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INSIGHTS

Meningeal lymphatics can influence stroke outcome

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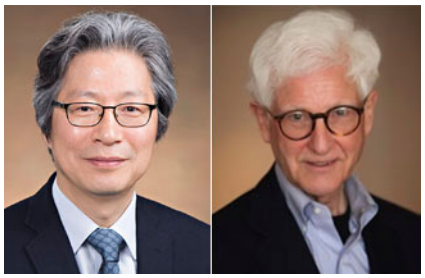
Meningeal lymphatics are conduits for cerebrospinal fluid drainage to lymphatics and lymph nodes in the neck. In this issue of *JEM*, Boisserand et al. (https://doi.org/10.1084/jem.20221983) provide evidence that expansion of meningeal lymphatics protects against ischemic stroke.

Recent evidence indicates that lymphatic vessels in the dural connective tissue layer of the meninges covering the brain and spinal cord can influence the severity and progression of multiple conditions including stroke, traumatic brain injury (TBI), Alzheimer's disease, autoimmune encephalomyelitis, and brain tumors (Da Mesquita et al., 2018; Esposito et al., 2019; Hussain et al., 2023; Song et al., 2020; Yanev et al., 2020). Meningeal lymphatics have long been recognized as important routes for drainage of cerebrospinal fluid (CSF) (Koh et al., 2005). Because CSF production and turnover, totaling 500 ml per day in humans, is essential for normal brain function, disturbances in CSF outflow can be detrimental to health and can worsen conditions that result in cerebral edema (Bolte et al., 2020; Esposito et al., 2019; Hussain et al., 2023; Tsai et al., 2022; Yanev et al., 2020). Meningeal lymphatics also serve as conduits for central nervous system (CNS) antigens in CSF to reach lymph nodes that mediate immune responses affecting the brain and spinal cord (Louveau et al., 2015; Song et al., 2020).

Meningeal lymphatics have particular significance in the outcome of stroke, which is one of the leading causes of death worldwide and can lead to disability and dementia. Ischemic stroke occurs when cerebral artery blockage prevents oxygenated blood from reaching the supplied brain region, whereas hemorrhagic stroke results from a ruptured cerebral blood vessel. Hypertension, smoking, high cholesterol, and diabetes are among the risk factors. The incidence of stroke can be lessened by preventive measures including risk factor reduction, lifestyle changes, and anti-platelet or anticoagulant drugs, but the latter have their own adverse effects. Therefore, the search for new preventive and therapeutic measures continues.

In this issue of *JEM*, Boisserand et al. provide evidence that adeno-associated virus (AAV) delivery of vascular endothelial growth factor-C (VEGF-C) into the CSF is protective against ischemic stroke in a mouse model (Boisserand et al., 2024). AAV-VEGF-C expanded the meningeal lymphatic network and increased CSF drainage to cervical lymph nodes of healthy adult mice. AAV-VEGF-C also improved the outcome of acute ischemic stroke after transient occlusion of the middle cerebral artery and induced a wide range of molecular changes in lymphatic endothelial cells, neurons, and immune cells in the brain. Of the multiple changes, increased CSF outflow was considered most important because ligation of the deep cervical lymphatics completely abolished the neuroprotective effects in stroke.

The results of the AAV-VEGF-C gain-of-function approach are consistent with earlier findings from a loss-of-function study, where $Vegfr3^{wt/mut}$ mutant mice with impaired VEGF-C/VEGFR3 signaling had fewer meningeal lymphatics, larger cerebral infarcts, and worse stroke outcome after middle cerebral artery occlusion (Yanev et al., 2020).



Insights from Gou Young Koh and Donald M. McDonald.

A major limitation of the gain-of-function approaches used by Boisserand et al. (2024) was that infusion of VEGF-C protein was ineffective after ischemia and, therefore, was not a useful treatment for stroke. Another limitation was that expansion of meningeal lymphatics by AAV-VEGF-C did not reduce the amount of cerebral edema after middle cerebral artery occlusion. Further studies are needed to determine whether other meningeal lymphatic gain-of-function approaches can benefit patients when used after ischemic stroke.

Another earlier study using the same mouse model implicated lymphatics in greater stroke severity by serving as routes for mediators from injured brain that activate inflammatory changes in draining lymph nodes (Esposito et al., 2019). Increases in inflammatory cytokines and lymphatic endothelial cell proliferation in

