclerosis: Neuroimmune crosstalk and therapeutic targeting. In: *Cell* 186 (7), S. 1309–1327. DOI: 10.1016/j.cell.2023.03.008.

Charvet, Benjamin; Pierquin, Justine; Brunel, Joanna; Gorter, Rianne; Quétard, Christophe; Horvat, Branka et al. (2021): Human Endogenous Retrovirus Type W Envelope from Multiple Sclerosis Demyelinating Lesions Shows Unique Solubility and Antigenic Characteristics. In: *Virologica Sinica* 36 (5), S. 1006–1026. DOI: 10.1007/s12250-021-00372-0.

Choi, Sung S.; Lee, Hong J.; Lim, Inja; Satoh, Jun-ichi; Kim, Seung U. (2014): Human astrocytes: secretome profiles of cytokines and chemokines. In: *PloS one* 9 (4), e92325. DOI: 10.1371/journal.pone.0092325.

Chung, Won-Suk; Clarke, Laura E.; Wang, Gordon X.; Stafford, Benjamin K.; Sher, Alexander; Chakraborty, Chandrani et al. (2013): Astrocytes mediate synapse elimination through MEGF10 and MERTK pathways. In: *Nature* 504 (7480), S. 394–400. DOI: 10.1038/nature12776.

Clarke, Laura E.; Liddelow, Shane A. (2017): Neurobiology: Diversity reaches the stars. In: *Nature* 548 (7668), S. 396–397. DOI: 10.1038/548396a.

Colonna, Marco; Butovsky, Oleg (2017): Microglia Function in the Central Nervous System During Health and Neurodegeneration. In: *Annual review of immunology* 35, S. 441–468. DOI: 10.1146/annurev-immunol-051116-052358.

Colton, Carol A.; Wilcock, Donna M. (2010): Assessing activation states in microglia. In: *CNS & neurological disorders drug targets* 9 (2), S. 174–191. DOI: 10.2174/187152710791012053.

Compston, Alastair (2005): Decade of the brain. In: *Brain : a journal of neurology* 128 (Pt 8), S. 1741–1742. DOI: 10.1093/brain/awh593.

Cordiglieri, Chiara; Farina, Cinthia (2010): Astrocytes Exert and Control Immune Responses in the Brain. In: *CIR* 6 (3), S. 150–159. DOI: 10.2174/157339510791823655.

Curtin, François; Perron, Hervé; Kromminga, Arno; Porchet, Hervé; Lang, Alois B. (2015): Preclinical and early clinical development of GNbAC1, a humanized IgG4 monoclonal antibody targeting endogenous retroviral MSRV-Env protein. In: *mAbs* 7 (1), S. 265–275. DOI: 10.4161/19420862.2014.985021.

D'Amelio, F. E.; Smith, M. E.; Eng, L. F. (1990): Sequence of tissue responses in the early stages of experimental allergic encephalomyelitis (EAE): immunohistochemical, light microscopic, and ultrastructural observations in the spinal cord. In: *Glia* 3 (4), S. 229–240. DOI: 10.1002/glia.440030402.

Deaton, Aimée M.; Bird, Adrian (2011): CpG islands and the regulation of transcription. In: *Genes & Development* 25 (10), S. 1010–1022. DOI: 10.1101/gad.2037511.

Duperray, Alain; Barbe, Delphin; Raguenez, Gilda; Weksler, Babette B.; Romero, Ignacio A.; Couraud, Pierre-Olivier et al. (2015): Inflammatory response of endothelial cells to a human endogenous retrovirus associated with multiple sclerosis is mediated by TLR4. In: *International Immunology* 27 (11), S. 545–553. DOI: 10.1093/intimm/dxv025.

Dutta, Ranjan; Trapp, Bruce D. (2014): Relapsing and progressive forms of multiple sclerosis: insights from pathology. In: *Current opinion in neurology* 27 (3), S. 271–278. DOI: 10.1097/WCO.0000000000000094.

Escartin, Carole; Galea, Elena; Lakatos, András; O'Callaghan, James P.; Petzold, Gabor C.; Serrano-Pozo, Alberto et al. (2021): Reactive astrocyte nomenclature, definitions, and future directions. In: *Nature neuroscience* 24 (3), S. 312–325. DOI: 10.1038/s41593-020-00783-4.

Farina, Cinthia; Aloisi, Francesca; Meinl, Edgar (2007): Astrocytes are active players in cerebral innate immunity. In: *Trends in immunology* 28 (3), S. 138–145. DOI: 10.1016/j.it.2007.01.005.

Fulmer, Clifton G.; VonDran, Melissa W.; Stillman, Althea A.; Huang, Yangyang; Hempstead, Barbara L.; Dreyfus, Cheryl F. (2014): Astrocyte-derived BDNF supports myelin protein synthesis after cuprizone-induced demyelination. In: *J. Neurosci.* 34 (24), S. 8186–8196. DOI: 10.1523/JNEUROSCI.4267-13.2014.

Garson, J. A.; Tuke, P. W.; Giraud, P.; Paranhos-Baccala, G.; Perron, H. (1998): Detection of virion-associated MSRV-RNA in serum of patients with multiple sclerosis. In: *The Lancet* 351 (9095), S. 33. DOI: 10.1016/S0140-6736(98)24001-3.

Giannetti, Paolo; Politis, Marios; Su, Paul; Turkheimer, Federico; Malik, Omar; Keihaninejad, Shiva et al. (2014): Microglia activation in multiple sclerosis black holes predicts outcome in progressive patients: an in vivo (11)C(R)-PK11195-PET pilot study. In: *Neurobiology of disease* 65, S. 203–210. DOI: 10.1016/j.nbd.2014.01.018.

Göttle, Peter; Förster, Moritz; Gruchot, Joel; Kremer, David; Hartung, Hans Peter; Perron, Hervé; Küry, Patrick (2019): Rescuing the negative impact of human endogenous retrovirus envelope protein on oligodendroglial differentiation and myelination. In: *Glia* 67 (1), S. 160–170. DOI: 10.1002/glia.23535.

Göttle, Peter; Schichel, Kira; Reiche, Laura; Werner, Luisa; Zink, Annika; Prigione, Alessandro; Küry, Patrick (2021): TLR4 Associated Signaling Disrupters as a New Means to Overcome HERV-W Envelope-Mediated Myelination Deficits. In: *Front. Cell. Neurosci.* 15, S. 777542. DOI: 10.3389/fncel.2021.777542.

