



Review

Aquaporin and brain diseases☆☆☆


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ABSTRACT

Background: The presence of water channel proteins, aquaporins (AQPs), in the brain led to intense research in understanding the underlying roles of each of them under normal conditions and pathological conditions.

Scope of review: In this review, we summarize some of the recent knowledge on the 3 main AQPs (AQP1, AQP4 and AQP9), with a special focus on AQP4, the most abundant AQP in the central nervous system.

Major conclusions: AQP4 was most studied in several brain pathological conditions ranging from acute brain injuries (stroke, traumatic brain injury) to the chronic brain disease with autoimmune neurodegenerative diseases. To date, no specific therapeutic agents have been developed to either inhibit or enhance water flux through these channels. However, experimental results strongly underline the importance of this topic for future investigation. Early inhibition of water channels may have positive effects in prevention of edema formation in brain injuries but at later time points during the course of a disease, AQP is critical for clearance of water from the brain into blood vessels.

General significance: Thus, AQPs, and in particular AQP4, have important roles both in the formation and resolution of edema after brain injury. The dual, complex function of these water channel proteins makes them an excellent therapeutic target. This article is part of a Special Issue entitled Aquaporins.

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1. Aquaporin expression and distribution in normal brain

1.1. Aquaporins: subgroups and expression in brain

The aquaporin (AQP) protein family is identified by six membrane spanning domains with intracellular carboxyl (C) and amino (N) termini and a molecular weight around 30 kDa. A common characteristic between all AQP is a consensus motif, Asn-Pro-Ala, which is strongly implicated to play a key role in pore formation [1]. The 13 members of the AQP family are ubiquitously distributed throughout the mammalian tissues and can be categorized into three subgroups: i) *Aquaporins* (AQP0, 1, 2,

4, 5, 6 and 8) considered as “pure water channel family” are primarily only permeable to water. AQP1, 4, 5, 6 and 8 have been described in the brain cells in rodents and primates [2–4]. AQP4 is the most studied since 1994, when the first description of the presence of the messenger ribonucleic acid (mRNA) in the hypothalamic supraoptic nuclei and perivascular brain regions was published (Figs. 1, 2) [5]. ii) The second subgroup, *Aquaglyceroporins* (AQP3, 7, 9 and 10) contribute to water diffusion, as well as glycerol, urea, and some monocarboxylates which can facilitate lactate diffusion [1]. AQP3 and 9 have been observed in brain cells (Fig. 1). iii) Finally, a new subgroup was proposed as the *Super-aquaporins*, including AQP11 and 12, which are localized in the cytoplasm and possibly involved in regulation of intracellular water transport, organelle volume and intra-vesicular homeostasis [1]. Only AQP12 has so far been described in some brain neurons, and its role is not yet well understood. As mentioned in the special issue, AQPs have a variety of diffusion properties, mostly investigated outside of the brain. The organization of AQPs in homo-tetramers (Fig. 3) gives formation to a central pore, through which water, ions and/or gases flow, depending on the AQP subtype. Recently, the central pore was shown to be permeable for gases O₂, CO₂ and nitric oxide for AQP1, 4 and 5 [6–9]. This unique property is opening a new range of physiological functions for the AQPs such as the regulation of CO₂ permeability. In the rest of the review, we will discuss the distribution of AQP1, 4 and 9 and the potential pathophysiological involvement of these three AQPs in brain disorders.

Abbreviations: AQP, aquaporin; Kir4.1, inwardly rectifying potassium channel 4.1; OAP, orthogonal array of particles; DWI, diffusion weighted imaging; T2WI, T2-weighted imaging; DTI, diffusion tensor imaging; ADC, apparent diffusion coefficient; MRI, magnetic resonance imaging; siAQP4, siRNA targeting AQP4; BBB, blood–brain barrier; SCI, spinal cord injury; jTBI, juvenile traumatic brain injury; MS, multiple sclerosis; NMO, neuromyelitis optica; EAE, experimental autoimmune encephalomyelitis

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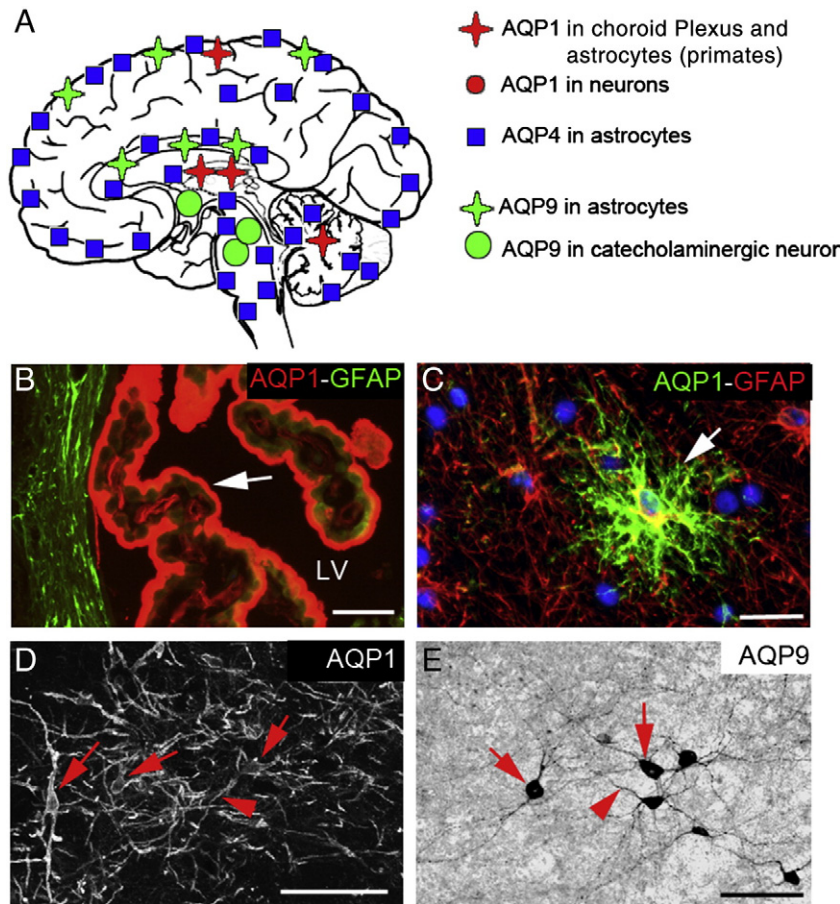


Fig. 1. Aquaporin 1, 4 and 9 distributions in the brain. (A) Schematic drawing of aquaporin 1, 4 and 9 distributions in the brain. AQP1 is mainly observed in the choroid plexus and in some neurons. AQP1 is present in some astrocyte in primates. AQP4 is present in astrocytes in all brain structures with different levels and patterns of expression (see Fig. 2). AQP9 is mostly present in astrocytes and catecholaminergic neurons. (B) AQP1 immunostaining (red) in choroid plexus epithelium (arrow) located in the lateral ventricle (LV). The border of the LV is outlined by the GFAP staining (green), specific marker of the astrocytes. (C) AQP1 staining (green) in monkey cerebellum is co-localized (arrow) with GFAP staining (in red), suggesting the presence of AQP1 in a sub-population of astrocytes in primates. (D) AQP1 labeling is present in some neurons of the septum in the rat brain. (E) AQP9 immunoreactivity in catecholaminergic neurons of the ventral tegmental area. Bars: B, D = 50 μ m; C, E = 40 μ m.

