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Incorporating meningeal immunity into vaccine development

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Manisha Menon[1], Colin N. Haile[2] & David J. Dowling[1,3]

Meningeal immunity, a less-studied aspect of neuroimmune interactions, has recently come under the spotlight owing to the characterization of meningeal lymphatic vessels draining of the central nervous system (CNS). This changing landscape has begun to impact how vaccinologists and immunologists approach early pre-clinical vaccine development, including decentering the antigen, with a greater focus on the role of formulation science and route of delivery. Here, we review the current state of how concepts emerging from meningeal immunity are being incorporated into modern vaccine innovation, discovery, and development.

The multi-layer meninges is an immune rich compartment

The meninges, a multi-layer of membranes – the pia mater, arachnoid mater, dura mater and the more recently discovered subarachnoid lymphatic-like membrane (SLYM)[1] surround the brain and spinal cord, encompassing the cerebrospinal fluid[2,3]. While the brain parenchyma is made up of two types of brain cells, neurons and glial cells, with some evidence also of tissue resident memory T cells (T_{RM}) to fight potential viral infections[4], the meninges contain a wide repertoire of immune cells[2,5]. These constitute meningeal immunity, which is primarily concerned with immune surveillance of the CNS, and are also involved in postinjury CNS recovery[6], protection from viral[7] and fungal infections[8] and even higher brain function[9,10]. As such, the meninges is a potential target for current and future research employing active immunization.

Brief overview of tenets of meningeal immunity

The meninges is a multi-layered membrane that covers the brain and is rich in immune cells like antigen-presenting cells, as well as T and B cells[2,11,12]. The outermost layer closest to the skull is the tough membranous dura mater, which contains dural sinuses – capillaries that carry blood from brain to heart. The middle layer, arachnoid mater, holds cerebrospinal fluid (CSF) within the sub-arachnoid space. Pia mater is closest to the brain parenchyma and is a highly vascularized membrane. The arachnoid and pia mater together make the leptomeninges ('thin' membrane). The outermost dura mater and the underlying subdural layers of the meninges have a rich subset of immune cells – macrophages, but also dendritic cells, monocytes, T cells and B cells. The macrophages called border associated macrophages (BAMs) showed self-renewal capacity following depletion[13]. Apart from dural and subdural meningeal layers, BAMs are also present at choroid plexus, another brain border tissue.

The meninges came to the forefront as an immune-rich environment with potential to be modulated for vaccine development and therapeutics with the re-discovery of lymphatic vessels in the dura[14,15]. Even though presence of a lymphatic system was proposed in the meninges 200 years ago, it was widely discredited, and hence the more recent re-discovery made the scientific community look at the meningeal layers, especially the dura, as an immune hub[2,5].

Unlike the brain parenchyma, meningeal layers - especially the dura mater vasculature - are semi-permeable to solutes, small molecules and also immune cells, permitting efficient immune surveillance. The leptomeningeal vessels in the subarachnoid space are made up of endothelial tight junctions, whereas dural sinuses are fenestrated and are more permissive to CNS antigens, immune cells and the occasional pathogen[2,7,16], potentially explaining the presence of antibodies at the dural sinuses to protect against pathogens[8]. The blood-meningeal barrier (BMB) is a semi-permeable barrier where meningeal blood vessels allow CSF antigens through, enabling sampling and presentation by antigen-presenting cells (APCs) at the dural sinuses to be recognized by patrolling T cells. Studies have shown that T cells at dura[5] and deeper leptomeningeal spaces[17] eventually flow to deep cervical lymph nodes via dural lymphatic drainage[14,15]. T cells at

[1]Precision Vaccines Program, Boston Children's Hospital, Boston, MA, USA. [2]Department of Psychology/TIMES, University of Houston, Houston, TX, USA. [3]Department of Pediatrics, Harvard Medical School, Boston, MA, USA. e-mail: david.dowling@childrens.harvard.edu

meninges can have pro- and anti-inflammatory implications – a study in rats that show autoimmune CNS lesions form when T cells at leptomeningeal vessels encounter phagocytes presenting myelin antigens, thereby triggering release of pro-inflammatory cytokines[17], and evidence of Interleukin 4-producing (IL4-producing) T cells that accumulate at the meninges, maintain an anti-inflammatory milieu for myeloid cells and maintain memory and cognition in mice[9,10].

Recently, a fourth meningeal membrane has been discovered in the subarachnoid space in mice and humans, called SLYM (Subarachnoid Lymphatic-like Membrane)[1]. The authors postulate that SLYM contains myeloid cells, CD45$^+$ cells, a lymphatic system and structurally and functionally controls flow of CSF solutes across a low permeability barrier membrane within SLYM. Further research however would be needed to understand more about this fourth layer and its qualifications as a bona fide meningeal layer before the established dogma can be updated. It is not fully understood whether SLYM has its own lymphatic system and whether it can be classified as distinct from the arachnoid mater[3].

