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Reimagining the meninges from a neuroimmune perspective: a boundary, but not peripheral

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Abstract

Recent advances in neuroscience have transformed our understanding of the meninges, the layers surrounding the central nervous system (CNS). Two key findings have advanced our understanding: researchers identified cranial bone marrow as a reservoir for meningeal immune cells, and rediscovered a brain lymphatic system. Once viewed merely as a protective barrier, the meninges are now recognized as a dynamic interface crucial for neuroimmune interactions. This shift in perspective highlights their unique role in maintaining CNS balance, shaping brain development, and regulating responses to injury and disease. This review synthesizes the latest insights into meningeal anatomy and function, with a focus on newly identified structures such as dural-associated lymphoid tissues (DALT) and arachnoid cuff exit (ACE) points. We also examine the diverse immune cell populations within the meninges and their interactions with the CNS, underscoring the emerging view of the meninges as active participants in brain immunity. Finally, we outline critical unanswered questions about meningeal immunity, proposing directions for future research. By addressing these knowledge gaps, we aim to deepen our understanding of the meninges' role in brain health and disease, potentially paving the way for novel therapeutic approaches.

Keywords Meninges, Meningeal immunity, Brain, Central nervous system, Neurodegenerative disease, Neuroinflammatory disease

Background

For decades, The central nervous system (CNS) was considered an "immune-privileged" site, isolated from peripheral immune influences [1, 2]. This view was primarily based on the presence of the blood-brain barrier, which restricts immune cell entry into the CNS under normal conditions. In this paradigm, microglia were seen as the only permanent immune cells in healthy brain parenchyma, separate from the body's wider immune system. However, the discovery of lymphatic structures [3–5] within the meninges has challenged this concept of CNS immune privilege, necessitating a reevaluation of the meninges' role in CNS and immune functions. Recent technological advancements have revolutionized

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neuroimmune research, providing unprecedented insights into meningeal composition and function. These include in vivo and live cell imaging, meningeal whole-mount immunohistochemistry, advanced tissue clearing techniques (vDISCO, wildDISCO, fMOST), mass cytometry, fate mapping, and genetic manipulation methods [6–14]. Notably, single-cell RNA sequencing and spatial transcriptomics have enabled precise mapping of immune cell heterogeneity and distribution within the meninges, often leading to reinterpretation of previous findings [6, 15–19].

This surge in neuroimmune research has fundamentally reshaped our understanding of the meninges [1, 20, 21]. It is now clear that significant interactions occur between the brain and the peripheral immune system, with the meninges serving as a critical interface. No longer viewed as a mere protective barrier or "wall," the meninges are now recognized as a "bridge" – a dynamic microenvironment hosting diverse immune cell populations with specialized roles. This functional neuroimmune interface plays a crucial role in maintaining brain homeostasis in both health and disease. The importance of the meninges in CNS immunity and neuropathobiology research continues to grow. Meningeal innate and adaptive immunity not only provide surveillance and defense against pathogens but also contribute to broader CNS functions. Recent studies have revealed that meningeal-derived signals, including cytokines, chemokines, and growth factors, influence brain development, neuronal connectivity, learning, and memory. These findings underscore the wider impact of meningeal immunity on overall CNS function and plasticity, extending far beyond its traditional protective role [22–29].

The meninges reimagined: from barrier to neuroimmune hub

The meninges form a crucial barrier and interface between the CNS and the peripheral environment [21]. This protective layer shields the CNS from physical trauma and pathogen infiltration, situated between the brain and the surrounding bony structures, including the skull and facial bones [30]. The meninges comprise three distinct layers: the outermost dura mater, the middle arachnoid mater, and the innermost pia mater. The arachnoid and pia mater are collectively known as the leptomeninges. In addition to the meninges, the CNS parenchyma is protected by several other barriers, including the blood-brain barrier (BBB), the blood-meningeal barrier (BMB), and the blood-cerebrospinal fluid (CSF) barrier [31, 32]. These barriers typically consist of a single layer of cells connected by tight junctions (TJs), which restrict the free movement of molecules and cells. This selective permeability renders these barriers impermeable to immune cells and most large circulating molecules.

These barrier systems effectively divide the CNS into distinct regions, each with varying levels of accessibility for immune cells and signaling molecules, creating the brain's unique immune landscape. The main compartments include: (1) Cranial bone marrow; (2) Dural meninges; (3) Leptomeninges; (4) Parenchymal perivascular space (Virchow-Robin space); (5) Choroid plexus; (6) Brain ventricles [30, 33, 34] (Fig. 1A). In this review, we will focus on the meninges and the cranial bone marrow, providing an in-depth analysis of their anatomical structures and functions. This examination will shed light on the complex interplay between these structures and their role in maintaining CNS homeostasis and immune regulation.

Dura

The dura, the outermost layer of the meninges, is a robust and dense membrane that envelops the brain [21, 30]. It comprises two distinct layers: the outer periosteal layer, which adheres tightly to the skull, and the inner meningeal layer, lined with flat cells adjacent to arachnoid layer. The vessels channels that collect venous blood from the brain, known as venous sinuses, pass between these two layers [34, 35]. The dura features prominent folds that extend between the cerebral and cerebellar hemispheres. These include the falx cerebri, which separates the cerebral hemispheres, and the tentorium cerebelli, which lies between the cerebrum and cerebellum. These structures play a crucial role in limiting brain movement within the CSF, thereby providing mechanical protection. Notably, the dura is richly vascularized and contains an intricate lymphatic network. It also possesses extensive innervation, making it a highly responsive and dynamic structure. This combination of vascular, lymphatic, and neural elements underscores the dura's importance not only as a protective layer but also as an active participant in maintaining brain homeostasis and mediating neuroimmune interactions.

Dural blood vessels

The meninges, particularly the dura, possess a specialized vascular network crucial for immune surveillance [34]. Intracranial vessels can be divided into four main types: around the skull (pericranial type), within skull bones (diploic type), in the brain's coverings (meningeal type), and inside the brain parenchyma (cerebral type) [36–38]. In primates, the dura and skull are perfused by the anterior, middle, and posterior meningeal arteries, which originate from the external carotid artery.

In rodents, the setup is different: the middle meningeal artery comes from the pterygopalatine or stapedial arteries, which branch off from the internal or common