1.2. AQP1: location in choroid plexus epithelial cells and in subpopulations of neurons

AQP1 was primarily observed within epithelial cells of the choroid plexus in primate and rodent brains and has been proposed to play a role in cerebrospinal fluid formation [10]. In addition to this location, AQP1 has been found in the dorsal horn of the spinal cord and the trigeminal sensory ganglia have been identified as new cellular locations for AQP1 with a possible role in nociception [11]. More recently, AQP1 has been observed in some neuronal processes in the septum with increased expression after brain injuries (Fig. 1D) [12]. AQP1 distribution seems to present differences in its location between species, such as its expression in a subpopulation of astrocytes within the non-human primate (Fig. 1C) [13], which has not been reported for rodents. This suggests that AQP1 in non-human primates may have an additional role in brain water homeostasis [13], and the anatomical differences between rodents and primates could implicate physiological differences as well (Fig. 1B–D).

1.3. AQP4: water mobility in the brain

AQP4 is the most abundant water channel found in all brain structures in contact with the cerebral vascular compartment. A high concentration of AQP4 is found on astrocyte endfeet (Fig. 2) [3,14] and most studies have focused on the cerebral cortex where AQP4 expression is

highly documented on astrocyte endfeet in contact with all blood vessels (Fig. 2B–D). However, astrocyte AQP4 distribution differs significantly within brain structures, such as the hippocampus (Fig. 2G,H) [15], the corpus callosum (Fig. 2D,E), the cerebellum (Fig. 2F1, F2) [16], the magnocellular nuclei of the hypothalamus [3,17], and the brainstem (Fig. 2I). The difference in astrocyte AQP4 distribution pattern suggests that AQP4 has multiple physiological functions in addition to water homeostasis. In fact, AQP4 is also possibly involved in cell adhesion [18], similar to the involvement of AQP0 in cell adhesion of epithelial cells in the ocular lens [19]. AQP4 has also been found expressed in both gray matter and white matter in spinal cord of rats and mice [20,21]. As observed in the cortex and other brain structures, intense AQP4 staining was found in the astrocytic endfeet surrounding the blood vessels (Fig. 4A). Additional AQP4 has been detected in astrocyte processes and cell bodies (Fig. 4A) in accordance with previous work [20]. In peripheral nerves, AQP4 was found in astrocyte processes in contact with Ranvier nodes and blood vessels [22].

AQP4 is mostly associated with water homeostasis in association with the function of the astrocyte in spatial potassium buffering [23]. Interestingly, the location of the AQP4 follows the distribution of the inwardly rectifying potassium channel 4.1 (Kir4.1) [24]. Although no functional relationship was clearly shown between AQP4 and Kir4.1, the absence of the AQP4 resulted in delaying the reuptake of the extracellular potassium in epileptic conditions possibly due to an increase of the extracellular space [25]. High presence of the AQP4 in corpus