Glymphatic system and meningeal lymphatics

The CNS is largely separated from the peripheral lymphatic system, however brain interstitial fluid (ISF) has an efficient waste disposal system which is essential for healthy brain function. A glial lymphatic ('glymphatic') system within the brain drains ISF to the CSF in the subarachnoid space. From the subarachnoid space, CSF eventually reaches the deep cervical lymph node, and a dural lymphatic system is involved in the process potentially presenting antigens in CSF to the immune compartments[14,15,18–21]. There is evidence that CSF from the subarachnoid space can cross directly into the dura across the arachnoid mater along veins linking to dural sinuses that act as 'hot spots'[22]. Meningeal lymphatic vessels at the skull base also drain CSF[18]. Dysfunction in meningeal lymphatic flow can cause neurodegenerative effects. Indeed, age is a common risk factor for neurodegeneration and lymphatic flow disruption, potentially leading to age-related cognitive decline and disease pathologies like Alzheimer's disease (AD), as shown in mice[23].

Current role of vaccination route and delivery systems in meningeal immunity

How immune cells reach the meninges is an intriguing question. Evidence shows that T cells and plasma cells at the dural venous sinuses associate with systemic vasculature and travel to the dura from distal sites[5,8]. Other cell subclasses, like myeloid cells, may originate from skull bone marrow niches proximal to the dura. The following sections detail various routes that populate the meninges with immune cells and can be harnessed for development of potential meningeal vaccination strategies (Fig. 1). Different indications for meningeal vaccination such as protection from pathogens, substance misuse and age-dependent neurodegenerative diseases like AD, by harnessing the dural lymphatic and vascular networks and meningeal immune cell populations, are reviewed later.

The gut-meningeal axis

Several studies have shown plasma cells localized along the dura and brain originate in the gut. For example, Multiple sclerosis (MS) is an autoimmune inflammatory disease whereby the presence of gut-derived IgA$^+$ plasma cells (PCs) in the CNS reduces incidence of experimental autoimmune encephalomyelitis (EAE) in a mouse model that is Interleukin-10 (IL-10)-dependent[24]. Further, MS relapse in human patients is associated with gut microbiota-derived IgA$^+$ B cells with an accompanying upregulation of *IL-10* expression in B cells in the CNS[25]. Post-mortem analysis of human brains demonstrated that gut microbiota-derived IgA$^+$ B cells are co-localized with active MS lesions, suggesting a link between gut microbiota and autoimmune diseases.[25]. It is possible that IgA$^+$ B cells play an immune regulatory role in an IL-10-dependent manner to temper MS-associated neuroinflammation. Immune cells like IgA-producing plasma cells position themselves at the dural sinus junctions, where they prevent entry of pathogens into the brain, for example the fungal pathogen *Candida albicans*[8]. The meningeal B cells here were clonally similar to intestinal B cells, suggesting a gut origin for the plasma cells at the dural sinuses. It is unclear whether the IgA-producing B cells at the dura differentiated at the gut and migrated to the dural spaces, or whether the plasmablasts differentiated into plasma cells at the dura. However, this and other studies linking gut, diet, immune cells[26] and specifically central nervous system (CNS) immune cells[24,25] suggests that an oral vaccine can potentially generate immune subsets that originate at the gut and target to the dura mater and protect against pathogens. The dura mater sinuses are fenestrated and have reduced blood flow rate, potentially increasing vulnerability to pathogen entry near the brain and hence an evolutionary connection between gut-sourced pathogens and immune cells at the dura mater might have developed.

T cells and blood-meningeal axis

T cells are also a distinct population whose origin at the meninges is not fully understood. There is, however, some evidence of the cells originating in systemic circulation and traveling to the meningeal spaces[2,10].