Grainger, David J.; Reckless, Jill; McKilligin, Elaine (2004): Apolipoprotein E Modulates Clearance of Apoptotic Bodies In Vitro and In Vivo, Resulting in a Systemic Proinflammatory State in Apolipoprotein E-Deficient Mice. In: *The Journal of Immunology* 173 (10), S. 6366–6375. DOI: 10.4049/jimmunol.173.10.6366.

Grajchen, Elien; Wouters, Elien; van de Haterd, Britt; Haidar, Mansour; Hardonnière, Kévin; Dierckx, Tess et al. (2020): CD36-mediated uptake of myelin debris by macrophages and microglia reduces neuroinflammation. In: *Journal of neuroinflammation* 17 (1), S. 224. DOI: 10.1186/s12974-020-01899-x.

Gruchot, Joel; Herrero, Felisa; Weber-Stadlbauer, Ulrike; Meyer, Urs; Küry, Patrick (2023a): Interplay between activation of endogenous retroviruses and inflammation as common pathogenic mechanism in neurological and psychiatric disorders. In: *Brain, Behavior, and Immunity* 107, S. 242–252. DOI: 10.1016/j.bbi.2022.10.007.

Gruchot, Joel; Lein, Ferdinand; Lewen, Isabel; Reiche, Laura; Weyers, Vivien; Petzsch, Patrick et al. (2022): Siponimod Modulates the Reaction of Microglial Cells to Pro-Inflammatory Stimulation. In: *International journal of molecular sciences* 23 (21). DOI: 10.3390/ijms232113278.

Gruchot, Joel; Lewen, Isabel; Dietrich, Michael; Reiche, Laura; Sindi, Mustafa; Hecker, Christina et al. (2023b): Transgenic expression of the HERV-W envelope protein leads to polarized glial cell populations and a neurodegenerative environment. In: *Proceedings of the National Academy of Sciences* 120 (38), e2308187120. DOI: 10.1073/pnas.2308187120.

Gruchot, Joel; Reiche, Laura; Werner, Luisa; Herrero, Felisa; Schira-Heinen, Jessica; Meyer, Urs; Küry, Patrick (2024): Molecular dissection of HERV-W dependent microglial- and astroglial cell polarization. In: *Microbes and infection*, S. 105382. DOI: 10.1016/j.micinf.2024.105382.

Gruchot, Joel; Weyers, Vivien; Göttle, Peter; Förster, Moritz; Hartung, Hans-Peter; Küry, Patrick; Kremer, David (2019): The Molecular Basis for Remyelination Failure in Multiple Sclerosis. In: *Cells* 8 (8). DOI: 10.3390/cells8080825.

Guttenplan, Kevin A.; Liddelow, Shane A. (2019): Astrocytes and microglia: Models and tools. In: *The Journal of experimental medicine* 216 (1), S. 71–83. DOI: 10.1084/jem.20180200.

Guttenplan, Kevin A.; Weigel, Maya K.; Prakash, Priya; Wijewardhane, Prageeth R.; Hasel, Philip; Rufen-Blanchette, Uriel et al. (2021): Neurotoxic reactive astrocytes induce cell death via saturated lipids. In: *Nature* 599 (7883), S. 102–107. DOI: 10.1038/s41586-021-03960-y.

Han, Rafael T.; Kim, Rachel D.; Molofsky, Anna V.; Liddelow, Shane A. (2021): Astrocyte-immune cell interactions in physiology and pathology. In: *Immunity* 54 (2), S. 211–224. DOI: 10.1016/j.immuni.2021.01.013.

Hartung, Hans-Peter; Derfuss, Tobias; Cree, Bruce Ac; Sormani, Maria Pia; Selmaj, Krzysztof; Stutters, Jonathan et al. (2022): Efficacy and safety of temelimab in multiple sclerosis: Results of a randomized phase 2b and extension study. In: *Multiple sclerosis (Houndmills, Basingstoke, England)* 28 (3), S. 429–440. DOI: 10.1177/13524585211024997.

Hauser, Stephen L.; Oksenberg, Jorge R. (2006): The neurobiology of multiple sclerosis: genes, inflammation, and neurodegeneration. In: *Neuron* 52 (1), S. 61–76. DOI: 10.1016/j.neuron.2006.09.011.

Healy, Luke M.; Stratton, Jo Anne; Kuhlmann, Tanja; Antel, Jack (2022): The role of glial cells in multiple sclerosis disease progression. In: *Nature Reviews Neurology* 18 (4), S. 237–248. DOI: 10.1038/s41582-022-00624-x.

Hemmer (2023): Hemmer B. et al. Diagnose und Therapie der Multiplen Sklerose, Neuromyelitis-optica-Spektrum-Erkrankungen und MOG-IgG-assoziierten Erkrankungen, S2k-Leitlinie, 2023, in: Deutsche Gesellschaft für Neurologie (Hrsg.), Leitlinien für Diagnostik und Therapie in der Neurologie. Online: www.dgn.org/leitlinien (abgerufen am TT.MM.JJJJ). Online verfügbar unter https://register.awmf.org/assets/guidelines/030-050l_S2k_Diagnose-Therapie-Multiple-Sklerose-Neuromyelitis-Optica-Spektrum-MOG-IgG-assoziierte-Erkrankungen_2024-01.pdf, zuletzt geprüft am 09.09.2024.

Heppner, Frank L.; Greter, Melanie; Marino, Denis; Falsig, Jeppe; Raivich, Gennadij; Hövelmeyer, Nadine et al. (2005): Experimental autoimmune encephalomyelitis repressed by microglial paralysis. In: *Nature medicine* 11 (2), S. 146–152. DOI: 10.1038/nm1177.

Horng, Sam; Therattil, Anthony; Moyon, Sarah; Gordon, Alexandra; Kim, Karla; Argaw, Azeb Tadesse et al. (2017): Astrocytic tight junctions control inflammatory CNS lesion pathogenesis. In: *The Journal of clinical investigation* 127 (8), S. 3136–3151. DOI: 10.1172/JCI91301.

Hou, Jinxing; Bi, Huiru; Ge, Qiting; Teng, Huajian; Wan, Guoqiang; Yu, Bin et al. (2022): Heterogeneity analysis of astrocytes following spinal cord injury at single-cell resolution. In: *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 36 (8), e22442. DOI: 10.1096/fj.202200463R.