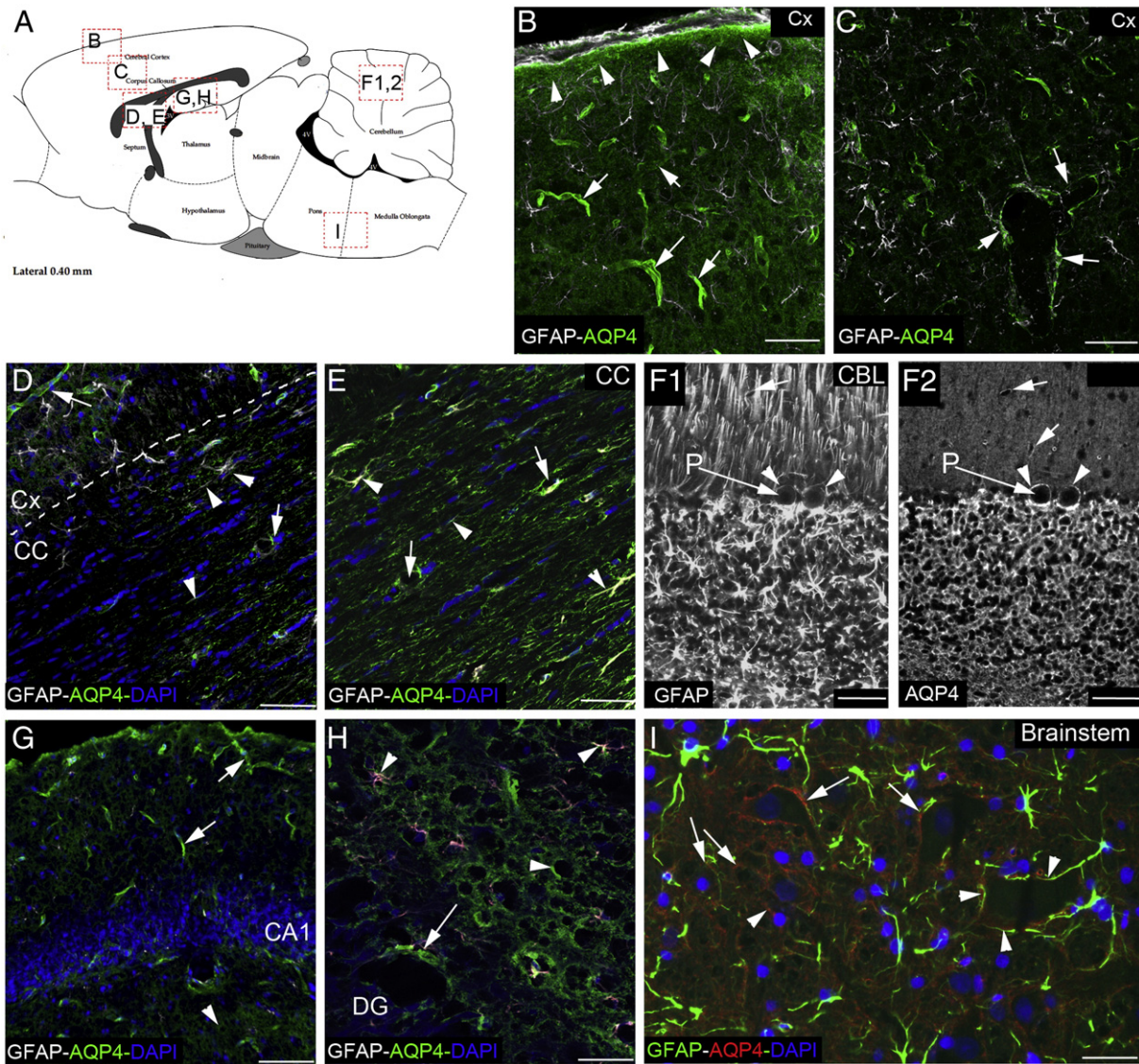


Fig. 2. Variety in astrocyte AQP4 distribution. (A) Sagittal drawing of rat brain with the location of the pictures of AQP4 distribution in different brain areas. (B) AQP4 labeling (green) in the parietal cortex (Cx) is abundant on the glia limitans (arrow heads), revealed in gray by the GFAP staining. The AQP4 labeling is underlining the blood vessels (arrows) by its concentration in the astrocyte endfeet stained by the anti-GFAP (gray). (C) AQP4 distribution in the deeper cortex layer showed the “polarization” of the AQP4 labeling around the blood vessels (arrows). The double staining of AQP4 and GFAP exhibits its presence on the astrocyte endfeet (arrows). (D) AQP4 staining (green) in the corpus callosum (CC) is abundant around the blood vessel (arrows) and at distance from the perivascular space (arrowheads). In the white matter structure, AQP4 exhibits a different pattern of staining, with distribution of the protein on the astrocyte processes (gray, GFAP staining), possibly in association with node of Ranvier. (E) AQP4 staining (green) at higher magnification in the corpus callosum (CC) shows the co-localization with GFAP staining (gray, arrows and arrowheads). In contrast with the Cx, the AQP4 staining is spread in all the structure with “patchy” distribution, following the direction of the neuronal processes. (F1, F2) GFAP (F1) and AQP4 (F2) staining in the cerebellum differs from the cortex, with an abundant staining around all the neurons of the granular layer as well as around the Purkinje cell bodies (P, arrowheads). AQP4 is observed around the blood vessels in the molecular layer, where the radial glia is located. (G) AQP4 labeling (green) in the location of CA1 of the hippocampus outlines the blood vessels but it is also present in astrocyte processes in the stratum radiatum layer. (H) AQP4 labeling (green) in proximity of the dentate gyrus (DG) in the hippocampus. AQP4 is around the blood vessels (arrow) and in astrocyte processes remote of the perivascular space (arrowheads). (I) AQP4 labeling (red) shows staining around the neuronal cell bodies (arrows) as well as in contact with blood vessels (arrowheads). AQP4 (red) is co-localized with the GFAP staining (green). Bars: B, C, D, E, H = 50 μ m; F1, F2 = 40 μ m; G = 100 μ m; I = 25 μ m.

callosum on astrocyte processes is possibly associated with buffering the osmotic pressure in contact with the node of Ranvier. The fine regulation of the water homeostasis and the osmotic pressure is required for efficient axonal conductance. Indeed, this may be an important role for AQP4 regarding some recent developments; AQP4 could also be involved in diffusion of gases such as CO_2 along with water (Fig. 3). In the cerebral cortex, astrocytic AQP4 has been recently associated with a general bulk flow to facilitate the perivascular flow and possibly involved in the clearance of some proteins such as beta amyloid [26]. Although the mechanism is unclear, these recent results confirm the

importance of the AQP4 in the water movements associated with clearance of potentially harmful substances in the central nervous system. AQP4 was also proposed to play a potential role for detecting variations of the plasma osmotic pressure in the hypothalamic nuclei [3].

One of the interesting features for AQP4 is its tendency to form a high density of orthogonal arrays of particles (OAPs) [27]. Organization of OAPs is based on two isoforms of AQP4: long (AQP4-m1) and short splice variants (AQP4-m23) (Fig. 5). The ratio of AQP4-m1 to AQP4-m23 determines the size of these OAPs [27], as the AQP4-m23 isoform stabilizes the OAP structure (Fig. 5A) [27,28]. An increase in the AQP4-

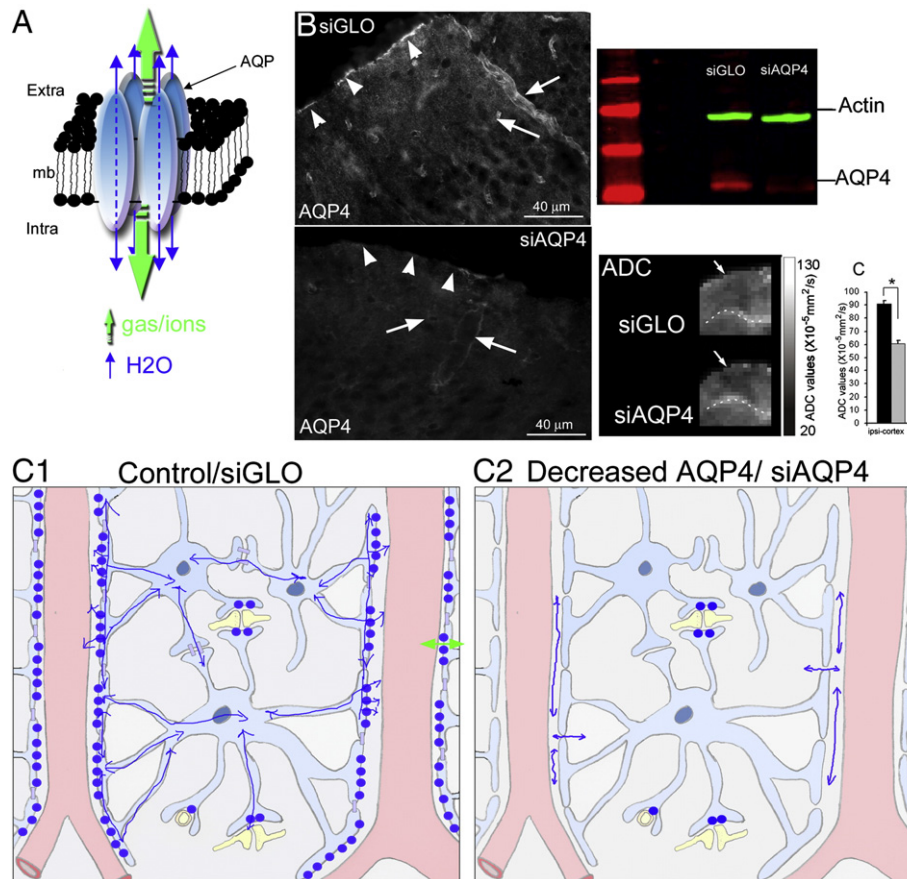


Fig. 3. AQP4 water diffusion in the brain. (A) Schematic drawing of the aquaporin homo-tetramer assembly within the lipid membrane resulting in a central pore permeable to cations and gases (green arrows). Each individual aquaporin facilitates bi-directional water movement depending on the osmotic gradient (blue arrows, adapted from [33]). (B) AQP4 labeling in siGLO and siAQP4 treated rats showed a significant decrease in the intensity of AQP4 staining in the glia limitans and perivascular astrocyte in the cortex of siAQP4 treated rats. AQP4 Western blot (red) showed a significant decrease of the intensity in siAQP4 compared to siGLO treated rats. Decrease of the AQP4 is associated with a decrease of the water diffusion. Enlarged ADC images focusing on the contralateral cortex showed the decrease in ADC in siAQP4 compared to siGLO treated rats. The quantification showed a 50% decrease of the ADC signals where the AQP4 expression is decreased by 30% (modified from [35]). (C) Schema depicting the location of the AQP4 in brain cortex in control/siGLO condition (C1) and after siAQP4 injection (C2). The presence of AQP4 facilitates the water movement in the astrocyte network (C1), and after silencing AQP4 (C2), lower ADC values are caused by decreased water permeability due to a decreased number of AQP4 channels in perivascular space (C2).

m1 variant has also been shown to disrupt the structure of the OAPs (Fig. 5) [27,28]. Although the exact functional role of the OAPs remains unknown under normal conditions as well as the consequences of OAPs disruption are not yet determined.