Direct visualization of meningeal whole mounts in mice by confocal microscopy shows almost half the CD3$^+$ T cells at the dural sinuses interact with APCs[5]. APCs capture circulating CSF antigens and present them to patrolling T cells at the dural sinuses. With regards to T cell immunity at the meninges, there appears to be conflicting observations on inflammation. In a Lewis rat model of EAE (Experimental Autoimmune Encephalomyelitis), CD4 T cells specific to myelin basic protein (MBP) can travel along the leptomeningeal vessels in the pia mater and interact with antigen presenting cells presenting CNS autoantigens, initiating autoimmune reactions[17]. However, there are also studies that show anti-inflammatory effects and improved cognitive function for interleukin-4 (IL-4) producing meningeal CD4 T cells[9]. Spatial memory tests using the Morris Water Maze led to an accumulation of IL4-producing CD4 T cells in meningeal spaces that may play a role in hippocampal-dependent spatial learning. These CD4 T cells can ameliorate inflammatory effects of myeloid cells, and T cell depletion in the meninges (as shown by FTY720 treatment) enhances a pro-inflammatory milieu. Hence, Th2 cytokines Like IL-4 and IL-10 (produced by plasma cells)[24] appear to have an anti-inflammatory effect in the CNS and vaccine design can, via use of adjuvants for example, try to tailor the desired immune response needed. Furthermore, there appears to be uneven involvement of the various meningeal layers and T cells in autoimmunity as shown in mouse models of CNS autoimmunity and patients with MS[27]. Indeed, T cells at the leptomeninges were more activated as compared to T cells at the dura and antigen-presenting cells presented antigens less efficiently like myelin and other autoantigens at the dura.

Skull bone marrow origin and homeostasis

There is ample evidence that immune cells at the meninges can also originate much closer to home – at bone marrow niches in the skull, where progenitor cells produce myeloid populations and B cells in the meninges under homeostatic conditions, and after CNS inflammation and injury[6,12,28,29]. A calvarial (bone flaps overlying the brain) bone marrow origin for meningeal immune cells was shown using wild-type (WT) and GFP-UBC (Green fluorescent protein under human ubiquitin C promoter) mice[6]. Parabiotic pairing of the two strains showed that even 60 days after joining the circulatory systems, many myeloid cells in the WT meninges had non-GFP origins, suggesting very low infiltration of the WT meninges with systemic circulation-derived myeloid population. Furthermore, bone-flap transfer (containing calvarial bone marrow) of GFP mice to WT mice directly showed GFP-positive

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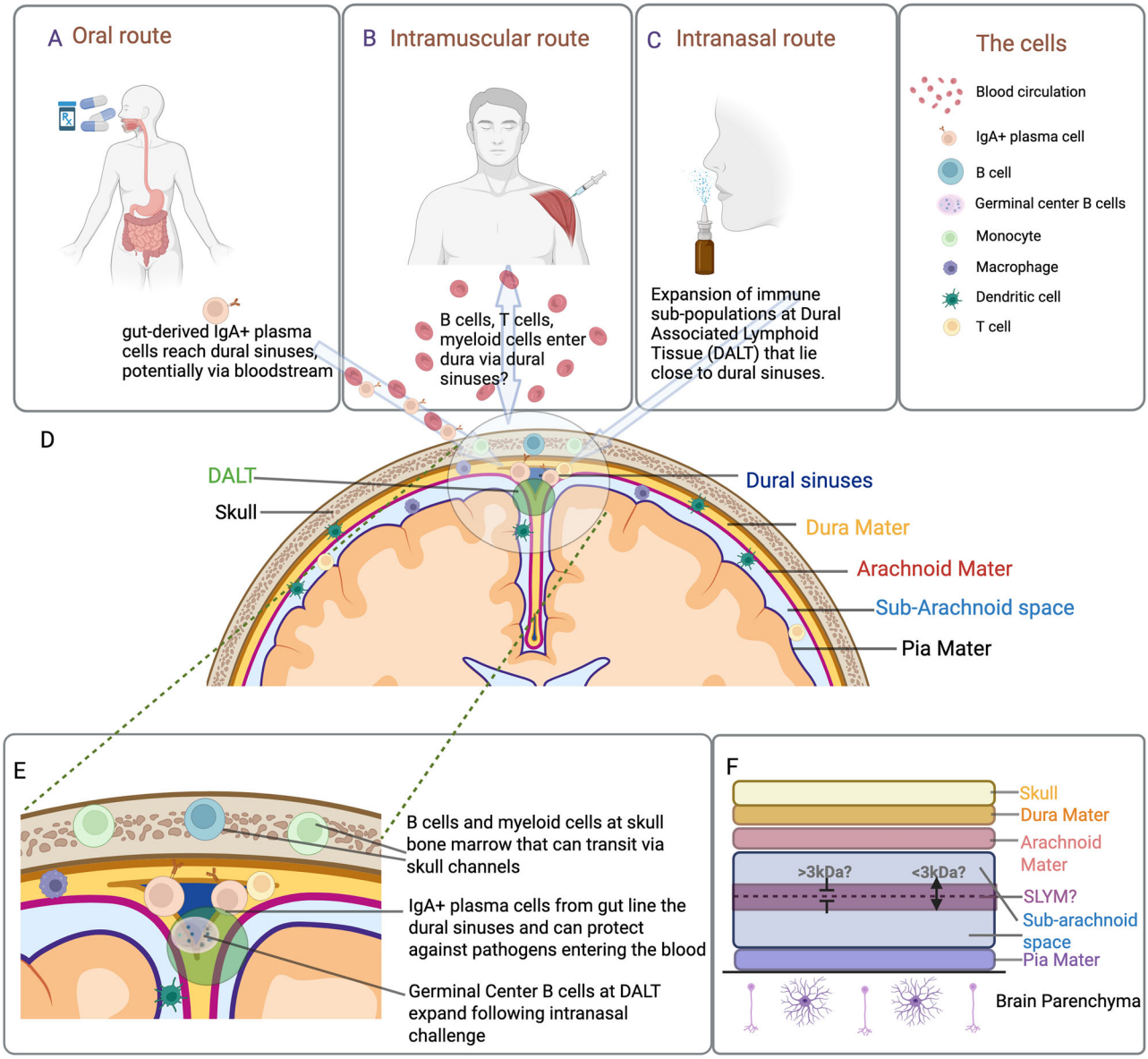