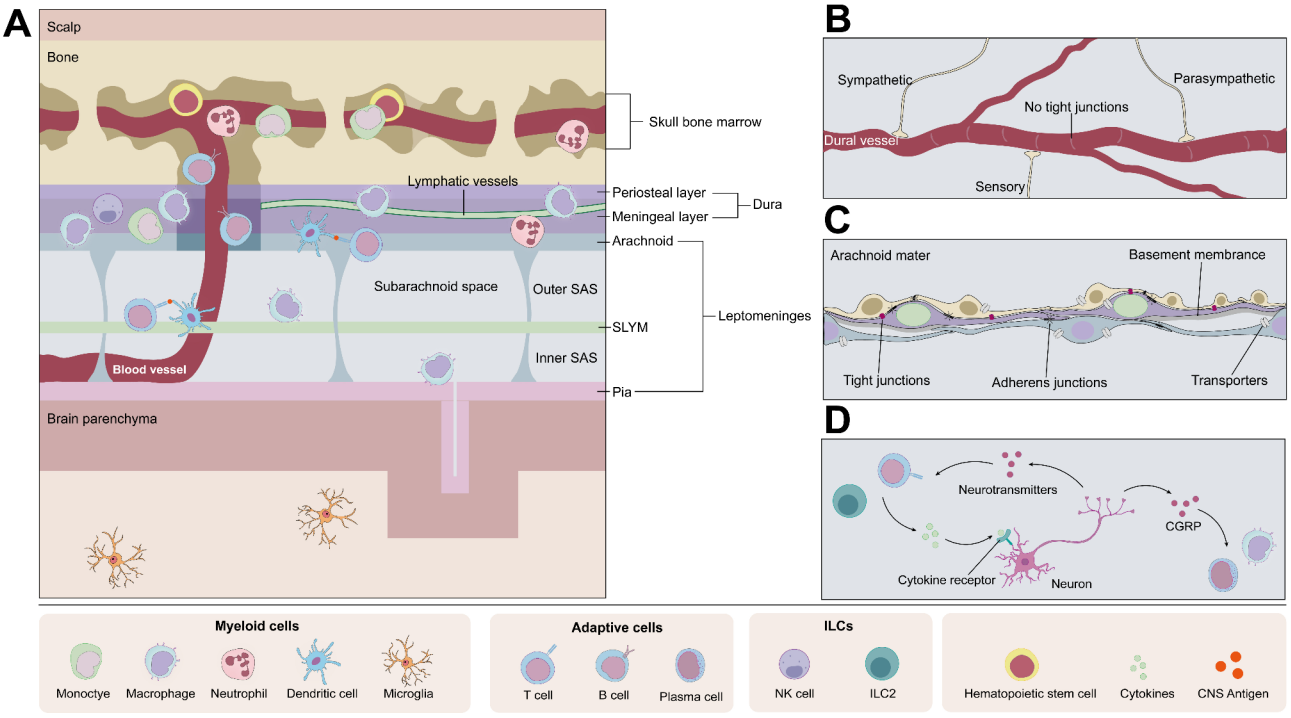

Fig. 1 Structure and immune function of meninges. **A** The meninges, located between the skull and the brain, are composed of three layers: the dura mater, the arachnoid mater, and the pia mater. The dura mater contains a rich distribution of nerves, blood vessels, and lymphatic vessels. The arachnoid mater is thin and transparent, lacking blood vessels and nerves, and it envelops the subarachnoid space, which is filled with cerebrospinal fluid (CSF). The subarachnoid lymphatic-like membrane (SLYM), situated between the arachnoid mater and the pia mater, is proposed to be a potential fourth meningeal layer. The SLYM divides the subarachnoid space into external and internal compartments. It plays a role in restricting the exchange of most small peptides and proteins between the superficial and deep layers of the subarachnoid space, and is also involved in meningeal immunity and fluid drainage. Numerous immune cells are found within the dura mater. In contrast, the leptomeninges and CSF contain fewer immune cells in both number and variety. **B** The barrier-like structure of the dura mater vasculature is permeable, allowing bidirectional exchange of blood-borne molecules and immune cells with the peripheral circulation. This feature renders the meninges an exceptional platform for immune surveillance. The dura mater is innervated by a network of peripheral nerve fibers, which project to both vascular and non-vascular targets. These fibers can modulate the contraction of dural blood vessels, thereby regulating their permeability. Signals arising from changes in the meninges, such as mechanical deformation or pH variations, can also be transmitted to the central nervous system. **C** Cells of the arachnoid mater are tightly joined together by tight junctions, preventing molecules and cells from freely passing from the dura mater into the subarachnoid space. This stringent barrier effectively isolates the dura mater from the rest of the central nervous system. Furthermore, arachnoid barrier cells express an abundance of transporters and efflux pumps, which support the regulatory function of this barrier layer over molecular movement. **D** Neuro-immune crosstalk: Immune cells within the meninges can influence behavior by secreting cytokines that act on neurons, thereby maintaining and modulating neuronal activity. Additionally, these immune cells are capable of responding to neurotransmitters and neuropeptides released by neurons

carotid arteries. The dura's vascular network is characterized by anastomosing vessels, including arteries, veins, and fenestrated capillary beds. The primary arterial supply comes from the middle meningeal artery, which nourishes the vascular network between the periosteal dura and the skull.

A distinctive feature of dural blood vessels is the presence of endothelial cells that exhibit a lower expression of TJs, particularly Claudin-5 and occludin [31, 39]. This results in a fenestrated pattern that allows for rapid exchange of large molecules between blood and surrounding tissues, as demonstrated by the quick leakage of dextran and horseradish peroxidase from dural vessels [40, 41]. This increased permeability allows for easier movement of cells and molecules between the meninges and the bloodstream (Fig. 1B). The dura also contains large venous structures, known as dural venous sinuses, which drain blood from both the dura and the brain. The endothelial cells lining these sinuses highly express leukocyte adhesion molecules, including ICAM1 and VCAM1 [16]. The combination of large vascular surface area, high expression of adhesion molecules, low tight junction levels, and relatively slow blood flow [42] creates an environment conducive to cellular and molecular exchange.

These vascular characteristics contribute to the immunological function of the dural vessels, allowing immunoactive soluble factors and cells to enter the dura. Consequently, the steady-state meninges, particularly the dura, harbor a diverse array of immune cells, including macrophages, neutrophils, T cells, B cells, antigen-presenting cells, mast cells, plasma cells, and innate

lymphoid cells [16, 17, 43–45]. The distribution of these immune cells within the dura is not uniform; they tend to accumulate in specific areas, notably along the walls of the dural venous sinuses. This unique vascular architecture and its associated immune cell populations underscore the dura's role not just as a protective barrier, but as a dynamic immunological interface between the CNS and the periphery.

Lymphatic vasculature in the dura

The discovery of meningeal lymphatic vessels (mLVs) within the dura in 2015 marked a significant milestone in our understanding of the CNS immunity and fluid dynamics [3, 4]. These vessels express classical lymphatic endothelial cell markers, including VEGFR3, Prox1, Podoplanin, Lyve1, CD31, and CCL21, confirming their lymphatic nature. Meningeal lymphatics form an extensive drainage network that connects the CNS parenchyma to the peripheral immune system. This network comprises dorsal mLVs on the dorsal skull, basal mLVs [5, 46] on the skull base, and an interconnected system within the cavernous sinus [47] (Fig. 2D). These vessels serve crucial functions in draining CSF, antigens, and immune cells, thereby clearing waste and coordinating immune responses in the CNS [48].

The anatomical distribution of mLVs correlates with their distinct morphological characteristics and functions. Dorsal mLVs, which run parallel to dural venous structures like the superior sagittal (SSS) and transverse sinuses (TS), have small, unbranched lumens without valves and discontinuous endothelial cell connections [3, 4]. In contrast, basal mLVs, located along the parietal sinuses (PSS) and sigmoid sinuses (SS), feature larger, branched lumens with valves and oak leaf-shaped endothelial cells [5, 46]. Both the dorsal mLV and the basal mLV can drain CSF out of the brain. However, the basal mLV is morphologically superior and is located adjacent to the subarachnoid space, so it is considered to be more effective in draining large molecules from CSF. Together with dural venous sinuses, these lymphatic vessels form dural immune centers, facilitating CSF sampling, antigen uptake, and presentation to T cells [16]. This arrangement plays a crucial role in establishing the neuroimmune interface. Additionally, the potential interconnection between meningeal lymphatics and the lymphatic systems of the nasal and nasopharyngeal regions suggests a continuous drainage and immune defense network extending from the CNS to the upper respiratory tract.

The mLVs network has been implicated in various neurological conditions, including aging [49], Alzheimer's disease [50, 51], Parkinson's disease [52], traumatic brain injury [53], subarachnoid hemorrhage [54], CNS viral infections [55], and brain tumors [56]. Changes in mLVs transport capacity can significantly influence disease progression. For instance, the deterioration of mLVs function with age or in Alzheimer's disease may contribute to cognitive impairment and exacerbate neurological damage. Moreover, the extensive distribution of mLVs within the dura offers promising avenues for transcranial nerve modulation therapies. Treatments targeting the lymphatic system show great potential for managing neurological conditions. Enhancing lymphatic drainage to clear toxic molecules from the CNS could improve cognitive function in age-related disorders. Future directions in neurological disease treatment may include developing non-invasive modalities to regulate mLVs function, such as pharmacological interventions and photostimulation techniques [51, 57, 58]. This evolving understanding of mLVs has fundamentally altered our perspective on intracranial fluid dynamics and brain immunity, opening new avenues for research and potential therapeutic strategies in neurological disorders.

