The Microcirculation—Fantastic Voyage

Is There a Cerebral Lymphatic System?

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The brain is unique among virtually all somatic organs in its lack of a conventional lymphatic vasculature.1–3 In the periphery, the lymphatic circulation facilitates the clearance of extracellular proteins and excess fluid from the interstitium, a role critical to tissue homeostasis and function.4,5 Yet within the brain, despite its complex architecture and high metabolic activity and neural cells’ sensitivity to changes in the extracellular environment, no specialized organ-wide anatomic structure has yet been identified that facilitates the efficient lymphatic clearance of extracellular solutes and fluid from the brain parenchyma.

Current Understanding of Brain Interstitial Solute Clearance

For small molecules and hydrophobic compounds, efflux across the blood–brain barrier is relatively unrestricted. Molecules that are substrates for specific blood–brain barrier transporters are also readily cleared from the brain.6,7 Other compounds must be cleared from the brain interstitium to the cerebrospinal fluid (CSF) compartment, where they are ultimately eliminated to the bloodstream via arachnoid granulations or to peripheral lymphatics along cranial veins.1,8,9 However, the distances between much of the brain tissue and the CSF compartments are too great for efficient clearance by simple diffusion, particularly for large molecules (such as peptides and proteins) with low diffusion coefficients.6 Rather, the clearance of these interstitial solutes from the brain is attributed to bulk flow, by which convective currents of interstitial fluid (ISF) sweep solutes along at a high rate that is largely independent of molecular size.1,2,6,7

In a controversial series of studies, Grady et al10,11 suggested that brain ISF may exchange with CSF along paravascular routes surrounding cerebral blood vessels. Because these findings seemed to be subsequently refuted by Cserr et al,12,13 such retrograde movement of CSF into the brain parenchyma is now thought to be of comparatively minor physiological importance.1 However, if a substantial amount of CSF moves through the brain interstitium, and if this flux occurs along defined anatomic pathways, this would fundamentally alter our understanding of how CSF facilitates the clearance of interstitial solutes and metabolic wastes from the brain.

Glympathic Pathway: A Paravascular Pathway for Interstitial Solute Clearance

In two recent studies,14,15 we define for the first time a brain-wide anatomic pathway that facilitates the exchange of CSF and ISF and the clearance of interstitial solutes from the brain. This pathway consists of 3 elements: a para-arterial CSF influx route; a para-venous ISF clearance route; and a transparenchymal pathway that is dependent on astroglial water transport via the astrocytic aquaporin-4 (AQP4) water channel (represented in Figure 1A).

Using in vivo 2-photon and ex vivo confocal imaging of small-molecular-weight fluorescent CSF tracers, we found that a large proportion (>40%)14 of subarachnoid CSF rapidly enters the brain parenchyma along paravascular spaces surrounding penetrating arteries throughout the brain. CSF tracer entered the brain initially through the Virchow–Robin space, then followed the arterial vascular smooth muscle basement membrane to reach the basal lamina of the brain capillary bed. At all levels of this paravascular route, CSF tracer entered into the interstitial space, reflecting the exchange of CSF and ISF.14 Para-arterial CSF influx extended throughout the brain and seemed to occur along virtually all penetrating arteries. ISF clearance pathways, in contrast, were restricted to a specific group of large-caliber draining veins. Fluorescent tracer injected directly into the interstitium of the cortex, striatum, or thalamus was cleared medially to the internal cerebral veins and great vein of Galen and ventrolaterally to the caudal rhinal vein.14

The astroglial AQP4 water channel is expressed in a highly polarized manner in perivascular astrocytic endfeet that immediately bound these paravascular CSF influx and ISF clearance pathways (Figures 1A and 2A).16,17 We proposed that these perivascular water channels may facilitate the convective bulk flow of fluid from the para-arterial CSF influx pathway through the interstitium, and along the para-venous clearance route. To test this, we evaluated paravascular CSF influx in global Aqp4 knockout mice by both in vivo 2-photon and ex vivo fluorescence imaging. Compared with wild-type controls, CSF influx into and through the parenchyma of Aqp4-null mice was dramatically reduced.14 Similarly, when we evaluated the rate of interstitial solute clearance from the brain using a radiotracer clearance assay, we found that interstitial solute clearance was reduced by ≈70% in Aqp4-null mice.
As detailed in our recent study,14 these findings demonstrate that AQP4-dependent bulk flow couples CSF influx along the para-arterial pathway to ISF clearance along the para-venous route, forming an organ-wide system that facilitates the clearance of interstitial solutes from the brain parenchyma. On the basis of this glial dependence and the functional and structural homology to the peripheral lymphatic system, we have termed this glio-vascular pathway the glymphatic system (Figure 1A).