Fig. 1 | How various vaccination routes can potentially influence different immune subpopulations at the meninges. The figure above goes over three vaccination routes: Intramuscular, oral and intranasal. Success of an oral route of vaccination (**A**) would depend on formulation and ability for immune cells to cross the gut mucosa into the bloodstream. IgA+ plasma cells that get activated in the gut can patrol dural sinuses and protect the brain. Antigens presented via an intramuscular injection (**B**) can reach the meninges via the bloodstream. An intranasal route of vaccination (**C**), potentially via a nasal spray, can cause expansion of germinal center B cells following infection or potentially vaccination, at DALT that lie in close proximity to dural sinuses and the skull bone marrow. This can be beneficial for vaccines against opioid overdose to generate anti-fentanyl IgA+ plasma cells at the meninges. **D** The immune repertoire at the meninges, with a zoomed in section (**E**) that shows myeloid and B cells at the skull bone marrow, IgA+ B cells at the dural sinuses and germinal center B cells at DALT. There is also evidence of T cells at dural sinuses. **F** Schematic showing the layers of the meninges, including the newly discovered SLYM (Subarachnoid Lymphatic-like Membrane). SLYM is hypothesized to be separated into two subarachnoid membranes which limits transfer of CSF solutes above 3 kDa. Created in BioRender. Menon, M. (2025) https://BioRender.com/ mfhvrp8.

myeloid cells in the WT dura mater. The local presence of these cells means that during a CNS or spinal cord injury – using optic and spinal nerve crush injury models – monocytes and neutrophils can be rapidly deployed to the meninges and CNS parenchyma[6]. Preferential recruitment of neutrophils from skull bone marrow versus tibial bone marrow following murine models of stroke and meningitis also suggest skull bone marrow providing the immune cells needed for repair following cranial injury[29].

Presence of skull channels and the transit of CSF via these channels to skull bone marrow, shown via intracisternal injection of fluorescent tracers in mice[30] might facilitate timely access of CNS antigens by immune cells. Intracisternal injection of GFP+ *Streptococcus pneumoniae*, clinically dominant cause of bacterial meningitis, led to travel of the bacteria into the calvaria bone marrow[30], suggesting a path by which any potential CNS danger signals can be presented to the bone marrow for immune cell deployment.

Following a viral infection like COVID-19, patients with long COVID had persistently high levels of Spike protein in the skull bone marrow[31]. Acute COVID-19 patients also had elevated levels of Tau in the CSF, suggesting a link between COVID-19 and neuroinflammation. Controlled mouse studies with SARS-CoV2 infection showed development of a neuroinflammatory milieu in the skull marrow, meninges and brain cortex. Interestingly, immunization of mice with the mRNA Cominarty vaccine from BioNTech/Pfizer led to reduced accumulation

of Spike protein in multiple tissues, including the brain and skull bone marrow,[31], suggesting a potential link between intramuscular vaccination routes and control of neuroinflammation.

Even though a stronger focus of study in the field has been on bone marrow in the dorsal part of the skull, lymphatic vessels at the base of the skull also drain CSF[18]. It is important to note here that meningeal layers exist beyond covering the brain and also extend down the spinal cord[2]. Also, more research on skull bone marrow and meningeal immunity in humans need to be done to better understand and design vaccination strategies.