Ikeshima-Kataoka, Hiroko (2016): Neuroimmunological Implications of AQP4 in Astrocytes. In: *International journal of molecular sciences* 17 (8). DOI: 10.3390/ijms17081306.

Jack, Carolyn S.; Arbour, Nathalie; Manusow, Joshua; Montgrain, Vivianne; Blain, Manon; McCrea, Ellie et al. (2005): TLR signaling tailors innate immune responses in human microglia and astrocytes. In: *Journal of immunology (Baltimore, Md. : 1950)* 175 (7), S. 4320–4330. DOI: 10.4049/jimmunol.175.7.4320.

Jensen, Cathy J.; Massie, Ann; Keyser, Jacques de (2013): Immune players in the CNS: the astrocyte. In: *Journal of Neuroimmune Pharmacology* 8 (4), S. 824–839. DOI: 10.1007/s11481-013-9480-6.

JM Charcot (1868): Leçons de 1868; Manuscrits des leçons de JM Charcot.

John, Gareth R.; Lee, Sunhee C.; Brosnan, Celia F. (2003): Cytokines: powerful regulators of glial cell activation. In: *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry* 9 (1), S. 10–22. DOI: 10.1177/1073858402239587.

Kalinin, Sergey; Boullerne, Anne I.; Feinstein, Douglas L. (2022): Serum levels of lipocalin-2 are elevated at early times in African American relapsing remitting multiple sclerosis patients. In: *Journal of Neuroimmunology* 364, S. 577810. DOI: 10.1016/j.jneuroim.2022.577810.

Kanemaru, Kazunori; Kubota, Jun; Sekiya, Hiroshi; Hirose, Kenzo; Okubo, Yohei; Iino, Masamitsu (2013): Calcium-dependent N-cadherin up-regulation mediates reactive astrogliosis and neuroprotection after brain injury. In: *Proceedings of the National Academy of Sciences of the United States of America* 110 (28), S. 11612–11617. DOI: 10.1073/pnas.1300378110.

Kany, Shinwan; Vollrath, Jan Tilmann; Relja, Borna (2019): Cytokines in Inflammatory Disease. In: *International journal of molecular sciences* 20 (23). DOI: 10.3390/ijms20236008.

Katoozi, Shirin; Skauli, Nadia; Rahmani, Soulmaz; Camassa, Laura M. A.; Boldt, Henning B.; Ottersen, Ole P.; Amiry-Moghaddam, Mahmood (2017): Targeted deletion of Aqp4 promotes the formation of astrocytic gap junctions. In: *Brain structure & function* 222 (9), S. 3959–3972. DOI: 10.1007/s00429-017-1448-5.

Kaur, C.; Hao, A. J.; Wu, C. H.; Ling, E. A. (2001): Origin of microglia. In: *Microscopy research and technique* 54 (1), S. 2–9. DOI: 10.1002/jemt.1114.

Khalil, M.; Renner, A.; Langkammer, C.; Enzinger, C.; Ropele, S.; Stojakovic, T. et al. (2016): Cerebrospinal fluid lipocalin 2 in patients with clinically isolated syndromes and early multiple sclerosis. In: *Multiple sclerosis (Houndmills, Basingstoke, England)* 22 (12), S. 1560–1568. DOI: 10.1177/1352458515624560.

King, Andrew; Szekely, Boglarka; Calapkulu, Eda; Ali, Hanan; Rios, Francesca; Jones, Shalmai; Troakes, Claire (2020): The Increased Densities, But Different Distributions, of Both C3 and S100A10 Immunopositive Astrocyte-Like Cells in Alzheimer's Disease Brains Suggest Possible Roles for Both A1 and A2 Astrocytes in the Disease Pathogenesis. In: *Brain sciences* 10 (8). DOI: 10.3390/brainsci10080503.

Koch-Henriksen, Nils; Thygesen, Lau Caspar; Stenager, Egon; Laursen, Bjarne; Magyari, Melinda (2018): Incidence of MS has increased markedly over six decades in Denmark particularly with late onset and in women. In: *Neurology* 90 (22), e1954-e1963. DOI: 10.1212/WNL.0000000000005612.

Koistinaho, Milla; Lin, Suizhen; Wu, Xin; Esterman, Michail; Koger, Deanna; Hanson, Jeffrey et al. (2004): Apolipoprotein E promotes astrocyte colocalization and degradation of deposited amyloid-beta peptides. In: *Nature medicine* 10 (7), S. 719–726. DOI: 10.1038/nm1058.

Kotter, Mark R.; Stadelmann, Christine; Hartung, Hans-Peter (2011): Enhancing remyelination in disease--can we wrap it up? In: *Brain : a journal of neurology* 134 (Pt 7), S. 1882–1900. DOI: 10.1093/brain/awr014.

Krasemann, Susanne; Madore, Charlotte; Cialic, Ron; Baufeld, Caroline; Calcagno, Narghes; El Fatimy, Rachid et al. (2017): The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases. In: *Immunity* 47 (3), 566-581.e9. DOI: 10.1016/j.immuni.2017.08.008.

Kremer, David; Förster, Moritz; Schichel, Tanja; Göttle, Peter; Hartung, Hans-Peter; Perron, Hervé; Küry, Patrick (2015): The neutralizing antibody GNbAC1 abrogates HERV-W envelope protein-mediated oligodendroglial maturation blockade. In: *Multiple Sclerosis Journal* 21 (9), S. 1200–1203. DOI: 10.1177/1352458514560926.

Kremer, David; Gruchot, Joel; Weyers, Vivien; Oldemeier, Lisa; Göttle, Peter; Healy, Luke et al. (2019): pHERV-W envelope protein fuels microglial cell-dependent damage of myelinated axons in multiple sclerosis. In: *Proceedings of the National Academy of Sciences of the United States of America* 116 (30), S. 15216–15225. DOI: 10.1073/pnas.1901283116.

Kremer, David; Schichel, Tanja; Förster, Moritz; Tzekova, Nevena; Bernard, Corinne; van der Valk, Paul et al. (2013): Human endogenous retrovirus type W envelope protein inhibits oligodendroglial precursor cell differentiation. In: *Annals of Neurology* 74 (5), S. 721–732. DOI: 10.1002/ana.23970.

Kurtzke, J. F. (2000): Multiple sclerosis in time and space--geographic clues to cause. In: *Journal of Neurovirology* 6 Suppl 2, S134-40.