Dural-associated lymphoid tissues (DALT)

Recent research by Fitzpatrick et al. has revealed a network of organized lymphoid structures within the dura of mice and humans, termed dural-associated lymphoid tissues (DALT) [59] (Fig. 2A). This groundbreaking study employed advanced techniques, including micro-CT imaging, single-cell RNA sequencing, and B cell receptor (BCR) sequencing, to provide a comprehensive description of these structures. DALT is intricately interwoven with the fenestrated vasculature of the dura, with its most complex components located in the rostral-rhinal and basal olfactory venous hubs. The rostral-rhinal DALT, also known as the rostral-rhinal venolymphatic hub, is directly connected to cranial marrow vessels via diploic veins. It also receives vascular input from multiple sources, including the rostral-rhinal sinuses, olfactory sinus, superior sagittal sinus (SSS), bridging veins, and cortical veins. These venolymphatic hubs host diverse immune cell populations, including various B cell subsets, plasma cells, T follicular helper (TFH) cells, and T follicular regulatory (TFR) cells. During pathogen invasion, interactions between germinal center B cells and TFH cells, mediated by IL21-IL21R and CD28-CD86 pathways, are intensified. B cells activated within DALT subsequently migrate to the sinus wall, bolstering local immunity in the venous sinus.

This discovery presents a novel model for understanding CNS immunosurveillance, highlighting DALT's strategic anatomical position for acquiring both local and whole-brain antigens. DALT can harvest antigens from blood and nasal passages to generate localized immune responses, supporting the production of class-regulated, high-affinity antibodies in response to viral challenges. These venous lymphoid hubs facilitate rapid expansion of

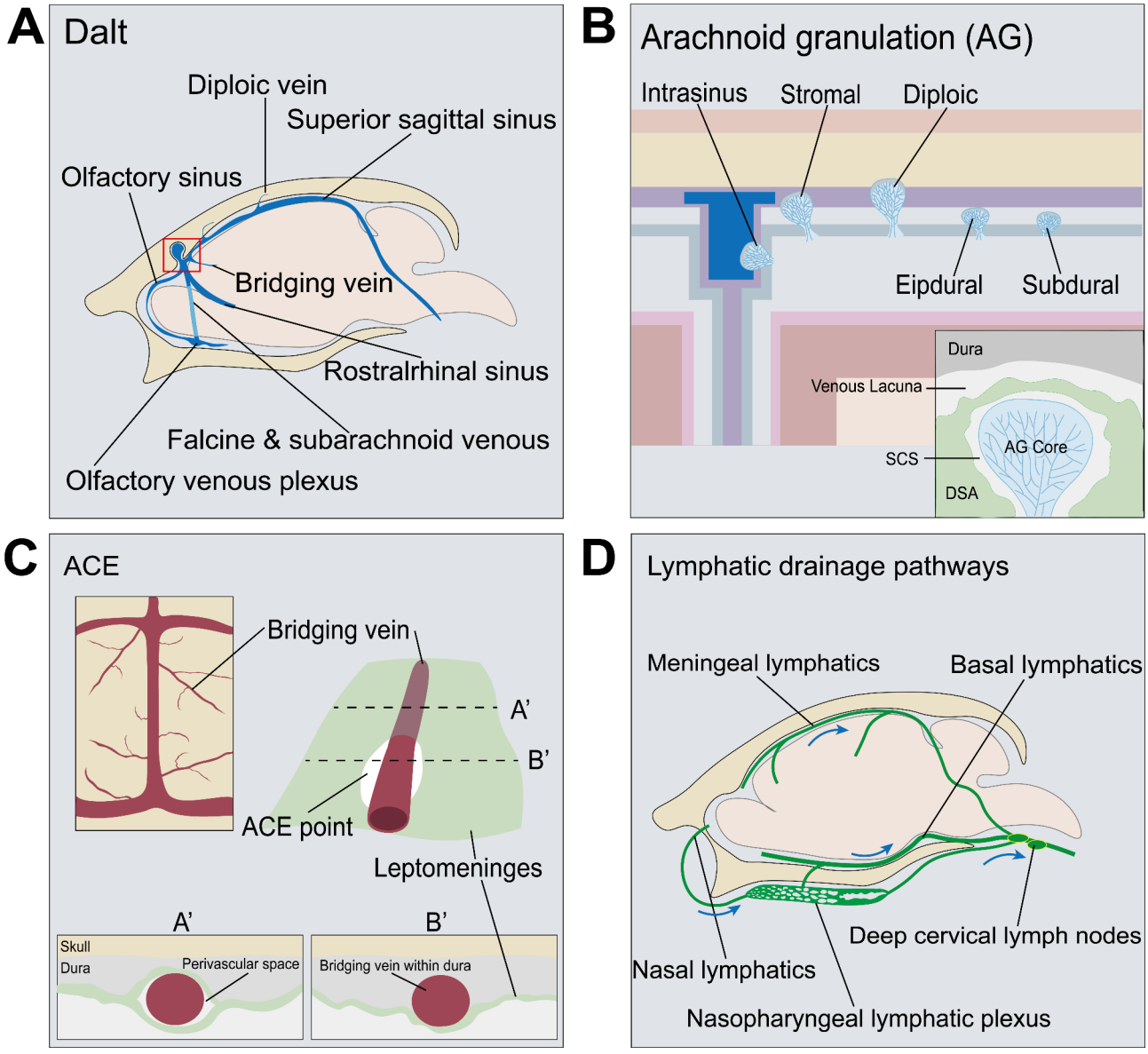

Fig. 2 Specialised structures at the meningeal interface. **A** Dural-Associated Lymphoid Tissues (DALT). DALT represents a structured lymphoid network interwoven with the barrier-like vasculature within the dura mater. The red box highlights the rostral-rhinal hub, which connects with multiple veins and the bone marrow of the skull. **B** Arachnoid granules. The arachnoid mater forms numerous villous protrusions on both sides of the superior sagittal sinus, which extend into the sinus and are referred to as arachnoid granulations. These structures may be involved in the reabsorption of cerebrospinal fluid (CSF) into the venous blood. Arachnoid granulations can be classified into five types based on their morphology and their relationship with the dura mater, each contributing to varying degrees to CSF flow and meningeal immunity. **C** Arachnoid cuff exit (ACE) points. As the bridging vein traverses the arachnoid barrier, it creates discontinuities, forming channels that connect the subarachnoid space with the dura mater. A' illustrates that the bridging vein is located within the arachnoid mater. B' shows that at the ACE point, the bridging vein is positioned outside the arachnoid mater. **D** Cranial lymphatic structures. Cerebral lymphatics can be categorized into meningeal lymphatics, basal mLVs, and nasal lymphatics and nasopharyngeal lymphatic plexus. Through these channels, CSF, carrying immune cells, cytokines, and metabolic waste, is drained from the subarachnoid space into the deep cervical lymph nodes (dCLN)

antigen-specific immune responses, potentially offering defense against foreign pathogens threatening the CNS parenchyma. The role of DALT in supporting humoral immunity within the meninges challenges the traditional view of the meninges as a simple barrier. Instead, it reveals a complex, immunologically active interface between the CNS and the periphery. This paradigm shift in our understanding of meningeal structures opens new avenues for research into CNS immunity and potential therapeutic approaches for neurological disorders.

Dural nerve

The meninges, particularly the dura, are characterized by extensive neural innervation [60, 61]. This innervation network comprises peripheral sensory and autonomic nerves that target both vascular and non-vascular structures. The trigeminal nerve, primarily its first branch, is the main supplier to the dura, with additional contributions from its other branches. Notably, the tentorial nerve, a branch of the first division, innervates the upper dura mater, the superior sagittal sinus, the transverse sinus, and the parietal branches of the middle meningeal artery.

Trigeminal ganglion projections extend to the middle cerebral artery (MCA) and form intricate loops with the middle meningeal artery (MMA) [62]. These neural fibers co-innervate various meningeal blood vessels and resident cells (Fig. 1B). Interestingly, some trigeminal nerve afferents form loops that terminate in the cranial bone marrow or traverse the skull to end in the external periosteal layer, potentially facilitating communication across the meningeal barrier.

The meninges constitute a highly sensitive sensory system. Sensory fibers within the meninges respond to changes in temperature, pH, and mechanical pressure, providing critical feedback about meningeal status [55, 63]. These afferents serve as key sensory transducers in proprioception and vestibular sensation, capable of detecting physiological meningeal deformation and possibly changes in intracranial pressure. This sensitivity may play a protective role during head impacts, with abnormal meningeal stretching potentially signaling to prevent injury during rapid head movements.