Summary of meningeal immune cells' routes and origins

With T cells and IgA-positive plasma cells that migrate from distal sites and more local-origin myeloid cells and B cells that can sample CNS-derived antigens, the meninges potentially holds a unique and important position – defending the CNS from outside dangers like pathogens and CNS injury, and 'dangers' within the body like neuroinflammation. Various types of immune cells involved in different roles – BAMs at dura against viral infections[7], myeloid cells derived from skull bone marrow mobilized following CNS injury[6], IgA+ plasma cells at dural sinuses defending CNS against fungal infections[8] and meningeal CD4 T cells producing IL-4 to promote memory and cognition[9], to name a few – can be harnessed to generate immune response following vaccination. Discovery of a dural lymphatic system[14,15] that drains macromolecules from the brain – via CSF – into deep cervical LNs, suggest a route by which both CSF antigens from brain and pathogenic antigens from distal sites that arrived via the dural sinuses, can be presented at secondary lymphoid organs. How the CSF traverses across the meningeal layers is not fully understood. A recent study in mice showed that CSF from the subarachnoid space can cross directly into the dura across the arachnoid mater, but through 'hot spots' along veins linking to dural sinuses[22]. From there, they drain to deep cervical LNs. With the dural sinuses also being locations where IgA+ plasma cells localize[8,32] and T cell-APC interactions[5] can occur and being dubbed 'immune hubs'[5], this evidence of CSF drainage via the dural sinuses where it can potentially be sampled by nearby immune cells, is an interesting discovery. Whether such meningeal dural 'hot spots' exist in humans remains to be determined.

Though not yet extensively studied, vaccination can potentially influence development of a robust immune repertoire at the meninges. In mouse studies assessing the effects of an adjuvanted (dmLT or LTA1) conjugate vaccine (CRM^{197}-FEN) against fentanyl, sublingual and intranasal delivery showed significant anti-fentanyl IgG and IgA in the serum[33]. Whether this adjuvanted conjugate vaccine leads to increased anti-fentanyl immunoglobulins in the meninges remains to be determined. Even though the authors didn't look at meningeal IgA, we speculate that increased serum IgA via mucosal routes of immunization may correlate with increased mucosal IgA at the meninges. Challenge with nasal antigens have recently shown to cause expansion of immune aggregates at dural sinuses[34].

Routes of vaccination and meningeal immunity

Based on current knowledge in the field, we offer here an evidence-based discussion on how three different vaccination routes – intramuscular (IM), intranasal (IN) and oral – can potentially influence meningeal immunity (Fig. 1).

Oral route

Studies linking the gut and meninges would form the basis for development of an oral vaccine. One strong indication would be against pathogens that may inadvertently be ingested, especially in immune vulnerable populations like infants and children. An oral route may be easier to administer than an injection in younger populations. IgA+ plasma cells derived from the gut line the dural sinuses in mice under homeostatic conditions and prevent entry of blood-borne pathogens[8]. Even though this data postulates a general shield-like protection by IgA +plasma cells at CNS portals like the dural sinuses where blood flow is slower[35], a similar mechanism could be employed to also generate antigen-specific antibody responses that reach the meninges from gut after oral routes of vaccination, potentially via the bloodstream[24]. Interestingly, there is evidence of gut-derived IgA+ plasma cells in the CNS[25] that produce IL-10[24] and reduce neuroinflammation in models of EAE in mice and MS in humans. It remains to be determined if vaccination strategies against autoimmune diseases can employ the oral route, even though this may make the case for a link between diet and management of autoimmune disease symptoms[36].

Intramuscular route

Injection of a vaccine via the muscle reaches the intended target via the bloodstream. Whether the antigens via intramuscular route can enter circulation, transit via the gut for antigen priming and then reach the dura via sinuses, remains to be determined. There is evidence a fungal pathogen when injected intravenously in mice, increased gut-derived IgA+ plasma cell accumulation over homeostatic baseline at the dural sinuses and protected against fungal infection of the brain[8]. Moreover, systemic Lymphocytic choriomeningitis virus (LCMV) infection in neonates reaches dura and eventually the brain via dural perisinus regions[7], suggesting a direct entry to the dura from systemic circulation in conditions that lack mature MHCII+ macrophages[37], like in neonates. It may be possible to harness this route for delivery of vaccine antigen to dural immune cells for priming against CNS pathogens that can infect neonates. Further, intravenous injection of vesicular stomatitis virus (VSV) in mice activates immune subpopulations in the meningeal dura, called Dural associated lymphoid tissue (DALT)[34].

Intranasal route

Evidence shows that immune populations are activated at the dura following intranasal antigen challenge in mice. DALT are immune cell clusters at dura, the most prominent of which are present at the rhostral rhinal hub of the dura[34] closely interlaced with fenestrated blood vessels, skull bone marrow and another venous hub at the skull base called rhostral-rhinal venolymphatic hub. Following intranasal VSV challenge, there is expansion of germinal center (GC) subsets and somatic hypermutation at the dural rhostral rhinal hub. More research, however, is needed to validate if intranasal vaccine strategies can also efficiently target human DALT across human diseases.

