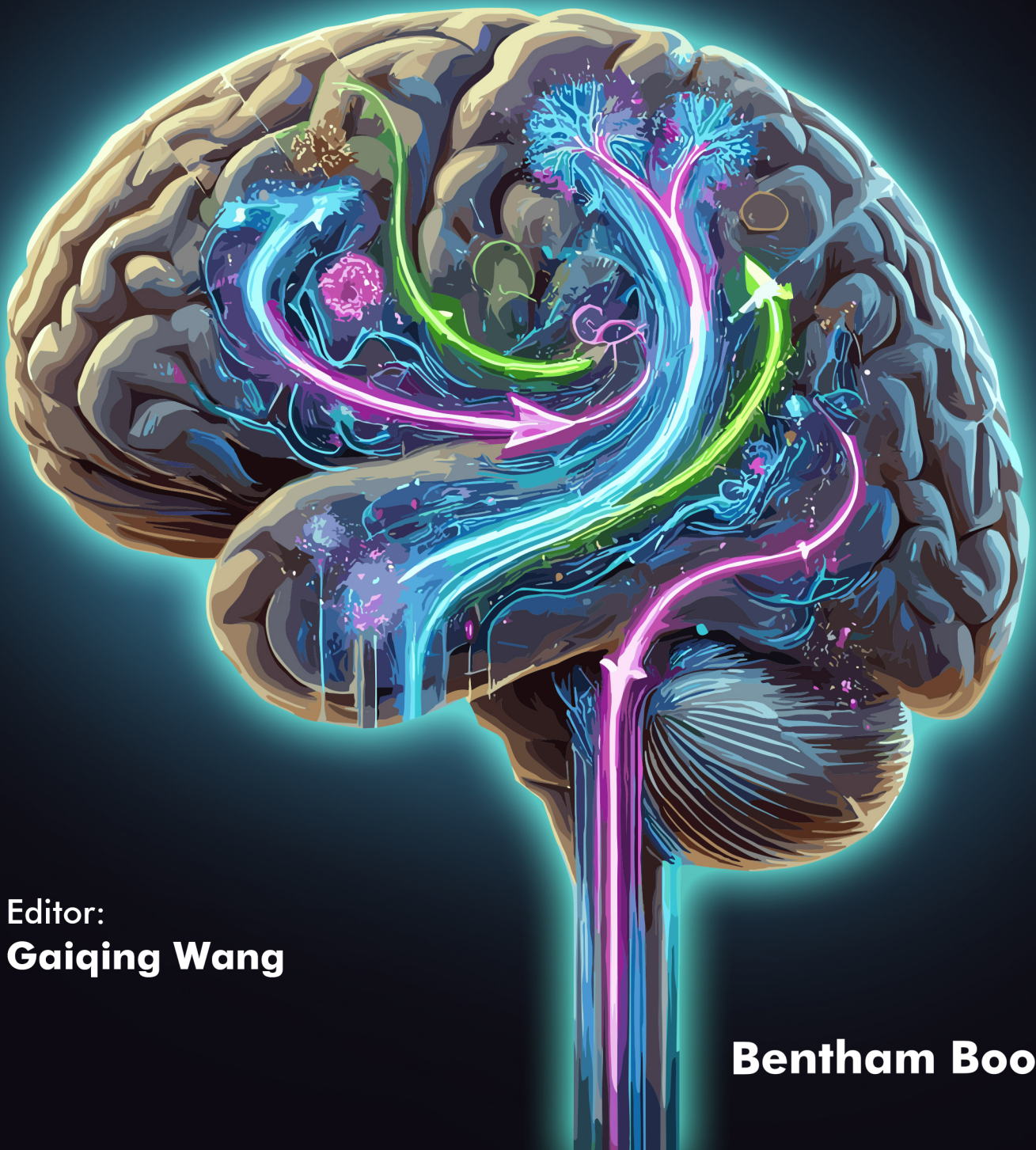
# WASTE CLEARANCE IN THE BRAIN



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# Waste Clearance in the Brain

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**Waste Clearance in the Brain**

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# PREFACE

Welcome to "Endogenous Waste Clearance System in Brain: Exploration and Understanding in Neuroscience". This book aims to delve into the vast and intricate systems within the brain, unveiling their mysteries. The endogenous brain clearance system is a crucial component for maintaining clarity and efficient function in the brain, yet it is an aspect that has rarely been deeply studied and understood.

The author of this book, Gaiqing Wang, is a seasoned expert in the field of neuroscience. Through years of research and practice, he has gathered profound insights into this mysterious system. In this digital age, we are fortunate to present this profound topic in the form of an e-book, making it easily accessible to readers and allowing them to delve into the internal workings of the brain.

The endogenous brain clearance system plays a critical role in maintaining the health and functionality of the brain. This system involves various processes and mechanisms that work together to remove waste products and maintain a balanced and efficient neural environment. The brain generates waste products as a natural part of its cellular activities. These waste products include proteins, cellular debris, metabolic by-products, and all foreign agents. Efficient waste clearance is essential for preventing the accumulation of toxic substances that can interfere with neural function and contribute to neurodegenerative diseases.

Understanding the intricate interplay between glial cells, the glymphatic system, the blood-brain clearance system, and the biological basis of waste clearance provides insights into the overall health and resilience of the brain. Dysregulation of these processes has been implicated in various neurological disorders, making the study of the endogenous brain clearance system crucial for advancing our understanding of brain health and disease. Ongoing research in this field holds promise for the development of therapeutic interventions targeting the enhancement of these clearance mechanisms to promote brain health, prevent aging, and mitigate cognitive decline.

In the process of completing this book, I want to sincerely thank all those who contributed to this project, especially researchers, peer reviewers, and supporters. Thank you for your hard work and professional advice, which have enriched and deepened the content of this book.

May this e-book serve as a starting point for a profound understanding of the endogenous brain clearance system, inspiring more individuals to engage in this captivating field. I hope readers, through this book, can appreciate the beauty of the complexity within the brain and make their own contributions to future research and discoveries.

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# INTRODUCTION

## WASTE CLEARANCE IN THE BRAIN

## BACKGROUND AND PURPOSE

Accumulations of neurotoxic substances were numerous and diverse, but there has been a similar clearance pathway in the central nervous system (CNS). Neurodegeneration has been hypothesized to result from an imbalance between waste production and clearance; this mechanism is also the leading cause of aging and age-related chronic diseases. As the most metabolically active organ in the body, there is a recognized need for the development of methods for improving the clearance of waste products and neurotoxins from the brain.

As the most metabolically active organ system in the human body, the CNS needs an efficient way of clearing its waste, given that just the brain, although representing 2% of the entire body mass, is responsible for approximately 25% of the global metabolism. Waste clearance is an essential process for brain homeostasis, which is required for the proper and healthy functioning of all cerebrovascular and parenchymal brain cells. Related brain waste clearance systems include the vascular system, blood-brain barrier (BBB), scavenger cells and receptors, transporters and pathways, cerebrospinal fluid, perivascular lymphatic drainage pathways, and the newly characterized meningeal lymphatic vessels. Any functional abnormality of perivascular spaces (PVS) or other related clearance systems might lead to the accumulation of brain waste. We describe the interplay of the BBB, interstitial fluid (ISF), and PVS within the brain parenchyma for brain waste clearance directly into the blood and/or cerebrospinal fluid (CSF). We also discuss the relevant role of the CSF and its exit routes in mediating waste clearance. Recent discoveries of the glymphatic system and meningeal lymphatic vessels and their relevance to brain waste clearance are highlighted. Controversies related to brain waste clearance research and potential future directions are presented. The currently proposed brain waste clearance systems are summarized in the chapter.

Previously, we mostly explored how phagocytes and their receptors could play a role in clearing hematoma following intracerebral hemorrhage (ICH). There are, however, several related or concomitant systems involved in the process, such as the BBB, Virchow-Robin spaces (VRS), and the CSF circulation. Moreover, despite the absence of classical lymphatic vessels in the brain, a sort of lymphatic drainage has been previously subsumed on the basis of communication between CSF, ISF, brain tissue, and the cervical lymph nodes. Furthermore, there are perineural and meningeal lymphatic drainage pathways from the brain to the cervical lymph nodes. These processes play a very significant role in maintaining homeostasis and ultimately contribute to the immune surveillance of the brain. It is, however, suspected that their role might be complementary because any functional abnormality of a given system may lead to the accumulation of brain waste and, therefore, can contribute to the progress of neurodegenerative and cerebrovascular pathology.

We summarize the currently proposed brain waste clearance systems in this book. The relative contribution of each of these systems has been illustrated.

# INTRODUCTION

As the most metabolically active organ in the body, there is a recognized need for pathways that can remove waste proteins and neurotoxins from the brain. Previous research has indicated potential associations between the clearance system in the brain and pathologic conditions of the central nervous system (CNS) due to its importance, which has attracted considerable attention recently. Many of us have concerns about the deposition of abnormal constitutes in the brain, but we often overlook the essential issue of the clearance pathway. Interactions between deposits of abnormal constitutes and the clearance pathway are complicated, and it could be necessary to create the optimal balance among brain protection, neurological outcome, social recovery, economic burden, and social burden. The rapid ageing of the global population is increasing the demand for medical services. The impact it will have on the deposition of metabolic wastes in the brain is tremendous, and we are only just now discovering the pathway for waste clearance in neural tissue and how function is regulated.

Hazardous Waste Deposits and Health Risks aims to point out critical areas of Waste Clearance Pathway: The Regulation and Removal of Hazardous Wastes Outside the Brain. The book focuses on several aspects of the clearance pathway for hazardous waste deposits in the brain, from waste sources to its consequences, for brain protection and neurological recovery. The author underlines that the potential handling of waste deposits can have an effect on neurologic disorders and public health. In this scenario, sound regulation of waste clearance plays a critical role in brain protection.

Furthermore, this book explains how the source of hazardous waste deposits can be positively implicated in the brain. It addresses the correlation between abnormal constitutes deposits and various acute and chronic diseases in the brain and also illustrates how response biomarkers can be considered as early signals of a hazardous waste invader and increased risk of adverse chronic health effects, outlining a possible new approach in prevention and intervention on brain dysfunction. Such approaches are very useful for preventive health surveillance programs, especially in the realm of various neurological disorders, including ICH, cerebral small vessel disease (CSVD), brain tumors, neurodegeneration, and pathogen infections.

Recent advances in common waste/garbage clearance systems in the brain, and, in particular, glymphatic and meningeal lymphatic systems, have necessitated the elucidation of metabolic waste transport processes and efflux in neurological disorders. This is also the case with several exotic and endogenic substance conditions, which include hematoma, tumors, pathogenic infections, metal deposition, protein aggregation, and abnormal protein deposits in the brain. Unfortunately, current knowledge about the mechanism of waste clearance in the brain and targeting the pathway for brain waste sediment clearance with potential strategy is insufficient. So, the author elaborates on the source and generations of waste deposits, their consequences and hazards, the various stages in the removal of waste outside the brain, and potential therapeutic/regulated strategies targeting waste deposits.

# PART 1

# THE SOURCE OF WASTE IN THE BRAIN

Production of brain waste, including debris accumulation in the form of dead cells, protein folding or aggregation, metal deposition, polymer formation, lipid peroxidation, protein oxidation, and inactivation of antioxidant enzymes, along with the aging trend, are discussed in this part. The sectionindicates that age affects the activity of antioxidant enzymes and elevates the levels of lipid peroxidation and protein oxidation in brain tissues in a time-dependent manner, accompanied by changes in antioxidant status [1]. The waste production in the brain can be described as age-related cabinets not cleaned thoroughly for a long time.

# CHAPTER 1

# Entrance of Exotic Pathogens into the Brain

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**Abstract:** CNS infections are life-threatening diseases caused by viral, bacterial, parasitic, and fungal microorganisms, including meningitis, encephalitis, and brain abscess. These infections are linked to significant illnesses and death rates. CNS is characterized by a specific structure and function. Despite a unique system of brain barriers and an autonomous immune system, CNS is very susceptible to microorganisms, which may invade directly *via* the BBB, blood, or less frequently by reverse axonal transport.

**Keywords:** BBB, CNS infections, Pathogenic microorganisms, Viral encephalitis bacterial infection.

## INTRODUCTION

Pathogenic microorganisms in the brain include viruses, bacteria, fungi, prions, parasites, toxoplasma, and so on. Invasion of the CNS by microorganisms is a severe and often fatal event in many infectious diseases. It can result in long-term consequences such as deafness, blindness, cerebral palsy, hydrocephalus, cognitive impairment, or permanent neurological dysfunction in survivors. Pathogens can cross the BBB through transcellular and paracellular migration or within infected macrophages. Once breached, the BBB allows pathogens to be detected by antigen-presenting cells through Toll-like receptor binding. This triggers the activation of nuclear factor kappa B and mitogen-activated protein kinase pathways, leading to leukocyte infiltration, proliferation, and the expression of proteins involved in inflammation and immune responses [2].

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## Viral Encephalitis

Viruses are a frequent cause of encephalitis. Common or important viruses causing encephalitis include herpesviruses, arboviruses, enteroviruses, parechoviruses, mumps, measles, rabies, Ebola, lymphocytic choriomeningitis virus, and henipaviruses. Other viruses may cause an encephalopathy. The most common etiologies are herpesviruses 1 and 2 (HSV-1 and HSV-2), non-polio enterovirus, and arboviruses (in Brazil, dengue, Zika, and chikungunya). Other relevant etiologies are seasonal influenza, cytomegalovirus (CMV), Epstein–Barr virus (EBV), human herpesvirus 6 (HHV-6), and the re-emergent measles [3]. Herpes simplex encephalitis (HSE) can occur at all ages during infancy and childhood, with a peak incidence during the first year of life. Its pathogenesis remains unsolved, although defects in the innate immune response have been observed in a few cases.

## Bacterial Infection of CNS

The complex process of bacteria and activated polymorphonuclear leukocyte transfer to the subarachnoid space, which is devoid of natural immune defense mechanisms, initiates an inflammatory response that subsequently spreads to the brain tissue. Consequences of these changes include damage to the BBB, development of vasogenic cerebral edema, and intracranial pressure-volume disturbances leading to impaired CNS perfusion.

CNS infections of bacteria may involve the meninges, brain and/or spinal cord. The most common etiologic agents are *Streptococcus pneumoniae*, group B *Streptococci, Neisseria meningitidis, Haemophilus influenzae*, and *Listeria monocytogenes*.

Bacterial infections of CNS are neurologic emergencies. Prompt recognition and treatment are essential not only to prevent mortality but also to decrease neurologic sequelae [4].

Patients with conditions that impair B-lymphocyte function are especially vulnerable to meningitis caused by encapsulated bacterial pathogens. The clinical presentation of bacterial meningitis is similar in both healthy individuals and those with compromised B-lymphocyte immunity. Individuals with compromised T-lymphocyte function who develop meningitis are more likely to have infections caused by Listeria or *Cryptococcus* rather than Toxoplasma or CMV. Conditions affecting host defenses, along with the treatment methods applied, can lead to clinical manifestations in the central nervous system that resemble CNS infections.

Several bacterial infections affect the central nervous system, encompassing meningitis, brain abscess, cranial and spinal epidural abscess, subdural empyema, and suppurative dural sinus thrombophlebitis.

Despite advancements in vaccination, *Streptococcus pneumoniae* and *Neisseria meningitidis* remain the primary causative organisms of community-acquired bacterial meningitis. The tetravalent meningococcal vaccine used in the United States covers serogroups A, C, W, and Y, excluding serotype B, responsible for a third of meningococcal cases in industrialized countries. Pneumococcal vaccines reduce acute otitis media and pneumonia incidence but are not specific meningitis vaccines. Identifying predisposing conditions aids in predicting the meningeal pathogen, such as *Streptococcus pneumoniae* in pneumonia or *Streptococci* spp. in cases with otitis, sinusitis, or mastoiditis history. Neurosurgical procedure-related meningitis is often caused by *staphylococci*, gram-negative bacilli, and anaerobes. Recognizing anaerobes as potential pathogens requires adding metronidazole to the empiric regimen; for instance, *Listeria monocytogenes* in pregnant patients, adults over 55, and those with impaired immunity. Ampicillin is crucial in these cases, and gentamicin might be used for critically ill patients suspected of *Listeria monocytogenes* meningitis, although its use should generally be minimized [4].

## Neuroinfections Caused by Fungi

The invasion of the CNS is primarily influenced by the host's immune status and the virulence of the fungal strain. Individuals with compromised T-lymphocyte or macrophage function are susceptible to developing CNS infections caused by intracellular pathogens. Among these, fungi, particularly Aspergillus, are the most common. CNS Aspergillus infections typically present as mass lesions, such as brain abscesses or cerebral infarcts, with meningitis being a rare presentation. In contrast, *Cryptococcus neoformans* often presents as meningitis and rarely as a cerebral mass lesion, even in the presence of cryptococcal elements. Both Aspergillus and *Cryptococcus* CNS infections indicate weakened host defenses and are rare in immunocompetent individuals [5]. Fungal infections pose a substantial morbidity risk in immunocompromised individuals, and their impact on the central nervous system can have fatal consequences.

The frequency of fungal infections is on the rise annually, particularly among high-risk groups, including individuals with HIV, AIDS patients, transplant recipients, and those undergoing immunosuppressive therapies like chemotherapy or corticosteroids. Additionally, patients with hematological disorders and chronic illnesses are more susceptible.

CHAPTER 2

# Deposition of Abnormal Proteins in the Brain

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**Abstract:** A common feature of neurodegenerative diseases is the abnormal accumulation of misfolded proteins in the brain, such as amyloid beta (Aβ), tau, α-synuclein, fused in sarcoma (FUS), and TAR DNA-binding protein 43 (TDP-43), which lead to selective neuronal degeneration and dysfunction. Dysfunction in the removal of these misfolded proteins from the brain, which is thought to be a major cause of neurodegenerative diseases and a major therapeutic target for their cure.

**Keywords:** Abnormal deposits, Amyloid beta (Aβ), Neurodegenerative diseases, Tau protein, α-synuclein.

## DEPOSITION OF ABNORMAL TAU PROTEIN IN THE BRAIN

Tau proteins, prevalent among microtubule-associated proteins in the brain, which are classified as intrinsically disordered proteins, are abundant in CNS neurons; they primarily uphold microtubule stability in axons, with lower expression of microtubule-associated proteins in brain-resident immune cells, such as astrocytes and oligodendrocytes, and play crucial roles in cell signaling, synaptic plasticity, and the regulation of genomic stability.

In the adult human brain, at least six isoforms of tau protein are expressed, derived from alternative splicing of messenger RNA from the microtubule-associated protein tau (MAPT) gene. Tauopathies are categorized based on the prevalence of tau protein isoforms within cytoplasmic inclusions and are classified based on their composition: those with inclusions mainly composed of 3-repeat tau (3R-tauopathies), those primarily consisting of 4-repeat tau (4R-tauopathies), and those displaying an equal ratio of 3R to 4R tau. Prominent tauopathies include Alzheimer's disease, frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), progressive supranuclear

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palsy (PSP), corticobasal degeneration (CBD), and Pick's disease (PiD). Additionally, rarer tauopathies encompass argyrophilic grain disease (AGD), postencephalitic parkinsonism (PEP), parkinsonism-dementia complex of Guam (PDCG), tangle-dominant dementia, and the newly recognized category of globular glial tauopathies (GGTs). These tauopathies can be further divided into three main groups based on their tau pathology (refer to Table **1**) [8].

Nevertheless, tau neuropathology seldom occurs in isolation and is often accompanied by the deposition of at least one other amyloidogenic protein, such as α-synuclein or huntingtin, in the majority of tauopathies. This suggests that tau likely plays a crucial pathological role in these disorders, which are characterized by multiple pathologies [8].

**Table 1. (Michalicova, Majerova *et al.* 2020).**
**The first subgroup of tauopathies is represented by 3R/4R tauopathies, such as Alzheimer's disease (AD), frontotemporal dementia, Parkinsonism linked to chromosome 17, Parkinsonism dementia complex of Guam (Lytico-bodig disease), chronic traumatic encephalopathy, postencephalitic Parkinsonism, atypical Parkinsonism of Guadeloupe, primary age-related tauopathy, or diffuse neurofilament tangles with calcification.**
**Three-repeat tauopathies include Pick's disease and some forms of frontotemporal dementia and Parkinsonism linked to chromosome 17, and the four-repeat tauopathies belong to progressive supranuclear palsy, corticobasal degeneration, argyrophilic grain disease, globular glial tauopathies, or aging-related tau astrogliopathy.**

| Gene | Protein | Disorder |
|---|---|---|
| *PANK2* | Pantothenate kinase 2 | Pantothenate kinase-associated neurodegeneration (PKAN) |
| *PLA2G6* | Calcium-independent phospholipase $A_2$ group VIa = iPLA2VIa | Phospholipase $A_2$-associated neurodegeneration (PLAN), including infantile neuroaxonal dystrophy (INAD) |
| *C19orf12* | C19orf12 | Mitochondrial membrane protein-associated neurodegeneration (MPAN) |
| *WDR45* | WD40-repeat protein 45 | Beta-propeller protein-associated neurodegeneration (BPAN) |
| *CoASY* | Coenzyme A synthase | CoA synthase protein-associated neurodegeneration (CoPAN) |
| *FA2H* | Fatty acid 2 hydroxylase | Fatty acid-2 hydroxylase-associated neurodegeneration (FAHN) |
| *ATP13A2* | Cation-transporting ATPase 13A2 | Kufor–Rakeb disease |
| *CPL* | Ceruloplasmin | Aceruloplasminemia |
| *FTL* | Ferritin light chain | Neuroferritinopathy |

A healthy BBB is crucial for proper neuronal function, while healthy neurons are essential for sustaining the local environment of the neurovascular unit (NVU). Neurons are intricately connected to brain capillaries, which were previously

described as a layer of endothelial cells forming the vessel or capillary wall. However, recent understanding has integrated the BBB as a key component of the NVU, a dynamic system essential for brain function.