The meningeal nerve supply is composed of thousands of sympathetic, parasympathetic, and sensory fibers, some of which are myelinated. This network forms a localized chemical defense system. Trigeminal nerve projections to the dura and pons contain vasoactive neuropeptides such as calcitonin gene-related peptide (CGRP), substance P, and pituitary adenylate cyclase-activating polypeptide (PACAP) [55, 64]. When activated, these neuropeptides are released to detect and limit tissue damage by modulating vascular function and signaling the brain. Dural blood vessels exhibit heightened sensitivity to mechanical and chemical stimuli compared to deeper brain vessels. Their responsiveness is complex: increased luminal pressure, neurotransmitters like norepinephrine, and neuropeptides such as neuropeptide Y can induce vasoconstriction. Conversely, electrical stimulation, certain neuropeptides (including CGRP and substance P), acetylcholine, histamine, and serotonin can promote vasodilation.

There are numerous immune cells and neural cells within the meninges, and their direct physical contact offers intriguing new research avenues for the concept of neuroimmune (Fig. 1D). Under normal circumstances, macrophages in the meninges detect, attack, and recruit other immune cells to combat bacterial invasion. The study by Chiu et al. highlights a critical neuroimmune axis in the meninges where bacteria exploit neurochemical signaling to suppress immune responses [65]. Specifically, bacteria like Streptococcus pneumoniae release toxins that activate pain neurons within the meninges. The Nav1.8+nociceptors are activated, leading to the release of the signaling molecule CGRP by these pain neurons. CGRP downregulates the expression of chemokines by meningeal macrophages through receptor activity-modifying protein 1 (RAMP1), inhibiting neutrophil recruitment and thereby suppressing the host's immune defenses, exacerbating bacterial meningitis. This intricate interplay between neural and immune components underscores the complex regulatory mechanisms at work in the meningeal microenvironment. Furthermore, the activity of resident mast cells in the meninges is tightly regulated by neurotransmitters and neuropeptides to maintain central nervous system homeostasis [66].

Arachnoid

The arachnoid mater, the middle layer of the three meningeal layers, is a complex structure composed primarily of avascular connective tissue. It consists of multiple cell layers, with an inner layer 1 to 4 cells thick [18, 67]. A distinctive feature of the arachnoid mater is its trabeculae, formed by fibroblast-like cells [15, 65], which span the subarachnoid space and extend to the pia mater. The subarachnoid space (SAS), a CSF-filled cavity between arachnoid and pia, serves critical functions. It houses large arteries that penetrate the brain parenchyma and provides a cushioning effect, protecting the brain from impact injuries. Moreover, CSF within SAS is crucial for brain buoyancy, effectively reducing the brain's weight and preventing compression under its own mass.

Compared to the dura, a key characteristic of the arachnoid mater is its relative impermeability. This property stems from its unique cellular composition and junctional complexes. In mice, arachnoid barrier (AB) cells originate from mesenchymal precursor cells but begin expressing E-cadherin and Claudin 11 around embryonic day 14 (E14) [15, 68]. The arachnoid mater forms an extensive barrier through tight junctions (TJs) containing Claudin-11 and adherens junctions (AJs) containing E-cadherin [68–70]. This barrier effectively separates and regulates the dura and subarachnoid space, restricting bidirectional molecular movement between them (Fig. 1C). For instance, 10 kDa dextran and chemokines (8–10 kDa) are blocked by the arachnoid mater, preventing their movement from the dura to the SAS.

While the arachnoid's impermeability is fundamental in safeguarding the CNS, it is important to acknowledge

that it also demonstrates a degree of selective permeability, particularly in the context of its cellular transport mechanisms. The presence of various transporters and efflux pumps in arachnoid barrier cells enhances their regulatory function. These include ATP-binding Cassette (ABC) Transporters such as ABCB1 (P-glycoprotein), ABCC4 (MRP4), and ABCG2 (breast cancer resistance protein), as well as slc22a6 and slc22a8 [71–77]. This array of transporters likely contributes to the selective permeability of the arachnoid barrier, allowing for finely tuned regulation of substance exchange between the blood and CSF. This complex structure and function of the arachnoid mater underscore its critical role in maintaining the unique environment of the central nervous system, balancing protection with selective permeability.

Arachnoid granules

While the arachnoid barrier creates a partition between the CNS and the dura, communication between these compartments is essential for toxin molecule clearance and immune surveillance. In humans and other large mammals, this communication occurs through arachnoid granules (AGs), which are specialized protrusions of the arachnoid mater. Arachnoid granules, also known as arachnoid villi or Pacchionian granulations, were first described by the Italian anatomist Antonio Pacchioni [78]. These structures have traditionally been described as penetrating the dura mater and facilitating CSF drainage into the dural venous system, but this understanding continues to evolve, reflecting a broader range of functions [79, 80]. Interestingly, AGs do not appear in smaller mammals like rodents, implying significant differences in fluid dynamics and immune interactions between species. This absence may suggest different pathways for CSF clearance and immune surveillance in these species.

Recent research by Shah et al. has provided detailed structural and cellular analyses of AGs, expanding our understanding of their potential roles beyond simple CSF drainage [81] (Fig. 2B). This study suggests that AGs may function as lymphatic conduits, connecting the bone marrow to the arachnoid stroma. This connection could play crucial roles in lymphatic-lymphatic coupling, CSF antigen clearance, homeostasis maintenance, and mechanisms underlying neurological diseases. AGs are classified into five types based on their association with the dura and venous sinuses: intrasinus, stromal, transdural, epidural, and subdural. Structurally, AGs consist of an outer capsule and an inner stromal core, encapsulated by a membrane composed of arachnoid cells. These structures exhibit significant heterogeneity in size, shape, location, and internal architecture.

Internally, AGs present as spongy cavities comprising complex lacunae, crypts, and sinuses. These cavities are rich in diverse immune cells, including macrophages, neutrophils, mast cells, dendritic cells, CD4+and CD8+T lymphocytes, B lymphocytes, and plasma cells. Notably, stromal AGs embedded in the dural matrix show an enrichment of immune cells, particularly MHCII+antigen-presenting cells and CD4+T cells. This cellular composition suggests potential antigen-presenting cell interactions within these structures. The architecture of AGs undergoes significant age-related changes, including increased size, enhanced lobulation, and loss of vesicle integrity. These structural alterations may have important implications for AG function over the lifespan.

In essence, AGs serve as sophisticated filtering channels for CSF, cellular debris, and immune cells. This multifaceted role suggests that AGs play a more complex part in brain-immune interactions than previously thought. Their unique structure and cellular composition position them as key players in maintaining CNS homeostasis and potentially in the pathogenesis of neurological disorders. This evolving understanding of AG function opens new avenues for research into CNS-immune system interactions and may provide novel insights into the mechanisms underlying various neurological conditions.

Arachnoid cuff exit (ACE) points

The arachnoid mater's relative impermeability and transport capacity enable it to regulate the exchange of molecules and factors between the CNS and its surroundings. However, the mechanisms by which the arachnoid barrier balances separation and communication have remained largely elusive. Recent research by Leon C. D. Smyth and colleagues has unveiled a unique structure within the arachnoid mater that sheds light on this complex interplay. Blood draining from the CNS must pass through bridging veins to reach the venous sinuses. These veins traverse the subarachnoid space and penetrate the arachnoid barrier, creating discontinuities in this otherwise impermeable layer. At these points of penetration, the arachnoid barrier undergoes specialization, forming distinct anatomical structures known as arachnoid cuff exit (ACE) points [82] (Fig. 2C).