Incorporating meningeal immunity into vaccine development for specific groups

Role of adjuvants and vaccines against substance use disorders

Adjuvants when included as part of the formulation can improve innate and adaptive immune responses elicited by vaccines[38–40]. Adjuvanted formulations are ideal to boost response in target populations with sub-optimal immune responses (newborns, older adults, those with metabolic diseases, diabetes, and cancer)[40–42]. However, while enhancing immunogenicity, it is equally important to maintain localized antigen presentation, to avoid systemic effects of the vaccine and to provide potential added benefits that some vaccines have via the depot effect. To this end, an efficacious vaccine formulation will have a targeted route of administration for controlled antigen presentation. Across different routes of administration, vaccines can harness the unique benefits of distinct immunities mounted by targeted tissues. An optimal vaccine formulation takes into account target tissues, route and schedule of administration and optimal combination of antigen and adjuvant. Most current adjuvantation strategies utilize particulate formulation delivery systems and/or agonists for patten recognition receptors (PRRs). To influence meningeal immunity, nociceptors modulation, known to naturally

BOX 1
Meninges and nociception

Nociceptors are peripheral somatosensory neurons whose activation causes pain. They communicate with immune cells in meninges via neuropeptides. Following infection of mice with *S. pneumoniae* and *S. agalactiae*, bacteria reach the meninges and brain over several hours. The bacteria cause release of the neuropeptide CGRP from meningeal nerve endings, activating trigeminal nociceptors and causing pain as a physical response to the infection. In mice lacking receptors for the neuropeptides, bacterial invasion was mitigated. Furthermore, scRNAseq analysis of $CD45^+$ cells from dura mater showed an upregulation in macrophage populations in bacterial infected mice versus non-infected, suggesting a role for macrophages in protection against bacterial invasion[62]. As such, neuropeptide modulation may become an additional mode to influence meningeal immunity. Further research may, however be needed to integrate nociception and meningeal immunity.

occur via neuropeptides, may become an adjuvantation modality (Box 1).

As a crucial hub for myeloid and lymphoid immune cells near the brain, the meninges has great potential for immune modulation via vaccine adjuvants. Its proximal location to nasal passages could prove the meninges to be an ideal candidate for vaccine formulations that target mucosal surfaces. A crucial potential application is protection against substance use disorders, where IgA is implicated as a correlate of protection, particularly against fentanyl (FEN)[33,43] and cocaine[44]. In mouse studies with FEN-derived haptenated vaccines adjuvanted with *E Coli* heat-labile toxin derivatives dmLT and LTA1, intranasal boosting of IM-primed immune responses increased anti-fentanyl IgG and IgA in the serum[33]. However, ELISPOT assays showed only anti-fentanyl IgG-antibody secreting cells (ASCs), and not IgA ASCs in the tibial bone marrow, suggesting a non-tibial bone marrow source for the anti-fentanyl IgA. Due to IgA's protective role in the meninges and CNS against infection and inflammation[8,24], vaccine formulations that improve IgG subclasses and IgA responses is a viable target. To tie these observations to pharmacological results, in FEN challenge studies in mice[33] and rats[43], vaccination with adjuvanted anti-FEN vaccines successfully prevented FEN entry into the brain. Targeting mucosal routes of vaccine delivery - like intranasal, sublingual and oral - for meningeal vaccines can be an effective strategy. It is possible that protection from FEN challenge is from IgA and IgG not necessarily located at the meninges. However, other studies have shown presence of IgA at the dural sinuses in mice that protects against pathogens, like the protection of CNS against intravenous injection of *Candida albicans* - a fungal pathogen that causes meningoencephalitis in neonates and immunocompromised individuals[8]. The potential of IgA accumulating at the meninges and guarding the venous sinuses, against an intravenous route of FEN entry into the body, is an attractive possibility. Even for an intranasal route of FEN entry, an intranasal vaccine that lines mucosal surfaces with anti-FEN IgA may likely be protective[33]. Antibody conjugates against small molecule stimulants like cocaine and nicotine and opioids like FEN can potentially prevent the molecules from crossing the blood-brain and blood-meningeal barriers[33,43–45] by generating drug-specific antibodies[33,44,45]. A big challenge will be to understand how and whether the meninges and IgA levels in humans play a role in vaccine-induced protection against substance use disorders. Studies in non-human primates have looked at IgG levels in serum, but not yet IgA[46,47].

Due to the close proximity of the meninges to the brain, antigen-specific T cells and antibodies generated by a 'prime' vaccine dose can potentially reach the meninges via the systemic circulation and CNS lymphatics, and be boosted by a subsequent dose, especially a mucosal route of delivery like intranasal[48]. Such a heterologous combination of vaccine delivery, could theoretically activate distinct adaptive immune subsets at or near the meninges.