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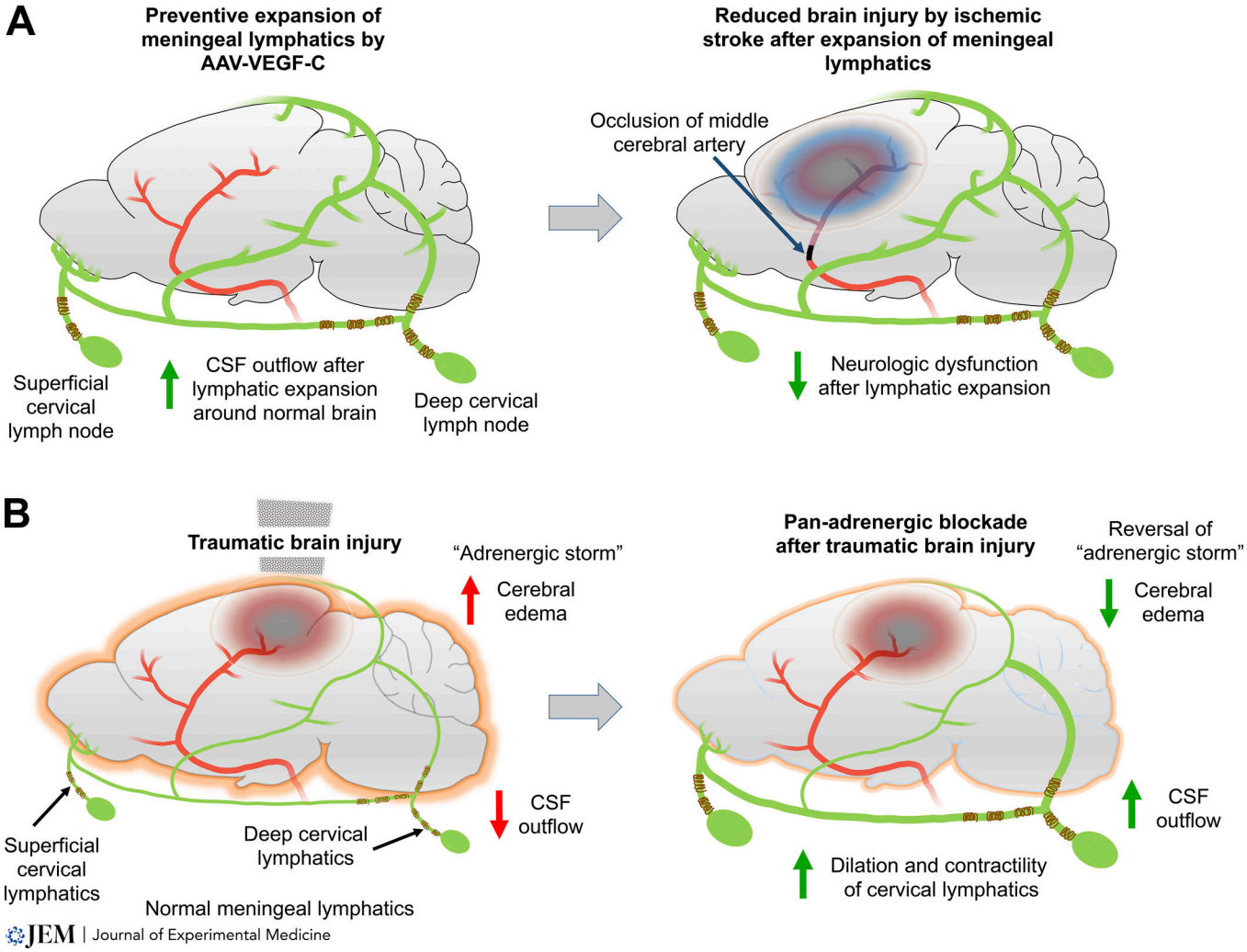

Meningeal lymphatics can influence stroke outcome. The beneficial contributions of meningeal lymphatics to the outcome of stroke and TBI are illustrated. (A) AAV delivery of VEGF-C to the CSF expands the meningeal lymphatic network and increases CSF drainage to cervical lymph nodes of adult mice and improves neurologic function after ischemic stroke resulting from transient occlusion of the middle cerebral artery (Boisserand et al., 2024). As an infusion of VEGF-C protein is ineffective after ischemia, the approach is not useful after stroke treatment (Boisserand et al., 2024). (B) Another recent study (Hussain et al., 2023) reports that cerebral edema occurring in a head concussion model of TBI results from suppression of CSF outflow caused by excessive systemic release of noradrenaline. This adrenergic storm reduces the contractility of cervical lymphatics, which in turn reduces CSF outflow and promotes cerebral edema. In support of this mechanism, the study (Hussain et al., 2023) reports that pan-adrenergic receptor blockade increases contractility of cervical lymphatics, restores CSF outflow, and reduces cerebral edema in mice with TBI. However, the characterization of CSF drainage through superficial and deep cervical lymphatics and lymph nodes and the feasibility of safely treating TBI patients with pan-adrenergic antagonists deserve further consideration.

superficial cervical lymph nodes were interpreted as brain/lymph node responses to middle cerebral artery occlusion. These changes were blocked by inhibiting VEGFR3 signaling with MAZ51, which reduced infarct size but had no significant effect on neurological outcome. Removal of superficial cervical lymph nodes had similar effects. However, the contributions of meningeal lymphatics and deep cervical lymph nodes—a primary destination of CSF drainage—were not examined.