ACE points serve as critical control checkpoints at the CNS gateway, allowing direct fluid and cell exchange between the dura and the SAS. These openings facilitate CSF drainage while simultaneously restricting molecular entry from the dura into the SAS. This bidirectional control mechanism highlights the sophisticated nature of the arachnoid barrier's regulatory function. Importantly, ACE points are closely associated with meningeal lymphatic vessels. Once CSF reaches the dura through these points, it can be rapidly drained by the lymphatic system. This association suggests a potential role for ACE points in facilitating the clearance of large molecular waste from the subarachnoid space into the dura. Such a function could be particularly significant in conditions

like traumatic brain injury (TBI) or subarachnoid hemorrhage (SAH), where efficient debris clearance is crucial. Moreover, ACE points enable cellular transport, providing a direct route for immune cells to move from the dura into the subarachnoid space. This cellular trafficking is mediated by laminin, an important molecule that guides the migration of myeloid cells to ACE points in the subarachnoid space. This discovery offers a novel explanation for the interaction between the seemingly barrier-isolated CNS and the peripheral immune system.

The identification of ACE points represents a significant advance in our understanding of CNS-immune system communication. These structures provide a nuanced mechanism for controlled exchange between compartments previously thought to be strictly separated. This finding not only enhances our comprehension of normal CNS physiology but also opens new avenues for investigating pathological conditions and potential therapeutic interventions targeting these unique anatomical features.

Pia

The pia mater, the innermost of the three meningeal layers, intimately adheres to the surface of the brain parenchyma. This delicate membrane follows the contours of the brain and spinal cord, extending into the fissures and grooves of their surfaces [34, 83]. Structurally, the pia mater consists of a single layer of flat fibroblasts that penetrate the sulci of the cerebral cortex [18]. Beneath the pia mater lies the limiting glial layer, composed of the parenchymal basement membrane and astrocyte end-feet. This layer functions as an additional barrier for the CNS parenchyma, selectively blocking immune cells while allowing the passage of fluids and low-molecular-weight tracers from the CSF into the brain parenchyma.

The pia mater is characterized by its rich vascularization. It envelops the vessels in the subarachnoid space (SAS), but at points where these vessels penetrate the brain parenchyma, a separation occurs between the SAS, the CNS parenchyma, and the perivascular space [34, 84]. This arrangement creates the Virchow-Robin space around the penetrating arteries of the brain, which exhibits semi-permeable properties. Arachnoid vessels traverse the cortex, establishing connections with the CNS parenchymal vasculature [85]. Notably, pial blood vessels possess barrier properties, featuring tight junctions that express occludin and claudin proteins, as well as adherens junctions linked to actin filaments [34]. This intricate setup of the pia mater and related structures is key in controlling the flow between brain fluid and tissue. The selective permeability of this barrier system allows for the maintenance of the unique CNS microenvironment while facilitating essential physiological processes.

SLYM

In 2023, Møllgård et al. reported the discovery of a 'fourth layer of the meninges' - the subarachnoid lymphatic-like membrane (SLYM) [86–88]. This finding expands our current understanding of the meningeal interface structure. The SLYM is positioned between the arachnoid membrane and the brain, specifically interfacing with the pontine region (Fig. 1A). The SLYM is described as being composed of tightly connected cells forming a quasi-impermeable barrier and loosely organized collagen fibers.

The SLYM's unique structure allows it to prevent the exchange of CSF solutes larger than 3 kDa between the inner and outer spaces of the arachnoid membrane. This property effectively divides the SAS into two functional zones: superficial and deep. At steady state, the SLYM's relative impermeability separates 'clean' and 'dirty' CSF, potentially synergizing with the glymphatic system to enhance brain waste removal efficiency. Moreover, the SLYM harbors resident immune cells, including macrophages and dendritic cells. Sharing immunophenotypic characteristics with lymphatic vessels, the SLYM may serve as a site for immune cell recruitment and proliferation. However, traumatic rupture or pathological degeneration of the SLYM can disrupt its barrier properties, leading to the mixing of 'dirty' and 'clear' CSFs and reducing brain waste removal efficiency. Such disruption may also allow pathogens and myeloid cells to enter the CNS parenchyma, exacerbating neuroinflammation and potentially inducing long-term inflammatory responses.

The existence and function of SLYM structures have long evaded clear exploration [88]. Early studies referred to similar structures using terms such as "epipial layer," "intermediate meningeal layer," or "outer pial layer." Indeed, the earliest description of "epipial tissue" in the human spinal cord dates back to Key & Retzius over a century ago. However, the recent proposal of the SLYM concept has faced skepticism due to the lack of clear identification with the ABC layer.

Current understanding of the SLYM is limited by several factors. Its extreme thinness makes it susceptible to loss or collapse during tissue processing, and it may be confused with arachnoid trabeculae or blood vessels. These challenges highlight the need for advanced methods to study the SLYM both in vitro and in vivo. Future research should focus on mapping the fine structure of the SLYM throughout the CNS to confirm its existence and elucidate its properties. Moreover, investigating the potential role of the SLYM in neuroinflammatory and neurodegenerative diseases, particularly concerning CSF dynamics, waste removal, and CNS immune responses, may provide valuable insights into CNS physiology and pathology. This discovery of the SLYM represents a significant advancement in our understanding of meningeal

structure and function. As research progresses, it may reshape our comprehension of CNS-immune interactions and open new avenues for therapeutic interventions in neurological disorders.

CNS fibroblasts

Fibroblasts are pivotal cells that produce and maintain a diverse range of extracellular matrix (ECM)-rich connective tissues, supporting vital organ functions across the skin, lungs, and skeletal muscle [89, 90]. They create essential niches and provide positional information to neighboring cells via microarchitectural, biomechanical, and biochemical cues within the ECM. In addition, fibroblasts regulate the secretion of soluble mediators, including cytokines, growth factors, and metabolites. Emerging evidence highlights significant functions beyond these conventional roles, especially within the CNS.

The CNS contains numerous fibroblasts, strategically positioned within the meninges, perivascular spaces, and choroid plexus. Despite their prevalence, the identification of fibroblasts has proven complex due to the absence of specific molecular markers, often resulting in confusion with pericytes, smooth muscle cells (SMCs), and other mesenchymal entities. Unlike the extensive investigations conducted in peripheral tissues, the characterization of fibroblast heterogeneity and their potential roles within the CNS has commenced only recently. Recent advancements in scRNA sequencing have elucidated the diverse gene expression profiles of the CNS fibroblasts, indicating distinct functional specializations. The CNS fibroblasts derive from varied origins and display molecular diversity that varies by region within the CNS [18, 90, 91]. Fibroblasts exhibit a complex profile of markers, sharing some with other cell types like CNS pericytes and SMCs. Specifically, PDGFRβ and chondroitin sulfate proteoglycan NG2 is common to both fibroblasts and CNS pericytes, whereas PDGFRα is found in both fibroblasts and oligodendrocyte precursor cells [90, 92–94]. The use of transgenic mouse models, such as Col1a1-GFP mice [94] and PDGFRβ-CreER mice [95], has played a crucial role in differentiating fibroblasts, particularly through their collagen expression, highlighting differences in postnatal development and lineage compared to pericytes. These models have been pivotal in identifying the developmental emergence of Col1a1 + perivascular fibroblasts, suggesting distinct developmental paths from those of pericytes.

Common Markers exist between fibroblasts. Fibroblasts typically express markers related to the production of ECM components, such as Col1a1, indicative of their role in collagen production and structural support. Markers like PDGFRα and PDGFRβ are common in fibroblast populations, reflecting their roles in growth, repair, and fibrotic processes. Fibronectin and Laminin are commonly expressed in fibroblasts, contributing to their ECM structural matrix and interaction properties [15, 89, 90].

With the application of scRNA-seq to the study of CNS fibroblasts, meningeal fibroblasts in different compartments have been found to have transcriptional heterogeneity, suggesting unique roles for these cells during different developmental stages or regional specializations. As research progresses, new markers are discovered, and the understanding of fibroblast biology becomes more nuanced. Earlier studies might have focused on broad markers, while recent studies take advantage of advancements in technology to discover specific subtypes. In a study of human leptomeninges, human Leptomeningeal Fibroblasts characterized by high expression of Lama2 (laminin-alpha 2), Slc4a4 and Slc7a2 [96]. Meanwhile, in another study of adult mouse brain and meningeal Fibroblasts, adult mouse leptomeningeal Fibroblasts characterized by high expression of Lama1 (laminin-alpha 1), Slc7a11 and Slc47a1 [18]. This highlight both the evolutionary conservation of meningeal fibroblasts in maintaining core cellular functions and their divergence to meet species-specific physiological and environmental challenges. These molecular markers and fibroblast subtypes highlight the complexity and specialization of fibroblast populations within the leptomeninges, offering insights into their physiological roles and potential implications in disease contexts. At the same time, however, it should also be noted that there are large differences between the results of different transcriptomics studies on fibroblasts Possible reasons for this result include differences in fibroblast signatures from one study to another, limited by differences in methodology, the state of the cells studied, anatomical focus, and the natural heterogeneity within the fibroblast population.