Küry, Patrick; Greiner-Petter, Regine; Cornely, Christiane; Jürgens, Tim; Müller, Hans Werner (2002): Mammalian Achaete Scute Homolog 2 Is Expressed in the Adult Sciatic Nerve and Regulates the Expression of Krox24, Mob-1, CXCR4, and p57kip2 in Schwann Cells. In: *J. Neurosci.* 22 (17), S. 7586–7595. DOI: 10.1523/JNEUROSCI.22-17-07586.2002.

Küry, Patrick; Nath, Avindra; Créange, Alain; Dolei, Antonina; Marche, Patrice; Gold, Julian et al. (2018): Human Endogenous Retroviruses in Neurological Diseases. In: *Trends in molecular medicine* 24 (4), S. 379–394. DOI: 10.1016/j.molmed.2018.02.007.

Kutzelnigg, Alexandra; Lucchinetti, Claudia F.; Stadelmann, Christine; Brück, Wolfgang; Rauschka, Helmut; Bergmann, Markus et al. (2005): Cortical demyelination and diffuse white matter injury in multiple sclerosis. In: *Brain* 128 (Pt 11), S. 2705–2712. DOI: 10.1093/brain/awh641.

Kwon, Hyuk Sung; Koh, Seong-Ho (2020): Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes. In: *Translational Neurodegeneration* 9 (1), S. 42. DOI: 10.1186/s40035-020-00221-2.

Lampron, Antoine; Larochelle, Antoine; Laflamme, Nathalie; Préfontaine, Paul; Plante, Marie-Michèle; Sánchez, Maria Gabriela et al. (2015): Inefficient clearance of myelin debris by microglia impairs remyelinating processes. In: *The Journal of experimental medicine* 212 (4), S. 481–495. DOI: 10.1084/jem.20141656.

Lander, E. S.; Linton, L. M.; Birren, B.; Nusbaum, C.; Zody, M. C.; Baldwin, J. et al. (2001): Initial sequencing and analysis of the human genome. In: *Nature* 409 (6822), S. 860–921. DOI: 10.1038/35057062.

Lassmann, Hans (2014): Mechanisms of white matter damage in multiple sclerosis. In: *Glia* 62 (11), S. 1816–1830. DOI: 10.1002/glia.22597.

Lassmann, Hans; van Horssen, Jack; Mahad, Don (2012): Progressive multiple sclerosis: pathology and pathogenesis. In: *Nature Reviews Neurology* 8 (11), S. 647–656. DOI: 10.1038/nrneurol.2012.168.

Lavie, Laurence; Kitova, Milena; Maldener, Esther; Meese, Eckart; Mayer, Jens (2005): CpG methylation directly regulates transcriptional activity of the human endogenous retrovirus family HERV-K(HML-2). In: *Journal of virology* 79 (2), S. 876–883. DOI: 10.1128/JVI.79.2.876-883.2005.

Lee, De-Hyung; Geyer, Eva; Flach, Anne-Christine; Jung, Klaus; Gold, Ralf; Flügel, Alexander et al. (2012): Central nervous system rather than immune cell-derived BDNF mediates axonal protective effects early in autoimmune demyelination. In: *Acta Neuropathologica* 123 (2), S. 247–258. DOI: 10.1007/s00401-011-0890-3.

Lee, S. J.; Benveniste, E. N. (1999): Adhesion molecule expression and regulation on cells of the central nervous system. In: *Journal of Neuroimmunology* 98 (2), S. 77–88. DOI: 10.1016/S0165-5728(99)00084-3.

Li, Qingyun; Barres, Ben A. (2018): Microglia and macrophages in brain homeostasis and disease. In: *Nature reviews. Immunology* 18 (4), S. 225–242. DOI: 10.1038/nri.2017.125.

Li, Tao; Niu, Jianqin; Yu, Guangdan; Ezan, Pascal; Yi, Chenju; Wang, Xiaorui et al. (2020): Connexin 43 deletion in astrocytes promotes CNS remyelination by modulating local inflammation. In: *Glia* 68 (6), S. 1201–1212. DOI: 10.1002/glia.23770.

Li, Xiaohui; Ding, Zhibin; Liu, Kexin; Wang, Qing; Song, Lijuan; Chai, Zhi et al. (2023): Astrocytic phagocytosis of myelin debris and reactive characteristics in vivo and in vitro. In: *Biology of the cell* 115 (12), e202300057. DOI: 10.1111/boc.202300057.

Liddelow, Shane A.; Barres, Ben A. (2016): Regeneration: Not everything is scary about a glial scar. In: *Nature* 532 (7598), S. 182–183. DOI: 10.1038/nature17318.

Liddelow, Shane A.; Barres, Ben A. (2017): Reactive Astrocytes: Production, Function, and Therapeutic Potential. In: *Immunity* 46 (6), S. 957–967. DOI: 10.1016/j.immuni.2017.06.006.

Liddelow, Shane A.; Guttenplan, Kevin A.; Clarke, Laura E.; Bennett, Frederick C.; Bohlen, Christopher J.; Schirmer, Lucas et al. (2017): Neurotoxic reactive astrocytes are induced by activated microglia. In: *Nature* 541 (7638), S. 481–487. DOI: 10.1038/nature21029.

Liddelow, Shane A.; Marsh, Samuel E.; Stevens, Beth (2020): Microglia and Astrocytes in Disease: Dynamic Duo or Partners in Crime? In: *Trends in immunology* 41 (9), S. 820–835. DOI: 10.1016/j.it.2020.07.006.

Lin, Ruihe; Iacovitti, Lorraine (2015): Classic and novel stem cell niches in brain homeostasis and repair. In: *Brain research* 1628 (Pt B), S. 327–342. DOI: 10.1016/j.brainres.2015.04.029.

Linker, Ralf A.; Lee, De-Hyung; Demir, Seray; Wiese, Stefan; Kruse, Niels; Siglienti, Ines et al. (2010): Functional role of brain-derived neurotrophic factor in neuroprotective autoimmunity: therapeutic implications in a model of multiple sclerosis. In: *Brain* 133 (Pt 8), S. 2248–2263. DOI: 10.1093/brain/awq179.

Lublin, F. D.; Reingold, S. C. (1996): Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. In: *Neurology* 46 (4), S. 907–911. DOI: 10.1212/wnl.46.4.907.

Magistretti, Pierre J. (2006): Neuron-glia metabolic coupling and plasticity. In: *Journal of Experimental Biology* 209 (Pt 12), S. 2304–2311. DOI: 10.1242/jeb.02208.