CNS tumor therapy

Meningeal lymphatic vessels (MLVs) are present on the dorsal and basal part of the dura and act as a conduit of immune cells between CNS and deep cervical lymph nodes (dCLNs)[18]. An interesting emerging application of meningeal lymphatics modulation is in control of intracranial tumors like glioblastoma) by enabling fluid drainage to CLNs and immune cell trafficking to the tumor microenvironment[49–51]. In mouse models of glioma and melanoma, MLV remodeling was observed by RNA-seq analysis showing changed expression of genes involved in lymphatic drainage and immune function[51]. Vascular endothelial growth factor (VEGF) treatment can enhance MLV drainage, potentially increasing accessibility of tumor antigens to dCLNs[49]. Intact MLV is required for transport of immune cells like dendritic cells from the tumor site to DLNs and for trafficking of cytotoxic CD8 T cells (CTLs) (Box 2) from DLNs to the tumor, as shown by MLV laser ablation which prevented CTL recruitment even in the presence of anti-PD1/CTLA-4 anti-tumor therapy[51]. Hence, modulation of dorsal meningeal lymphatics can open new therapeutic venues for CNS tumors.

Infectious diseases

Meningeal immune cell composition and localization can change with age, which can potentially protect against pathogens that infect the brain[7,37]. The meninges, as an immune-rich barrier tissue overlying the CNS, can protect against viral and bacterial infections from reaching the brain. BAMs in the dura are identified by their expression of MHCII and CD38[52], and are involved in innate immune protection of the CNS. Evidence suggests age-specific roles for $MHCII^{hi}$ BAMs in protecting the CNS from viral infection[7]. Indeed, in neonate mice, absence of these macrophages at the meningeal dural sinuses render them more susceptible to infections like meningitis. An 'immune armor' of $MHCII^{-hi}$ mature macrophages that are recruited from the blood stream into the dural sinuses of the meninges in adult mice can protect against systemic viral infection of brain and CNS[7], via secretion of the cytokine interferon γ. This innate immune system-mediated protection against CNS infection can be protective before antigen-specific adaptive immunity is initiated. Further evidence suggests that MHC-II+ meningeal macrophages (present in adult mice, but not neonates) are needed to protect against fatal viral meningitis caused by lymphocytic choriomeningitis virus (LCMV)[37]. Knowledge of the difference in immune cell makeup at the dura in young versus older mice can help instruct future research to overcome age-related immune insufficiencies to fight deadly infections like meningitis in children. Results from various antigen administration routes are also helpful to instruct future vaccination strategies against infectious diseases that affect the CNS. CSF drains via several channels[30] that connect the glymphatics to the skull bone marrow and eventually drain to cranial lymph nodes. Injection of a GFP+ strain of *Streptococcus pneumoniae* into cisternae magna of mice showed time-dependent development of GFP signal in the skull bone marrow via skull channels, suggesting that CSF containing CNS antigen can prime immune cells at the skull bone marrow[30]. This potential immune cell priming can be exploited for vaccination strategies inducing CNS antigen-primed plasma and myeloid cells present in the skull bone marrow, which can be deployed faster than immune cells from distal tibial bone marrow. More studies are needed to elucidate potential meningeal vaccination strategies against infectious diseases in humans.

BOX 2

CD8⁺ T cells and anti-tumor responses at skull bone marrow

Anti-tumor CD8⁺ cells discovered in cranial samples of newly diagnosed glioblastoma patients[63] were clonally similar to CD8⁺ T cells in the adjacent cranial bone marrow, suggesting that a tumor-specific immune response developed locally. Additionally, higher fraction of CD8⁺ effector and T_{EM} cells at the cranial bone marrow expressed Sphingosine 1 Phosphate Receptor (S1PR1), a T cell egress marker, as compared to T cells at the distal bone marrow, suggesting the more local-to-the-tumor bone marrow at the skull may deploy anti-tumor CD8⁺ T cells first. This localized recruitment of effector CD8 + T cells can potentially compensate for T cell dysfunction observed at distal bone marrow in glioblastoma patients[64], suggesting a need to focus on cranial bone marrow T cell populations for cranial-specific anti-tumor responses.

Age-associated neuroinflammation and Multiple sclerosis

Changes in meningeal lymphatics have been linked to neuroinflammation with age[23], which could in turn influence localization of immune cells at the meninges. Confocal imaging and CyTOF have shown more CD3 + T cells in old dura mater versus young dura mater. T cells in older mice, however, were more in dural non-sinus sites than at dural sinuses, suggesting a parenchymal origin rather than vascular, whereas in younger mice, T cells localize to the dural sinuses, and are present in systemic circulation[5]. Hence, homeostatic T cell migration appears impacted in older mice and it will be interesting to determine if this has implications in neurodegenerative diseases that worsen with age.