The NVU relies on intricate interactions between endothelial cells, neurons, pericytes, mast cells, and glial cells to maintain brain homeostasis and ensure proper neuronal activity. Furthermore, it also includes circulating immune cells and peripheral tissue cells, connected through humoral secretions that influence the physical, biochemical, and immune processes of CNS barriers. Nearly every neuron is thought to have its own capillary, underscoring the critical role neurons play in tightly regulating metabolism, blood flow, and permeability within the brain. As a result, pathological processes in neurons inevitably disrupt the functioning of the NVU [8].

The association between neuropathological hallmarks like intra- and extracellular protein aggregates and chronic neuroinflammation is well established. Chronic neuroinflammation impacts the BBB in several ways: it increases vascular permeability, induces structural changes in brain capillaries such as fragmentation and thickening, leads to pericyte atrophy, causes the accumulation of laminin in the basement membrane, enhances permeability to small molecules and plasma proteins, promotes immune cell migration, alters transport systems, and influences the BBB's role as a signaling interface (Fig. **1**). Inflammatory mediators are crucial in regulating the transmigration of cells from blood to the brain, sustaining inflammation and thereby worsening the disease pathology. The structural and functional alterations of the BBB result in gradual synaptic and neuronal dysfunction [8].

Previous studies have demonstrated a correlation between tangle formation and neuroinflammation in Alzheimer's disease (AD) as well as in non-AD human tauopathies like tangle-predominant dementia, Guam parkinsonism dementia, PSP, and CBD. Additionally, neuroinflammation associated with tau deposition has been extensively documented in transgenic mice expressing human mutant tau or in transgenic rats expressing misfolded truncated tau protein [8].

Research has demonstrated that, unlike Aβ peptides, truncated tau does not directly harm brain endothelial cells. However, the impact of tau is mediated through the activation of glial cells. Furthermore, tau-induced activation of glial cells results in increased expression of endothelial adhesion molecules and enhanced transport of leukocytes across the BBB [8].

CHAPTER 3

# Harmful Effects of Metal Deposits in the Brain

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**Abstract:** Metal deposits in the brain can lead to various harmful effects, which can depend on factors such as the type of metal, its amount and location of deposition, and the individual's overall health. These effects may include:

1. Neurotoxicity: Metals like lead, mercury, and arsenic can directly damage neurons and interfere with their normal functioning, leading to neurological symptoms and cognitive impairments.

2. Inflammation: Metal deposits can trigger an inflammatory response in the brain, leading to tissue damage and exacerbating neurodegenerative processes.

3. Oxidative stress: Metals can induce oxidative stress, causing an imbalance between free radicals and antioxidants in brain cells, which can lead to cellular damage and dysfunction.

4. Impaired neurotransmitter function: Metals can disrupt neurotransmitter systems in the brain, affecting communication between neurons and leading to cognitive and behavioral changes.

5. Compromised blood-brain barrier: Some metals can weaken the blood-brain barrier, allowing harmful substances to enter the brain more easily and exacerbating neuronal damage.

6. Increased risk of neurodegenerative diseases: Metal deposition has been linked to an increased risk of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS).

7. Cellular dysfunction: Metals can interfere with cellular processes and signaling pathways, leading to cellular dysfunction and contributing to neurological disorders.

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It is crucial to minimize exposure to toxic metals through proper safety measures and environmental regulations. If metal deposition in the brain is suspected or confirmed, seeking medical attention is important to evaluate the extent of damage and determine appropriate treatment strategies to mitigate harmful effects.

**Keywords:** Copper accumulation, Hazardous metals, Iron overload, Manganism.

## IRON OVERLOAD IN THE BRAIN

In the CNS, iron plays a crucial role in various essential processes, such as oxygen transportation, oxidative phosphorylation, myelin production, and the synthesis and metabolism of neurotransmitters. Iron is primarily bound within ferritin and neuromelanin in healthy aging, with selective accumulation occurring in specific brain regions and cell types.

However, abnormal iron homeostasis can lead to cellular damage due to the production of hydroxyl radicals, which can oxidize and modify lipids, proteins, carbohydrates, and DNA. This oxidative stress and cellular damage are often associated with neurodegenerative diseases where iron accumulation in specific brain regions exceeds normal levels seen in healthy aging.

The role of iron accumulation in neurodegenerative diseases remains unclear as to whether it is a primary event or a secondary effect. Nevertheless, aging is a significant risk factor for neurodegeneration, and age-related accumulation of iron may contribute to the neurodegenerative processes. Different iron complexes can accumulate in brain regions associated with motor and cognitive impairments during aging, and alterations in iron homeostasis are observed in diseases like Alzheimer's disease(AD) and Parkinson's disease(PD), leading to changes in cellular iron distribution and accumulation.

Understanding the relationship between iron dysregulation and neurodegenerative diseases is crucial for developing targeted interventions that could potentially mitigate the harmful effects of iron accumulation and oxidative stress in these conditions.

Fig. (**5**) summarizes the current understanding of iron homeostasis in the brain. Iron initially enters the brain by crossing the vascular endothelial cells of the blood-brain barrier, primarily bound to transferrin *via* the transferrin-transferrin receptor 1 (TFR1) system. TFR1 is highly expressed on the luminal side of endothelial cells. Once inside the brain, iron is released into the cytoplasmic iron pool and is believed to be exported at the abluminal membrane, although the specific pathways involved, such as ferroportin or other transporters, are not yet fully elucidated.

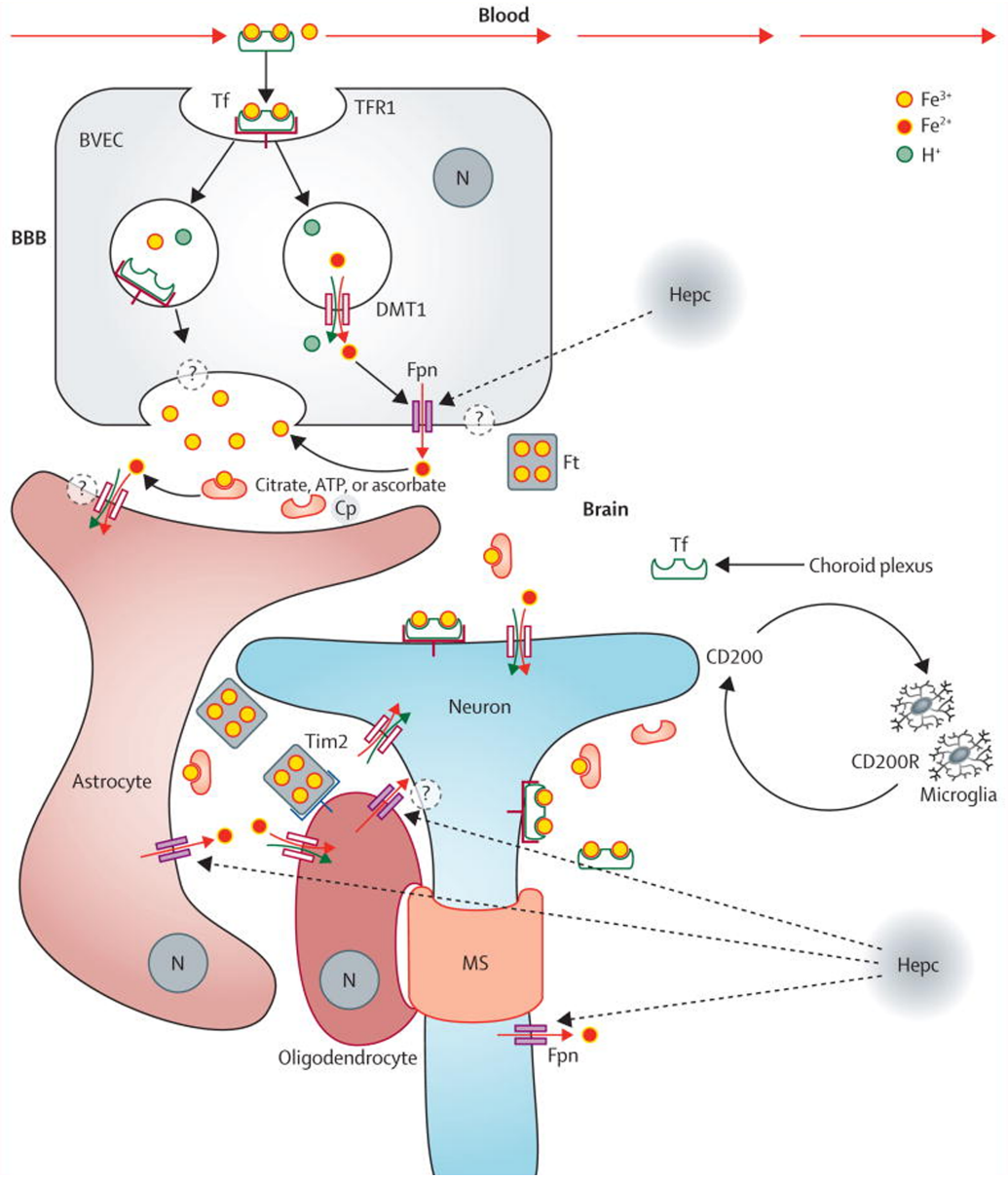


**Fig. (5).** Brain iron metabolism.

Iron enters the endothelial cells of the blood–brain barrier as a low molecular weight complex, or *via* transferrin receptor-1 mediated endocytosis of transferrin, or independently as non-transferrin-bound iron. Transferrin receptors line the lumen of the brain and bind circulating differic–transferrin facilitating iron uptake

# PART 2

# RESPONSE TO WASTE DEPOSITION IN THE BRAIN

Brain function is intricately linked to the regulation of cerebral blood flow (CBF), which ensures the delivery of oxygen and nutrients while removing cellular waste. This regulatory process involves the response of cells to various stimuli, including both external and internal factors. Cell-surface expression profiles play a crucial role in this response, as they dictate how cells interact with their environment and modulate their activity accordingly. Additionally, the production of immune factors in neuronal microenvironments contributes to the adaptation of brain cells to local changes, further influencing CBF regulation and overall brain function.

In our comprehensive overview, we delve into the functions of brain cells, focusing on the profiles of cell-surface receptors, transcriptional signatures, and potential functional phenotypes of microglia and astrocyte subsets, as highlighted in recent studies. We explore the evidence supporting their putative physiological roles, including immunological surveillance, waste clearance mechanisms, and regulation of vascular permeability within the neurovascular unit (NVU).

Furthermore, we examine how brain cells respond to insults, noting that their original functions may initially serve a neuroprotective role during acute phases but may become deleterious with prolonged stimuli. This transformation and accumulation of brain cells in response to insults are discussed in detail. Additionally, we review the cytokines produced by brain cells and address key issues related to the overall function of the NVU, emphasizing its critical role in maintaining brain homeostasis and responding to various physiological and pathological conditions.

# CHAPTER 4

# Response to Foreign Pathogens in the Brain

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**Abstract:** The brain's defense against foreign invaders involves a specialized immune system called the neuroimmune system. It includes microglia, astrocytes, and other immune cells. Microglia detect and respond to abnormalities, while astrocytes help maintain the blood-brain barrier and participate in immune signaling. Together, these cells protect the brain from pathogens and maintain its health. Understanding the neuroimmune system is crucial for combating brain infections and inflammatory disorders.

**Keywords:** Inflammation, Microglia activation, Phagocytosis, Reactive astrocytes.

## INTRODUCTION

In recent decades, it has become evident that monocytes, once believed to have fixed responses to foreign stimuli, play crucial and balanced roles in both protecting against and contributing to diseases and immune responses. This balance is especially critical in vital organs like the CNS, where minor changes in immune factors and microenvironments can lead to severe diseases or lasting neurological damage.

Viral encephalitis is a significant example, where monocytes recruited from the bone marrow play a key role in the disease's clinical impact by transforming into macrophages or dendritic cells in the CNS to carry out immune functions. Unlike the adaptive immune response's lymphocyte populations, which are antigen-specific, the infiltration of macrophages in viral encephalitis can lead to immunopathological damage, seizures, and even death. While it is well-known that macrophages and dendritic cells are prominent in the inflamed CNS during

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viral encephalitis, how the CNS mobilizes monocytes from the bone marrow to migrate and the specific factors driving their differentiation pathways *in vivo* are still not fully understood [25].

## CELL REACTION TO FOREIGN PATHOGENS IN THE BRAIN

When pathogens like bacteria, viruses, or fungi invade the brain, various cells within the CNS react to fight off the infection. The main cells responsible for this immune response in the brain are microglia and astrocytes [26].

Microglia, as resident immune cells in the CNS, play a crucial role as the initial defense against pathogens. Upon detecting foreign invaders, microglia become activated and move towards the infection site. Activated microglia release cytokines, chemical signals that attract other immune cells to the affected area. They also produce ROS and nitric oxide (NO) to eliminate pathogens. Furthermore, microglia engage in phagocytosis, which involves engulfing and breaking down pathogens as well as clearing cellular debris from the CNS [26].

Astrocytes, characterized by their star-shaped appearance, are essential glial cells that contribute significantly to brain homeostasis and neuronal function support. In response to infection, astrocytes undergo activation and secrete cytokines and chemokines, which attract immune cells to the infected area. Additionally, astrocytes assist in the formation and maintenance of the BBB, a protective barrier that limits the passage of pathogens and harmful substances from the bloodstream into the brain [26].

Recent research has highlighted the importance of microglia-astrocyte crosstalk in glial functions. Studies show that signals from both microglia and astrocytes play crucial roles in shaping the behaviors of these cells during CNS insults or injuries. Microglia, which constantly monitor changes in the CNS environment and maintain tissue stability, not only function as the CNS's primary immune cells but also influence the innate immune activities of astrocytes. Likewise, microglia play a role in determining the functions of reactive astrocytes, which can range from protective to harmful to neurons. On the other hand, astrocytes regulate microglial behaviors, including motility and phagocytosis, through their secreted molecules. This bidirectional communication between microglia and astrocytes is crucial for maintaining normal neuronal functions and responding to pathological conditions in the CNS.

During brain infections, additional immune cells, such as lymphocytes and monocytes, are recruited. Lymphocytes, including T cells and B cells, contribute to adaptive immunity by recognizing particular antigens on pathogens and orchestrating a focused immune reaction. Monocytes can transform into

macrophages upon brain entry, participating in phagocytosis and clearing pathogens [27].

Indeed, the immune cell response in the brain is meticulously regulated to strike a balance between eliminating pathogens and preventing excessive inflammation, which could harm nearby brain tissue. Any disruption in this immune response regulation can result in neuroinflammatory disorders like meningitis or encephalitis.

## INFLAMMATORY RESPONSE TO FOREIGN PATHOGENS IN THE BRAIN

When foreign pathogens infiltrate the brain, the immune system is activated to defend brain tissue, leading to a response called neuroinflammation. This response involves the activation of immune cells like microglia and astrocytes, which release pro-inflammatory substances such as cytokines, chemokines, and reactive oxygen species. These substances help eradicate pathogens and kickstart the immune response [28].

The inflammatory response aims to draw immune cells to the infection site, increase the permeability of the BBB, and aid in clearing pathogens. Nevertheless, prolonged or excessive neuroinflammation can harm brain tissue and contribute to various neurological disorders [28].

Inflammation within the brain can result in neuronal damage, disturbances in normal brain functioning, and the release of excitotoxic substances that can exacerbate neuronal harm. Moreover, heightened inflammation may generate toxic molecules that compromise the integrity of the BBB and elevate the likelihood of neurological disorders.

To manage neuroinflammation, the brain employs specialized mechanisms to regulate the immune response and mitigate inflammation-induced damage. Anti-inflammatory agents like interleukin-10 and transforming growth factor-beta are released to counter pro-inflammatory signals, preserving a balanced immune response.

Inflammasomes serve as internal sensors for various types of pathogens and danger signals, whether from the host or external sources. When activated, they trigger an innate immune response by releasing inflammatory cytokines such as interleukin (IL)-1β and IL-18. Additionally, they can induce pyroptosis, a form of cell death that leads to the release of further inflammatory mediators. Microglia are the primary innate immune cells in the brain responsible for inflammasome activation. However, other cell types within the CNS, including astrocytes and

CHAPTER 5

# Reaction to Abnormal Protein Precipitation in the Brain

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**Abstract:** The response to abnormal protein deposits in the brain involves a complex interaction among various components of the immune system. Initially, the immune response aims to clear the protein aggregates. However, in neurodegenerative diseases, chronic inflammation and immune dysregulation can occur, leading to additional damage.

**Keywords:** Autophagy, Inflammatory response, Microglia activation, Phagocytosis.

## PHAGOCYTIC RESPONSE TO ABNORMAL PROTEIN DEPOSITS IN THE BRAIN

Neurodegenerative disorders like AD, PD, and HD are marked by the buildup of protein aggregates such as amyloid-beta plaques, tau tangles, alpha-synuclein aggregates, and mutant huntingtin protein. Phagocytosis, the process of engulfing and clearing these abnormal protein aggregates and damaged neurons, plays a crucial role in combating these diseases. However, the persistent accumulation of these aggregates can trigger inflammation and neuronal damage, contributing to the progressive degeneration of the nervous system [30].

Phagocytic cells in the CNS, like microglia and infiltrating macrophages, play a vital role in clearing abnormal protein aggregates and cellular debris associated with neurodegenerative disorders. These cells identify and engulf these atypical structures using specialized receptors like scavenger receptors and complement receptors. Following engulfment, the phagocytes utilize lysosomal enzymes to break down the internalized material, aiding in the clearance process [30].

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**Gaiqing Wang (Ed.)**

In neurodegenerative disorders, the ability of phagocytic cells such as microglia to effectively clear protein aggregates and cellular debris can be compromised, leading to the build-up of these harmful substances and subsequent neuronal damage. Several factors contribute to this impaired phagocytic capacity, including genetic mutations, age-related decline in phagocytic activity, and sustained inflammation in the brain.

Recent research has shed light on the essential roles played by microglia, the brain's primary phagocytes, and their associated receptors in controlling the accumulation of neurotoxic substances like Aβ and myelin debris in neurodegenerative diseases. However, the specific intracellular mechanisms that regulate the neuroprotective functions of microglia are not yet fully understood [30].

Studies have indeed demonstrated that enhancing phagocytosis can be beneficial in the context of neurodegenerative disorders. Strategies aimed at modulating the activity of phagocytic cells or promoting the recruitment of new phagocytes to affected brain regions show promise in clearing protein aggregates and reducing neuronal damage.

Various approaches are being investigated to boost phagocytosis in neurodegenerative diseases. These include the development of immunomodulatory drugs that can enhance the phagocytic activity of microglia and other immune cells. Gene therapy techniques are also being explored to introduce genes that can stimulate phagocytosis or improve the function of phagocytic cells.

Furthermore, stem cell-based therapies hold the potential for replacing dysfunctional phagocytes with healthy ones or promoting the differentiation of stem cells into phagocytic cells that can effectively clear protein aggregates and cellular debris in the brain.

Overall, these strategies aim to harness the natural phagocytic capacity of immune cells in the brain to combat the accumulation of harmful substances and mitigate neuronal damage in neurodegenerative disorders.

## AUTOPHAGY IN THE BRAIN WITH ABNORMAL PROTEIN AGGREGATION

The pathological changes observed in AD include both macroscopic and microscopic alterations in the brain. Macroscopically, AD is associated with brain atrophy, particularly noticeable in the hippocampal formation, temporal lobes, and parietotemporal cortices. This atrophy is often accompanied by cortical thinning,

enlargement of the brain ventricles, and abnormalities in the white matter, all of which can be visualized using magnetic resonance imaging (MRI).