Soluble amyloid β (Aβ) is present in the ISF of the healthy young brain and the failure of Aβ clearance is thought to underlie the deposition of Aβ plaques associated with Alzheimer disease progression.10,11 We next evaluated whether soluble Aβ constitutes one of the solutes cleared from the brain interstitium along the glymphatic pathway. When fluorescently labeled Aβ was injected into the cortex or striatum, it accumulated around the same paravascular pathways observed with other fluorescent tracers.14 We also measured the clearance of radiolabeled Aβ injected directly into the striatum of wild-type and Aqp4-null mice. In Aqp4-null mice, radiolabeled Aβ clearance was reduced by ≈65% compared with wild-type mice, suggesting that AQP4-dependent bulk flow along the glymphatic pathway constitutes a key mechanism of clearance of soluble Aβ from the brain interstitium.14

**Effect of Diffuse Gliotic Injury on Glymphatic Pathway Function**

Reactive astrogliosis is a cellular response to injury common to many mechanistically distinct forms of brain injury, including ischemic and traumatic brain injury, and is characterized by changes in astrocyte morphology and molecular expression patterns.19–21 Although more severe ischemic and traumatic brain injury is accompanied by glial scar formation, low-intensity injury frequently results in diffuse and long-lasting reactive astrogliosis. This is reflected in 2 recent studies from our group. In a mouse model of diffuse microinfarction exhibiting only low-level aggregate ischemic burden, widespread reactive astrogliosis was evident throughout the cortex and striatum for up to a month after injury.18 Similarly, in a mild traumatic brain injury model, widespread cortical and subcortical reactive gliosis was evident for at least 1 month after injury in the absence of frank tissue destruction.22

Changes in AQP4 expression are often observed in conjunction with reactive astrogliosis. After ischemic or traumatic brain injury, widespread reactive astrogliosis (glial fibrillary acidic protein [GFAP] immunoreactivity) is observed throughout the ipsilateral cortex. In regions of reactive astrogliosis, AQP4 localization is severely perturbed, exhibiting a loss of polarization to the endfoot process and increased somal labeling. Similar expression patterns are observed after diffuse microinfarction.18
In our own studies of microinfarction and mild traumatic brain injury, changes in AQ4P expression within regions of diffuse reactive gliosis are more complex. General AQ4P expression is elevated in gliotic regions 7 days after diffuse microinfarction, but normalizes by 14 days after injury. The distribution of AQ4P expression, however, remains perturbed for at least 1 month after injury. Rather than the highly polarized perivascular localization observed in healthy brain, AQ4P in reactive astrocytes exhibits a marked reduction in polarity, with lower perivascular AQ4P immunoreactivity and higher somal AQ4P immunoreactivity. Similar patterns of AQ4P dysregulation are also observed in reactive astrocytes after mild traumatic brain injury (Figure 2B).

In light of the critical role that perivascular AQ4P plays in the lymphatic clearance of interstitial solutes, including soluble Aβ, changes in AQ4P localization after diffuse injury may have critical implications for the pathogenesis of conditions, such as vascular dementia and traumatic brain injury. We propose that mislocalization of AQ4P from the perivascular endfeet to the astrocytic soma prevents the efficient directional flux of water into and out of the paravascular spaces that contribute to interstitial solute clearance (Figure 1B). This may cause the widespread failure of waste clearance from the diffusely gliotic brain tissue, resulting in the accumulation of neurotoxic metabolites, such as Aβ, in addition to the extracellular fluid volume.

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**Disclosures**

None.

**References**

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