Fibroblasts in the CNS play multiple critical roles [90]: contribute to the maintenance of tissue structural integrity and immune dynamics; they regulate structural expansion and promote neurogenesis during CNS development. Notably, their involvement goes beyond traditional structural roles to include functions like mechanosensation and facilitation of immune cell interactions. Similar to the role of stromal cells in other organs, CNS fibroblasts interact with macrophages, potentially influencing inflammation and immune responses. Perivascular and choroid plexus fibroblasts might offer structural support while modulating macrophage activity and immune responses through cytokine interaction. Moreover, perivascular fibroblasts play a critical role in regulating solute exchange and preserving the structural integrity of brain vasculature. Here, we will specifically focus on detailing the functions of meningeal fibroblasts.

Meningeal fibroblasts display diverse transcriptomic profiles and functions based on their specific locations within the meninges. These fibroblast subtypes are actively involved in vascular transport, cell-matrix adhesion, ECM organization, and cellular communication [96]. Notably, meningeal fibroblasts are essential for the CNS development, facilitating the structuring of the glia limitans and supporting neuronal migration. By interacting with immune cells and producing ECM, meningeal fibroblasts contribute to neurogenesis and immune regulation. Dural fibroblasts producing key elements for B cell development, such as Cxcl12, which interacts with CXCR4 expressed by early meningeal B cells. This relationship suggests that dural fibroblasts may support early B cell survival and differentiation through the CXCL12-CXCR4 axis [17]. And dural fibroblasts play a role in maintaining suture patency during cranial expansion to accommodate brain growth [97, 98]. However, the full role of fibroblast-like cells (FLCs) in dural function during adulthood remains largely unexplored.

Fibroblasts in the brain are crucial for their roles in fibrotic responses during injury and inflammation. After CNS injuries like spinal cord injury and TBI [6, 93, 95], fibroblasts proliferate and secrete ECM components, leading to fibrotic scar formation. These scars can impede axon regeneration and recovery following the CNS injuries. So, recognized as negative factors for recovery, fibroblasts offer potential therapeutic targets for managing CNS diseases. The potential of fibroblasts to transform into other cell types post-injury opens new therapeutic avenues, similar to successful tissue repair strategies in skin and liver. Current research focuses on reprogramming fibroblasts into functional cells, leveraging their plasticity for interventions targeting tissue repair and regeneration after injury diseases. In chronic inflammation, such as multiple sclerosis, fibroblasts play a role in forming tertiary lymphoid organs, influencing disease progression and neuroinflammatory responses [99]. In neurodegenerative diseases like Amyotrophic Lateral Sclerosis and Alzheimer's, fibroblasts exhibit altered expression patterns and secretions, thereby potentially facilitating disease advancement by modifying the immune environment [100].

CNS fibroblasts, with their unique roles compared to peripheral fibroblasts, are a promising research area, particularly through advanced techniques like spatial transcriptomics and lineage tracing. These methods are vital for understanding fibroblast diversity and their functional specialization. Though recent discoveries have advanced our knowledge, significant gaps remain in understanding their developmental biology and roles beyond fibrotic scarring. Exploring interactions with other CNS cells, such as astrocytes and microglia, is crucial for uncovering their roles in CNS signaling, maintenance, and disease treatment. As we delve deeper, fibroblasts may emerge as pivotal entities in the maintenance, defense, and repair of neural environments, redefining their significance in health and disease.

The skull-meninges interface: a novel paradigm in neuroimmunology

This review introduces a crucial structure in neuroimmunology: the skull-meninges channels. In primates, including humans, the diploe – the space between the outer and inner plates of the skull – contains a complex network of thin-walled diploic veins [101]. Studies in both rodents and humans indicate that this diploic space houses hematopoietic bone marrow, which harbors monocyte-dendritic cell progenitors (MDPs) and monocyte-committed progenitors (cMoPs), maintaining a local pool of monocytes [13, 14].

In 2018, researchers discovered small transosseous vessels connecting the skull and the meninges, linking the dural venous system with the diploic venous system [102]. The cranial cavity structures that surround these vessels are known as skull-meninges channels (Fig. 1A). Detailed observations of these structures have been made using advanced techniques such as microCT [13], two-photon imaging [7, 103], and vDISCO [9]. In the calvaria of adult mice, approximately 1,000 channels connect the dura with the calvarial bone marrow. These channels measure about 80–100 μm in length and 20 μm in diameter [13, 102]. Many immune cells in the dura come from skull and spine bone marrow, not blood. These cells likely travel along blood vessel surfaces and through tiny bone channels to reach the dura. The bone marrow in the calvaria and vertebrae plays a crucial role in supplying monocytes and neutrophils to the dura under normal conditions. Following brain injury or during neuroinflammation, it provides these cells to both the meninges and brain parenchyma via vascular tunnels connecting the bone marrow and dura mater. Further studies of bone marrow niches at the skull base have revealed similar channels for CSF tracer drainage and perivascular conduits, mirroring observations in the dorsal skull [104]. These findings underscore the extensive interconnectivity between the skull's bone marrow and the meninges, suggesting a novel pathway for immune cell trafficking and CSF circulation in the central nervous system. Notably, in some specific regions the cranial bone marrow is partly connected to the DALT, potentially providing immune cells such as B cells to this structure.

Recent scRNA-seq studies have revealed that the skull and vertebrae house both developing and mature immune cells, mirroring the composition found in the tibial niche. While these immune cells share similar transcriptomes across locations, subtle yet significant differences exist between immune cells from the calvaria and

tibia [104]. In cranial bone marrow, hematopoietic stem cells show downregulation of proliferation genes, while monocytes and macrophages exhibit reduced expression of genes related to reactive oxygen species production. Additionally, neutrophils display decreased expression of genes associated with myeloid cell differentiation. The skull-meninges channels enable direct communication between the skull and meninges, facilitating immune cell migration independent of systemic circulation. The origin of these cells may influence their function in CNS injury [105]. Cells derived from local bone marrow may play diverse roles depending on their developmental history and interactions with the stromal niche as they traverse the dura [14, 102, 104, 106].

Skull bone marrow: a unique source of immune cells in neuroinflammation

Recent studies have revealed important functional differences among monocyte subpopulations based on their origin. Blood-derived monocytes tend to exhibit pro-inflammatory characteristics, while bone marrow-derived monocytes from adjacent tissues show more regulatory properties. This distinction is particularly evident in experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis, suggesting that the source of these immune cells may significantly influence their function in neuroinflammatory conditions [107]. Interestingly, neutrophils from the cranial bone marrow show unique properties compared to those from other parts of the body. After SAH, cranial neutrophils are more likely to form neutrophil extracellular traps (NETs) than neutrophils from the femur. This increased potential for NETosis, a special form of neutrophil death with release of NETS, was observed in both healthy mice and those with SAH, suggesting an inherent difference in the function of cranial neutrophils [108]. New research indicates that immune cells from skull bone marrow migrate into the brain after injury, revealing a new role for cranial marrow in brain trauma inflammation. In TBI, researchers have observed a rapid shift in the behavior of hematopoietic stem cells in nearby cranial bone marrow [109]. These stem cells quickly differentiate into myeloid cells, likely as a response to the injury. Furthermore, mouse models of the CNS trauma have revealed the presence of myeloid progenitor cells in the meninges during neuroinflammation, which are absent under normal conditions. In cases of bacterial meningoencephalitis, an interesting pattern of monocyte recruitment has been observed. Dural monocytes are recruited from the adjacent cranial bone marrow through a mechanism that does not depend on the CCR2 receptor, which is typically involved in monocyte migration. The skull-meninges channels may have implications beyond normal immune function. Cancer cells, particularly those from breast cancer and melanoma, can potentially use this pathway to spread from bone to the meninges [110, 111]. This finding opens up new avenues for understanding and potentially preventing the spread of certain cancers to the CNS.