Microscopically, AD is characterized by the accumulation of Aβ proteins, leading to the formation of parenchymal senile plaques, also known as neuritic plaques. Additionally, Aβ can accumulate in the walls of cerebral capillaries and arteries, a condition referred to as cerebral amyloid angiopathy (CAA). Another hallmark of AD is the aggregation of hyperphosphorylated tau proteins, which form intracellular structures called neurofibrillary tangles (NFTs) and neuropil threads.

Interestingly, the severity of CAA, the presence of NFTs, and the extent of synapse loss are closely correlated with the degree of cognitive decline in AD patients. It is worth noting that these neuro-pathological changes are not exclusive to AD but are also observed in individuals with mild cognitive impairment (MCI), as well as in asymptomatic individuals, indicating that these changes may begin years or even decades before the onset of cognitive symptoms [31].

Neurodegenerative disorders like AD, PD, and HD have key molecular causes:

- Protein Misfolding: Certain proteins misfold and clump together in the brain, causing harm.
- Mitochondrial Problems: Issues with cell energy sources can damage neurons.
- Protein Clearance Issues: Cells struggle to remove harmful proteins, leading to buildup.
- Brain Inflammation: Persistent immune activation damages neurons over time.
- Oxidative Stress: Imbalance in cell oxidation damages brain cells.

These mechanisms drive disease progression and are targets for potential treatments.

Autophagy is vital for cells as it clears out damaged or unneeded components, ensuring cellular health. In neurodegenerative diseases, disrupted autophagy contributes significantly to disease development and progression.

Autophagy and apoptosis are fundamental processes that help maintain cellular balance. Autophagy targets damaged cellular components for recycling, while apoptosis is a programmed cell death pathway with distinct morphological features such as cell rounding, membrane blebbing, and nuclear changes. Autophagy involves the formation of double membranes and vesicles that deliver materials to lysosomes for degradation. In contrast, apoptosis is characterized by cell shrinkage, chromatin condensation, and the formation of apoptotic bodies that are cleared by nearby cells or immune cells.

CHAPTER 6

# Reaction to Abnormal Metal Accumulation in the Brain

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**Abstract:** The immune response to metal deposits in the brain can vary depending on the type of metal and specific circumstances. Generally, the immune system identifies metal deposits as antigens and triggers immune cells to eliminate them.

**Keywords:** Anti-inflammation, Neuroinflammation, Phagocytosis.

## PHAGOCYTOSIS IN THE BRAIN WITH METAL ACCUMULATION

Metal accumulation in the brain, also known as metal neurotoxicity, can result from various sources, such as environmental exposure, occupational hazards, or genetic disorders. Metals like lead, mercury, aluminum, and iron can amass in the brain, disrupting its normal functions and potentially causing neurological disorders.

When metals accumulate in the brain, microglia can become activated and engage in phagocytosis to clear the accumulated metals. This activation of microglia is part of the brain's defense mechanism to eliminate harmful substances. However, prolonged or excessive activation of microglia and phagocytosis can also contribute to neuroinflammation and the development of neurodegenerative diseases [37].

Phagocytosis in the brain during metal accumulation is indeed a complex process involving interactions among microglia, metals, and neighboring brain cells. A deep understanding of the mechanisms and outcomes of phagocytosis in the context of metal neurotoxicity is essential for devising therapeutic approaches to

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mitigate the detrimental effects of metal buildup and the resulting neurological disorders [37].

Iron is known to influence the synthesis, post-translational modification, and aggregation of α-synuclein, particularly in the context of PD. Moreover, glial cells, especially activated astrocytes and microglia, are implicated in iron accumulation in PD. The contributions of glial cells are largely mediated by the factors they release, including neurotrophic factors, pro-inflammatory factors, lactoferrin, and others yet to be fully determined. Studies have highlighted the role of microglial cells, the immune effectors in the brain, in maintaining iron homeostasis. Microglial cells accumulate iron during development and play a role in the myelination process. Notably, in neurodegenerative diseases, there is an observed increase in iron accumulation, with iron-positive microglial cells found at lesion sites. Excessive iron within activated microglia has been linked to increased release of pro-inflammatory cytokines and free radicals, contributing to neuroinflammation [38].

Activated microglia demonstrate directed migration toward areas of pathology, guided by chemo-attractant gradients that recruit additional microglia to the site of damage. Astrocytes play a crucial role in maintaining brain iron balance, situated near the blood-brain barrier to uptake iron from circulation and distribute it within the CNS. Iron entry pathways include DMT1 and the Tf-TfR mediated process. Astrocytes express the glycosylphosphatidylinositol-anchored form of caeruloplasmin, which is closely associated with ferroportin. One hypothesis suggests that astrocyte-secreted hepcidin may regulate FPN1 on brain microvascular endothelial cells (BMVECs), leading to FPN1 internalization and degradation, thus modulating iron transport across the BBB. However, it remains unclear whether astrocytes possess the capacity to accumulate and store iron [38].

## INFLAMMATORY RESPONSE TO METAL ACCUMULATION IN THE BRAIN

Indeed, studies have demonstrated that metal accumulation in the brain can induce an inflammatory response, leading to the production of pro-inflammatory cytokines and reactive oxygen species. These inflammatory molecules can activate microglia, initiating an immune response and aiding in the phagocytosis of accumulated metals.

Astrocytes express several key proteins involved in iron regulation, including Iron Regulatory Proteins (IRPs) such as IRP1 and IRP2, along with DMT1+ and Iron Regulatory Elements (IREs). Increased expression of IRP regulatory proteins can lead to an elevation in the +IRE form of DMT1, influencing iron transport.

The pro-inflammatory cytokine IL-6 is notably involved as a positive regulator of hepcidin mRNA expression, achieved through the activation of the JAK/STAT3 pathway. Previous research has proposed a mechanism where IL-6 released from activated microglia triggers astrocytes to release hepcidin. Hepcidin, in response, signals to neurons *via* the hepcidin-FPN1 axis, preventing the release of iron. This process effectively enhances iron storage within brain cells by facilitating the internalization of the ferroportin-hepcidin complex [38].

Neuroinflammation frequently co-occurs with brain iron accumulation, observed in several neurodegenerative disorders like PD, AD, HD, FRDA, and MS. Although the exact causes of this iron overload remain unclear, its presence within glial cells and neurons can intensify the inflammatory response.

One of the key pathological aspects of iron is its ability to induce oxidative stress, a significant feature in neurodegeneration. Through processes like the Fenton reaction, iron can generate highly reactive radicals, such as hydroxyl radicals, leading to damage in DNA, proteins, and lipids, ultimately resulting in cell death.

A term proposed to describe a specific type of cell death dependent on iron is ferroptosis. This process is characterized by the accumulation of lipid peroxides and has been associated with the pathophysiology of various neurodegenerative diseases [38].

Astrocytes are now recognized for their crucial role in supporting learning, memory consolidation, and protecting the CNS from metal toxicity, including copper neurotoxicity. Moreover, there is growing acknowledgment of the involvement of microglia in the pathogenesis of sporadic AD. It has been suggested that cognitive decline, which can be influenced by modifiable lifestyle factors, may be mediated through the regulation of aberrant microglia activation in aging and the subsequent reduction of neuroinflammation.

Interestingly, the hypothesis of central nervous system Cu dyshomeostasis fits well within both of these approaches. It can aid in elucidating the pathogenesis of AD and also serve as a source for identifying novel drug targets, particularly for a specific subgroup of AD patients [39]. Astrocytes play a critical role in responding to oxidative stress by producing antioxidative molecules such as metallothionein (MT)-1/2, which are rich in cysteine and possess antioxidative properties. MTs bind to metals like Zn and Cu, contributing to metal homeostasis and detoxification in the brain. They also regulate the Zn-mediated transcriptional activation of various genes. Recent studies have shown that MTs can prevent Cu-induced aggregation of α-synuclein, a protein implicated in neurodegenerative diseases like Parkinson's.

# PART 3

# RELATED OR CONCOMITANT BRAIN WASTE CLEARANCE SYSTEMS

**Abstract:** The brain possesses multiple interrelated systems for clearing waste products and maintaining its health. These systems include the neurovascular unit (NVU), glymphatic system, lymphatic system, and phagocytic system. They collaborate to efficiently remove waste products from the brain, which is crucial for its proper function and well-being. Any dysfunction or impairment in these clearance mechanisms can result in the buildup of waste materials, potentially leading to the onset or progression of neurodegenerative diseases.

**Keywords:** Clearance system, Glymphatic system, Lymphatic system, Neurovascular unit (NVU), Phagocytic system.

The accumulation of neurotoxic substances is diverse and numerous, but the CNS has various pathways for clearing waste products. These pathways, working in conjunction, remove waste substances and uphold brain health and function. Neurodegeneration is believed to stem from an imbalance between waste production and clearance, a process also implicated in aging and age-related chronic diseases. Given that the brain is the most metabolically active organ, there is a recognized imperative for developing strategies to enhance the clearance of waste products and neurotoxins from the brain.

CNS requires an efficient waste clearance mechanism, especially considering that the brain, despite representing only 2% of the body mass, accounts for about 25% of global metabolism. Waste clearance is crucial for maintaining brain homeostasis, which is essential for the proper functioning of all cerebrovascular and parenchymal brain cells. Key brain waste clearance systems include the BBB, scavenger cells and receptors, various transporters and pathways, CSF, perineural lymphatic drainage pathways, and the recently discovered meningeal lymphatic vessels. Any dysfunction in perivascular spaces (PVS) or related clearance systems can lead to the accumulation of brain waste.

The interplay among the BBB, interstitial fluid (ISF), and PVS within the brain parenchyma facilitates brain waste clearance directly into the blood and/or CSF. Additionally, the CSF and its exit routes play a crucial role in mediating waste clearance. Recent advancements have shed light on the glymphatic system and meningeal lymphatic vessels, highlighting their significance in brain waste clearance.

Although we have mainly focused on how scavenger cells and their receptors can aid in clearing hematoma following ICH, it is essential to recognize the broader context of brain waste clearance systems. Further research and exploration of these systems can pave the way for a better understanding of CNS health and potential therapeutic interventions [42 - 50]. There are, however, several related or concomitant systems involved in the process, such as the BBB, Virchow-Robin spaces (VRS), and the CSF circulation. Moreover, despite the absence of classical lymphatic vessels in the brain, a sort of lymphatic drainage has been previously subsumed on the basis of communication between CSF, ISF, brain tissue, and the cervical lymph nodes. Furthermore, there are perineural and meningeal lymphatic drainage pathways from the brain to the cervical lymph nodes. These processes play a very significant role in maintaining homeostasis and ultimately contribute to the immune surveillance of the brain. It is, however, suspected that their role might be complementary because any functional abnormality of a given system may lead to the accumulation of brain waste and, therefore, can contribute to the progress of neurodegenerative and cerebrovascular pathology.

We summarize the currently proposed brain waste clearance systems in this book (Table **5**). The relative contribution of each of these systems has been illustrated [51]. Their roles are believed to be complementary, as any dysfunction in a particular system can result in the buildup of brain waste like β-amyloid. This accumulation can potentially contribute to the advancement of neurodegenerative and cerebrovascular diseases such as AD and CAA [51].

**Table 5. (Gouveia-Freitas and Bastos-Leite 2021).**
**Currently proposed brain waste clearance systems.**

| System | Components and Clearance Location |
|---|---|
| "Paravascular pathway" and "glymphatic" system | Subarachnoid space → periarterial, periarteriolar, and pericapillary spaces → interstitial space → perivenous spaces → venous blood/cervical lymph nodes (*e.g.*, along the venous walls) |
| "Intramural periarterial drainage" (IPAD) pathway | Interstitial space → basement membranes of the capillaries in the pericapillary spaces, and basement membranes within the *tunica media* of the arterioles and arteries in the periarteriolar and periarterial spaces → cervical lymph nodes (along the arterial walls) |
| Blood-brain barrier | Direct vascular transport (*e.g.*, transport of β-amyloid *via* the LRP1) |
| Scavenger cells | Intracellular and extracellular brain waste degradation |
| Cerebrospinal fluid | Arachnoid *villi* and granulations → venous blood;<br>Blood-CSF barrier at the choroid plexuses;<br>Lymphatic pathways |
| Perineural (cranial and spinal) lymphatic drainage | Subarachnoid space → perineural space (*e.g.*, peri-olfactory lymphatic drainage → cribriform plate → nasal lymphatics) → cervical lymph nodes |
| Meningeal lymphatic vessels | Subarachnoid space → meningeal lymphatic vessels → cervical lymph nodes |

*CSF*, cerebrospinal fluid; *LRP1*, low-density cholesterol receptor-related protein-1

The accumulation of neurotoxic substances stems from various and numerous reasons, posing a significant challenge in treating neurodegenerative disorders. Aβ accumulation is theorized to occur due to an imbalance between its production and clearance, which is also a primary factor in aging and age-related chronic illnesses. Given that the brain is the most metabolically active organ, there is a critical need for pathways that efficiently clear waste proteins and neurotoxins from the brain [52].

The failure of Aβ clearance is gaining recognition as a crucial aspect in the development of AD. It is essential to comprehend the mechanisms through which Aβ is eliminated from the brain and to explore new methodologies for studying this process in well-characterized patients and healthy individuals. Given that Aβ accumulation can occur in presymptomatic individuals long before AD symptoms appear, insights into Aβ clearance can potentially lead to interventions that reduce Aβ deposits, delaying or even preventing the onset of the disease [31].

Soluble Aβ is eliminated from the brain through several clearance pathways, such as enzymatic breakdown and uptake by cells, transportation across the BBB and blood-cerebrospinal fluid barrier (BCSFB), flow within the ISF, and absorption of CSF into the circulatory and lymphatic systems [31].

In the early 2000s, studies on mice revealed that the majority (around 75%) of extracellular Aβ (e-Aβ) is cleared through the BBB, with a smaller portion (about 10%) being cleared by ISF bulk flow. However, recent two-photon imaging studies have indicated that ISF bulk flow, facilitated by astroglial aquaporin-4 (AQP4) channels and known as the glymphatic system, plays a more significant role in eAβ clearance than previously believed. Additionally, the discovery of meningeal lymphatic vessels has introduced another potential clearance pathway. While the specific contributions of each system to overall clearance remain unclear, they collaborate in expelling eAβ from the brain. Any alterations in these systems can contribute to changes in the pathophysiology and accumulation of lesions observed in AD [31].

The brain's clearance of soluble waste involves multiple overlapping systems that can be categorized based on where the waste is cleared from and where it is cleared into. Protein waste can be cleared from either the intracellular or extracellular compartment, which includes the ISF around neurons and the CSF surrounding the brain. These proteins can then be eliminated through enzymatic breakdown or cellular uptake, exported into the bloodstream or lymphatic system, or circulated back into the CSF (Table **6**). The exact roles and contributions of different clearance systems in the brain remain uncertain. While traditional understanding suggests that BBB clearance is the primary method, recent research on perivascular CSF circulation has cast doubt on this perspective. As a result, understanding the brain's clearance systems has become a crucial focus for future scientific investigations [31].

**Table 6. (Tarasoff-Conway, Carare *et al.* 2015).**
**Clearance systems in the brain.**

| **Clearance System** | **Source** | **Destination** | **Factors Affecting Clearance System** | **Clearance Pathways** |
|---|---|---|---|---|
| Blood–brain barrier clearance | ISF | Blood | Transporter expression and activity; Ligand affinity and competition; Vascular integrity | Efflux transporters and mediators; Influx transporters and mediators |
| Degradation clearance | | | | |
| Intracellular | ICS | Degradation | Enzyme expression and activity; Ligand affinity and competition; Initiation of intracellular degradation pathways | Ubiquitin–proteasome pathway;Autophagy–lysosome pathway; Endosome–lysosome pathway;Proteases |

*(Table 6) cont.....*

| | | | | |
|---|---|---|---|---|
| Extracellular | ISF | Degradation or cellular uptake | Enzyme expression and activity;Ligand affinity and competition;Activation of cellular uptake | Proteases;<br>Glial phagocytes |
| ISF bulk flow clearance | | | | |
| CSF sink | ISF | CSF sink (subarachnoid space, ventricles) | Intrinsic ISF fow rate | ISF efflux into CSF sink |
| Perivascular drainage | ISF | Periarterial space to peripheral lymph | *APOE*ε4;* Immune complex deposition; Arterial age; Arterial pulsation (hypothetical) | ISF efflux into basement membrane of capillary and arterial walls |
| Perivascular glymphatic | ISF | Perivenous space to peripheral lymph or ventricles | Molecular size; Arterial pulsation; *AQP4* expression and localization; Sleep | CSF influx into periarterial space; CSF–ISF exchange within interstitium; CSF–ISF efflux along perivenous space |
| CSF absorption clearanc | | | | |
| Circulatory | CSF | Blood | CSF production; BCSFB transporters; Arachnoid villi resistance | Arachnoid villi integrity and BCSFB efflux and influx transporters and mediators |
| Lymphatic | CSF | Peripheral lymph | Lymphatic absorption of CSF | Perivascular space<br>Perineural space |
| Meningeal lymphatic vessels | CSF | Lymph | Unknown | Subarachnoid CSF into meningeal lymphatic vessels |

Abbreviations: APOE*ε4, apolipoprotein E ε4 allele; AQP4, aquaporin-4; BCSFB, blood–CSF barrier; CSF, cerebrospinal fluid; ICS, intracellular space; ISF, interstitial fluid.

The brain's clearance system has garnered significant interest in neuroscience due to its role in removing metabolic waste and toxins. Despite the brain's high metabolic rate, it lacks a traditional lymphatic system, making the clearance system a crucial area of study since the 1960s. Recent years have seen a growing recognition of the clearance system's importance in CNS diseases. However, a comprehensive understanding and structural characterization of the entire clearance system, along with its connections, remain limited in the research literature. While some studies have focused on the glymphatic system, there is a need to explore the relationships between other brain structures and the clearance system for potential clinical interventions targeting waste accumulation in brain disorders. This article aims to address these gaps by systematically reviewing the literature on key structural components, including nerve cells and extracellular vesicles (EVs) (Fig. **13**) [52].

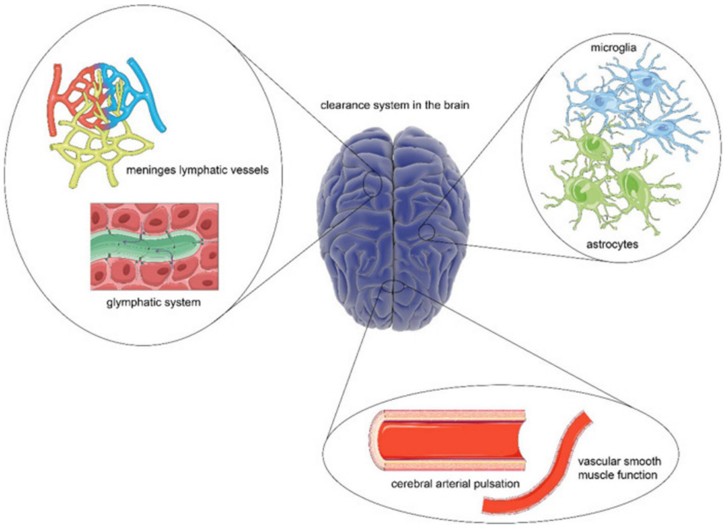


**Fig. (13).** A comprehensive overview of the clearance system in the brain.

As the scarcity of lymphoid tissue, the brain has a unique clearance system to eliminate metabolic waste. The construction of a framework based on the glymphatic system and meningeal lymphatic vessels provides a platform for cerebrospinal fluid (CSF) flow, which is essential for the exchange and transport of metabolic waste. Besides, the driving force for CSF flow is provided by cerebral arterial pulsation and smooth muscle function. While the steady states of the process are guaranteed by astrocytes and microglia.

In summary, the endogenous clearance system in the brain encompasses the transvascular clearance system across the BBB, the cerebral lymphatic drainage system, and the degradation and metabolism carried out by phagocytes. This intricate system facilitates the removal of abnormal proteins, metabolic wastes, metals, and various molecules from the brain into the blood.

The transvascular clearance system operates by transporting substances across the vascular endothelium *via* the BBB, allowing for the elimination of waste products from the brain to the bloodstream. Similarly, the cerebral lymphatic drainage system acts akin to a purification circuit, discharging cell debris and metabolic waste products into the cervical lymph nodes through the exchange of interstitial fluid and cerebrospinal fluid. The protein AQP4 plays a crucial role in facilitating these scavenging processes.

Additionally, microglia, functioning as phagocytes, are vital components of the cerebral clearance system. They serve as the brain's garbage disposal cells, aiding in the removal of cellular debris and other waste materials to uphold brain health and functionality.

# CHAPTER 7

# The Function of Neurovascular Unit in Clearance System

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**Abstract:** The neurovascular unit (NVU) encompasses multiple cellular elements such as the BBB, astrocytes, microglia, and pericytes. Together, these components form a system that aids in the removal of waste products, toxins, and surplus neurotransmitters from the brain tissue. The NVU's functions include circulatory regulation, perivascular drainage, and phagocytic degradation clearance systems. Its pivotal role lies in preserving brain homeostasis and thwarting the buildup of detrimental substances that might otherwise impede regular brain function.