1.4. AQP9: potential involvement in astrocyte glucose energy metabolism

AQP9, an aquaglyceroporin, facilitates the diffusion of water as well as glycerol, urea and monocarboxylates and has been identified in both rodent and primate brains [13]. AQP9 is present in astrocytes [29,30], endothelial cells of subpial vessels [30] and catecholaminergic neurons (Fig. 1E) [13,30,31]. AQP9 is expressed as 2 isoforms, one of 26 kDa found on the inner membrane of mitochondria and one of 30 kDa anchored in the cell membrane [32]. Recently, AQP9 has been shown to be involved in the astrocyte glucose energy metabolism by facilitating glycerol diffusion [33]. Interestingly, brain AQP9 is downregulated with increase of blood insulin concentration and thus an increase in AQP9 was observed in the catecholaminergic nuclei in diabetic rats [34], further suggesting that it likely participates in neuronal energy balance by facilitating glycerol diffusion. As suggested in our recent work, AQP9 can facilitate diffusion of glycerol and monocarboxylates, which may serve as energy substrates in the CNS [33].

2. AQP, water movement and neuroimaging

Diffusion weighted imaging (DWI), T2-weighted imaging (T2WI) and more recently diffusion tensor imaging (DTI) are used for diagnosis/prognosis in patients with brain diseases. The computed T2 value is believed to represent the water content within brain tissues; where increased T2 values correspond to water accumulation in pathological conditions [35]. The quantitative DWI parameter, the apparent diffusion coefficient (ADC), is believed to reflect water mobility within brain tissues. Traditionally, decreased ADC values are supposed to reflect decreased extracellular space due to cellular swelling [35] in brain disorders. The magnitude of these magnetic resonance imaging (MRI) changes has been correlated with patient outcomes [35] indicating that ADC/T2 values are precious biomarkers that can be used to monitor patients and treatment modalities. More recently, DTI is becoming a new imaging modality to detect changes of water diffusion directionality. However so far, the cellular and molecular events underlying these imaging changes remain unclear [36]. The decrements in ADC early after stroke onset are very likely a combination of astrocytic and neuronal dendritic swelling [37] supplemented by decreased AQP4 expression [38,39]. In fact, ADC values were shown to be reduced in normal animals after cortical siRNA targeting AQP4 (siAQP4) injection (Fig. 3B), with no significant change in T2 [38]. Increased ADC was correlated

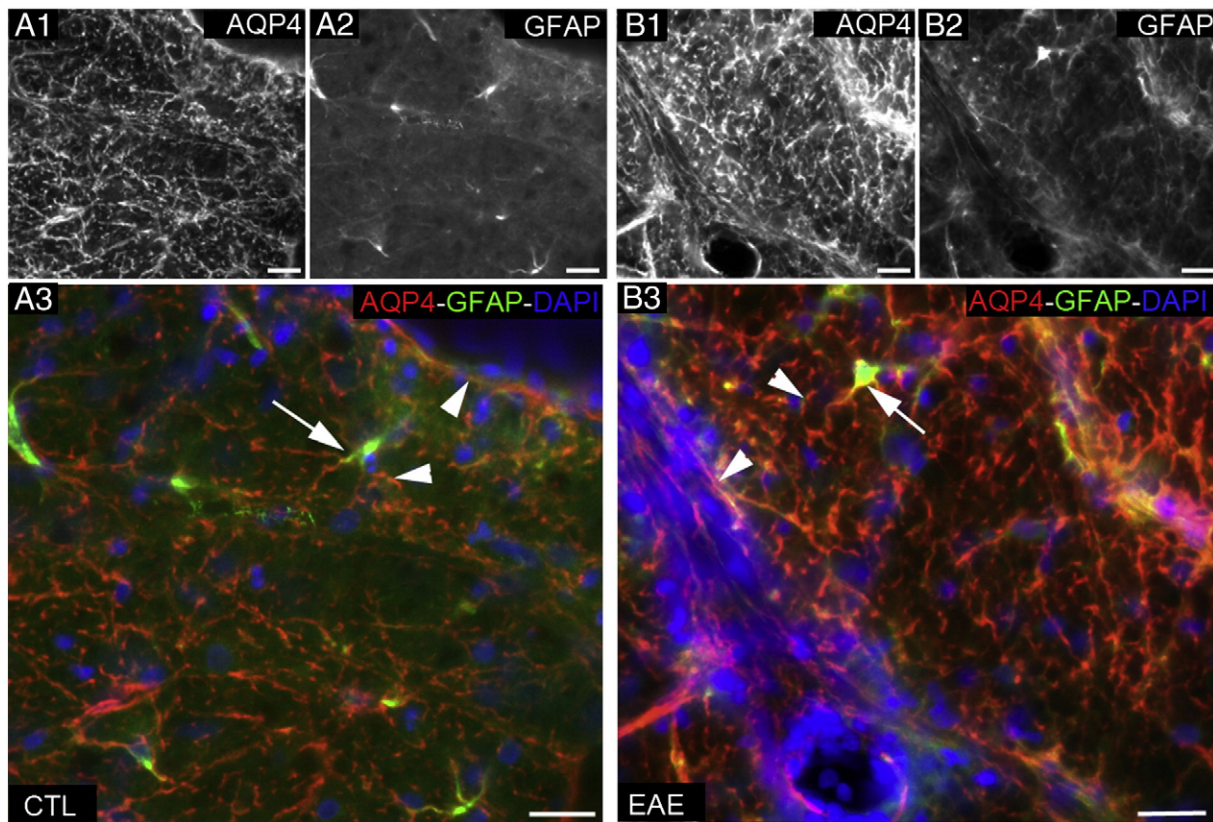


Fig. 4. AQP4 expression in spinal cord in normal and EAE rats. The co-staining AQP4 (A1, B1, in red A3, B3) and GFAP (A2, B2, in green A3, B3) showed in the spinal cord the abundant presence of AQP4 in astrocytes around the blood vessel and in processes (arrowheads, A3, B3). Additional positive AQP4 staining is observed in the astrocyte cell bodies (arrows, A3, B3). As described in literature, AQP4 expression is dramatically increased in the spinal cord of the EAE rats (B) compared to the control (CTL, A). Bars: A, B = 50 μ m.

with AQP4 increases in experimental hydrocephalus and focal neuro-inflammatory models [40,41].

DTI takes into account the non-uniform directionality of water flow (anisotropy) in the brain especially in the white matter tract. This anisotropy has mainly been attributed to myelinated neuronal axons in the white matter tract. Recent evidences in TBI model have suggested the possible role of astrocytes and glial scars in DTI signal changes [42]. Interestingly, increased anisotropy in cortex was connected with astrogliosis and not with axonal changes [42]. This concept is also reinforced by another study showing a direct correlation with changes in DTI signals associated with hypertrophic astrocytes and increase of AQP4 [43]. All together, these new observations support the idea that the changes in the astrocytic AQP4 will possibly be reflected in neuroimaging (Fig. 3B–C).