Mameli, Giuseppe; Astone, Vito; Arru, Giannina; Marconi, Silvia; Lovato, Laura; Serra, Caterina et al. (2007): Brains and peripheral blood mononuclear cells of multiple sclerosis (MS) patients hyperexpress MS-associated retrovirus/HERV-W endogenous retrovirus, but not Human herpesvirus 6. In: *The Journal of general virology* 88 (Pt 1), S. 264–274. DOI: 10.1099/vir.0.81890-0.

Mameli, Giuseppe; Poddighe, Luciana; Astone, Vito; Delogu, Giuseppe; Arru, Giannina; Sotgiu, Stefano et al. (2009): Novel reliable real-time PCR for differential detection of MSRVenv and syncytin-1 in RNA and DNA from patients with multiple sclerosis. In: *Journal of Virological Methods* 161 (1), S. 98–106. DOI: 10.1016/j.jviromet.2009.05.024.

Mandolesi, Georgia; Gentile, Antonietta; Musella, Alessandra; Centonze, Diego (2015): IL-1β dependent cerebellar synaptopathy in a mouse mode of multiple sclerosis. In: *Cerebellum (London, England)* 14 (1), S. 19–22. DOI: 10.1007/s12311-014-0613-0.

Manghera, Mamneet; Douville, Renée N. (2013): Endogenous retrovirus-K promoter: a landing strip for inflammatory transcription factors? In: *Retrovirology* 10, S. 16. DOI: 10.1186/1742-4690-10-16.

Masutani, Hiroshi; Bai, Jie; Kim, Yong-Chul; Yodoi, Junji (2004): Thioredoxin as a neurotrophic cofactor and an important regulator of neuroprotection. In: *Molecular neurobiology* 29 (3), S. 229–242. DOI: 10.1385/MN:29:3:229.

Matthews, Paul M. (2019): Chronic inflammation in multiple sclerosis - seeing what was always there. In: *Nature Reviews Neurology* 15 (10), S. 582–593. DOI: 10.1038/s41582-019-0240-y.

Mayo, Lior; Trauger, Sunia A.; Blain, Manon; Nadeau, Meghan; Patel, Bonny; Alvarez, Jorge I. et al. (2014): Regulation of astrocyte activation by glycolipids drives chronic CNS inflammation. In: *Nature medicine* 20 (10), S. 1147–1156. DOI: 10.1038/nm.3681.

McCarthy, K. D.; Vellis, J. de (1980): Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue. In: *The Journal of cell biology* 85 (3), S. 890–902. DOI: 10.1083/jcb.85.3.890.

Michailidou, Iliana; Naessens, Daphne M. P.; Hametner, Simon; Guldenaar, Willemijn; Kooi, Evert-Jan; Geurts, Jeroen J. G. et al. (2017): Complement C3 on microglial clusters in multiple sclerosis occur in chronic but not acute disease: Implication for disease pathogenesis. In: *Glia* 65 (2), S. 264–277. DOI: 10.1002/glia.23090.

Montalban, Xavier (2024): 2024 revisions of the McDonald criteria. September 2024- ECTRIMS København. 2024- ECTRIMS København. ECTRIMS København, September 2024.

Murakami, Masaaki; Hirano, Toshio (2012): The molecular mechanisms of chronic inflammation development. In: *Frontiers in immunology* 3, S. 323. DOI: 10.3389/fimmu.2012.00323.

Muzio, Luca; Viotti, Alice; Martino, Gianvito (2021): Microglia in Neuroinflammation and Neurodegeneration: From Understanding to Therapy. In: *Frontiers in neuroscience* 15, S. 742065. DOI: 10.3389/fnins.2021.742065.

Nagappan, Palaniappan Ganesh; Chen, Hong; Wang, De-Yun (2020): Neuroregeneration and plasticity: a review of the physiological mechanisms for achieving functional recovery postinjury. In: *Military Medical Research* 7 (1), S. 30. DOI: 10.1186/s40779-020-00259-3.

Negi, Neema; Das, Bimal K. (2018): CNS: Not an immunoprivilaged site anymore but a virtual secondary lymphoid organ. In: *International reviews of immunology* 37 (1), S. 57–68. DOI: 10.1080/08830185.2017.1357719.

Nelson, P. N.; Carnegie, P. R.; Martin, J.; Davari Ejtehadi, H.; Hooley, P.; Roden, D. et al. (2003): Demystified. Human endogenous retroviruses. In: *Molecular pathology : MP* 56 (1), S. 11–18. DOI: 10.1136/mp.56.1.11.

Nugent, Alicia A.; Lin, Karin; van Lengerich, Bettina; Lianoglou, Steve; Przybyla, Laralynne; Davis, Sonnet S. et al. (2020): TREM2 Regulates Microglial Cholesterol Metabolism upon Chronic Phagocytic Challenge. In: *Neuron* 105 (5), 837-854.e9. DOI: 10.1016/j.neuron.2019.12.007.

O'Gorman, Cullen; Lucas, Robyn; Taylor, Bruce (2012): Environmental risk factors for multiple sclerosis: a review with a focus on molecular mechanisms. In: *International journal of molecular sciences* 13 (9), S. 11718–11752. DOI: 10.3390/ijms130911718.

Ohtani, Hitoshi; Liu, Minmin; Zhou, Wanding; Liang, Gangning; Jones, Peter A. (2018): Switching roles for DNA and histone methylation depend on evolutionary ages of human endogenous retroviruses. In: *Genome research* 28 (8), S. 1147–1157. DOI: 10.1101/gr.234229.118.

Orihuela, Ruben; McPherson, Christopher A.; Harry, Gaylia Jean (2016): Microglial M1/M2 polarization and metabolic states. In: *British Journal of Pharmacology* 173 (4), S. 649–665. DOI: 10.1111/bph.13139.

Orthmann-Murphy, Jennifer L.; Abrams, Charles K.; Scherer, Steven S. (2008): Gap junctions couple astrocytes and oligodendrocytes. In: *Journal of molecular neuroscience : MN* 35 (1), S. 101–116. DOI: 10.1007/s12031-007-9027-5.

Owens, T.; Tran, E.; Hassan-Zahraee, M.; Krakowski, M. (1998): Immune cell entry to the CNS--a focus for immunoregulation of EAE. In: *Research in immunology* 149 (9), 781-9; discussion 844-6, 855-60. DOI: 10.1016/s0923-2494(99)80005-4.