**Keywords:** Astrocytic endfeet, Blood-brain barrier, Neurovascular unit, Perivascular drainage, Pericyte.

## BLOOD-BRAIN BARRIER (BBB)

BBB consists of a seamless endothelial layer encircled by astrocytic foot processes and pericytes. This complex structure collaboratively controls the entry of substances from the bloodstream into the brain. The BBB works in tandem with the glymphatic system for waste clearance and serves as a primary barrier in segregating blood cells, external pathogens, and circulatory waste materials from entering the brain, thereby upholding brain homeostasis (Fig. **14**) [52]. The BBB employs carrier-mediated transport across the epithelium to facilitate waste efflux and clear cellular components, effectively preventing the CNS from accumulating metabolites and xenobiotics. This barrier is further recognized as comprising a fundamental functional entity known as the NVU (Fig. **15**) [43]. NVU is a complex assembly comprising neurons, vascular cells like endothelial cells (EC) and pericytes, and glial cells including astrocytes and microglia.

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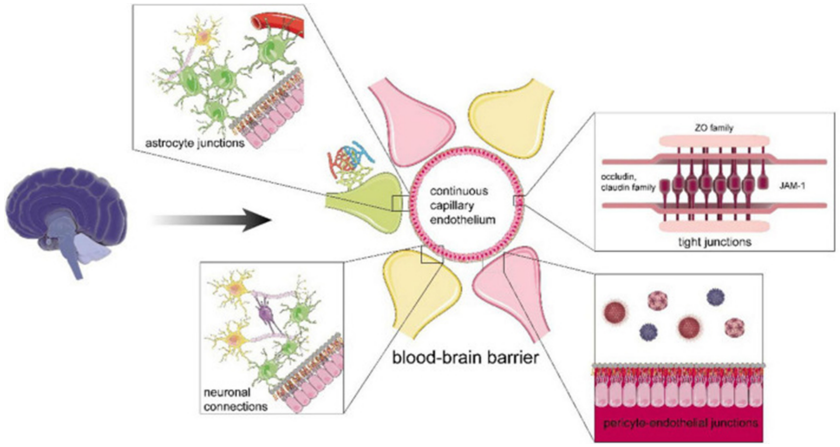


**Fig. (14).** The functional structure of the blood-brain barrier (BBB).

The BBB includes continuous capillary endothelium with tight junction (TJ), adherens junctions and gap junctions, pericyte-endothelial junctions, astrocyte junctions, neural connections, intact basement membrane, and glial membrane. The TJ is abundant in TJ proteins. For instance, claudin and scaffolding protein, such as zonula occludens family, form the backbone of TJ, occludin helps maintain the integrity and stability of TJ, and junctional adhesion molecule A mediates cell adhesion to restrict epithelial permeability. In terms of astrocytes, they can strengthen the BBB by junctions with ECs, communicate with myelinated nerve fiber and synapses, and support them. For neurons, their connections are enhanced by glial cells, such as oligodendrocytes, which can assist efficient jump transmission of bioelectrical signals and maintain the normal function of neurons.

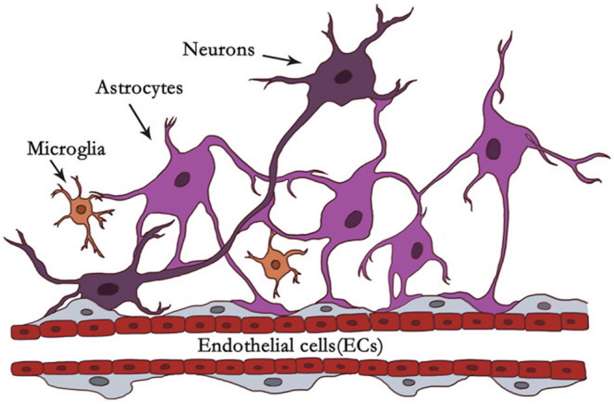


**Fig. (15).** Illustration of microglia and astrocytes in NVU.

In the context of the NVU, astrocytes are located in the center between neurons and endothelial cells (ECs). Astrocytes are closely associated with neurons and blood vessels as versatile cells. Astrocytes communicate with neuronal pre- and postsynaptic terminals to help modulate synaptic transmission. It has been reported that one astrocyte can supervise over 100,000 synapses. Astrocytes extend end-feet processes to cover the surface of cerebral blood vessels with a ratio of ~99% to modulate CBF or the BBB. Astrocytes can be organized into syncytial structures of up to 100 units by gap junctions to facilitate long-range signaling. Microglia account for about 5–15% of all cells in the human brain. Under physiological or pathological conditions, they scan their environment through scavenging functions. Microglia firstly react to brain insults like "pioneers," monitoring and transmitting "danger." Astrocytes with dominant quantity may be "reserve forces" and amplify the neuroinflammation, owing to their syncytium of the structure, and function, and strategic position to mobilize peripheral immunity.

Increasing evidence supports the pivotal role of NVU in regulating the permeability of the BBB, the functioning of the cerebrovascular network, and neurogenesis processes.

In pathological scenarios, the malfunction or breakdown of NVU constituent cells and associated structures, such as tight junctions (TJ), can lead to neurological disorders and functional deficits. The "blood-brain barrier" section will delve into the mechanisms through which these cellular components, along with their junctions, execute the barrier function of the BBB and facilitate the clearance of waste products through the elimination system [52].

The BBB and the glymphatic system share the common goal of removing waste from CSF and ISF in the brain, and they mutually promote each other's functions through cooperation. However, they differ in their specific mechanisms due to structural differences.

The BBB relies on cellular barriers and tight connections, along with selective channels for substances like proteins, to primarily execute waste clearance. In contrast, the glymphatic system separates waste from circulating CSF by promoting the convective movement of CSF between the PVS and interstitial space, achieving clearance in a different way.

When the clearance capacity of BBB transporters reaches saturation, the glymphatic system can take on a scavenging role. Additionally, the convection activity in the glymphatic system can enhance the responsiveness of AQP4 water channels and promote BBB function, particularly in astrocytes.

# CHAPTER 8

# Phagocytosis and Scavenger Receptors in the Brain

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**Abstract:** Phagocytosis is a vital process where immune cells called phagocytes engulf and digest foreign particles, pathogens, and cellular debris. This process is crucial for tissue homeostasis and infection defense. Scavenger receptors, found on immune cells like microglia, recognize and bind to diverse ligands such as pathogens, altered self-molecules, and cellular debris. They are pivotal in facilitating phagocytosis and clearing these substances from the body.

**Keywords:** CD36/CD47/CD163, Low-density lipoprotein receptor-related protein-1(LRP1), Microglia, Macrophages, Scavenger receptors.

## INTRODUCTION

We reviewed the endogenous garbage cleaning system, also known as scavenger receptors, which plays an important role in the regulation of hematoma resolution in ICH [44].

Microglia and macrophages (MMΦ) form the primary phagocytic system and serve as the frontline defense against brain injuries, including the cleanup of hematomas. Thus, the effectiveness of their phagocytic function is crucial for limiting damage caused by ICH. When lesions occur, resident microglia and peripheral macrophages are swiftly mobilized to the site, which initiates the release of mediators and recruits other immune cells. While microglia are essential for removing hematomas and clearing debris, they also contribute to ongoing inflammation.

Activated microglia and macrophages can have both neuroprotective and neurotoxic effects. On one hand, they express pro-inflammatory factors that can exacerbate neuronal damage. On the other hand, they also exhibit neuroprotective

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properties. However, attempts to treat stroke with anti-inflammatory agents have often been unsuccessful, possibly due to the broad suppression of microglia and macrophages, which compromises their normal defensive functions in the brain.

It is crucial to note that acute inflammation has protective roles, whereas chronic inflammation tends to worsen injuries. Therefore, experimental stroke therapies should shift from broadly suppressing microglia and macrophages towards a more nuanced approach that balances their protective and toxic phenotypes.

Phagocyte activation during hematoma resolution releases pro-inflammatory mediators and free radicals that can be toxic to neighboring cells, leading to secondary brain injury. However, promoting phagocytosis in a timely and efficient manner can help limit the toxic effects of persistent blood products on surrounding tissue, potentially aiding recovery after ICH. Recent studies have shown that enhancing microglia and macrophage-mediated phagocytosis accelerates hematoma clearance and improves functional outcomes after ICH.

Scavenger receptors, expressed on microglia, astrocytes, or endothelial cells, play a significant role in regulating phagocytosis in microglia and macrophages [44].

As shown in Fig. (**18**), endogenous scavenger receptors, a significant subset of innate pattern recognition receptors, primarily function in endocytosis and the recognition of exogenous invaders. They are pivotal for maintaining cerebral homeostasis and regulating phagocytosis. This section outlines the recently identified roles of scavenger receptors in clearing hematomas after intracerebral hemorrhage (ICH) [44].

Phagocytosis and scavenger receptors are crucial mechanisms within the body's clearance system and are responsible for removing foreign particles, cellular debris, and potentially harmful substances. Their roles are paramount in maintaining tissue homeostasis and ensuring proper immune function.

## CD36

CD36 is a well-known integral protein found on the cell membranes of microglia and macrophages, serving as a type II scavenger receptor. It plays a crucial role in mediating the recognition and phagocytosis of various particles and substances. When cells lack phagocytic abilities, introducing CD36 through transfection can confer phagocytic functions.

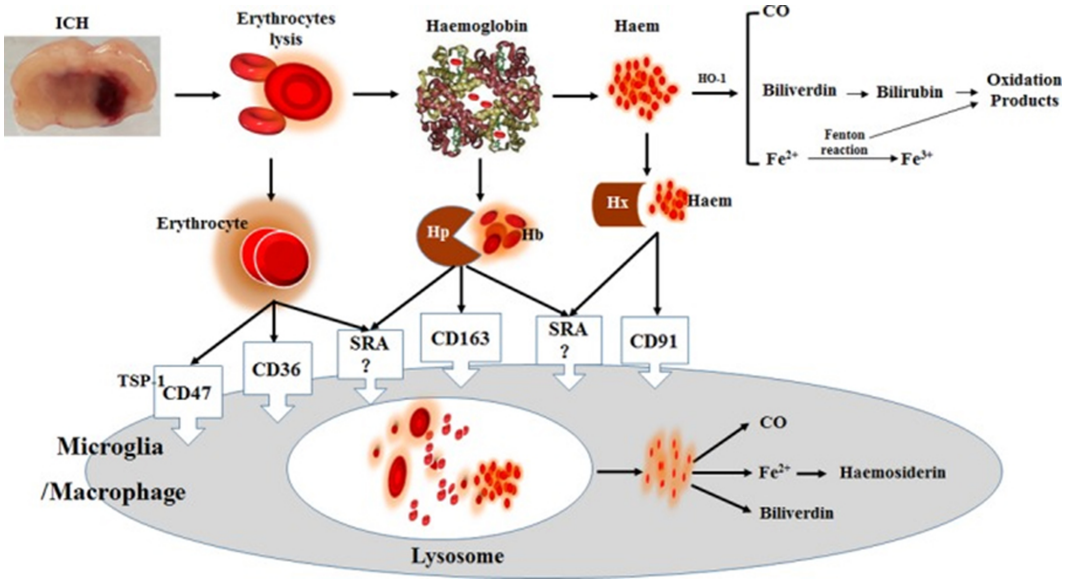


**Fig. (18).** The potential endogenous scavenger receptors (such as CD36, CD47, SRA, Hp–Hb–CD163 and Hx–haem–CD91) following ICH.

Additionally, the levels of CD36 influence erythrocyte adhesion, and it may also have a role in signal transduction in platelets and monocytes. CD36, in conjunction with thrombospondin, is involved in several cell adhesion processes, such as thrombin-induced platelet aggregation and the adhesion of platelets to monocytes [44].

In short, CD36 plays a crucial role in the brain's clearance system, promoting the removal of harmful substances and maintaining brain health. Understanding the mechanisms underlying CD36-mediated clearance may have implications for the development of therapeutic strategies for neurodegenerative diseases.

## CD47

CD47 is a transmembrane protein expressed in various cell types, including microglia/MMΦ, oligodendrocytes, and erythrocytes. Known as a "don't eat me" signal, CD47 positively regulates erythrocyte lifespan by inhibiting phagocytosis through interaction with signal regulatory protein (SIRPα) on normal/healthy erythrocytes. It plays a crucial role in the clearance of aged erythrocytes and can act as a regulator of target cell phagocytosis on other cell types.

During aging, CD47 undergoes a conformational change that leads to thrombospondin (TSP-1) binding, converting CD47 into an "eat me" signal recognized by SIRPα, thereby promoting phagocytosis. The conformation of

# CHAPTER 9

# The Role of Cerebrospinal Fluid in the Clearance System

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**Abstract:** The cerebrospinal fluid (CSF) system is vital for maintaining the brain's environment and clearing waste. The BBB-CSF barriers regulate the movement of molecules between the blood and brain fluids. CSF flow is driven by arterial pulsation and may be influenced by vascular muscle. The glymphatic system helps drain interstitial fluid, especially during sleep. Anesthesia may impair CSF circulation, affecting brain waste clearance. Meningeal lymphatic vessels also assist in CSF clearance and immune cell movement. Understanding these processes can lead to new treatments for neurological disorders.

**Keywords:** Blood-brain barrier (BBB), Cerebrospinal fluid (CSF), Glymphatic system, Neurological disorders, Sleep.

## ROLE OF CEREBROSPINAL FLUID

The brain consists of four fluid compartments: CSF, interstitial fluid, intracellular fluid, and the blood vasculature (Fig. **20**). The blood-brain barrier and blood-CSF barrier are vital for maintaining the brain's extracellular environment by regulating the composition of different fluid compartments. The blood-brain barrier is composed of endothelial cells lining blood vessels, while the blood-CSF barrier is primarily formed by choroid plexus epithelial cells. Unlike other brain capillaries, the capillaries in the choroid plexus lack tight junctions, making them more permeable to macromolecules.

The fluid compartments in the brain consist of intracellular fluid (ICF) (60-68%), interstitial fluid (ISF) (or extracellular fluid) (12-20%), blood (10%) and the cerebrospinal fluid (CSF) (10%) [5, 10]. The blood is separated from the CSF and

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interstitial fluid by the blood brain barrier (BBB) and blood-CSF barrier, respectively. Tight junctions between the blood endothelial cells constitute the BBB, restricting macromolecules to move freely from the blood to the brain parenchyma. Fluid and solutes in the perivascular space located between endothelial cells and astrocytic endfeet, expressing the water channel aquaporin-4 (AQP4) diffuses into the brain parenchyma. The blood-CSF barrier is formed by tight junctions between the choroid plexus epithelial cells. Macromolecules from the blood can move freely between the fenestrated endothelial cells to the interstitial fluid but is restricted by tight junctions in the choroid plexus epithelial cells, which therefore are believed to be the main players in determining CSF composition.

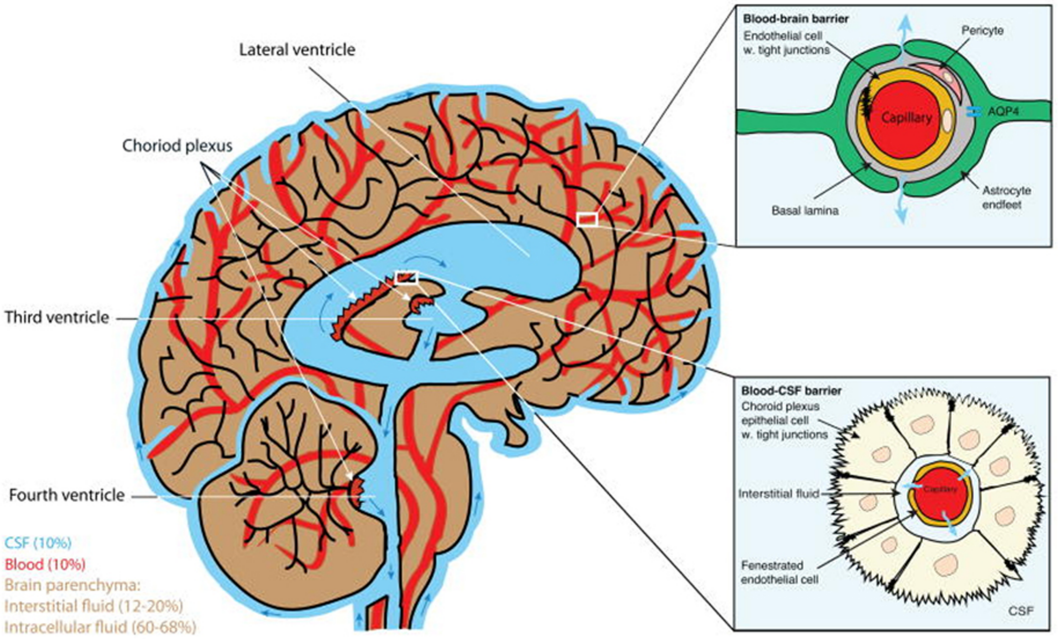


**Fig. (20).** Schematic representation of the brain's fluid compartments and barriers.

However, the choroid plexus epithelial cells are connected by tight junctions, which control the trans-epithelial transport of macromolecules. This regulation by epithelial transporters determines which macromolecules can enter the CSF from the bloodstream (Fig. **20**). CSF, produced by the choroid plexus, surrounds the brain ventricles and spinal cord, playing a crucial role in maintaining normal biological function through its clearance system. Understanding the driving forces behind CSF flow is essential for developing a comprehensive framework.

Research has confirmed that CSF flow direction follows the pulsation of cerebral arteries, particularly the leptomeningeal arteries. This highlights the significance of arterial pulsation, such as that driven by the cardiac pump, as a driving force for CSF movement. Observations on CSF flow velocity have also indicated a pulsatile flow pattern that aligns with the cerebral arterial pulse.

However, debates arise regarding whether arterial pulsation alone is sufficient to support efficient CSF flows. Some arguments suggest that vascular smooth muscle function also plays a crucial role in regulating CSF pressure and dynamics. Increased reactivity of vascular smooth muscle may influence the clearance of metabolic waste within the brain. As research in the clearance system progresses, understanding the interplay between arterial pulsation, vascular smooth muscle function, and CSF dynamics becomes increasingly important [53].

The circulation of CSF plays a crucial role in the brain's clearance system. According to the “glymphatic system” hypothesis, CSF enters the brain through periarterial spaces, moves into the interstitium *via* perivascular astrocytic AQP4, and then drives the drainage of ISF and its solutes through perivenous pathways. Previous research has indicated that this circulation of CSF in the brain is particularly active during sleep or under general anesthesia.

Recent evidence suggests that maintaining a regular sleep schedule is essential for optimal brain clearance system efficiency. As neurodegenerative diseases like AD are closely linked to the brain's clearance system, improving sleep quality may serve as a preventive measure against such diseases. However, the impact of anesthesia on CSF circulation remains a topic of debate. Notably, a study by Gakuba *et al.* utilized MRI and near-infrared fluorescence imaging in mice to investigate the effects of general anesthesia on intracranial CSF circulation.

Contrary to expectations, their findings indicated that CSF circulation was more active during wakefulness but significantly impaired under general anesthesia. This suggests that the effects of anesthesia on the brain's clearance system may vary depending on the dosage used. However, clinical studies exploring whether anesthesia can be utilized to modulate the function of the brain clearance system are still lacking [52].

The primary production site for CSF is believed to be the blood-cerebrospinal fluid barrier (BCSFB) at the choroid plexus. This barrier consists of fenestrated endothelium and choroid plexus epithelium, which are modified ependymal cells with tight junctions. The choroid plexus is located in the brain ventricles and plays a crucial role in both producing CSF and clearing solutes from the ventricular CSF.

# CHAPTER 10

# The Role of Brain Vasculature and the Perivascular Space

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**Abstract:** The brain vasculature encompasses a network of blood vessels responsible for supplying oxygen and nutrients and eliminating waste from the brain. This network includes arteries, veins, and capillaries. The perivascular space is a fluid-filled region surrounding these blood vessels within the brain, situated between the vessel walls and the brain tissue. Functioning as part of the glymphatic system, the perivascular space serves as a pathway for clearing waste products from the brain by facilitating their transport along the blood vessels and subsequent drainage out of the brain.

**Keywords:** Brain vasculature, Perivascular space, Waste clearance.

## ROLE OF BRAIN VASCULATURE AND THE PERIVASCULAR SPACE

The brain's vasculature possesses distinctive characteristics that set it apart from the vasculature in the rest of the body. Its arterial cerebral circulation comprises an anterior and posterior circulation, fed by the internal carotid arteries and vertebral arteries, respectively. The anterior circulation, which includes the middle and anterior cerebral arteries, connects with the posterior circulation (basilar artery and posterior cerebral arteries) through anterior and posterior communicating arteries at the circle of Willis. This arterial configuration allows for perfusion of different brain regions: the anterior circulation supplies the newer brain areas, like the neocortex of the cerebral hemispheres from the circle of Willis, while the posterior circulation nourishes the brainstem and cerebellum.