3. Edema process: role of the AQPs

Most brain diseases (e.g. stroke, traumatic brain injury, brain tumors, brain inflammation) present the hallmark of edema, which is water accumulation resulting from brain osmotic homeostasis dysfunctions. The main consequence of edema is the swelling of the brain, which aggravates the secondary injuries such as decrease of brain perfusion. Edema has been known in the clinic and pre-clinical science for many years but the molecular and cellular events in edema formation/resolution are still poorly understood. Moreover, there is no efficient treatment to prevent or limit edema formation or expansion in brain disorders. Thus, the discovery of the brain AQPs was a beacon of hope in the development of new therapy to battle the edema process. The knowledge gathered in the past 15 years on AQPs as a potential drug

target for edema is summarized in the following parts after a short introduction on the edema build-up phase.

3.1. Edema build-up phase: anoxic, ionic and vasogenic edema

For 40 years, cerebral edema has been traditionally divided into 2 major classes: cytotoxic and vasogenic [36]. Classically, *cytotoxic edema* is defined by intracellular water accumulation without blood–brain barrier (BBB) disruption while *vasogenic edema* appears after BBB disruption, leading to a diffusion of proteins from the blood to the tissue followed by water accumulation in the extracellular space [36]. However, this classical subdivision has been challenged by the recent knowledge in molecular changes during the edema formation and BBB properties, and the classical subdivision represents a simplified view of a more complex pathological process. In regard to vascular brain injuries, the recent cellular and molecular events behind edema suggest that the edema build-up phase can be divided into 3 major types: *anoxic*, *ionic* and *vasogenic edema* [36,44,45]. *Anoxic edema* is characterized as swelling of the astrocytes and neuronal dendrites that occur within minutes (Fig. 5B) after oxygen and glucose deprivation in the context of cerebrovascular disease. The depletion of oxygen and energy nutrients induces major changes in the cellular ionic gradients due to the absence of efficient energy dependent co-transporters. This leads to a massive entry of ions into cells which can be observed in phenomenon such as a slow rise in extracellular K^+ concentration [46,47], followed by water entry into the cells, which induces cellular swelling in astrocytes first, and then in neuronal dendrites (Fig. 4B). Then, anoxic edema quickly evolves to become *ionic edema*. The absence of oxygen and nutrients also alters the ionic gradients of endothelial cells, including transcapillary flux of Na^+ [45,48] with tissue swelling (Fig. 5B).

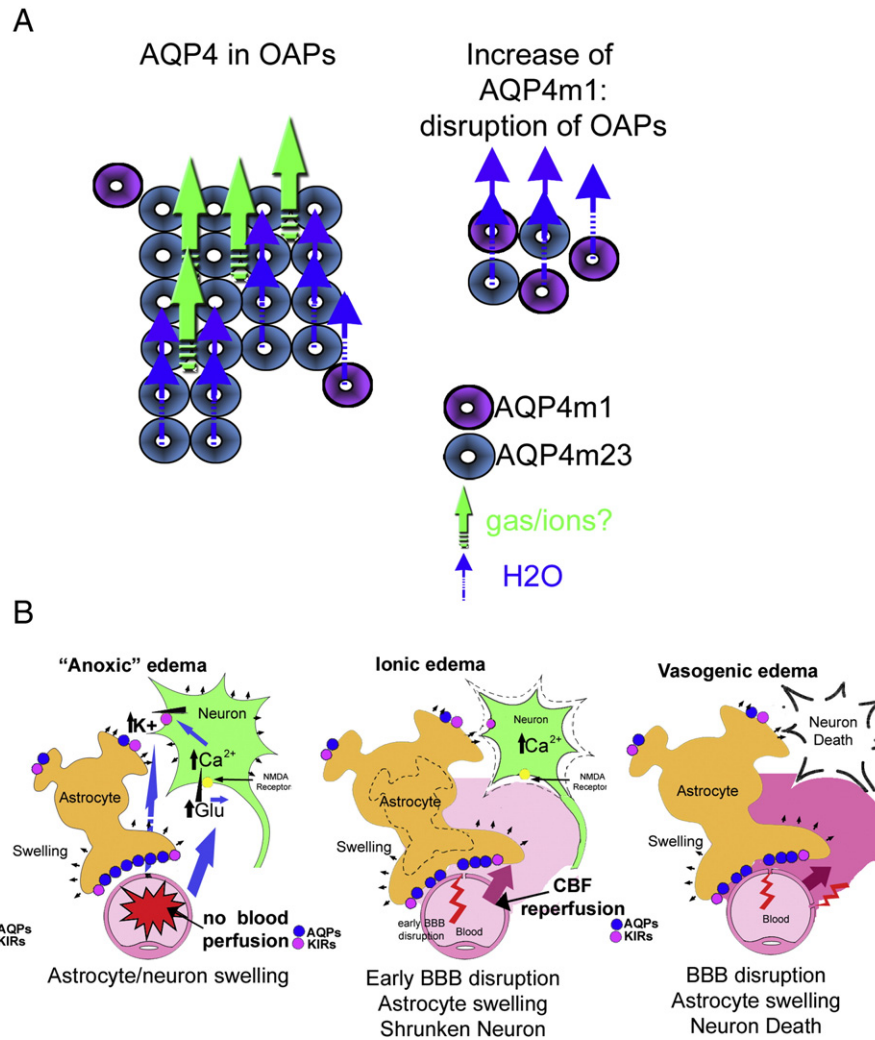


Fig. 5. Disorganization of AQP4 in orthogonal particles after brain injuries and edema process. (A) AQP4-m1 (purple circles) and AQP4-m23 (blue circles) isoforms contribute together to form orthogonal array particles (OAPs) in astrocyte endfeet in contact with the blood vessels. It was previously shown that higher expression of AQP4-m23 contributes to the formation of large OAPs. However, increase of AQP4-m1 induced disruption of OAPs with a reduction of the size. This modification is observed in pathological conditions such as stroke. Recent knowledge on AQP leads us to hypothesize that the large OAPs contribute to gas and cation diffusion in the astrocyte membranes through central pores (green arrows) (modified from [33]). (B) Schematic drawing of the events happening during edema formation with 3 different edema phases: anoxic, ionic and vasogenic edema. During the injury with decrease of brain perfusion, the first minutes are characterized by anoxic edema. Anoxic edema is characterized by a swelling of the astrocytes and the neuronal dendrites caused by a disruption of the cellular ionic gradients and the entry of ions followed by water entry and leading to cellular swelling. During the ionic edema, astrocytes become swollen and neuronal death starts to occur resulting in shrinkage of the neurons, shear stress and endothelial dysfunctions on the non-perfused vascular tree, which results in early transient leakage of the BBB. Vasogenic edema is a result of disruption of the tight junctions between the endothelial cells, leading to increased permeability of the cerebral blood-vessels to albumin and other plasma proteins, further contributing to swelling of astrocytes and subsequent neuronal cell death (adapted from [33]).