All of these effects reflect the contribution of meningeal lymphatics to CSF drainage that is vital for protecting, nourishing, and clearing neurotransmitters, metabolites, antigens, amyloid-β, tau, and other protein aggregates (Wichmann et al., 2022). CSF circulates within and around the brain and spinal cord, turning over three to five times per day (Koh et al., 2005). Although some CSF clearance routes are debated, CSF is known to drain from the subarachnoid space through meningeal lymphatics around olfactory nerves in the cribriform plate and other cranial nerves (Koh et al., 2005; Proulx, 2021) and over the dorsal and basolateral surfaces of the brain (Ahn et al., 2019; Aspelund et al., 2015; Louveau et al., 2015; Ma et al., 2017). Ablation of meningeal lymphatics by photodynamic therapy with light-activated verteporfin increases the accumulation of amyloid-β in mouse models of Alzheimer's disease (Da Mesquita et al., 2018), albeit this treatment has the confounding effect of recruiting inflammatory leukocytes that contribute to the changes (Tammela et al., 2011).

Less is known of the contribution of meningeal lymphatics to the severity of hemorrhagic stroke, but lymphatic function has been manipulated in mouse models of

intracerebral hemorrhage (Tsai et al., 2022). Here, the gain-of-function approaches of intracisternal infusion of one dose of VEGF-C or seven daily oral doses of the phosphodiesterase-3 inhibitor cilostazol, a vasodilator administered to increase lymphatic drainage, were compared to the loss-of-function approaches of photodynamic ablation or deep cervical lymphatic ligation. Cilostazol was administered after the hemorrhage, but the other approaches were used as preventions. The gain-of-function approaches reduced hematoma volumes and improved behavioral performance, consistent with beneficial contributions of lymphatics, whereas the loss-of-function approaches had the opposite effects.

TBI from accidents ranging from vehicle collisions to sports injuries is the most frequent form of brain injury in humans and can cause diverse health issues, behavioral changes, and cognitive dysfunction through effects on the brain parenchyma and surrounding meninges. Particularly harmful consequences of TBI are increased intracranial pressure and cerebral edema, which increase the risk of death tenfold and worsen the outcome of patients who survive the initial injury. Bolte et al. (2020) reported that CSF outflow through meningeal lymphatics to deep cervical lymph nodes was reduced immediately after TBI and could take 2 mo to recover in a head concussion model in mice (Bolte et al., 2020). Abnormally elevated intracranial pressure was considered responsible for the reduction in CSF outflow.

The authors of a recent study (Hussain et al., 2023) claim that cerebral edema in the same head concussion model of TBI results from suppression of CSF outflow caused by systemic release of noradrenaline. According to this view, the "adrenergic storm" after TBI reduces the contractility of cervical lymphatics, which in turn reduces CSF outflow and promotes cerebral edema. In support of this mechanism, the authors report that pan-adrenergic receptor blockade increased contractility of cervical lymphatics, restored CSF outflow, and reduced cerebral edema in mice with TBI followed by three daily intraperitoneal doses of the α_1-adrenergic receptor antagonist prazosin combined with α_2-adrenergic antagonist atipamezole and broad β-adrenergic receptor antagonist propranolol. However, the characterization of CSF drainage through superficial and deep cervical lymphatics and lymph nodes and the feasibility of safely treating TBI patients with pan-adrenergic antagonists deserve further consideration. Nonetheless, these findings raise the possibility of reducing cerebral edema and neurological deficits after TBI by increasing cervical lymphatic contractility to augment CSF outflow.

These new approaches for reducing stroke sequelae (Boisserand et al., 2024) or treating TBI in mice (Hussain et al., 2023) deserve additional work to rigorously establish efficacy, safety, and underlying mechanisms in experimental animals and humans. One objective should be to define the specific lymphatic routes for CSF outflow and the relative contribution of each to overall CSF clearance. Despite solid documentation of lymphatic contributions to CSF clearance (Ahn et al., 2019; Aspelund et al., 2015; Koh et al., 2005; Louveau et al., 2015; Ma et al., 2017; Proulx, 2021), the connections between the subarachnoid space and intracranial and extracranial lymphatics involved in CSF outflow have yet to be fully defined. Most studies have focused on dorsal meningeal lymphatics, which contribute less to CSF outflow than lymphatics around the olfactory and other cranial nerves.

Another objective should be to understand better CSF outflow regulation through cervical lymphatics, including the contributions of lymphatic valves, smooth muscle, and autonomic innervation to unidirectional CSF flow. A more complete understanding of the autonomic regulation of cervical lymphatic pumping is also needed. A related objective should be to develop a safe and efficacious approach for local control of rhythmic contractility of cervical lymphatics within the physiological range to increase CSF outflow without systemic drug administration.

A further objective should be to expand meningeal lymphatics that contribute to CSF drainage to deep cervical lymph nodes by activating local endogenous VEGF-C/VEGFR3 signaling. Activation of VEGF-C production by meningeal stromal cells could be beneficial, because VEGF-C is produced by fibroblasts, vascular smooth muscle cells, and endothelial cells, whereas VEGFR3 is expressed only by lymphatics. Further work will be needed to develop approaches for promoting VEGF-C production in these cells.

Collectively, these findings illustrate that meningeal lymphatics contribute to CSF clearance and CNS immune surveillance under normal and diverse pathological conditions. They also identify multiple strategies that can potentially prevent or reduce the consequences of stroke by manipulating the number and function of meningeal lymphatics.

Acknowledgments

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