On the cortical surface, cerebral arteries extend into pial arteries that traverse the CSF-filled subarachnoid space and subpial space. These pial arteries dive deeper into the brain parenchyma, transitioning into penetrating arterioles (Fig. **21**), and

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perivascular space, also known as the Virchow-Robin space, is a unique feature of the CNS vasculature. It is filled with CSF and is bordered by a layer of leptomeningeal cells on both the inner wall facing the vessel and the outer wall facing the perivascular astrocytic endfeet. What sets the CNS vasculature apart is that all arterioles, capillaries, and venules within the brain parenchyma are surrounded by astrocytic vascular endfeet.

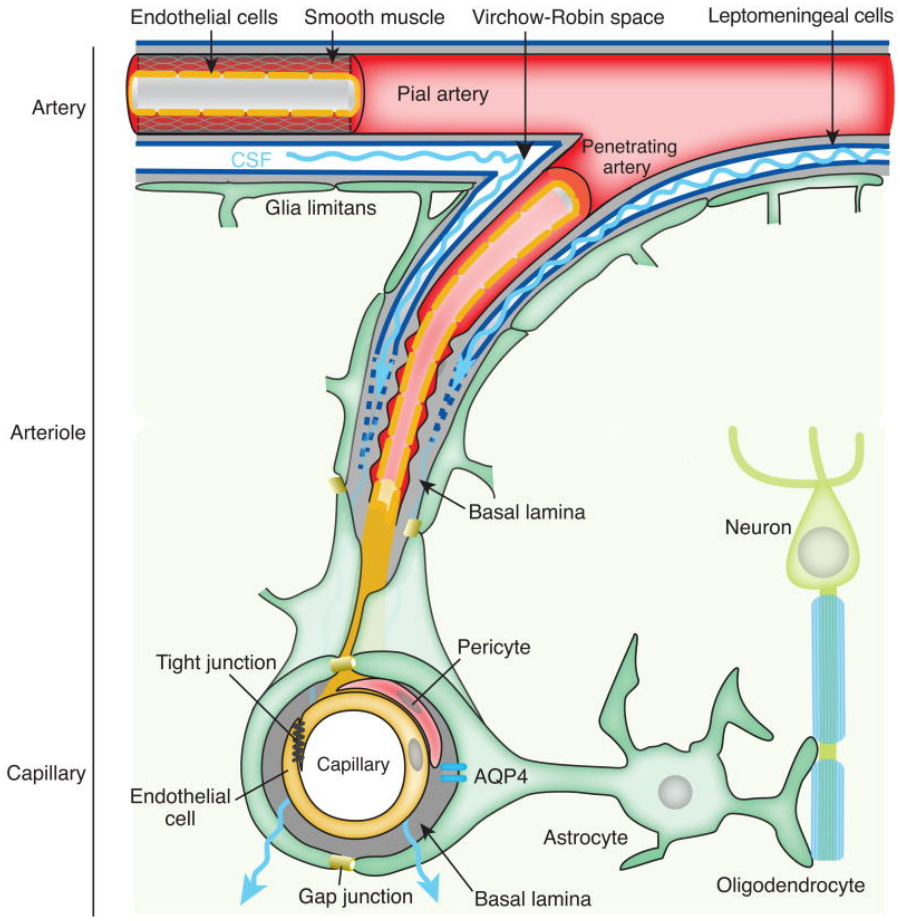


**Fig. (21).** The neurovascular unit.

The structure and function of the neurovascular unit allow bidirectional communication between the microvasculature and neurons, with astrocytes playing intermediary roles. Pial arteries in the subarachnoid space bathed in CSF become penetrating arteries upon diving into the brain parenchyma. The perivascular space around penetrating arteries is termed the Virchow-Robin space. As the penetrating arteries branch into arterioles and capillaries the CSF-

containing Virchow-Robin spaces narrow and finally disappear. However, the perivascular space extends to arterioles and capillaries to venules where it is made up by the basal lamina's extracellular matrix that provides a continuity of the fluid space between arterioles and venules. Astrocytic vascular endfeet expressing aquaporin-4 (AQP4) surround the entire vasculature and form the boundary of the perivascular spaces.

These vascular endfeet create the outer wall of the perivascular space, forming a donut-shaped tunnel around the vasculature. As penetrating arterioles narrow deeper into the brain parenchyma, the Virchow-Robin spaces merge with the basal lamina, and they disappear before reaching the capillary level. At the capillary level, the perivascular space consists solely of the basal lamina.

The basal lamina is a thin sheet of extracellular matrix primarily composed of laminin, fibronectin, and type IV collagen. It also contains heparin sulfate proteoglycan and other extracellular matrix components. The basal lamina separates endothelial cells, pericytes, and astrocytes. These cell types, along with smooth muscle cells and neurons, form the neurovascular unit and are tightly linked to the extracellular matrix of the basal lamina through adhesion molecules like integrins and dystroglycan.

The porous structure of the extracellular matrix in the basal lamina provides minimal resistance to the influx of CSF into the perivascular space. This arrangement plays a crucial role in the exchange of molecules and fluids between the blood vessels and the brain parenchyma, contributing to the maintenance of CNS homeostasis [53].

The perivascular spaces, beyond being pathways for CSF influx with low resistance, are vital for delivering energy substrates and regulating blood flow. In conditions like stroke, they also serve as sites where the innate inflammatory response and edema formation are triggered [53].

Blood from cerebral capillaries then flows into post-capillary venules, where the perivascular spaces are enlarged by the basement membranes of endothelial cells and astrocytes, facilitating CSF drainage. Unlike the distinct territories of anterior and posterior arteries in the arterial cerebral circulation, the drainage territories of central and cortical veins overlap considerably. This overlapping can extend from deep white and gray matter surrounding ventricles to the subcortical layers. The cortical veins, extending from the brain surface as pial veins, connect with the superficial cortical veins and deep veins, eventually draining into the superior sagittal sinus. From there, cerebral venous blood exits the brain *via* a confluence of sinuses that drain into the sigmoid sinuses and jugular veins [53].

# CHAPTER 11

# The Role of the Glymphatic System

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**Abstract:** The glymphatic system, a recently identified waste clearance pathway in the brain, is crucial for removing waste products and maintaining brain homeostasis. Similar to the lymphatic system in the body, the glymphatic system operates uniquely within the brain. Its main role is to clear various waste products, such as metabolites, proteins, and toxins, from the brain. AQP4 plays a pivotal role in facilitating the function of this system.

**Keywords:** AQP4, CSF, Glymphatic system, ISF, Waste clearance.

## ROLE OF THE GLYMPHATIC SYSTEM

Until 2012, it was widely believed that the brain, unlike other organs, was capable of recycling all its protein waste internally. Only a limited number of proteins were known to cross the blood-brain barrier, which did not include many major proteins produced or shed by brain cells. With no lymphatic vessels or apparent pathways for fluid export, it remained a mystery as to how protein waste could exit the mature brain parenchyma. The prevailing assumption was that the traditional cellular protein degradation pathways, such as autophagy and ubiquitination, were solely responsible for recycling proteins within the CNS [54].

The notion that the brain exclusively recycles its own waste was challenged by the revelation of the glymphatic system. This system is a well-organized transport network within the cerebrospinal fluid, bearing similarities to the lymphatic vessels found in peripheral tissues. One of its primary functions is to facilitate the removal of excess interstitial fluid and proteins from the brain (Fig. **22**) [54]. Indeed, the brain's cerebrospinal fluid (CSF) and peripheral lymph are jointly drained into the venous system, leading to the removal and recycling of protein

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waste by the liver. However, unlike peripheral tissues, the brain lacks distinct lymphatic vessels. Instead, fluid clearance from the brain occurs through the glymphatic system, a specialized network of fluid transport facilitated by the perivascular spaces formed by the vascular endfeet of astrocytes.

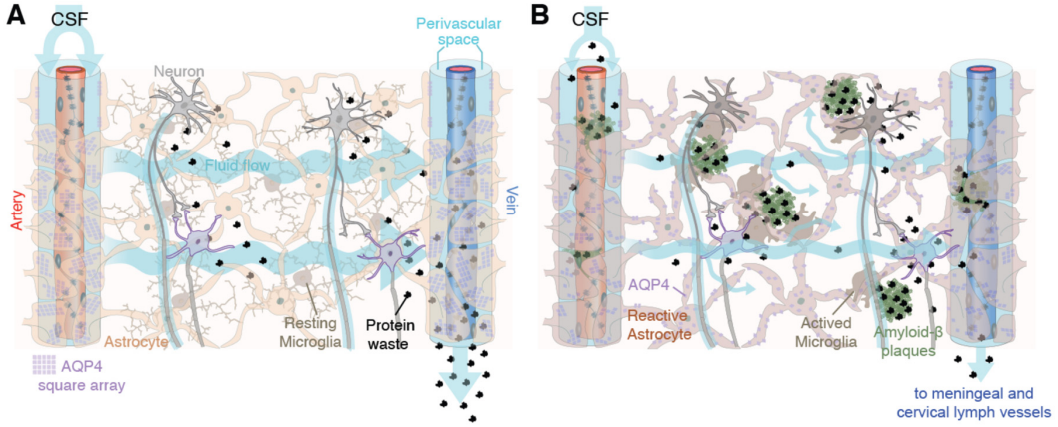


**Fig. (22).** The brain glymphatic system is a highly organized fluid transport system.

(A) The vascular endfeet of astrocytes create the perivascular spaces through which CSF enters the brain and pervades its interstitium. CSF enters these perivascular spaces from the subarachnoid space, and is propelled by arterial pulsatility deep into the brain, from where CSF enters the neuropil, facilitated by the dense astrocytic expression of the water channel AQP4, which is arrayed in nanoclusters within the endfeet. CSF mixes with fluid in the extracellular space and leaves the brain *via* the perivenous spaces, as well as along cranial and spinal nerves. Interstitial solutes, including protein waste, are then carried through the glymphatic system and exported from the central nervous system *via* meningeal and cervical lymphatic vessels. (B) Amyloid-ß plaque formation is associated with an inflammatory response, including reactive micro- and astrogliosis with dispersal of AQP4 nanoclusters. An age-related decline in CSF production, the decrease in perivascular AQP4 polarization, gliosis and plaque formation all impede directional glymphatic flow, and thereby impair waste clearance. Of note, vascular amyloidosis might be initiated by several mechanisms. Amyloid-ß might be taken up from the CSF by vascular smooth muscle cells expressing the low-density lipoprotein receptor-related protein 1 (LRP1) [111]. Alternatively, amyloid deposition might be initiated by the backflow of extracellular fluid containing amyloid-ß into the periarterial space from the neuropil – rather than proceeding on to the perivenous spaces - due to an increase in hydrostatic pressure on the venous side, or because of an inflammation-associated loss of AQP4 localization to astrocytic endfeet.

These endfeet envelop arteries, capillaries, and veins, acting as a secondary barrier throughout the cerebral vascular bed. The perivascular spaces are open tunnels filled with fluid, providing minimal resistance to flow. This contrasts with the densely packed architecture of adult brain tissue, known as the neuropil, where fluid movement is slow and restricted. The glymphatic system's perivascular tunnels are directly linked to the subarachnoid spaces surrounding the brain, allowing rapid CSF movement into deep brain regions driven by arterial wall pulsations.

Astrocytic endfeet, a major type of glial cells, encircle the perivascular spaces and serve as gateways for fluid influx into the neuropil. These endfeet are interconnected by gap junctions, and a significant portion of their membrane contains AQP4 water channels. Deleting AQP4 channels reduces the influx of CSF tracers and the efflux of solutes from the neuropil. Because of its functional resemblance to the peripheral lymphatic system, we termed this astrocyte-mediated brain fluid transport mechanism the glymphatic (glial-lymphatic) system [54].

Fluid transport through the glymphatic system exhibits directional polarization, with fluid influx along penetrating arteries facilitated by AQP4 and efflux along the peri-venous spaces and cranial/spinal nerves. Additionally, glymphatic clearance is temporally regulated, with fluid transport occurring primarily during non-rapid eye movement (NREM) sleep. This sleep-dependent regulation is evident in the correlation between CSF tracer influx and EEG slow wave activity, which is prominent during NREM sleep.

The glymphatic system's activity is closely tied to the sleep-wake cycle, as fluid flow diminishes significantly during wakefulness. EEG slow wave activity, a marker of sleep pressure that increases with sleep deprivation, indicates that waste removal is most efficient during early sleep stages and recovery sleep following extended wakefulness.

The wakeful state poses challenges for active parenchymal fluid flow due to its reliance on precise synaptic transmission. Active flow could lead to glutamate spillover during synaptic activity, potentially affecting the fidelity of synaptic transmission. Recent studies also reveal a circadian regulation of glymphatic flow, peaking during the sleep phase and decreasing during wakefulness, independent of light cycles. This rhythmicity is supported by the dynamic localization of AQP4 *via* the dystrophin-associated complex, linking glymphatic function to the molecular circadian clock [54].

# CHAPTER 12

# The Role of Meningeal Lymphatic Systems

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**Abstract:** The meningeal lymphatic system is a newly identified network of lymphatic vessels located within the meninges, the protective membranes enveloping the brain and spinal cord. This system is essential for clearing waste products, supporting immune responses, and ensuring the balance of brain functions.

**Keywords:** Deep cervical lymph nodes (dCLNs), CSF, ISF, Meningeal lymphatic vessels (mLVs), Waste clearance.

## ROLE OF MENINGEAL LYMPHATIC SYSTEMS

The meningeal lymphatic vessels (mLVs) have recently emerged as a critical component in the intricate circulation and exchange of soluble substances between the CSF and the ISF in the brain (Fig. **25**). In aging mammals, dysfunctional meningeal lymphatic vessels can accelerate the buildup of toxic amyloid beta protein in the brain, worsening AD pathology. The connection between meningeal lymphatic vessels and the movement of CSF and ISF suggests that changes in their activity could affect the accessibility of CSF-borne immune neuromodulators to the brain parenchyma. This alteration may influence the impact of these molecules on brain function [55].

The initial serious discussions and analyses of MLVs date back to the 1800s, pioneered by Paolo Mascagni. Early evidence of MLVs was obtained through autopsies, revealing lymphatic vessels within the human dura mater near the superior sagittal sinus. In 2015, groundbreaking studies by Aspelund *et al.* and Louveau *et al.* identified characteristic protein markers of lymphatic endothelial cells expressed in meningeal vessels. These studies provided strong evidence for

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the existence of the meningeal lymphatic system in the CNS of mice (Fig. **26**) [56]. In 2017, Absinta *et al.* achieved a significant milestone by visualizing lymphatic vessels in the dura mater of human beings using brain MRI. This innovative approach provided concrete evidence and demonstrated the existence of MLVs in humans, further solidifying our understanding of this crucial component of the CNS [52].

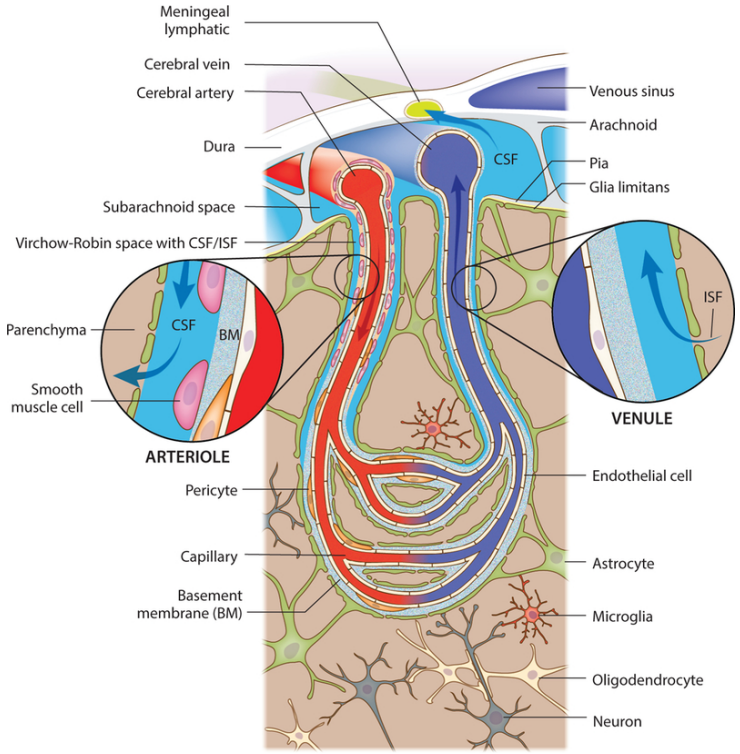


**Fig. (25).** Cytoarchitecture of the meninges, brain vasculature and pathways of paravascular recirculation.

A schematic representation of the brain meninges constituted by dura, arachnoid and pia layers. Lymphatic vessels that are present in the meningeal dura drain components of the cerebrospinal fluid (CSF) that fills the subarachnoid space. Arising from the brain surface, cerebral arteries extend into pial and then subpial arteries. Higher caliber pial arteries extend into smaller caliber arterioles (both wrapped by smooth muscle cells) that dive into the brain parenchyma. Clearly defined paravascular spaces of about 50–100 nm, the Virchow-Robin spaces, are filled with CSF that flows into deeper brain regions, along the arterioles and capillaries, and diffuses through the glia limitans into the parenchyma. Efflux of interstitial fluid (ISF) happens through paravenous spaces back into the subarachnoid CSF.

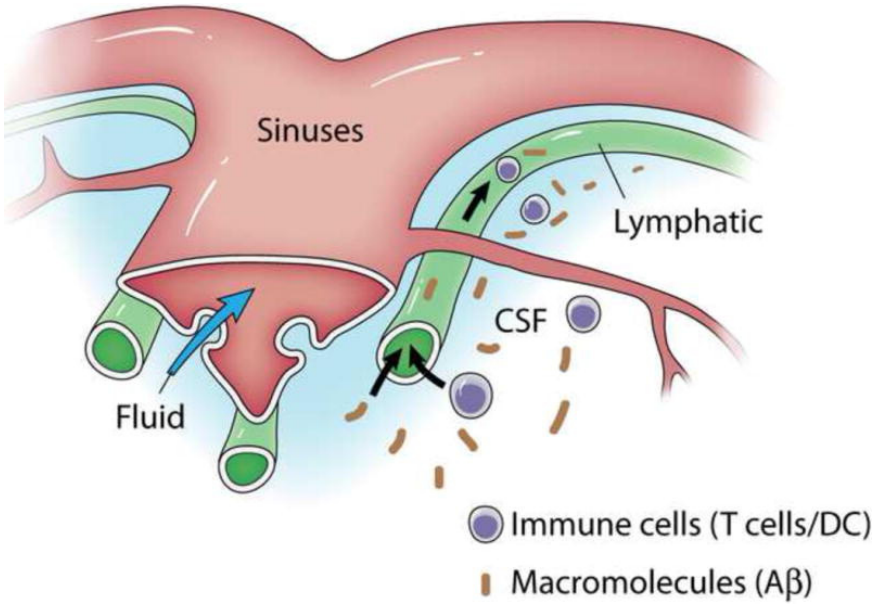


**Fig. (26).** The meningeal lymphatics enable the drainage of macromolecules and immune cells.

While the CSF fluid drains back to the bloodstream, the macromolecules and immune cells localized within the CSF drain primarily through the meningeal lymphatic vessels to reach the deep cervical lymph nodes.

The initial perception of MLVs was that they primarily resided at the base of the skull. However, the advent of MRI technology has allowed for a more detailed and precise characterization of MLVs. By employing 3D-rendering techniques on subtraction MRI images, researchers have been able to identify dural lymphatics that run parallel to the dural venous sinuses and alongside branches of the middle meningeal artery.

Moreover, MRI imaging has revealed the presence of arteries, veins, and cranial nerves that contribute to the drainage of various contents into the deep cervical lymph nodes (dCLNs). These contents include immune cells, CSF, and ISF from the subarachnoid space. This underscores the role of MLVs as the downstream pathway for draining soluble and cellular components present in the CSF.

In T2-fluid attenuated inversion recovery (FLAIR) MRI scans, the outflow of CSF through lymphatics can be observed, particularly flowing through the jugular foramen into the cisterna magna. Interestingly, most of this CSF flows towards the basal MLVs rather than the dorsal MLVs, eventually reaching the dCLNs from the basal MLVs [52].

# PART 4

# FACTORS CONTRIBUTING TO WASTE CLEARANCE IN THE BRAIN

Age-related vascular dysfunction is often linked to a decline in clearance and barrier functions, which can result in neuroinflammation. Proteins responsible for waste clearance in both the brain and peripheral circulation could serve as potential biomarkers and targets for drugs during the early stages of neurodegeneration. Investigating the regulatory mechanisms behind deficiencies in the clearance system may aid in pinpointing precise therapeutic targets for neurological disorders.