Suffering of endothelial cells therefore results in early transient leakage of the BBB in stroke [31,49] as well as in TBI [50]. This results in further entry of water through endothelial cells leading to brain swelling as observed from examples in stroke models within 30 min after reperfusion [31,49] associated with further increased BBB permeability [49,51]. *Vasogenic edema* follows this cascade of events (Fig. 5B) with increased permeability to plasma proteins such as albumin [36] due to a physical disruption of endothelial tight junctions, an extracellular matrix degradation and potentially an increased transendothelial cell transport by the transcytosis mechanism.

It is important to mention that this definition was proposed in the context of brain injuries involving acute cerebrovascular dysfunctions and may not be adequately adapted for other brain disorders such as brain tumors [52]. In this regard, it is important to underline that clinical treatments solely focusing on osmotic challenges are not efficient or sufficient for treatment of cerebral edema, because of the suggested complexity and diversity in the molecular mechanisms underlying the

edema formation process. Although the exact functional contributions of cerebral AQPs are not yet fully understood, because of their localization and their identity as water channel proteins, they most likely play vital roles in the cerebral edema process. We will now review the role of the AQPs within the context of this revised subdivision of edema formation.

3.2. Contribution of AQPs in edema formation and resolution

AQP1, 4 and 9 show changes in their levels of expression in several brain disorders in rodent as well as in human samples [12,29,53]. However, these studies were mostly focused on AQP4 because its level of expression changes follows the edema process for several brain disorders [12,31]. An important tool introduced several years ago to address the question of AQP4's involvement in the edema process has been the generation of AQP4 knockout (AQP4^{−/−}) mice by Dr. Verkman's group [54]. AQP4^{−/−} mice did not exhibit any major structural and

physiological modifications, except that AQP4 $-/-$ mice were associated with extracellular space expansion by about 20% compared to the wild-type (WT) [55–58]. However, it cannot be excluded that some astrocyte properties could be changed in the AQP4 $-/-$ mice, like glutamate reuptake by a decrease of the glutamate transporter GLT-1 [59]. These changes in molecular properties should be included in the interpretation of the results obtained from the experiments carried out in several rodent brain injury models. Interestingly AQP4 $-/-$ mice were tested in many rodent brain disorder models and often gave opposite outcomes in which WT sometimes fared better and other times worse. The results suggested by Dr. Verkman's group lead to hypothesize a dual role for AQP4 in the edema process: deleterious during edema formation and beneficial during the edema resolution phase [60]. However, the absence of available drugs to acutely and specifically block AQP4 did not allow the testing of the dual role hypothesis after brain injury until recently by using a siRNA approach *in vivo* [38,61].

3.2.1. AQP4 and edema build-up

Acute brain injuries including trauma [12,62], ischemia [31,39], and subarachnoid hemorrhage [53], each have distinct patterns of alteration in the level of AQP4 expression. Early after stroke, AQP4 expression is rapidly up-regulated in the astrocyte endfeet in contact with blood vessels, peaking at 1 h after stroke onset in a model of transient occlusion of the middle cerebral artery [31,49]. This increase in AQP4 is observed in the future lesion site and in peri-infarct in mouse stroke model, and the degree of its increase is temporally correlated with the degree of brain swelling [31,49]. These early changes are associated with the development of ionic brain edema (Fig. 5B) and the swelling of the astrocyte processes in stroke models [37]. However, increased AQP4 expression is not observed in more severe stroke models [63], prompting the hypothesis that under great tissue duress, the brain is not able to synthesize sufficient new AQP4 proteins during the early phase of reperfusion. Interestingly, the ratio of AQP4-m1 and AQP4-m23 is changed in the ischemic hemisphere, with higher induction of AQP4-m1 compared to AQP4-m23 [49], suggesting a disorganization of the OAPs in accordance with previous rat stroke data [64]. However, the functional consequences of AQP4-m1 increase and disorganization are not yet elucidated (Fig. 5A).

The complexity of the role of AQP4 in edema process is outlined by the variety of the changes in AQP4 expression, which seems to depend on both the degree of severity and the pathological model. Indeed, increased AQP4 [65–69] versus decreased AQP4 [62,70,71] is likely due to differences in injury type, rodent strains, and age at impact [12]. Moreover, several studies using the AQP4 $-/-$ mice showed discrepancies in the outcomes and interpretation of the role of AQP4 in edema process. For example, AQP4 $-/-$ mice reveal a protective role for AQP4 in spinal cord injury models with a decrease of edema formation and lesion size at early time point after injury [72]. Using a similar model and transgenic mice, a second set of experiments showed functional improvement for the WT mice compared to the AQP4 $-/-$ mice at long-term after contusion spinal cord injury (SCI) [73], suggesting that AQP4 plays either detrimental roles in the edema process or a protective role by facilitating the clearance of excess water. Several other contradictory results using the non-conditional AQP4 $-/-$ mice [73,74] indicate the limitations of using this genetic tool to solve the question of the pathophysiological role of AQP4 in edema formation. In our lab, we recently developed *in vivo* application of siRNA targeting AQP4 (siAQP4), showing specific AQP4 decrease with reduced water mobility [38,61]. This new tool is further developed as a molecular approach to address the question of the role of AQP4 in edema formation, which was tested in a model of juvenile traumatic brain injury (jTBI). In this model we showed that AQP4 expression is not changed at 24 h during the build-up of edema [12]. Our results suggest that stable AQP4 levels may contribute to water entry leading to cellular swelling (lower apparent diffusion coefficient (ADC)) and increased edema (increased T2) at proximity of the site of impact [12]. Interestingly,

application of siAQP4 after jTBI induced a reduction in edema formation associated with cognitive improvement at 2 months post-injury, suggesting that siAQP4 could be used as a specific drug to prevent edema formation by transiently decreasing the level of AQP4 expression [61]. This data show that the presence of AQP4 plays a deleterious role during the edema formation by facilitating the entry of water in the astrocytes.

3.2.2. Edema resolution in acute brain disease: role of AQP in water clearance

As mentioned previously, the data generated using AQP4 $-/-$ mice raised the hypothesis of a dual role for AQP4 in the edema process with a deleterious role of AQP4 in edema build-up and its beneficial role in water clearance during the edema resolution [75,76]. Despite the lack of a clear answer on the role of AQP4 in edema resolution, several results support this hypothesis. The first evidence is the infusion of saline solution in brain parenchyma that induced significant increase in the intracranial pressure in AQP4 $-/-$ mice compared to the WT [75]. In several pathological conditions, increased AQP4 was observed to be associated with edema resolution measured over time using MRI [12,36,38–41,61]. Frequently, AQP4 expression is increased after 48 h in stroke models [31,49], in TBI [12,65–69] and in neuroinflammatory lesion conditions [41]. Most of the time, the increase of the AQP4 is observed near the lesion site in perivascular astrocyte endfeet, astrocyte processes, and the glia limitans [12,31]. These changes may indicate that excess AQP4 could facilitate edematous fluid elimination through the subarachnoid space [41,77,78]. As observed in a jTBI model, increased AQP4 in the glia limitans may compensate for water accumulation at 1 and 3 days (higher T2), with a gradual increase of AQP4 at 3 days and normalization of both AQP4 and T2 values by 7 days [12]. In the rat neuroinflammatory lesion model, the ADC monitored time course study indicates a clear distinction between a minor AQP4 expression increase during the edema build-up phase and a shift to strong AQP4 expression during the edema resolution phase [41]. In fact, ADC values are significantly increased when AQP4 expression is at the peak of its expression [41].