The skull-brain axis: CSF as a key mediator of neuroimmune interactions

CSF plays a key role in mobilizing immune cells from the cranial bone marrow. In stroke models, researchers found that the sympathetic nervous system does not significantly influence the distribution of immune cells within the cranial bone marrow [112]. Instead, CSF appears to be the primary signal directing myeloid cell migration from the bone marrow to the meninges, likely by carrying specific chemical signals that activate different regions of the skull bone marrow. Researchers found that injecting AMD3100, a drug that blocks the CXCR4 receptor, into the CSF promotes the movement of the skull bone marrow-drive monocytes and neutrophils into the dura [14, 104]. These cells tend to accumulate in the sinuses near the bone marrow niches. Similarly, applying AMD3100 directly to the cranial bone marrow through a thinned skull also encourages the exit of these immune cells. In a mouse model of bacterial meningitis, CSF entering the cranial bone marrow stimulated the proliferation of myeloid cells [103]. Human studies using advanced imaging techniques, such as MRI and TSPO-PET, have confirmed that substances injected into the CSF can indeed reach the cranial bone marrow [13, 113]. These findings suggest that following the CNS injury or infection, CSF can penetrate the cranial bone marrow niches and mobilize both stem cells and mature immune cells. Following injury, CSF enters areas of cranial infiltration and mobilizes immune cells. These cells can play various roles depending on the stage of disease progression, potentially promoting inflammation or protecting neural tissue.

CSF factors influence the migration of immune cells from the skull bone marrow niches to the dura. Moreover, CSF can flow through these channels into the bone marrow cavity, stimulating hematopoiesis [103, 104, 113]. This bidirectional flow creates a dynamic system where CSF both influences and is influenced by the immune cells in the cranial bone marrow. In summary, these findings highlight the complex interplay between CSF, cranial bone marrow, and immune cells in CNS health and disease. They underscore the unique properties of the cranial immune environment and its potential significance in various neurological conditions.

From a therapeutic perspective, the unique characteristics of the skull-meninges channels offer potential opportunities for drug delivery. Researchers are exploring the possibility of using these channels as a direct route for administering treatments from the skull's bone marrow

to the brain, like the intracranial pathway (ICO) [114, 115]. This intracranial pathway could provide a novel method for treating various neurological conditions, potentially bypassing the blood-brain barrier [114, 115]. These findings highlight the unique properties of skull-derived immune cells and their potential significance in CNS health and disease. The direct connection between cranial bone marrow and the meninges represents a paradigm shift in our understanding of neuroimmunology, offering new perspectives on immune surveillance and response in the CNS.

Fluid connections: CSF-mediated communication between the brain, meninges, and immune system

Cerebrospinal fluid (CSF) is primarily produced by the choroid plexus, with human choroid plexus epithelial cells secreting about 650 milliliters daily [80, 116]. CSF circulation follows the "glymphatic" system [80, 117, 118]. According to this theory, CSF from the SAS or the Virchow-Robin spaces enters the brain via arterial pulsation through aquaporin 4 (AQP4) water channels on astrocyte end-fee [119]. Blood vessel pulsation allows perivascular fluid and its macromolecules (within a specific size range) to diffuse from arterial perivascular spaces into the parenchyma, then be reabsorbed in the perivascular spaces. As fluid moves from arterial to venous perivascular spaces, it "washes" the parenchymal tissue, removing waste products. The perivascular space contains various cell types, including astrocytes, endothelial cells, vascular smooth muscle cells, pericytes, fibroblast-like cells, and leptomeningeal mesothelial cells [120].

CSF outflow occurs through multiple pathways: perineuronal drainage along spinal and cranial nerves, dural lymphatics, arachnoid granulations, skull-meninges channels, and parasagittal spaces in the dura [80, 103]. In addition, the nasal lymphatics and nasopharyngeal lymphatic plexus are also drainage routes for CSF [121, 122]. Together they provide drainage pathway from various intracranial regions to the deep cervical lymph nodes (dCLNs) (Fig. 2D). Immune cells, CNS antigens, and other macromolecules can exit the CNS by traversing the olfactory nerves, entering the nasal mucosa, and subsequently being reabsorbed by nasal mucosal lymphatics before draining into the deep cervical lymph nodes.

Beyond barriers: the multifaceted role of CSF in CNS immune surveillance and signaling

CSF serves as a primary medium for immune cell migration into the brain parenchyma. In normal conditions, the brain fluid contains few innate immune cells, with effector and memory T cells being the most common immune cells present [123]. However, the precise migration patterns of these cells remain to be fully elucidated. Observations have shown effector T cells extravasating from cortical vessels and the choroid plexus (ChP), as well as migrating from dural extracellular spaces through the arachnoid [34, 41]. The relative significance of these pathways may vary depending on the specific disease context. In a steady state, T cells in the CSF are transient. They exit via dural lymphatics to reach the deep cervical lymph nodes, and through transcribrosal lymphatics to access the superficial cervical lymph nodes. Effector T cells engage in continuous circulation between the meninges and CSF [123, 124]. During this process, activated T cells adhere to the leptomeninges, while non-activated cells are released into the CSF.

Interactions between the meninges and brain parenchyma may occur through the binding of cytokines, secreted by meningeal immune cells, and other CSF-borne molecules to specific receptors expressed by various cell types in the brain parenchyma. However, the mechanism by which cytokines penetrate the arachnoid membrane to enter the CSF remains unclear. Although the dura does not directly contact the brain parenchyma, cytokines derived from dural T cells can regulate neuronal activity [124–127]. Once cytokines from the periphery and meninges enter the CSF, they can be transported through perivascular pathways into the brain parenchyma. CSF circulation throughout the brain parenchyma, driven by arterial pulsation and vascular movement, can activate neurons either directly or through indirect pathways [25, 128, 129]. Neurons in both the central and peripheral nervous systems express cytokine receptors, enabling them to respond differentially to immune cell-derived cytokines. In indirect pathways, cytokines are recognized by glial cells, which subsequently release cytokines that can modulate neuronal function. Conversely, immune cells express receptors for neurotransmitters and neuropeptides—signaling molecules originating from neurons—allowing neuronal signals to influence immune responses [130, 131].

Bridging brain and immunity: the multifaceted roles of CSF in CNS immune function

Antigen recognition in CSF likely plays a crucial role in immune surveillance. The dura, given its strategic position in relation to CSF outflow and sampling opportunities, serves as a key site for this surveillance [16, 104, 132]. The flow of CSF to the dura occurs directly, as evidenced by studies using various molecular tracers. These tracers, including dextran of different molecular weights (10, 70, and 2,000 kDa), ovalbumin (45 kDa), and 0.5 μm polystyrene beads, all appear in the dura at comparable rates, regardless of their diverse molecular properties [82]. The composition of CSF is dynamic, changing in response to different physiological and pathological conditions. This variability allows the meninges, through continuous CSF sampling, to effectively monitor brain

content. Consequently, this sampling mechanism may provide valuable insights into ongoing brain activity.

The flow of CSF to the dura is critical not only for waste removal but also for antigen presentation, B cell negative selection, and communication with adjacent cranial bone marrow. These regional hubs may facilitate immune surveillance of soluble CNS-derived antigens in the CSF. Evidence suggests that CSF preferentially flows along dural sinuses towards the dura, allowing CNS-rich antigens to accumulate [120]. Antigen-presenting cells (APCs) associated with dural sinuses can capture these CSF antigens and present them to circulating T cells, thereby promoting effector function and tissue retention [16]. CSF can also be collected by intracranial lymphatic vessels and drained to reach extracranial lymph nodes and nasal tissues [3, 119, 133]. This mechanism allows brain-derived antigens to directly interact with the peripheral immune system.