It is worth noting that advancements in multi-omics technologies have significantly contributed to research on neurodegenerative diseases. Enhancing waste clearance mechanisms holds promise as a therapeutic approach in the near future, offering a window of opportunity for effective treatment. This sectiondiscusses the synthesisof all existing regulators on glymphatic clearance, including sleep, aging, pulsation, the polarization of scavenger cells and AQP4, lifestyle choices such as sleep position, alcohol intake, exercise, omega-3 consumption, intermittent fasting, and chronic stress, in order to harness the power of this mass transport system, promote healthy brain aging, and possibly prevent neurodegenerative processes.

A comparative analysis of gene expression patterns in both physiological and pathological states of the clearance system can provide initial evidence of a connection between them (Table **8**) [52]. Applying systematic multiomics approaches to precision medicine and systems biology holds significant promise for enhancing patient care in cases of clearance system dysfunction. Additionally, target genes identified through multiomics studies can potentially be repurposed for drug development in ICH. This approach is approved to be cost-effective, faster, and more efficient than traditional drug development methods.

**Table 8. (Liu, Guo *et al.*, 2022).**
**Biomarkers related to the clearance system in the brain.**

| Biomarker | Organism | Phenotype (case and control) | Sample Size (case, control) | Cell or Tissue Type | Gender | Ethnicity | Age [Yrs, Mean (range) ±S.D.] | Outcome | PMID |
|---|---|---|---|---|---|---|---|---|---|
| Amyloid-β (Aβ) | Homo sapiens | People with disrupted Aβ aggregation | 24 | CSF | N.A. | N.A. | N.A. | 75% of substitutions in 798 possible single amino acid related to brain | 200139122 |
| p-tau | Homo sapiens | Chronic traumatic encephalopathy (CTE) | 6 | CSF | N.A. | N.A. | N.A. | p-tau is associated with the neurological disease that can develop independent of head trauma | 30506062 |
| p-tau | Homo sapiens | Temporal lobe epilepsy (TLE) | 19 | CSF | N.A. | N.A. | N.A. | p-tau is associated with the neurological disease that can develop independent of head trauma | 30506062 |
| NFT | Homo sapiens | Alzheimer's disease | 31 | CSF | N.A. | American | 75-101 | NTF is straightly related to AD | 14769913 |
| Integrin | Homo sapiens | Alzheimer's disease | 31 | CSF | N.A. | American | 75-101 | Integrin is regulated upto exceed extracellular matrix/cell adhesion/motility processes | 14769913 |
| tenascin | Homo sapiens | Alzheimer's disease | 31 | CSF | N.A. | American | 75-101 | Tenascin is regulated upto exceed extracellular matrix/cell adhesion/motility processes | 14769913 |

***(Table 8) cont.....***

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Collagen | Homo sapiens | Alzheimer's disease | 31 | CSF | N.A. | American | 75-101 | Collagen is regulated upto exceed extracellular matrix/cell adhesion/motility processes | 14769913 |
| Cadherin | Homo sapiens | Alzheimer's disease | 31 | CSF | N.A. | American | 75-101 | Cadherin is regulated upto exceed extracellular matrix/cell adhesion/motility processes | 14769913 |
| Proteoglycan | Homo sapiens | Alzheimer's disease | 31 | CSF | N.A. | American | 75-101 | Proteoglycan is regulated upto exceed extracellular matrix/cell adhesion/motility processes | 14769913 |
| Amyloid precursor protein | Homo sapiens | Alzheimer's disease | 31 | CSF | N.A. | American | 75-101 | Amyloid precursor protein is regulated upto exceed extracellular matrix/cell adhesion/motility processes | 14769913 |
| IADGs | Homo sapiens | Alzheimer's disease | 31 | CSF | N.A. | American | 75-101 | Histogenesis, apoptosis, phosphorylation, and lipid metabolism including prostaglandin synthesis, were overrepresented by up-regulated IADGs | 147699 |
| VEGF-C | Mus musculus | Prolonged meningeal lymphatic dysfunction | 10 | CSF | Male | C57BL/6J | 2 months | VEGF-C can enhance meningeal lymphatic drainage of CSF macromolecules, improving brain perfusion and learning and memory performancee | 200104182 |
| AQP-4 | Mus musculus | Moderate to severe traumatic brain injury (TBI) | 51 | CSF, ISF | Male | C57BL/6J | 8-12 months | Paravascular CSF-ISF exchange and interstitial solute clearance is dependent upon water transport *via* astroglial aquaporin-4 (AQP4) water channels | 25471560 |

***(Table 8) cont.....***

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Secretory carrier membrane protein 5 | Rat | Over-expression of SCAMP5 | 6 | Exoso-me | N.A. | N.A. | N.A. | Secretory carrier membrane protein 5 is an autophagy inhibitor that promotes the secretion of a-synuclein *via* exosome | 28700687 |
| Gfap (Glial fibrillary acidic protein) | Mus musculus | CB-NP exposed and control | 5 | Plasma | Female | N.A. | N.A. | Astrocyte activation and astrogliosis, brain tissues protein β-sheet content increase and the α-helix content decrease around blood vessels | 28700687 |
| Aquaporin-4 | Mus musculus | CB-NP exposed and control | 6 | Plasma | Female | N.A. | N.A. | Astrocyte activation and astrogliosis, brain tissues protein β-sheet content increase and the α-helix content decrease around blood vessels | 28700687 |
| SP-G | Homo sapiens | Central nervous system infections, brain hemorrhage | 121 | Cerebr-ospinal fluid | N.A. | N.A. | N.A. | Low in control subjects and patients suffering from aqueductal stenosis and highest in infections of the central nervous system and brain hemorrhage | 30032421 |
| Endothelial specific nitric oxide synthase (eNOS) | Mus musculus | Tumor-bearing mice and control | 9 | Plasma | Male | N.A. | N.A. | Genetic or pharmacological inhibition significantly suppressed meningeal lymphangiogenesis and dCLN Metastasis | 32094452 |
| VEGF-C | Mus musculus | Tumor-bearing mice and control | 9 | Plasma | Male | N.A. | N.A. | Promoting dCLN Metastasis and correlating meningeal lymphangiogenesis | 32094452 |
| CCL21 | Mus musculus | Tumor-bearing mice and control | 9 | Plasma | Male | N.A. | N.A. | Mediating the facilitation of antitumor immunotherapeutic effects induced by VEGF-C overexpression with CCR7 | 32094452 |

*(Table 8) cont.....*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| CCR7 | Mus musculus | Tumor-bearing mice and control | 9 | Plasma | Male | N.A. | N.A. | Mediating the facilitation of antitumor immunotherapeutic effects induced by VEGF-C overexpression with CCR7 | 32094452 |

The glymphatic transport of CSF relies on several mechanisms, including CSF movement along periarterial spaces, convective flow through brain tissue, and exit of ISF through perivenous spaces to the cervical lymphatic system. This process requires energy and is influenced by various factors.

The production of CSF by the choroid plexus creates pressure that guides the flow of fluid through the ventricular system to the subarachnoid space. Respiratory activity also plays a role in CSF movement, particularly through the aqueduct. Entry of CSF along the perivascular space of penetrating leptomeningeal arteries is crucial for glymphatic exchange and clearance functions.

Studies using reporter mice have shown that CSF tracers follow arteries along the pial surface and descend along penetrating arteries that dive perpendicularly into the brain to reach capillary beds. The entry of CSF along these penetrating arteries is driven by arterial pulsatility, specifically the pulse waves generated by smooth muscle cells along the length of the arteries.

Experiments with dobutamine, an adrenergic agonist, have demonstrated that increasing arterial pulsatility enhances CSF penetration into brain tissue. Conversely, dampening arterial pulsatility through carotid artery ligation reduces CSF-ISF exchange. This indicates that glymphatic activity is partly driven by arterial pulsatility, explaining why perivascular influx occurs predominantly around pulsating arteries rather than cerebral veins [53].

Overall, adopting a healthy lifestyle that includes adequate sleep, regular exercise, a balanced diet, and minimizing exposure to environmental toxins can promote brain waste clearance and lower the risk of neurodegenerative diseases.

# CHAPTER 13

# The Target Therapeutic Approach for the BBB

**Gaiqing Wang[1], Haiyun Chen[1], Juan Yang[1,*], Jing Wang[1], Bo Yan[1]** and **Minglei Chen[1]**

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**Abstract:** The blood-brain barrier (BBB) is crucial for maintaining the microenvironment needed for proper neuronal function. BBB breakdown can lead to immune cell infiltration and uncontrolled movement of molecules and ions, contributing to neurodegenerative diseases. Repairing the BBB is a key therapeutic strategy for treating neurological disorders, utilizing methods such as glucocorticosteroids (GCs) and mesenchymal stromal cells (MSCs). GCs can restore BBB integrity by regulating tight junction proteins (TJs), while MSCs show potential in promoting angiogenesis and BBB repair. Although these strategies are promising, further research is needed to determine their safety and effectiveness for clinical use.

**Keywords:** Blood-brain barrier (BBB), Glucocorticosteroids (GCs), Mesenchymal stromal cells (MSCs), Neurodegenerative diseases, Tight junction proteins (TJs).

## THERAPEUTIC APPROACH FOR THE BBB

An intact BBB is crucial for creating and preserving a microenvironment that enables the proper functioning of neuronal circuits. Its essential properties include regulating the passage of leukocytes across the BBB, which is necessary for immune surveillance and responding to brain infections, as well as clearing debris by macrophages following brain tissue damage. However, BBB breakdown can result in heightened infiltration of immune cells and uncontrolled movement of molecules and ions across the BBB, especially when tight junctions (TJs) are disrupted or transport processes are impaired. The mechanisms underlying BBB breakdown and the outcomes of a compromised barrier are diverse and multifaceted (Fig. **27**) [57].

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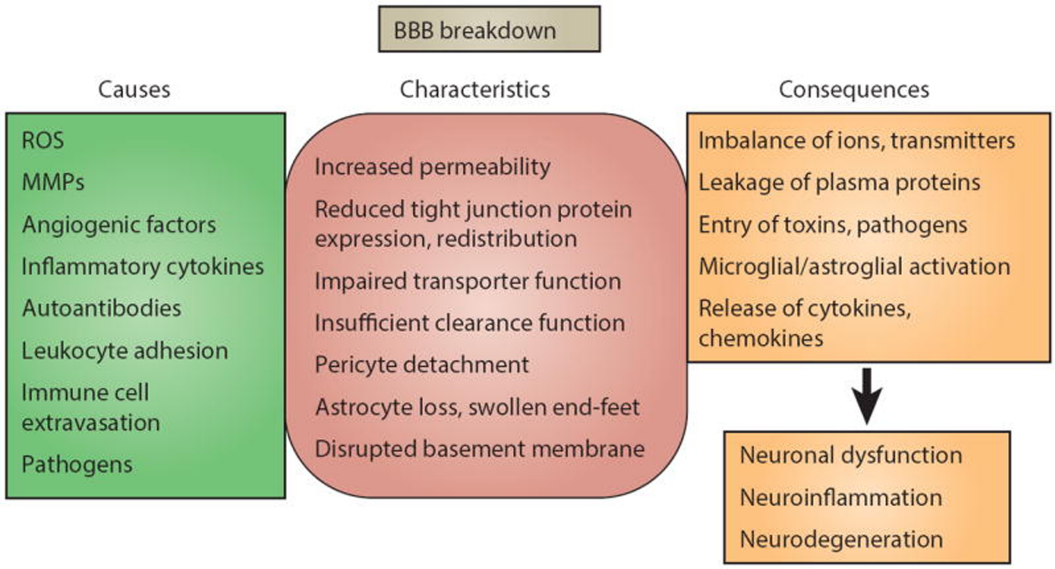


**Fig. (27).** Causes, characteristics and consequences of BBB breakdown.

Factors that can disrupt the BBB are varied, ranging from secreted elements to immune cells and pathogens. Compromised BBB integrity manifests mainly as increased barrier permeability. In addition to direct effects on endothelial cells, other members of the neurovascular unit can be affected, that is pericytes, astrocytes and basement membrane, which in turn aggravate impairment of BBB functions. Consequences vary from dysregulated molecular and ionic flux across the damaged BBB to the initiation of a central inflammatory response. Despite manifold causes, characteristics and consequences, BBB breakdown generally culminates in neuronal dysfunction, neuroinflammation, and neurodegeneration. Downstream pathological outcomes and potential for recovery are diverse.

ROS, reactive oxygen species; MMPs, matrix metalloproteinases.

The blood-brain barrier plays a crucial role in protecting neurons from systemic factors and maintaining a regulated internal environment necessary for proper synaptic and neuronal function. When the blood-brain barrier breaks down, it allows neurotoxic products, cells, and pathogens from the blood to enter the brain. This breakdown is associated with inflammatory and immune responses that can trigger various pathways leading to neurodegeneration.

Blood-brain barrier dysfunction in neurodegenerative disorders manifests as increased permeability, microbleeds, impaired glucose transport, dysfunctional P-glycoprotein activity, deposits of blood-derived products around blood vessels, cellular infiltration, and degeneration of pericytes and endothelial cells [58].

The successful delivery of therapeutic agents across the BBB depends on several factors. These include having functionally and structurally healthy blood vessels, normal vascularization, adequate blood flow, and the recruitment of transport systems such as solute carrier-mediated transport (CMT) or receptor-mediated transcytosis (RMT) to facilitate drug delivery to the CNS (Fig. **28**). Researchers have explored strategies that utilize existing solute CMT and RMT systems at the BBB to enhance the brain penetration and efficacy of neurotherapeutic agents (Fig. **29**). For instance, the large neutral amino acid transporter, a solute CMT system, facilitates the delivery of L-3,4-dihydroxyphenylalanine (L-DOPA) to the brain in PD. Additionally, the transferrin RMT system can transport therapeutic antibodies to the brain in various neurological conditions.

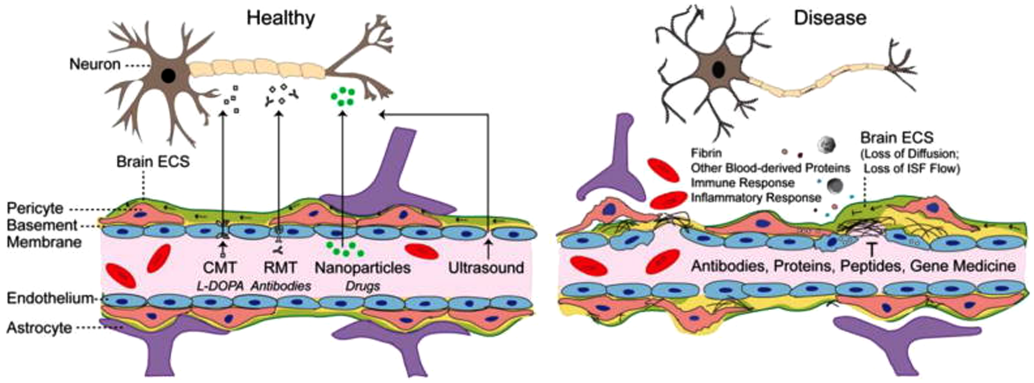


**Fig. (28).** Blood-brain barrier (BBB) dysfunction – implications for drug delivery.

In a healthy BBB (left), strategies to breach the barrier and deliver neuropharmaceuticals to brain rely on carrier-mediated transporters (CMT), receptor-mediated transporters (RMT), nanoparticles, and/or transient opening of BBB as for example by focused ultrasound. Under pathological conditions (right), the disrupted BBB leads to accumulation of blood-derived debris and cells into enlarged perivascular spaces. This blocks normal distribution of molecules throughout the CNS by concentration gradient-driven diffusion across brain extracellular spaces (ECS) and interrupts regionally formation of interstitial fluid (ISF) and ISF flow preventing the therapeutic antibodies, proteins, peptides, gene medicine and other drugs to efficiently reach their neuronal targets. See main text for details.

CHAPTER 14

# The Regulatory Mechanism Targeting Microglia

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**Abstract:** Microglia are vital immune cells in the central nervous system (CNS) responsible for maintaining brain balance and responding to injuries or infections. They are regulated by a variety of mechanisms involving interactions with different signaling molecules and cell types within the CNS. Ensuring proper regulation of microglial activity is essential for preserving brain health and preventing the onset of neuroinflammatory and neurodegenerative disorders.

**Keywords:** Anti-inflammation, Microglia activation, Microglial phenotype, Nuclear factor erythroid 2 (NF-E2)-related factor 2 (Nrf2), Pro-inflammation, Phagocytosis, Toll-like receptors (TLRs).

## INTRODUCTION

We have summarized the function and regulation of microglia following ICH. Microglia become activated in response to various pathological events or disruptions in brain homeostasis, with the nature of activation being highly diverse and context-dependent based on the specific stressor or pathology involved. The complex functional roles of microglia highlight the existence of distinct pro-inflammatory and anti-inflammatory functional states following ICH. The importance of defining microglial subtypes lies in identifying new functional states of microglia, understanding the impact of molecules on different microglial types, and discovering ways to modulate their functions in both healthy and diseased states.

Throughout the progression of ICH, many researchers continue to use the expression of M1/M2 markers and microglial polarization as indicators, although this may not provide a comprehensive mechanistic understanding of how microglial function evolves. Therefore, it would be intriguing to uncover the

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regulators and factors influencing the polarization of microglia towards either a neuroprotective or neurodestructive phenotype. This exploration can provide fresh insights into the pathogenic role of microglia following ICH [42].

Indeed, the regulatory mechanisms that target microglia are intricate and involve a combination of intrinsic and extrinsic factors. Ensuring proper regulation of microglial function is crucial for maintaining CNS homeostasis and preventing neuroinflammation and neurological diseases.

## ENDOGENOUS REGULATION MECHANISMS OF MICROGLIA IN ICH

Much of the literature has focused on the autoregulation of microglia during ICH progression. For instance, studies by Wu *et al.* revealed that soluble epoxide hydrolase expression is heightened in microglia after ICH, leading to neuroinflammatory responses by degrading anti-inflammatory epoxyeicosatrienoic acid. Other research has shown that microglial recruitment is linked to TWIK-related K+ channel 1 (TREK-1), which triggers the release of pro-inflammatory factors like IL-1β and TNF-α, along with cell adhesion molecules, post-ICH. Additionally, LRP1 in the neurovascular unit interacts with Mac-1 expressed by microglia to facilitate tissue plasminogen activator (tPA)-mediated activation of platelet-derived growth factor-cc (PDGF-cc). The activation of PDGF-cc and PDGF receptor-α signals can heighten blood-brain barrier permeability, contributing to deterioration after ICH [42].

Microglia have been observed to play a role in exerting anti-inflammatory and phagocytic effects on the hematoma, which contributes to neurological recovery following ICH. The relationship between regulatory T lymphocytes (Tregs) and the neuroinflammatory response after ICH has been elucidated. *In vitro* experiments have shown that Tregs influence microglial polarization towards an anti-inflammatory phenotype through the IL-10/GSK3β/PTEN axis as part of this regulatory process [42].

Gene expression is intricately regulated by genetic and epigenetic networks, and there is growing evidence highlighting the crucial roles of microRNAs (miRNAs) in microglial effects following ICH. For instance, miRNA-7 (miR-7) has been found to inhibit the expression of Toll-like receptor 4 (TLR4), triggering a secondary inflammatory response mediated by microglia after ICH. Further research has confirmed that compounds targeting TLR4 and miR-7, such as ligustilide (LIG) and senkyunolide H (SH), can provide neuroprotective effects against ICH. These compounds achieve this by inhibiting Prx1/TLR4/NF-κB signaling, primarily through the activation of microglia and astrocytes [42].

Recent studies have shown that inhibiting miRNA-222 can reduce microglia-mediated inflammatory responses and improve neurological function in preclinical mouse models of ICH. These studies identified integrin subunit β8 (ITGB8) as a direct target negatively regulated by miR-222 in microglial cells. This regulation leads to a decrease in inflammation and apoptosis. Additionally, miR-132 enhances the cholinergic blockade of the inflammatory response by targeting acetylcholinesterase (AChE). This action inhibits the activation of pro-inflammatory microglia and provides protection against neuronal death caused by ischemia [42].

Moreover, miRNAs play a crucial role in regulating the gene expression and autophagic activity of microglia, which are critical factors in autophagy. For instance, miRNA-144 targets mTOR by directly interacting with 3′ untranslated regions (UTRs), influencing hemoglobin-mediated activation of microglial autophagy and inflammatory responses. The role of autophagy in ICH has been challenging to determine definitively, as its function can be dualistic.

While many studies have suggested that autophagy can enhance protection against endoplasmic reticulum stress and reduce oxidative damage after ICH by clearing cellular waste and oxidative stress products, recent findings indicate that autophagy may also positively regulate inflammation following ICH [42].