Let us now consider the possibility of other AQPs playing a role in cerebral edema resolution. AQP9 protein is up-regulated in reactive astrocytes after 7 days along the infarct border after ischemic injury. However, AQP9 up-regulation does not correlate with the degree of brain swelling, in contrast to AQP4 [29,31]. Moreover, this pattern of changes was not observed in all brain pathology models [12]. In gerbils, AQP9 expression is found within hippocampal pyramidal neurons in CA1, CA2 and CA3 at 6 h after stroke onset [79], which contrasts with other stroke models [29,31]. All together, these results would suggest that AQP9 does not play a significant role in edema process after brain injury. As pyramidal neurons do not express this channel under normal physiological conditions it is likely that metabolic stressors may induce expression of this “energy” channel. However, the functional consequences of increased AQP9 in neurons and astrocytes have not yet been elucidated. The involvement of AQP9 in astrocyte energy metabolism suggests [33] that increased AQP9 may facilitate the use of glycerol as an alternate fuel and possibly assist in neuronal recovery [29,31].

AQP9 does not seem to play a role in edema resolution, but it is likely that AQP4 is working together with other astrocyte proteins such as connexin-43 (Cx43) and the potassium channel Kir4.1 to clear the excess water. Cx43 forms gap-junctions between the astrocytes and contributes to the diffusion of solutes including water within the astrocyte network. Interestingly, Cx43 is down-regulated after silencing RNA against AQP4 in primary astrocyte cultures [80], and it may decrease the connectivity between the astrocytes. Kir4.1 co-localizes with AQP4 in astrocyte endfeet [24] and cellular potassium reuptake is impaired in an AQP4 $-/-$ mice epilepsy model [25] and potassium has been linked with water flux during astrocyte swelling in a SCI [81]. It is highly possible that AQP4, Cx43, and Kir4.1, all expressed in astrocytes, are working in concert to address cerebral edema post-brain injury.

3.2.3. Chronic changes of brain AQP: relation with water homeostasis dysfunction?

AQP was linked to water homeostasis in physiology and pathophysiology. However, AQPs have been associated with other cell physiological functions such as cell migration [82] and gas diffusion [6]. AQP1, 4 and 9 expressions also increased in brain tumors and in peritumoral tissue [53,83]. The presence of the AQPs in astrocyte in peritumoral tissue could be associated with edema formation due to high changes of the tissue homeostasis and hyper-metabolism. In addition, presence of these AQPs could be associated with the properties of the gas diffusion in the clearance of excess CO₂ as well as the diffusion of O₂. Similarly in rodent SCI, AQP1 induction is observed in astrocytes and neurons [84]. In the same way, increase of AQP1 expression is observed in neuronal processes in the dorsolateral septum at 7 days, 1 month and 2 months post-jTBI [12]. While AQP1 expression in astrocytes could be linked to the secretion to cerebrospinal fluid during cyst formation [84], neuronal AQP1 expression was co-localized with growth associated protein-43 in the context of SCI [84]. AQP1 was also associated with cell migration and its presence in neurons suggests a role in plasticity and neurorepair after injury [84]. Neuronal septal tracts are involved in a number of cognitive pathways including pain [85,86] and AQP1 $-/-$ mice had reduced pain responses compared to WT [87]. Clinically, TBI patients experience varying degrees of chronic pain post-injury [88,89]; thus septal increase of AQP1 could be associated with pain experience although consensus has not been established for the role of AQP1 in the pain process [90]. These results illustrate the complexity of the pathophysiological roles of AQP1.

Long term changes of AQP4 have been observed as well: AQP4 expression remained increased up to 28 days in the border region of the remaining injury site in stroke model [91] and after SCI [21]. In fact, AQP4 is known to be involved in astrocyte migration in glial scar formation, by facilitating water entry necessary for filopodia formation [82], and to facilitate cell adhesion between astrocytes [18]. It is likely that the high expression of AQP4 in astrocytes would contribute to glial scar formation by facilitating the migrations and cell adhesion between astrocytes to form a new barrier in the border of the cavity formed post-injury.

In chronic brain diseases such as Alzheimer's disease and also post-jTBI, decrease of AQP4 expression was observed around the blood vessels in relation with beta amyloid deposition [50,92]. This reduction of AQP4 might affect the water homeostasis within the neurovascular unit. The decrease of the AQP4 has a consequence of reducing the perivascular flow and limiting the clearance of toxins such as beta amyloid [26].

4. AQP4 and neuroinflammation in autoimmune and neurodegenerative diseases

Neuroinflammation starts during the acute phase after brain injury and is also present at long term and in chronic brain pathologies like multiple sclerosis (MS). Neuroinflammation is a generic term encompassing complex molecular and cellular events at the BBB among the various brain diseases and injuries [93]. In parallel to microglia activation, astrocytes become activated and play a role in the neuroinflammation process with their involvement in astrogliosis. The role of astrogliosis is dual: could be beneficial or detrimental depending on the situation [94], much like microglial activation. The process of astrogliosis includes the hypertrophy of astrocytes with different morphological fates depending on the severity of the injury [95]. Interestingly, absence of AQP4 in astrocyte endfeet is associated with decrease of astrocyte migration toward the site of the injury and decrease of hypertrophy of astrocytes observed in astrogliosis [82,96]. As discussed above, AQP4 expression changes during the inflammatory process suggesting changes in water movement related to neuroinflammation [41,93]. The possible link between neuroinflammation and AQP4 was elucidated with research on neuromyelitis

optica (NMO) [97], in which serum antibodies were found to recognize the astrocytic AQP4.

4.1. AQP4 and neuroinflammation in neuromyelitis optica [97]

NMO is an autoimmune neurodegenerative disease affecting the myelin in the spinal cord and optic nerve. The clinical disease is often characterized by abnormal signals in the optic nerve and spinal cord; AQP4 has been recently identified as the target for NMO-immunoglobulin G (IgG) [98–100]. Interestingly, presence of the anti-AQP4 IgG in serum patient is used as a differential diagnosis, to differentiate NMO from multiple sclerosis pathology [98–100]. There is now discussion whether NMO-IgG specifically targets AQP4 within the OAP structures, rather than free AQP4 isoforms [77,101–104]. In fact, NMO-IgG can also bind to denatured monomers and native tetramers [103]. However, there is still an unanswered key question: Is the presence of the autoantibody against AQP4 the cause of the disease or a collateral consequence of some secondary pathological mechanism? Regardless of the answer, the presence of anti-AQP4 IgG remains to be a powerful and useful differential diagnostic tool in the daily clinical practice [105]. However, several clinical observations have also reported patients with myasthenia gravis suffering from auto-AQP4-antibody positive serum as well [106–112]. This points out the possibility of a common autoimmune mechanism for both diseases; and further points to the involvement of AQP4 in the peripheral immune system as well.