Immune cells

Recent advancements in scientific tools have revolutionized our understanding of the immune landscape within the meninges, the protective layers surrounding the CNS. Techniques such as two-photon intravital microscopy, high-dimensional cytometry, and flow and mass cytometry studies have revealed a complex and diverse immune environment [43]. In particular, scRNA sequencing and spatial transcriptomics have provided unprecedented insights, leading to continuous new discoveries in this field [134]. The healthy meninges harbor a wide array of immune cells, including various types of innate lymphoid cells, B cells, T cells, monocytes, macrophages, dendritic cells, mast cells, plasma cells, and neutrophils [17, 43, 104, 134, 135]. These immune cell populations are dynamic, undergoing changes in diversity and composition during aging, inflammation, and neurodegenerative conditions. Interestingly, the distribution of these immune cells is not uniform across the meningeal layers. The outermost layer, the dura, exhibits greater immune cell heterogeneity compared to the inner layers (leptomeninges). The leptomeninges contain fewer cell types and numbers, primarily consisting of macrophages, dendritic cells, and mast cells.

The unique position of the meninges, surrounding and in close proximity to the CNS, makes them ideal for immune surveillance and defense of the CNS and its boundaries [2, 21, 136](Fig. 1A). This complex immune environment can influence CNS function in numerous ways, many of which are only beginning to be understood. Meningeal cells play crucial roles in maintaining the barrier functions of CNS-associated structures [21, 120]. They regulate the excretion of CNS antigens and control the exchange of metabolites between the brain and its environment. Moreover, the immune system within the meninges is essential for supporting brain homeostasis and plasticity. Meningeal cells and the cytokines they release affect various functions of the brain. These include short-term memory, sensory responses, cognition, social abilities, and spatial learning [24, 25, 137, 138]. This highlights the far-reaching impact of the meningeal immune system on higher brain functions, extending well beyond traditional concepts of immune defense. For a comprehensive discussion of the interactions between meningeal immune and non-immune cells with the central nervous system in both physiological and pathological states, readers are directed to several excellent reviews beyond the scope of this article [1, 21, 27, 28, 120, 139–141].

Future directions in meningeal research: structures, functions, and technological advancements

Recent advances in neuroimmunology, genetics, and imaging techniques have revolutionized our understanding of meningeal immunity. The skull-meninges-CNS interface is increasingly understood as a tightly interconnected structure, crucial for both CNS homeostasis and disease pathogenesis [1, 2, 21]. However, as this field rapidly develops, new questions continually emerge, often outnumbering the answers we've found. Looking ahead, several key areas require further exploration.

A primary focus should be on comprehensively characterizing the cells within the meninges. This includes determining their types, numbers, locations, and origins. The composition of immune cells in the meninges likely accounts for significant immunological differences between the meningeal layers [17, 43, 104, 134, 135]. These variations may influence brain cell activity and overall health in both normal and disease states. Therefore, accurately identifying cell types and subpopulations under physiological conditions is crucial for gaining deeper insights into meningeal function [15]. An intriguing question is whether non-immune cells, such as meningeal fibroblasts, play specific roles in immune processes [15]. Additionally, there may be yet undiscovered cell types within the meninges. Developing high-throughput techniques to precisely locate cells within the meninges and track their dynamic changes will be invaluable. Such methods will enhance our understanding of complex cell-cell and cell-matrix interactions in this unique environment. Understanding the origins of meningeal cells is another critical area of research. Cell types and numbers in the meninges are known to change during various developmental stages and in pathological conditions. These cells may originate from diverse sources, including the yolk sac, peripheral blood, or through local proliferation [14, 112]. Identifying the signals that drive cell migration and differentiation in the meninges will

provide crucial insights into meningeal development and function [14, 108, 112].

Discovering more meningeal structures. The field of meningeal research is rapidly evolving, with recent years witnessing the discovery of novel structures such as the SLYM, DALT, and ACE points. These findings prompt the question: are there more undiscovered meningeal structures around the CNS? Continued exploration in this area could reveal additional components crucial to our understanding of meningeal biology.

Elucidating the functional roles of the meninges under various physiological and pathological conditions. The immune cells retained at the CNS interface play a vital role in regulating immune surveillance and defense [10, 119]. However, many questions remain unanswered. We need to investigate the nature of communication between immune cells residing in the meninges and the brain. In pathological conditions where the barrier properties of the meninges are compromised, it's crucial to understand the factors controlling the spread of inflammation from the meninges to the brain parenchyma. Future investigations should elucidate the mechanisms by which the brain communicates with cells residing in the meninges and the hematopoietic niches of cranial and vertebral bone marrow [82, 102, 107]. The extent to which meningeal immunity affects human cognition and behavior is another intriguing area for investigation [138]. Additionally, we need to unravel how molecules produced in the meninges acquire or transmit information to the CNS parenchyma. An important step forward will be to evaluate whether the preliminary results of meningeal immunity observed in experimental models of neurological diseases are comparable to those in humans. This comparative analysis will be crucial for translating these findings into clinical applications.

The development of more reliable tools and animal models. These will enable precise and elegant tracking of cell evolution in the meninges. Cutting-edge techniques such as single-cell transcriptomics [18, 96], spatial transcriptomics [19], and the emerging field of 3D spatial transcriptomics [142], along with other new tools, will provide a more comprehensive and precise understanding of meningeal biology. 3D spatial transcriptomics hold particular promise in overcoming current limitations, allowing for precise localization of cells within brain and the meningeal structures, offering unprecedented insights into the spatial organization of the meningeal immune landscape.

Finally, **developing more effective means of drug delivery and transport**. The challenge of drug delivery across the meningeal barrier remains a significant hurdle in treating CNS disorders, Future research should focus on [115]. As we advance in these research directions, we stand to gain a more nuanced and comprehensive understanding of the meninges, their structures, and their critical roles in CNS health and disease. This knowledge will be instrumental in developing new therapeutic strategies and improving our approach to CNS-related disorders.

Conclusion

Recent research has unveiled a complex array of specialized structures at the meningeal interface of the CNS. These include vascular meningeal structures such as the dural venous sinuses and the skull-meninges channels. Meningeal structures characterized by lymphatics, such as DALT, and the nasopharyngeal lymphatic plexus. And non-vascular or non-lymphatic related structures, such as ACE points, arachnoid granulations. These newly identified structures form an intricate network that supports CNS function, facilitates immune surveillance, and regulates CSF dynamics. By enabling the movement of cells and molecular factors across the CNS boundary, they are key players in maintaining CNS health and mediating immune responses. The insights gained provide new perspectives on neuro-immune interactions and open avenues for future research.

Abbreviations

AB	Arachnoid barrier
ACE	Arachnoid cuff exit points
AGs	Arachnoid granulations
AJs	Adherens junctions
APCs	Antigen-presenting cells
AQP4	Aquaporin 4
BBB	Blood-brain barrier
BCR	B cell receptor
BMB	Blood-meningeal barrier
CGRP	Calcitonin gene-related peptide
ChP	Choroid plexus
cMoPs	Monocyte-committed progenitors
CNS	Central nervous system
CSF	Cerebrospinal fluid
DALT	Dural-associated lymphoid tissues
dCLNs	Deep cervical lymph nodes
EAE	Encephalomyelitis
fMOST	Fluorescence micro-optical sectioning tomography
ICO	Intracranial pathway
MCA	Middle cerebral artery
MDPs	Monocyte-dendritic cell progenitors
mLVs	Meningeal lymphatic vessels
MMA	Middle meningeal artery
NETs	Neutrophil extracellular traps
PACAP	Pituitary adenylate cyclase-activating polypeptide
PSS	Parietal sinuses
SAH	Subarachnoid hemorrhage
SAS	Subarachnoid space
scRNA-seq	Single-cell RNA sequencing
SLYM	Subarachnoid lymphatic-like membrane
SS	Sigmoid sinuses
SSS	Superior sagittal
TBI	Traumatic brain injury
TJs	Tight junctions
TS	Transverse sinuses
vDISCO	Nanobody(VHH)-boosted 3D imaging of solvent cleared organs
wildDISCO	Immunolabeling of wildtype mice and DISCO clearing

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Author contributions
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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Competing interests
The authors declare no competing interests.

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