The anti-inflammatory functions of microglia are achieved through the integration of various signaling pathways, forming a complex network involved in multiple biological processes. Investigating this network of biological signaling pathways and understanding its molecular basis can lead to the development of novel interventions targeting these pathways to halt the pathological progression of ICH (Fig. **31**) [42].

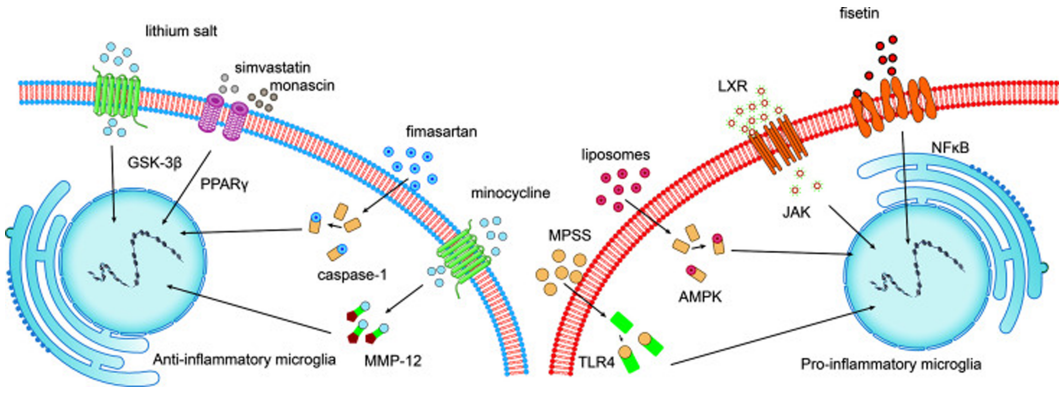


**Fig. (31).** Potential intervention strategies targeting the microglial phenotypic shift following ICH.

# CHAPTER 15

# The Regulatory Mechanism for Astrocyte Polarization

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**Abstract:** Astrocyte polarization is a multifaceted process governed by various mechanisms such as developmental and environmental cues, transcription factors, signaling pathways, epigenetic modifications, and interactions with other cells. Gaining insights into these regulatory mechanisms is vital for unraveling the significance of astrocytes in brain development and overall brain function.

**Keywords:** Astrocyte polarization, Circadian, Interactions, Regulation, Signaling pathway.

## INTRODUCTION

In recent times, there has been a surge of interest in astrocytes due to their crucial roles in maintaining homeostasis, regulating metabolism and clearance processes, and modulating synaptic plasticity and transmission. Recent findings have highlighted a direct connection between reactive astrocyte function induced by immune cells and subsequent neuronal and oligodendroglial cell death.

## EFFECT OF AD THERAPEUTICS ON ASTROCYTE FUNCTION

Astrocytes play a crucial role in modulating neuroinflammation within the CNS, releasing pro-inflammatory or anti-inflammatory cytokines based on the specific stimulus or injury type. Additionally, under normal conditions, astrocytes provide the structural foundation for the glymphatic system (Fig. **33**) [61]. The glymphatic system is a brain-wide fluid transport system responsible for clearing proteinaceous waste products like excess Aβ, tau, and α-synuclein, as well as metabolic byproducts. When activated by IL-1β, cultured fetal human astrocytes

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increase the production of several pro-inflammatory cytokines. Treating aged APPswe/PS1 mice with galantamine for 2 months reduces astrocyte production of pro-inflammatory cytokines TNF-α and IL-6. Neurotoxic astrocyte reactivity depends on microglia activation; thus, reducing microglia-related inflammation would also decrease astrocyte reactivity. Acetylcholine (ACh) has been shown to attenuate microglial cytokine production, promoting anti-inflammatory and neuroprotective pathways. In this context, the cholinergic system can be considered a modulator of astrocyte-associated neuroinflammation, both directly and indirectly. Astrocytes play a crucial role in supporting neurons by secreting neurotrophic factors, and stimulating choline receptors with AChEIs or ACh agonists may further promote the secretion of these factors [62].

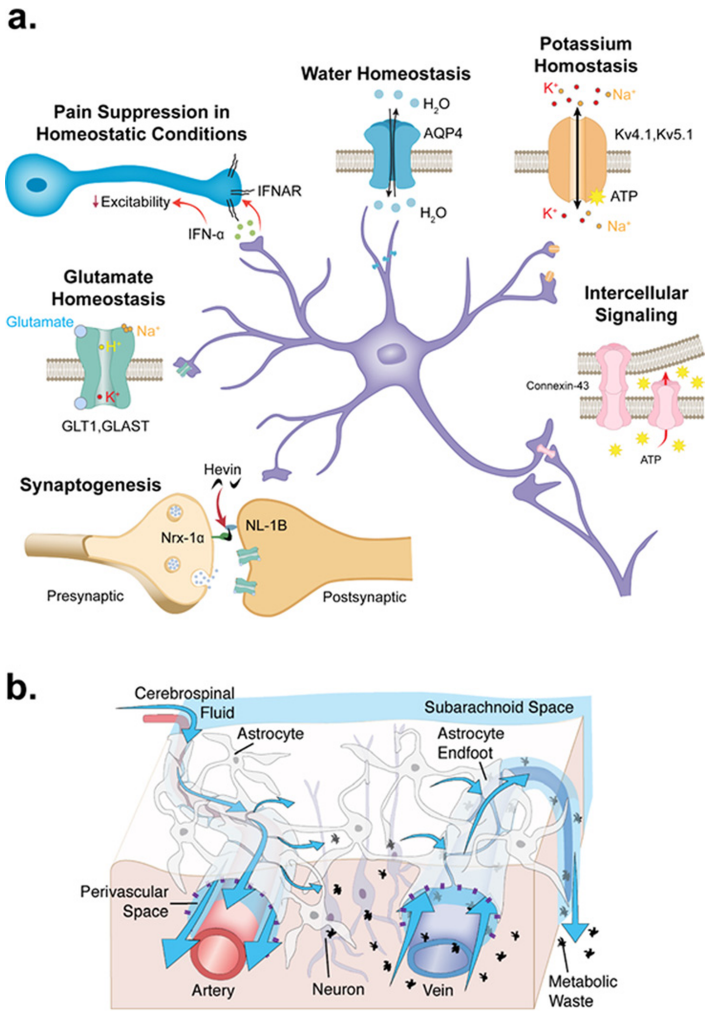


**Fig. (33).** Homeostatic functions of astrocytes.

Summary of the various supportive and neuroprotective roles of astrocytes under physiological conditions. Astrocytes wrap around synapses to provide structural support and insulation for synapses and further regulate synaptogenesis *via* the expression of adhesion molecules such as hevin. Astrocytes express glutamate transporters, potassium channels and water channels, allowing them to contribute to the maintenance of glutamate homeostasis, potassium homeostasis and water homeostasis, respectively. Astrocytes mediate long-range (intercellular) signaling *via* connexin-43-mediated gap-junction communication. Astrocytes in the spinal cord dorsal horn, the brain stem trigeminal nucleus and the sensory cortex (as well as in other regions) may also suppress physiological pain through the release of inhibitory molecules such as type-I interferons (IFN-I), which acts *via* its neuronal receptor, the interferon α/β receptor (IFNAR). The glymphatic system utilizes the perivascular spaces formed by the vascular endfeet of astrocytes, which plaster around the vasculature. Cerebrospinal fluid (CSF) is driven through the periarterial space by arterial pulsations. The vascular endfeet of astrocytes are particularly enriched with AQP4 channels, allowing water flow which facilitates dispersal of cerebrospinal fluid (CSF) into the brain tissue alongside interstitial fluid (ISF). The mixture of CSF and ISF flows through the extracellular space (blue arrows indicate fluid flow) to perivenous spaces and cranial nerve tracts, permitting fluid and wastes to collect in lymphatic and cervical lymphatic vessels.

NMDA receptor antagonists have a limited impact on modulating reactive astrocytes. Studies show that treatment with memantine for 10 days in 10-month-old APPswe mice does not reduce the intensity of GFAP+ immunoreactivity, which is a general measure of astrocyte reactivity associated with Aβ plaques. The influence of drugs targeting Aβ (such as enhancing clearance or inhibiting key enzymes) on astrocyte function needs careful evaluation. Additionally, tau pathology may influence glial cells (known as “astrogliotauopathy”), while glial cells may, in turn, affect tau pathology. However, the effects of treatments targeting tau (such as inhibiting tau aggregation or clearing phosphorylated tau) on glial function are still being investigated.

Another class of drugs that could potentially modulate inflammation in AD and have intriguing effects on astrocyte function are non-steroidal anti-inflammatory drugs (NSAIDs). Chronic treatment (9 months) with CHF5074, a novel γ-secretase modulator, in Tg2576 mice led to an increased presence of reactive astrocytes around Aβ plaques. These changes might indicate cytoskeletal reorganization, promoting migration to injury sites and potentially enhancing astrocyte-mediated neuroprotection (like phagocytosis of Aβ plaques). However, there is limited and substantiated information available regarding the impact of these drugs on astrocyte function (Fig. **34**) [62].

# CHAPTER 16

# The Regulatory Mechanism for Scavenging Pathways

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**Abstract:** Scavenging pathways are essential for cells to recycle and reuse cellular components. These pathways are regulated by a complex network of mechanisms that ensure efficient scavenging and recycling of cellular materials. The regulatory mechanisms involved are intricate and interconnected, employing multiple levels of control to facilitate the proper recycling and reuse of cellular components.

In our previous discussions, we outlined the upstream regulatory mechanisms and intervention strategies for scavenger receptors following ICH [44].

**Keywords:** Nuclear factor erythroid 2-related factor 2 (Nrf2), Peroxisome proliferator-activated receptor-γ (PPAR-γ), Regulatory mechanism, Scavenging pathways.

## NUCLEAR FACTOR ERYTHROID 2-RELATED FACTOR 2 (Nrf2)

Nrf2 is a critical regulator that helps cells deal with oxidative stress and inflammation. Under normal conditions, it is kept inactive in the cytoplasm by Keap1. When cells face stress, Nrf2 activates antioxidant genes, protecting against damage. It also plays a role in clearing harmful substances like phosphorylated tau.

Nrf2's activity is crucial for macrophage function, aiding in debris and tau clearance. It also regulates CD36, which is important for brain recovery after conditions like stroke or bleeding in the brain.

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Nrf2's activation reduces oxidative stress by helping remove red blood cells and increasing protective proteins like Hp and CD163. In experiments, Nrf2 is vital for clearing blood clots in the brain, highlighting its potential in post-bleeding recovery, as shown in Fig. (**39**) [44].

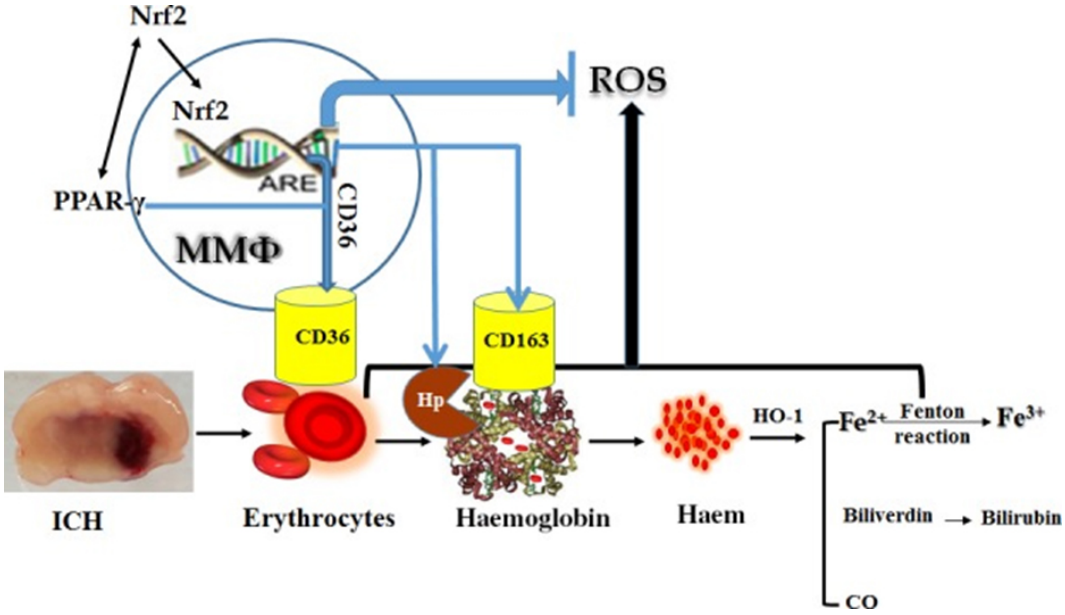


**Fig. (39).** The potential role of Nrf2 and the interaction of PPAR-γ with Nrf2 following ICH.

Overall, the activation of Nrf2 has been shown to enhance brain clearance mechanisms and promote brain health by increasing the expression of detoxifying enzymes, transport proteins, and amyloid-beta clearance enzymes, as well as reducing inflammation and oxidative stress. Therefore, Nrf2 activation is an area of interest for the development of therapeutic strategies to improve brain clearance and potentially treat neurodegenerative diseases such as AD.

## PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-Γ (PPAR-Γ)

PPAR-γ is a vital transcription factor in the nuclear hormone receptor superfamily, overseeing functions like reproduction, metabolism, development, and immune responses. It is found not only in fat cells but also in blood vessel tissues like vascular smooth muscle cells (VSMCs), endothelial cells, and macrophages. PPAR-γ and its activators play a protective role in various neurological disorders by reducing inflammation, oxidative damage, and cell death.

PPAR-γ does not just protect neurons, astrocytes, oligodendrocytes, and endothelial cells but also microglia and macrophages, both in laboratory studies and living organisms. PPAR-γ activators help macrophages by decreasing their inflammatory response while boosting their ability to engulf particles, both opsonized and unopsonized, *via* different receptors like Fc-γ and CD36.

These activators also increase the expression of CD36, aiding microglia and macrophages in clearing harmful cellular debris. They enhance the phagocytosis of red blood cells by microglia and reduce the production of harmful hydrogen peroxide during this process. However, it is worth noting that excessive activation of phagocytosis by PPAR-γ activators can lead to an inflammatory response and neurotoxicity, which is dose-dependent [44].

Several studies have demonstrated an interaction between PPAR-γ and Nrf2. Endogenous ligands for PPAR-γ can activate the expression of Nrf2, while Nrf2 also regulates the expression of PPAR-γ. Notably, Nrf2 controls the expression of CD36 independently of PPAR-γ. Knockdown of PPAR-γ leads to reduced Nrf2 expression and *vice versa*, indicating a mutual positive interaction between the two. PPAR-γ agonists can increase Nrf2 expression, and reducing PPAR-γ leads to decreased Nrf2 mRNA levels. This tight and positive two-way transcriptional interaction between PPAR-γ and Nrf2 may contribute to enhancing endothelial function.

The interaction between PPAR-γ and Nrf2 is relevant following ICH, which is illustrated in Fig. (**39**). Potential exogenous pharmacological/molecular manipulations direct at hematoma resolution after ICH (as illustrated in Fig. (**40**) [44].

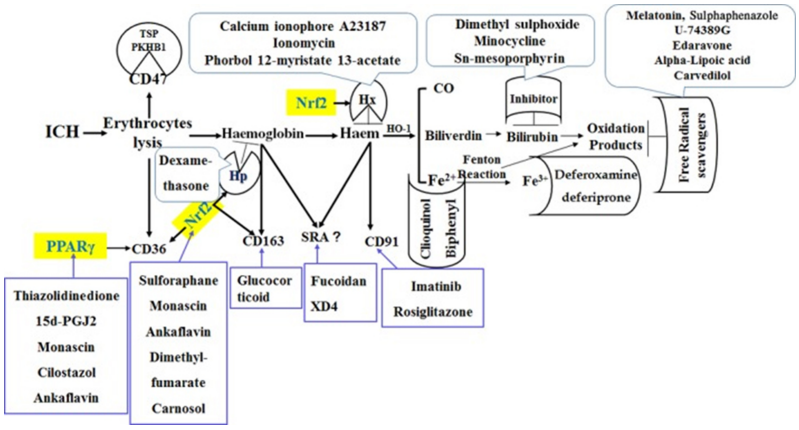


**Fig. (40).** Summary of current potential exogenous pharmacological/molecular manipulations direct at haematoma resolution after ICH.

CHAPTER 17

# The Regulatory Mechanism for Glymphatic System

**Gaiqing Wang[1,*], Haiyun Chen[1], Juan Yang[1], Jing Wang[1], Bo Yan[1]** and **Minglei Chen[1]**

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**Abstract:** The regulatory mechanism of the glymphatic system is a complex interplay involving multiple factors and processes. These include CSF production, arterial pulsations, astrocytic water channels (AQP4), the sleep-wake cycle, AQP4 polarization, and BBB permeability. Together, these factors ensure the efficient clearance of waste products from the brain, contributing to overall brain health.

**Keywords:** AQP4, CSF-ISF, Glymphatic system, Regulatory mechanism, Waste clearance pathway.

## INTRODUCTION

The glymphatic system functions as a glial-dependent pathway for waste clearance in the brain, substituting for the absence of lymphatic vessels. It is responsible for removing soluble waste proteins and metabolic byproducts from the brain. This system is like a "pseudo-lymphatic" network that spreads throughout the brain, playing a role in both replenishing and cleansing brain tissue.

Glymphatic clearance involves convective fluid transport on a larger scale, helping remove harmful metabolic waste products from the brain's interior. It acts as the brain's primary waste drainage pathway, with a perivascular network facilitating CSF transport. This network connects to a genuine lymphatic system associated with the meninges, cranial nerves, and large vessels exiting the skull. However, the details of how these systems anatomically and functionally connect are still not fully understood [67]. Regulating glymphatic clearance can potentially enhance the removal of aggregated proteins in diseases linked to protein deposition, thereby potentially slowing down or even reversing neurodegenerative processes.

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The glymphatic pathway is a well-structured fluid transport system. Initially, CSF from the subarachnoid space enters the brain through the perivascular spaces surrounding large leptomeningeal arteries. As the vascular tree branches out, CSF is then pushed into the brain tissue through the perivascular spaces surrounding penetrating arteries, which are also referred to as Virchow-Robins spaces (Fig. **21**). After entering the perivascular space, CSF moves across the glial basement membrane and the astrocytic endfeet that border the brain parenchyma. These astrocytic endfeet are rich in AQP4 water channels, which aid in the flow of CSF into the brain parenchyma, where it mixes with the ISF. Within the interstitial space, the fluid disperses in a directional manner, flowing towards the venous perivascular spaces and perineuronal spaces.

Ultimately, CSF exits through various routes, including along the perineural sheaths of cranial and spinal nerves, meningeal lymphatic vessels, and arachnoid granulations [68].

The glymphatic system and waste clearance process in the rodent brain was originally described as a 3-step serial process as follows [67]:

- CSF is constantly transported from the basal cisterns to cover the cerebral hemispheres in the subarachnoid space. From there, it enters the peri-arterial spaces driven by bulk flow.
- Within the peri-arterial spaces, CSF moves into the ISF space with the help of AQP4 water channels located on the astroglial endfeet. This process allows for the mixing of CSF with ISF and facilitates the removal of waste solutes.
- The CSF-ISF fluid mixture, along with interstitial waste solutes, is then transported towards the peri-venous compartment near the larger central veins. Eventually, it exits into lymphatic vessels and enters the systemic circulation (Fig. **41**) [67].

The latest insight regarding the glymphatic system revolves around the crucial function of AQP4 water channels in facilitating swift and substantial CSF movement from the perivascular region into the ISF space, aiding in waste drainage. This dependency on AQP4 water channels for CSF transport within brain parenchyma was highlighted through studies quantifying CSF solute transport and clearance abilities in mice lacking AQP4 water channels (AQP4−/−), compared to control subjects [67].