Paolicelli, Rosa C.; Sierra, Amanda; Stevens, Beth; Tremblay, Marie-Eve; Aguzzi, Adriano; Ajami, Bahareh et al. (2022): Microglia states and nomenclature: A field at its crossroads. In: *Neuron* 110 (21), S. 3458–3483. DOI: 10.1016/j.neuron.2022.10.020.

Pekna, Marcela; Pekny, Milos (2021): The Complement System: A Powerful Modulator and Effector of Astrocyte Function in the Healthy and Diseased Central Nervous System. In: *Cells* 10 (7). DOI: 10.3390/cells10071812.

Perron, H.; Geny, C.; Laurent, A.; Mouriquand, C.; Pellat, J.; Perret, J.; Seigneurin, J. M. (1989): Leptomeningeal cell line from multiple sclerosis with reverse transcriptase activity and viral particles. In: *Research in Virology* 140 (6), S. 551–561. DOI: 10.1016/S0923-2516(89)80141-4.

Perron, H.; Jouvin-Marche, E.; Michel, M.; Ounanian-Paraz, A.; Camelo, S.; Dumon, A. et al. (2001): Multiple sclerosis retrovirus particles and recombinant envelope trigger an abnormal immune response in vitro, by inducing polyclonal Vbeta16 T-lymphocyte activation. In: *Virology* 287 (2), S. 321–332. DOI: 10.1006/viro.2001.1045.

Perron, H.; Seigneurin, J. M. (1999): Human retroviral sequences associated with extracellular particles in autoimmune diseases: epiphenomenon or possible role in aetiopathogenesis? In: *Microbes and infection* 1 (4), S. 309–322. DOI: 10.1016/s1286-4579(99)80027-6.

Perron, Hervé; Germi, Raphaëlle; Bernard, Corinne; Garcia-Montojo, Marta; Deluen, Cécile; Farinelli, Laurent et al. (2012): Human endogenous retrovirus type W envelope expression in blood and brain cells provides new insights into multiple sclerosis disease. In: *Multiple sclerosis (Houndmills, Basingstoke, England)* 18 (12), S. 1721–1736. DOI: 10.1177/1352458512441381.

Pham, Hong; Ramp, Anton A.; Klonis, Nectarios; Ng, Sze Woei; Klopstein, Armelle; Ayers, Margaret M.; Orian, Jacqueline M. (2009): The astrocytic response in early experimental autoimmune encephalomyelitis occurs across both the grey and white matter compartments. In: *Journal of Neuroimmunology* 208 (1-2), S. 30–39. DOI: 10.1016/j.jneuroim.2008.12.010.

Pitt, D.; Werner, P.; Raine, C. S. (2000): Glutamate excitotoxicity in a model of multiple sclerosis. In: *Nature medicine* 6 (1), S. 67–70. DOI: 10.1038/71555.

Ponath, Gerald; Lincoln, Matthew R.; Dahlawi, Somiah; Mubarak, Mayyan; Sumida, Tomokazu; Airas, Laura et al. (2017a): Enhanced Astrocyte Responses are Driven by a Genetic Risk Allele Associated with Multiple Sclerosis: Cold Spring Harbor Laboratory.

Ponath, Gerald; Park, Calvin; Pitt, David (2018): The Role of Astrocytes in Multiple Sclerosis. In: *Frontiers in immunology* 9, S. 217. DOI: 10.3389/fimmu.2018.00217.

Ponath, Gerald; Ramanan, Sriram; Mubarak, Mayyan; Housley, William; Lee, Seunghoon; Sahinkaya, F. Rezan et al. (2017b): Myelin phagocytosis by astrocytes after myelin damage promotes lesion pathology. In: *Brain* 140 (2), S. 399–413. DOI: 10.1093/brain/aww298.

Prowse, Natalie; Hayley, Shawn (2021): Microglia and BDNF at the crossroads of stressor related disorders: Towards a unique trophic phenotype. In: *Neuroscience and biobehavioral reviews* 131, S. 135–163. DOI: 10.1016/j.neubiorev.2021.09.018.

Pugliatti, Maura; Sotgiu, Stefano; Rosati, Giulio (2002): The worldwide prevalence of multiple sclerosis. In: *Clinical neurology and neurosurgery* 104 (3), S. 182–191. DOI: 10.1016/s0303-8467(02)00036-7.

Ransohoff, Richard M.; Brown, Melissa A. (2012): Innate immunity in the central nervous system. In: *The Journal of clinical investigation* 122 (4), S. 1164–1171. DOI: 10.1172/JCI58644.

Rolland, Alexandre; Jouvin-Marche, Evelyne; Viret, Christophe; Faure, Mathias; Perron, Hervé; Marche, Patrice N. (2006): The envelope protein of a human endogenous retrovirus-W family activates innate immunity through CD14/TLR4 and promotes Th1-like responses. In: *Journal of immunology (Baltimore, Md. : 1950)* 176 (12), S. 7636–7644. DOI: 10.4049/jimmunol.176.12.7636.

Römer, Christine (2021): Viruses and Endogenous Retroviruses as Roots for Neuroinflammation and Neurodegenerative Diseases. In: *Frontiers in neuroscience* 15, S. 648629. DOI: 10.3389/fnins.2021.648629.

Rosati, G. (2001): The prevalence of multiple sclerosis in the world: an update. In: *Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology* 22 (2), S. 117–139. DOI: 10.1007/s100720170011.

Rosciszewski, Gerardo; Cadena, Vanesa; Murta, Veronica; Lukin, Jeronimo; Villarreal, Alejandro; Roger, Thierry; Ramos, Alberto Javier (2018): Toll-Like Receptor 4 (TLR4) and Triggering Receptor Expressed on Myeloid Cells-2 (TREM-2) Activation Balance Astrocyte Polarization into a Proinflammatory Phenotype. In: *Molecular neurobiology* 55 (5), S. 3875–3888. DOI: 10.1007/s12035-017-0618-z.

Rothhammer, Veit; Quintana, Francisco J. (2015a): Control of autoimmune CNS inflammation by astrocytes. In: *Seminars in immunopathology* 37 (6), S. 625–638. DOI: 10.1007/s00281-015-0515-3.

Rothhammer, Veit; Quintana, Francisco J. (2015b): Control of autoimmune CNS inflammation by astrocytes. In: *Seminars in immunopathology* 37 (6), S. 625–638. DOI: 10.1007/s00281-015-0515-3.