B cells at meninges can arise from bone marrow niches in the skull[28] or from the bloodstream[8]. With aging however, the number of blood-derived antigen-experienced B cells infiltrating the meninges increases, as shown by single-cell RNA sequencing (sc-RNAseq) which identified a B cell population called 'age associated B cells (ABCs)' in the dura mater and brain, which was clonally similar to antigen-experienced B cells in blood of aged mice[28]. The existence of this population may also have implications in age-associated neuroinflammation. Disrupted meningeal lymphatic flow have been linked to development of age-associated neuroinflammatory conditions like AD[53,54]. Removal of beta amyloid plaques via efficient glymphatic and dural lymphatic drainage is critical, and the disruption in this can exacerbate AD etiology[55]. Immune therapy for AD using monoclonal antibodies against amyloid beta (Aβ) aggregates have produced mixed results in the past[56]. In a murine model of AD called 5xFAD mice where meningeal lymphatics deteriorate with age and cause accumulation of Aβ, therapeutics that combine monoclonal antibody treatment with improved meningeal lymphatic flow using VEGF treatment was shown to more efficiently clear Aβ plaques[55]. Similarities in gene signatures were observed between microglia of mice with disrupted meningeal lymphatic flow and microglia of human AD patients, suggesting the importance of lymphatic flow in meninges and clearance of Aβ plaques. It is however, important to note that early attempts at vaccines against AD were discontinued due to development of meningoencephalitis in a subset of patients in a phase II clinical trial[57]. Safety will need to continue remaining the most important readout for any treatment against neurological conditions that involve meningeal immunity and lymphatic flow.

In another neurological disease model, experimental autoimmune encephalomyelitis (EAE), the mouse model for MS, disease severity was enhanced with VEGFR3-dependent lymphangiogenesis in the cribriform plate that forms the base of the skull[58], but not in dural lymphatics, suggesting heterogeneity in CNS lymphatics in neuroinflammation and the need to focus on the appropriate targets for therapy. Similarly, most people infected with Epstein-Barr virus (EBV) do not develop MS, suggesting that genetic predisposition and other environmental factors also play a role[59] including EBV infection. EBV infection can trigger an abnormal immune response, potentially leading to the activation of T cells that attack the brain and spinal cord. Preventing EBV infection via vaccination may therefore provide a promising basis for developing prophylactic dual EBV vaccines with beneficial non-specific effects towards MS prevention[60].

Concluding remarks and future perspectives

The meninges has emerged as a critical facilitator of both innate and adaptive immunity to protect the central nervous system and brain against pathogenic agents from external sources and self-antigens generated by the body. An immune cell-rich environment compared to the brain underneath, the meninges acts as an 'immunological barrier tissue'[2,5] and provides defensive forces in the form of dendritic cells, T cells and plasma cells. Evidence from several studies has shown the origin of immune cells at the meninges to be via systemic circulation, the gut or from bone marrow niches in the surrounding skull. Skull channels can transport myeloid cells to the dura and neutrophils to the brain, which is especially relevant following injuries like stroke. Harnessing these different routes could prove important to the development of vaccine-mediated meningeal immunity to defend against external infectious antigens and neurodegenerative self-antigens or for vaccines against opioid overdose. For example, delivery of vaccines via oral versus nasal passages may directly, though in different ways, impact availability of antigenic epitopes. While an oral route can deliver epitopes to the gut mucosa for development of antigen-specific IgA⁺ plasma cells that traffic to the meninges via blood, a nasally administered vaccine can potentially present epitopes directly at the dura which can then serve as a site for antigen-specific plasmablast and plasma cell development via the skull bone marrow (Fig. 1). Furthermore, discovery of DALT in the rhostral-rhinal hub of murine meninges that connect to the bone marrow is further proof of immune cells that expand following an intranasal challenge[34]. Traditional routes of vaccine delivery like IM can also potentially deliver antigen-primed immune cells to meninges via systemic routes. Since vaccine bio-availability is closely tied to formulation[61], a focus on formulation science in the context of meningeal immunity is critical.

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Author contributions

DJD conceived the project, secured funding, and supervised the study. MM and DJD wrote the first draft. CNH provided edits and critical feedback.

Competing interests

The authors declare the following competing financial interest(s): D.J.D. is named inventors on vaccine adjuvant patents assigned to Boston Children's Hospital. D.J.D. is on the scientific advisory board of EdJen BioTech and serves as a consultant with Merck Research Laboratories/ Merck Sharp & Dohme Corp. (a subsidiary of Merck & Co., Inc.). CNH is named as an inventor on a vaccine patent assigned to the University of Houston. D.J.D. and C.N.H. are co-founders of ARMR Sciences Inc. The rest of the authors do not have any competing interests.

Additional information

Correspondence and requests for materials should be addressed to David J. Dowling.

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