4.2. AQP4 and experimental autoimmune encephalomyelitis (EAE)

Experimental autoimmune encephalomyelitis (EAE) model is used to mimic MS pathophysiology. In this model, up-regulation of AQP4 was starting at 10 days until the onset (Fig. 4B) and peak of cerebellar enlargement and showed a significantly positive correlation between AQP4 and BBB disruption in the cerebellum, associated with a decrease of tight junction proteins such as occludin [113]. Accordingly, AQP4 $-/-$ mice showed a less severe clinical score and tissue inflammation after EAE and lipopolysaccharide injection compared to WT animals [114]. These results are in support of a deleterious role of AQP4 in MS pathophysiology. One of the possible explanation to the benefits of the absence of AQP4 is reduced production of the proinflammatory cytokines, tumor necrosis factor-alpha and interleukin-6, observed in AQP4 $-/-$ compared to WT mice astrocyte cultures [114]. AQP4 has also been related with the production of CD4⁺ and CD25⁺ T regulator cells [115]. Absence of AQP4 possibly interrupts the immunosuppressive regulators in Parkinson's disease, leading to increased microglial activation and a worse outcome due to more dopaminergic neuronal loss after induction of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [115]. The presence of the AQP4 in the spleen, thymus and lymph nodes suggests that the AQP4 not only is involved in the neuroinflammatory process but also could participate in the systemic immune response [115].

4.3. Neuroinflammation in brain injury: astrocytic AQP4

As discussed previously, AQP4 expression is frequently increased during the onset of neuroinflammatory period characterized by microglia activation and astrogliosis. In fact, several interesting recent data suggest a potential relationship between AQP4 and microglial activation, without a clear definition about the link. First, AQP4 $-/-$ mice are more vulnerable to seizures compared to WT one month after TBI [116]. This difference was explained by the neuroinflammatory response showing less astrogliosis and increased microglial activation in AQP4 $-/-$ compared to WT mice. Minocycline injection in AQP4 $-/-$ reversed the outcome by inhibition of the increase in microglia activation and decreased the severity of post-traumatic seizures [116]. Similar results were reported in cryo-injury models, where AQP4 $-/-$ mice presented increased microglia and reduced astrogliosis compared to WT. In this model, a decrease in the lesion volume and in neuronal

loss in AQP4^{−/−} mice compared to WT was reported at 1 day after injury whereas the opposite result was reported at 7 and 14 days [117]. In our lab, we have also observed that the siAQP4 treatment after jTBI showed a decrease in AQP4 associated with less BBB disruption, less edema, more NeuN positive cells, and better behavior outcomes compared to the control group up to 2 months post-injury [61]. As observed in the AQP4^{−/−} mice, we reported an increase in activated microglia and a decrease in astrogliosis around the lesion at 3 days post-injury in siAQP4 treated rats compared to control group. However, this difference is not present anymore at 2 months post-injury [61]. One possible explanation for this observation may be that changes in AQP4 expression are associated with changes in astrogliosis and microglia activation in acute brain injury. Astrogliosis may require the presence of AQP4 to facilitate the water movement necessary for migration [82,96] and hypertrophy. However, the mechanism behind the decrease of the AQP4 and activation of microglia is still unknown. One possible mechanism is a modification in the pattern of cytokine release in response to astrocytic AQP4 down-regulation or inhibition. The other hypothesis is in relation with the presence of stretch-activated chloride (Cl[−]) channels expressed in microglia and known to be activated by osmotic stress [118–120]. The activation of these channels contributes to the maintenance of the non-activated (ramified) phenotype of microglia [118]. Because AQP4 is responsible for water transport and osmotic pressure, we can hypothesize that inhibition of AQP4 through either genetic deletion or siRNA will modify the osmotic stress within the extracellular space surrounding the microglia. This would change the activation status of the swelling activated chloride channels, resulting in microglia activation. Another possibility lies in the cross talk that occurs between astrogliosis and microglia activation [121]. The decrease of reactive astrogliosis as a result of the absence of AQP4 may cause an increase in microglial activity. The exact link between microglial activation and decreased astrogliosis in absence of AQP4 needs further investigation. However, it is likely that at 3 days post-jTBI, increased microglia activation due to siAQP4 treatment is contributing to the beneficial effects observed on the cognitive outcome. Indeed, acute microglia activation was hypothesized to be beneficial in contrast to chronic microglia activation [122]. These changes of AQP4 in relation with neuroinflammation could also be correlated with imaging signal changes over time after brain injury or in chronic brain disorders.

5. Future developments: drugs against AQP4?

As mentioned previously, there is no specific inhibitor to block the AQP4 channel and such a compound is critical for evaluating the role of AQP4 and treatment of edema. Using siRNA strategy permitted to show the potential to use a specific inhibitor of AQP4 in jTBI and the contribution of astrocytic AQP4 in neuroimaging [54,61]. Although non-specific, a range of compounds already commercially available that may block AQP4 have been tested. Bumetanide blocks the AQP4 channel and water permeability in oocytes [123] and prevents edema formation after brain ischemia [48,124], which correlates with decreased AQP4 expression [124]. However bumetanide is also an inhibitor of Na–Cl–K co-transporter expressed in endothelial cells. These multiple sites of action of bumetanide are complicating in vivo validation and make teasing out solo effects of AQP4 difficult [48]. Therefore the benefits of bumetanide on edema could also be due to the inhibition of Na–Cl–K co-transporter expressed in endothelial cells. Acetazolamide (AZA), a sulfonamide carbonic anhydrase inhibitor was also proposed for a specific inhibitor of water permeability associated with AQP1 and AQP4 [125,126]. However, it was reported that AZA has no effect on water permeability [127,128]. Similarly, two other inhibitors belonging to sulfonamide carbonic anhydrase inhibitor class, methazolamide and valproic acid have also been tested but without clear effects on the water permeability [126,129]. Waiting to have a specific pharmacological drug to block AQP4, the siRNA strategy was used with success in normal brain to silence the AQP4 expression [38]. Decrease of AQP4 is

associated with a decrease of ADC values (Fig. 3) [38]. siAQP4 was used as treatment in jTBI with decrease of the edema formation post-TBI and functional improvement up to 2 months post-injury [61]. These results encourage us to use the siRNA strategy as a specific drug to prevent the edema formation. However, heterogeneous alterations in AQP4 expression demonstrate the complexity of modifying edema reduction. This strongly suggests testing the siAQP4 treatment strategy in different brain disorder models to find out which battles will be won with siRNA, and which ones should be avoided.

6. Conclusions

The pattern of AQP4 expression during brain disease reveals that AQP4 is a critical component regulating water movement in edema formation and resolution. It is important to note that the role of AQP4 in edema resolution is still unclear and debated. The role of AQP4 in edema formation or resolution might depend on the physiological conditions inducing brain injury. In acute developing injuries like trauma and ischemia, the AQP4 activation pattern seems to be different from more “chronic” evolving brain lesion development such as neuroinflammation. In such pathological conditions like in animal models of multiple sclerosis or systemic sepsis, the BBB forming cells are already exposed to molecular alterations induced by pro-inflammatory circulating cytokines. In such neuroinflammatory context, the responsiveness of AQP4 expression to the developing edema formation might be delayed compared to the instant AQP4 expression in acutely occurring brain injuries.

There are other brain-AQPs (AQP1 and 9) which have modified expression in brain disorders but little is known about their pathophysiological roles.

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