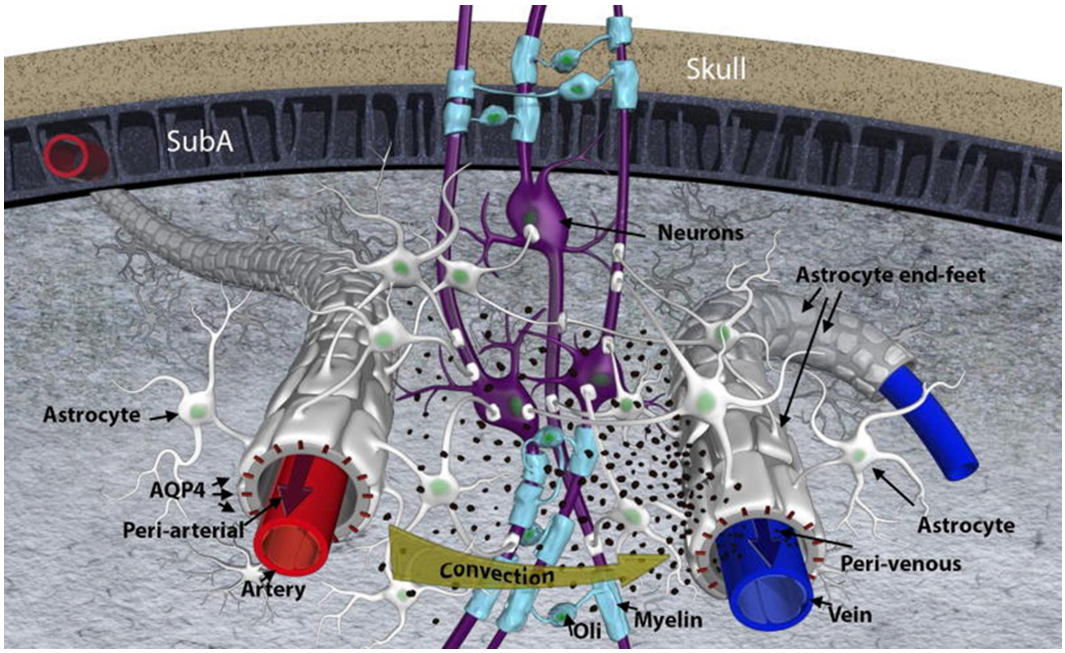


**Fig. (41).** Glymphatic transport and waste drainage concept.

Original concept of the glymphatic transport [1], highlighting the periarterial and the perivenous space, and the astrocytic endfeet with aquaporin 4 (AQP4) water channels and forming a sheath around the blood vessels. Cerebrospinal fluid is driven by convection through the periarterial space and is propelled across the astroglia end-feet to mix with interstitial fluid and waste products. From there the waste and excess fluids are driven towards the peri-venous space, to ultimately be directed towards the lymphatic vessels and general circulation for breakdown and clearance. The black particles represent 'waste' particles in the interstitial fluid (*e.g.*, amyloid-beta). SubA = subarachnoid space; Oli = oligodendrocyte; AQP4 = aquaporin 4 water channels.

Understanding the regulatory mechanisms of the glymphatic system is vital for devising therapeutic approaches aimed at enhancing waste clearance and potentially preventing or treating neurodegenerative diseases. Current research is primarily focused on identifying drugs or interventions capable of improving glymphatic function and overall brain health.

## CIRCADIAN RHYTHMS AND GLYMPHATIC SYSTEM

The glymphatic system becomes active during sleep, making sleep a crucial factor in glymphatic clearance. Recent evidence suggests that sleep deprivation (SD) and

# CHAPTER 18

# Effect Factors of Meningeal Lymphatics (MLVs)

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**Abstract:** The regulatory mechanism for meningeal lymphatics is not fully understood yet, but research is ongoing to uncover its functions and regulation. Several factors play a role in regulating these vessels, including:

Circadian Rhythm: There is evidence suggesting that MLVs exhibit circadian rhythms in their function. For example, studies have shown variations in the clearance of waste molecules from the brain *via* MLVs based on circadian rhythms.

CSF Flow: The flow of CSF is intricately connected with MLVs' function. Changes in CSF dynamics, such as pressure or flow rate, can influence the activity and permeability of MLVs.

Astrocytes: Astrocytes, a type of glial cell in the brain, have been implicated in the regulation of MLVs. They are known to play roles in maintaining brain homeostasis and responding to changes in the brain microenvironment, which can affect MLV function.

Signaling Molecules: Various signaling molecules, including cytokines, growth factors, and chemokines, are involved in the regulation of MLVs. These molecules can modulate the permeability, inflammation, and immune responses associated with MLVs.

Immune Cells: Immune cells, such as macrophages and lymphocytes, interact with MLVs and contribute to their regulation. These cells can influence the inflammatory status and immune surveillance within the meninges, affecting MLV function.

Understanding the complex interplay among these factors is crucial for unraveling the regulatory mechanisms of MLVs and their significance in brain health and disease. Ongoing research aims to elucidate these mechanisms further and explore potential therapeutic targets related to MLVs in conditions like neuroinflammation, neurodegenerative diseases, and brain injuries.

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**Keywords:** Circadian rhythm, CSF, Meningeal lymphatics vessels (MLVs), Regulation, Signaling molecules.

## INTRODUCTION

Until recently, it was unclear if the meningeal lymphatic system and glymphatic system collaborated to maintain CSF/ISF balance. Recent research has shown that the effectiveness of the glymphatic system, which involves the movement of CSF into the brain and the removal of waste molecules, is influenced by meningeal lymphatic function. This indicates a direct connection between these systems through brain fluids, even in the absence of an obvious anatomical link (Fig. **50**). Scientists have demonstrated through pharmacological, surgical, and genetic models that reduced drainage by meningeal lymphatic vessels leads to a decline in the entry of CSF molecules into the brain. Remarkably, in young adult mice, one month after the pharmacological removal of brain meningeal lymphatics, learning and memory impairments were observed without any noticeable effects on blood vessels [55].

Understanding the factors that influence meningeal lymphatics is crucial for developing therapies that target the lymphatic system. These therapies have the potential to treat or even prevent various neurological conditions.

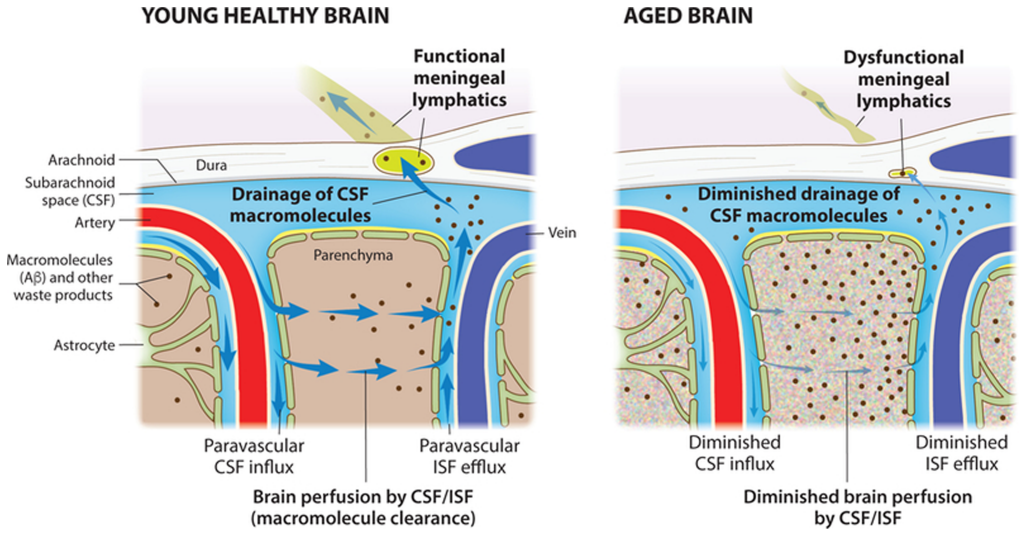


**Fig. (50).** Aging diminishes meningeal lymphatic drainage and paravascular recirculation of CSF.

Macromolecules Functional meningeal lymphatic vessels drain macromolecules (such as Aβ) from the CSF of the young and healthy brain. Influx of CSF through

the paravascular (glymphatic) route leads to brain perfusion by CSF/ISF and paravascular efflux of macromolecules from the parenchymal ISF back into the CSF. Dysfunction of meningeal lymphatic vessels with aging contributes to impairment of influx/efflux mechanisms and to poor recirculation of CSF content.

## AGING AND MLVs

Aging has been observed to have a detrimental impact on the function of lymphatic vessels in peripheral organs. Similarly, research has indicated that aging is linked to impaired functioning of meningeal lymphatic vessels in the CNS (Fig. **50**). Sequencing of meningeal lymphatic endothelial cells (LECs) from young adult and aged mice has unveiled significant differences in gene expression, indicating impaired immune-related function, cytoarchitecture, morphology, extracellular matrix, and response to growth factors in older mice. These changes were further supported by alterations in lymphatic vessel morphology, reduced capacity for CSF solute drainage into dCLNs, and decreased CSF solute outflow into supraclavicular lymph nodes (sCLNs) in old mice.

Using strategies to deliver VEGF-C, a growth factor affecting lymphatics, into the meningeal milieu of old mice enhanced lymphatic drainage into dCLNs and brain CSF influx. Furthermore, old mice treated with VEGF-C exhibited improved performance in learning and memory tests. This discovery of a meningeal lymphatic-glymphatic connection with implications for brain function in aging raises questions about the sequence of events leading to lymphatic and glymphatic dysfunction with age, their connections with CSF/ISF composition changes, blood-brain barrier integrity, and neural cell function, particularly microglia. Future studies should explore these connections in detail, including longitudinal characterization of lymphatic and glymphatic changes with aging (Fig. **51**) [55].

Age-related changes in meningeal lymphatics play a role in the buildup of toxic substances and inflammation in the brain, which are major factors in the development of neurodegenerative diseases. By understanding these changes and working on strategies to enhance meningeal lymphatic function, there is potential for therapeutic interventions that can help prevent and treat age-related cognitive decline and neurodegenerative conditions.

## CYTOKINE AND MLVS

Cytokines, which are produced by meningeal immune cells, can be released into the CSF. From there, they may diffuse into the brain *via* the glymphatic pathway, interacting with neurons and glial cells that possess cytokine receptors. This interaction can influence various cellular processes and contribute to the regulation of brain function and immune responses (Fig. **52**). Investigating

# PART 5

# CONCLUSION

The book provides an in-depth examination of the brain's waste clearance mechanisms, highlighting the crucial role these systems play in maintaining brain homeostasis and preventing neurological disorders. The authors delve into the complexities of the brain's waste management, exploring both well-known and newly discovered pathways and systems, such as the glymphatic and meningeal lymphatic systems.

**Key Points**

**IMPORTANCE OF WASTE CLEARANCE**

The accumulation of neurotoxic substances in the brain is a hallmark of various neurological disorders. Efficient waste clearance is vital for brain health and function.

**MECHANISMS OF CLEARANCE**

**Phagocytosis**

Microglia and astroglia play pivotal roles in scavenging and degrading waste.

**Blood-Brain Barrier (BBB)**

It acts as a selective filter to transport waste out of the brain.

**Glymphatic System**

It facilitates the clearance of waste through CSF and ISF dynamics.

**Meningeal Lymphatic System**

Recently discovered, this system aids in draining waste from the brain.

**PATHOLOGICAL IMPLICATIONS**

Dysfunctional clearance systems can lead to the accumulation of waste, contributing to neurodegenerative diseases, infections, and other neurological conditions.

## FUTURE DIRECTIONS

The book emphasizes the need for further research to fully understand these mechanisms and develop strategies to enhance waste clearance, potentially offering new therapeutic avenues for treating neurological disorders.

The book illustrates the physiological and pathological aspects of the brain's waste clearance systems, providing a comprehensive overview of the current understanding and highlighting areas for future research. By exploring the interplay between various clearance pathways and their roles in disease, the book underscores the significance of maintaining efficient waste clearance for overall brain health.

Improving waste clearance mechanisms in the brain holds immense potential for treating and preventing neurodegenerative diseases and other conditions that impact brain function. Enhancing the removal of waste products from the brain can potentially slow down or even reverse the progression of these debilitating conditions.

In conclusion, the cerebral waste clearance system plays a vital role in preserving brain health and function. Dysfunction in this system can lead to neurological disorders such as neurodegenerative diseases and aging-related cognitive decline. Current research efforts are aimed at unraveling the mechanisms of the cerebral clearance system and devising interventions to improve this crucial process.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| **ABC** | ATP-Binding Cassette |
| **Ach** | Acetylcholine |
| **AChE** | Acetylcholinesterase |
| **AD** | Alzheimer's Disease |
| **ADC** | Apparent Diffusion Coefficient |
| **AGD** | Argyrophilic Grain Disease |
| **AHR** | Aryl Hydrocarbon Receptor |
| **ALS** | Amyotrophic Lateral Sclerosis |
| **AMPK** | Adenosine Monophosphate-Activated Protein Kinase |
| **ANLS** | Astrocyte-Neuron-Lactate-Shuttle |
| **APP** | Amyloid Precursor Protein |
| **AQP4** | Aquaporin-4 |
| **ARE** | Antioxidant Response Element |
| **As** | Arsenic |
| **ASC** | Apoptosis-Associated Speck-Like Protein Containing A CARD |
| **αSyn** | Alpha-Synuclein |
| **Aβ** | Amyloid Beta |
| **BBB** | Blood–Brain Barrier |
| **BCSFB** | Blood–Cerebrospinal Fluid Barrier |
| **BDNF** | Brain-Derived Neurotrophic Factor |
| **BIN1** | Bridging Integrator 1 |
| **BIO** | 6-Bromoindirubin-3′-Oxime |
| **BMP** | Bone Morphogenic Proteins |
| **BMVECs** | Endothelial Cells |
| **Bold** | Blood Oxygen Level-Dependent |
| **CAA** | Cerebral Amyloid Angiopathy |
| **cAMP** | Phosphorylated Cyclic Adenosine Monophosphate |
| **CBD** | Corticobasal Degeneration |
| **CBF** | Cerebral Blood Flow |
| **Cd** | Cadmium |
| **CMT** | Carrier-Mediated Transport |
| **CN** | Calcineurin |

**Gaiqing Wang (Ed.)**

**CNS** Central Nervous System
**CR3** Complement Receptor 3
**CRD** Circadian Rhythm Disruption
**CREB** Camp Response Element Binding Protein
**CSF** Cerebrospinal Fluid
**CSVD** Cerebral Small Vessel Disease
**CTRP9** C1q/TNF-Related Protein 9
**Cu** Copper
**CX** Connexin
**DA** Dopamine
**DAM** Disease-Associated Microglia
**dCLNs** Deep Cervical Lymph Nodes
**DFA** Deferoxamine
**DLB** Dementia With Lewy Bodies
**DMN** Default Mode Network
**DMT1** Divalent Metal Transporter 1
**DTI** Diffusion Tensor Imaging
**EAE** Experimental Autoimmune Encephalomyelitis
**eAβ** Extracellular Aβ
**ECs** Endothelial Cells
**EEG** Electroencephalography
**ePVS** Enlarged Perivascular Space
**ER** Endoplasmic Reticulum
**Evs** Extracellular Vesicles
**Fals** Familial ALS
**Fe** Iron
**FGF2** Fibroblast Growth Factor2
**FLAIR** T2-Fluid Attenuated Inversion Recovery
**fMRI** Functional Magnetic Resonance Imaging
**Fpn** Ferritin Transporter
**FTDP-17** Frontotemporal Dementia With Parkinsonism Liked To Chromosome 17
**FTLD** Frontotemporal Lobar Degeneration
**FUS** Fused in Sarcoma
**GABA** Gamma Aminobutyric Acid
**GC** Glucocorticosteroid

**GCIs** Glial Cytoplasmic Inclusions
**GPX** Glutathione Peroxidase
**GR** Glucocorticoid Receptors
**GS** GSH Synthetase
**GSH** Glutathione
**GSK-3β** Glycogen Synthase Kinase-3beta
**Hb** Haemoglobin
**HBOP** Hyperbaric Oxygen Preconditioning
**HD** Huntington's Disease
**HDAC** Histone Deacetylase Three
**HLA** Human Leukocyte Antigen
**hnRNP** Heterogeneous Nuclear Ribonucleoprotein
**Hp** Haptoglobin
**HPA** Hypothalamic–Pituitary–Adrenal
**Hx** Haemopexin
**ICH** Intracerebral Hemorrhage
**IGF** Insulin-Like Growth Factor
**IL** Interleukin
**IREs** Iron Regulatory Elements
**IRPs** Iron Regulatory Proteins
**IRT** Iron-Regulated Transporter
**ISF** Interstitial Fluid
**ITGB8** Integrin Subunit B8
**JAK/STAT3** Janus Kinase/Signal Transducer and Activator Of Transcription 3
**Keap1** Kelch-Like ECH-Associated Protein 1
**LB** Lewy Bodies
**LCDs** Low-Complexity Domains
**LDLR** Low-Density Lipoprotein Receptor
**L-DOPA** L-3,4-Dihydroxyphenylalanine
**LECs** Lymphatic Endothelial Cells
**LIG** Ligustilide
**LLPS** Liquid-Liquid Phase Separation
**LNs** Lewy Neurites
**L-OOH** Hydroperoxy Lipids
**LRP1** Low-Density Lipoprotein Receptor-Related Protein-1

**LXR** Liver X Receptor
**MAPK** Mitogen-Activated Protein Kinase
**MAPT** Microtubule-Associated Protein Tau
**MCAO** Middle Cerebral Artery Occlusion
**MCI** Mild Cognitive Impairment
**MeHg** Methylmercury
**mHTT** Mutations in Protein Huntingtin
**mIgG** Murine Immunoglobulin G
**MLVs** Meningeal Lymphatic Vessels
**MMPs** Matrix Metalloproteinases
**MMΦ** Macrophages
**Mn** Manganese
**MOAs** Mode of Actions
**MR** Mineralocorticoid Receptors
**MRI** Brain Magnetic Resonance Imaging
**MSA** Multiple Systems Atrophy
**MSCs** Mesenchymal Stromal Cells
**mSOD** Mutant Human SOD
**MT** Metallothionein
**MT-III** Metallothionein III
**n3-PUFAs** Omega-3 Polyunsaturated Fatty Acids
**NCOA4** Nuclear Co-Activator 4
**NFTs** Neurofibrillary Tangles
**NF-κB** Nuclear Factor-Kb
**NLR** Nucleotide-Binding Domain Leucine-Rich Repeat
**NLRP3** NLR And Pyrin Domain Containing Receptor 3
**NMDA** N-Methyl-D-Aspartate
**NREM** Non-Rapid Eye Movement
**NVU** Neurovascular Unit
**OLGs** Oligodendrocytes
**OTUB1** Otubain-1
**Pb** Lead
**PCBP** Poly rC Binding-Protein
**PD** Parkinson's Disease
**PDCG** Parkinsonism Dementia Complex of Guam

**PDGF-cc** Platelet-Derived Growth Factor-CC

**PEP** Postencephalitic Parkinsonism

**PiD** Pick's Disease

**PPAR-γ** Peroxisome Proliferator-Activated Receptor Gamma

**PrP** Prion Protein

**PrPc** Prp in Cellular Isoform

**PrPsc** Prpc into Scrappie

**PRRs** Pattern Recognition Receptors

**PSP** Progressive Supranuclear Palsy

**PTMs** Posttranslational Modifications

**PVS** Perivascular Spaces

**RAGE** Receptor for Advanced Glycation End Products

**RBPs** RNA Binding Proteins

**REM** Rapid-Eye-Movement

**RLD** Right Lateral Decubitus

**RMT** Receptor-Mediated Transcytosis

**RNS** Reactive Nitrogen Species

**ROS** Reactive Oxygen Species

**S129** Serine 129

**sADC** Shifted Water Diffusion Coefficient

**SAH** Subarachnoid Hemorrhage

**SAP** Serum Amyloid P Component

**SAS** Subarachnoid Space

**SD** Sleep Deprivation

**SH** Senkyunolide H

**SIRP** Signal Regulatory Protein

**sLRP** Soluble Form of LRP

**SN** Substantia Nigra

**SNARE** Soluble N-Ethylmaleimide-Sensitive Factor Attachment Protein Receptor

**SNc** Substantia Nigra Pars Compacta

**SNP** Single Nucleotide Polymorphism

**SOCS2** Suppressor of Cytokine Signaling 2

**SOD** Superoxide Dismutase

**SRA** Scavenger Receptor A

**sRAGE** Soluble Form of RAGE

**SRs** Scavenger Receptors

**TBI** Transferrin-Bound Iron

**TDP-43** TAR DNA-Binding Protein 43

**TF** Transferrin

**TFR1** Transferrin Receptor1

**TGFβ** Transforming Growth Factor Beta

**Timp-1** Tissue Inhibitor of Metalloproteinases 1

**TJ** Tight Junction

**Tl+** Thallium

**Tlrs** Toll-Like Receptors

**TNF** Tumor Necrosis Factor

**TOM20** Translocase of Outer Membrane 20 Kda Subunit

**Tregs** Regulatory T Lymphocytes

**TREK-1** TWIK-Related K+ Channel 1

**Trem2** Triggering Receptor Expressed on Myeloid Cells 2

**TSP** Thrombospondin

**Utrs** 3′ Untranslated Regions

**V** Vanadium

**VEGF** Vascular Endothelial Growth Factor

**VEGF-C** Vascular Endothelial Growth Factor C

**VRS** Virchow-Robin Spaces

**Vsmcs** Vascular Smooth Muscle Cells

**Zn** Zinc

**Znt** Zn Transporter

**ZRT** Zinc-Regulated Transporter

**α2m** α2-Macroglobulin

# SUBJECT INDEX

## D



## E

## F



## G



## H



## I

## J



## L



## M

## N



## O



## P



## R



## S

## T



## V



## W



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