Sanmarco, Liliana M.; Polonio, Carolina M.; Wheeler, Michael A.; Quintana, Francisco J. (2021): Functional immune cell-astrocyte interactions. In: *The Journal of experimental medicine* 218 (9). DOI: 10.1084/jem.20202715.

Santello, Mirko; Volterra, Andrea (2010): Neuroscience: Astrocytes as aide-mémoires. In: *Nature* 463 (7278), S. 169–170. DOI: 10.1038/463169a.

Sawcer, Stephen; Ban, Maria; Maranian, Mel; Yeo, Tai Wai; Compston, Alastair; Kirby, Andrew et al. (2005): A high-density screen for linkage in multiple sclerosis. In: *American journal of human genetics* 77 (3), S. 454–467. DOI: 10.1086/444547.

Schartz, Nicole D.; Tenner, Andrea J. (2020): The good, the bad, and the opportunities of the complement system in neurodegenerative disease. In: *Journal of neuroinflammation* 17 (1), S. 354. DOI: 10.1186/s12974-020-02024-8.

Schindelin, Johannes; Arganda-Carreras, Ignacio; Frise, Erwin; Kaynig, Verena; Longair, Mark; Pietzsch, Tobias et al. (2012): Fiji: an open-source platform for biological-image analysis. In: *Nature methods* 9 (7), S. 676–682. DOI: 10.1038/nmeth.2019.

Sen, Monokesh K.; Mahns, David A.; Coorssen, Jens R.; Shortland, Peter J. (2022): The roles of microglia and astrocytes in phagocytosis and myelination: Insights from the cuprizone model of multiple sclerosis. In: *Glia* 70 (7), S. 1215–1250. DOI: 10.1002/glia.24148.

Silva Oliveira Junior, Markley; Schira-Heinen, Jessica; Reiche, Laura; Han, Seulki; Amorim, Vanessa Cristina Meira de; Lewen, Isabel et al. (2022): Myelin repair is fostered by the corticosteroid medrysone specifically acting on astroglial subpopulations. In: *EBioMedicine* 83, S. 104204. DOI: 10.1016/j.ebiom.2022.104204.

Smith, Kenneth J.; Lassmann, Hans (2002): The role of nitric oxide in multiple sclerosis. In: *The Lancet Neurology* 1 (4), S. 232–241. DOI: 10.1016/s1474-4422(02)00102-3.

Soelberg Sorensen, P. (2017): Safety concerns and risk management of multiple sclerosis therapies. In: *Acta Neurologica Scandinavica* 136 (3), S. 168–186. DOI: 10.1111/ane.12712.

Sofroniew, Michael V. (2015): Astrocyte barriers to neurotoxic inflammation. In: *Nature Reviews Neuroscience* 16 (5), S. 249–263. DOI: 10.1038/nrn3898.

Sofroniew, Michael V.; Vinters, Harry V. (2010): Astrocytes: biology and pathology. In: *Acta Neuropathologica* 119 (1), S. 7–35. DOI: 10.1007/s00401-009-0619-8.

Sørensen, T. L.; Tani, M.; Jensen, J.; Pierce, V.; Lucchinetti, C.; Folcik, V. A. et al. (1999): Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. In: *Journal of Clinical Investigation* 103 (6), S. 807–815. DOI: 10.1172/JCI5150.

Srinivasan, Radhika; Sailasuta, Napapon; Hurd, Ralph; Nelson, Sarah; Pelletier, Daniel (2005): Evidence of elevated glutamate in multiple sclerosis using magnetic resonance spectroscopy at 3 T. In: *Brain* 128 (Pt 5), S. 1016–1025. DOI: 10.1093/brain/awh467.

Stadelmann, Christine; Kerschensteiner, Martin; Misgeld, Thomas; Brück, Wolfgang; Hohlfeld, Reinhard; Lassmann, Hans (2002): BDNF and gp145trkB in multiple sclerosis brain lesions: neuroprotective interactions between immune and neuronal cells? In: *Brain* 125 (Pt 1), S. 75–85. DOI: 10.1093/brain/awf015.

Stocking, C.; Kozak, C. A. (2008): Murine endogenous retroviruses. In: *Cellular and molecular life sciences : CMLS* 65 (21), S. 3383–3398. DOI: 10.1007/s00018-008-8497-0.

Stratoulias, Vassilis; Venero, Jose Luis; Tremblay, Marie-Ève; Joseph, Bertrand (2019): Microglial subtypes: diversity within the microglial community. In: *The EMBO Journal* 38 (17), e101997. DOI: 10.15252/embj.2019101997.

Streit, Wolfgang J.; Conde, Jessica R.; Fendrick, Sarah E.; Flanary, Barry E.; Mariani, Christopher L. (2005): Role of microglia in the central nervous system's immune response. In: *Neurological research* 27 (7), S. 685–691. DOI: 10.1179/016164105X49463a.

Szpakowski, Sebastian; Sun, Xueguang; Lage, José M.; Dyer, Andrew; Rubinstein, Jill; Kowalski, Diane et al. (2009): Loss of epigenetic silencing in tumors preferentially affects primate-specific retroelements. In: *Gene* 448 (2), S. 151–167. DOI: 10.1016/j.gene.2009.08.006.

Takase, Ezgi Ozdemir; Yamasaki, Ryo; Nagata, Satoshi; Watanabe, Mitsuru; Masaki, Katsuhisa; Yamaguchi, Hiroo et al. (2024): Astroglial connexin 43 is a novel therapeutic target for chronic multiple sclerosis model. In: *Scientific reports* 14 (1), S. 10877. DOI: 10.1038/s41598-024-61508-2.

Tassoni, Alessia; Farkhondeh, Vista; Itoh, Yuichiro; Itoh, Noriko; Sofroniew, Michael V.; Voskuhl, Rhonda R. (2019): The astrocyte transcriptome in EAE optic neuritis shows complement activation and reveals a sex difference in astrocytic C3 expression. In: *Scientific reports* 9 (1), S. 10010. DOI: 10.1038/s41598-019-46232-6.

Thompson, Alan J.; Banwell, Brenda L.; Barkhof, Frederik; Carroll, William M.; Coetzee, Timothy; Comi, Giancarlo et al. (2018): Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. In: *The Lancet. Neurology* 17 (2), S. 162–173. DOI: 10.1016/S1474-4422(17)30470-2.

Toft-Hansen, Henrik; Füchtbauer, Laila; Owens, Trevor (2011): Inhibition of reactive astrocytosis in established experimental autoimmune encephalomyelitis favors infiltration by myeloid cells over T cells and enhances severity of disease. In: *Glia* 59 (1), S. 166–176. DOI: 10.1002/glia.21088.

Trapp, Bruce D.; Nave, Klaus-Armin (2008): Multiple sclerosis: an immune or neurodegenerative disorder? In: *Annual Review of Neuroscience* 31, S. 247–269. DOI: 10.1146/annurev.neuro.30.051606.094313.

van Horssen, Jack; van der Pol, Susanne; Nijland, Philip; Amor, Sandra; Perron, Hervé (2016): Human endogenous retrovirus W in brain lesions: Rationale for targeted therapy in multiple sclerosis. In: *Multiple Sclerosis and Related Disorders* 8, S. 11–18. DOI: 10.1016/j.msard.2016.04.006.

Venter, J. C.; Adams, M. D.; Myers, E. W.; Li, P. W.; Mural, R. J.; Sutton, G. G. et al. (2001): The sequence of the human genome. In: *Science (New York, N.Y.)* 291 (5507), S. 1304–1351. DOI: 10.1126/science.1058040.

Ventura, R.; Harris, K. M. (1999): Three-dimensional relationships between hippocampal synapses and astrocytes. In: *J. Neurosci.* 19 (16), S. 6897–6906. DOI: 10.1523/JNEUROSCI.19-16-06897.1999.

Virchow, R. (1856): Gesammelte Abbildung zur wissenschaftlichen Medizin.

Wagner, H-J; Munger, K. L.; Ascherio, A. (2004): Plasma viral load of Epstein-Barr virus and risk of multiple sclerosis. In: *European journal of neurology* 11 (12), S. 833–834. DOI: 10.1111/j.1468-1331.2004.00871.x.

Wallin (2017): The prevalence of multiple sclerosis in the united states: a population-based healthcare database approach. In: *ECTRIMS Online Libr* 26, S. 199999.

Wang, Dongwei; Ayers, Margaret M.; Catmull, Deanne V.; Hazelwood, Lisa J.; Bernard, Claude C. A.; Orian, Jacqueline M. (2005): Astrocyte-associated axonal damage in pre-onset stages of experimental autoimmune encephalomyelitis. In: *Glia* 51 (3), S. 235–240. DOI: 10.1002/glia.20199.

Wang, Xiuling; Liu, Zhongchun; Wang, Peigang; Li, Shan; Zeng, Jie; Tu, Xiaoning et al. (2018): Syncytin-1, an endogenous retroviral protein, triggers the activation of CRP via TLR3 signal

cascade in glial cells. In: *Brain, Behavior, and Immunity* 67, S. 324–334. DOI: 10.1016/j.bbi.2017.09.009.

Watkins, Lewis M.; Neal, James W.; Loveless, Sam; Michailidou, Iliana; Ramaglia, Valeria; Rees, Mark I. et al. (2016): Complement is activated in progressive multiple sclerosis cortical grey matter lesions. In: *Journal of neuroinflammation* 13 (1), S. 161. DOI: 10.1186/s12974-016-0611-x.

Werner, P.; Pitt, D.; Raine, C. S. (2001): Multiple sclerosis: altered glutamate homeostasis in lesions correlates with oligodendrocyte and axonal damage. In: *Annals of Neurology* 50 (2), S. 169–180. DOI: 10.1002/ana.1077.

Wishart, Claire L.; Spiteri, Alanna G.; Locatelli, Giuseppe; King, Nicholas J. C. (2023): Integrating transcriptomic datasets across neurological disease identifies unique myeloid subpopulations driving disease-specific signatures. In: *Glia* 71 (4), S. 904–925. DOI: 10.1002/glia.24314.

Xiang, Yangquan; Liang, Hongqing (2021): The Regulation and Functions of Endogenous Retrovirus in Embryo Development and Stem Cell Differentiation. In: *Stem cells international* 2021, S. 6660936. DOI: 10.1155/2021/6660936.

6 Danksagung

An dieser Stelle möchte ich allen beteiligten Personen meinen großen Dank aussprechen, die mich bei der Anfertigung meiner Dissertation unterstützt haben.

Zuerst möchte ich meinem Doktorvater Herr Prof. Patrick Küry für die Überlassung des interessanten Forschungsthemas, und die Aufnahme in sein Labor danken. Danke für deine hervorragende Betreuung und ständige Unterstützung.

Außerdem möchte ich mich bei Frau Prof. Christine Rose für die Übernahme des Zweitgutachtens und die gelungene Kommunikation bedanken.

Mein besonderer Dank gilt Dr. Joel Gruchot für die ausgezeichnete Betreuung und die enorme Unterstützung bei der Umsetzung der gesamten Arbeit. Dein großes Engagement, deine fachliche Expertise und deinen persönlichen Einsatz weiß ich sehr zu schätzen.

Auch möchte ich meinen Kolleg:innen der AG Küry meinen Dank aussprechen. Ihr habt mich auf diesem Weg mit gutem Rat, tatkräftiger Unterstützung und lieben Worten begleitet.

Nicht zuletzt möchte mich bei unseren technischen Assistentinnen Brigida Ziegler, Zippora Kohne und Birgit Blomenkamp-Radermacher bedanken, deren hervorragende Unterstützung ich sehr zu schätzen gelernt habe.

Für die finanzielle Unterstützung bedanke ich mich für das Promotionsstipendium der Forschungskommission der HHU.

Nicht nur für die Durchsicht und Kritik, sondern besonders für die stete Unterstützung in allen Bereichen bedanke ich mich bei meinem Freund Jannik, ohne den ich diese Arbeit nicht in dieser Art hätte erstellen können. Vielen Dank!

Ich danke meinen Freunden für ihre Unterstützung und Verständnis, ohne die diese Zeit nur halb so schön gewesen wäre.

Zuletzt danke ich meinen Eltern für ihre Geduld, Ermutigungen und stete Unterstützung während der Arbeit an dieser Dissertation. Noch mehr bedanken muss ich mich jedoch für die vorrangehende Zeit. Meinem Vater verdanke ich mein Interesse an der Wissenschaft und Forschung und meiner Mutter meine Neugier und den Glauben an mich selbst. Danke, dass ihr immer an mich glaubt und mir diesen Traum ermöglicht habt.