Aquaporin-4 Gene Disruption in Mice Reduces Brain Swelling and Mortality in Pneumococcal Meningitis

Marios C. Papadopoulos and A. S. Verkman‡

From the Departments of Medicine and Physiology, Cardiovascular Research Institute, University of California, San Francisco, California 94143-0521

The astroglial water channel aquaporin-4 (AQP4) facilitates water movement into and out of brain parenchyma. To investigate the role of AQP4 in meningitis-induced brain edema, *Streptococcus pneumoniae* was injected into cerebrospinal fluid (CSF) in wild type and AQP4 null mice. AQP4-deficient mice had remarkably lower intracranial pressure (9 ± 1 versus 25 ± 5 cm H₂O) and brain water accumulation (2 ± 1 versus 9 ± 1 µl) at 30 h, and improved survival (80 versus 0% survival) at 60 h, through comparable CSF bacterial and white cell counts. Meningitis produced marked astrocyte foot process swelling in wild type but not AQP4 null mice, and slowed diffusion of an inert macromolecule in brain extracellular space. AQP4 protein was strongly up-regulated in meningitis, resulting in a 5-fold higher water permeability (P_w) across the blood-brain barrier compared with non-infected wild type mice. Mathematical modeling using measured P_w and CSF dynamics accurately simulated the elevated lower intracranial pressure and brain water produced by meningitis and predicted a beneficial effect of prevention of AQP4 up-regulation. Our findings provide a novel molecular mechanism for the pathogenesis of brain edema in acute bacterial meningitis, and suggest that inhibition of AQP4 function or up-regulation may dramatically improve clinical outcome.

*Streptococcus pneumoniae* (pneumococcus) is the most common and aggressive meningal pathogen (1–3). The overall incidence of pneumococcal meningitis is rising (4, 5) and the emergence of penicillin-resistant *S. pneumoniae* makes treatment harder (6, 7). Even with effective antibiotic treatment, mortality from pneumococcal meningitis is 10–30%, with 30–90% survival (8, 9). The molecular mechanisms responsible for the formation and absorption of excess fluid in brain edema associated with meningitis are poorly understood.

The two main types of brain edema are cytotoxic and vasogenic (10). Cytotoxic edema, as occurs in ischemic stroke, refers to cell swelling that primarily affects astroglial cells. Vasogenic edema, as occurs in brain tumors, involves accumulation of excess fluid in the extracellular space of the brain parenchyma because of a leaky blood-brain barrier (11). Although both types of brain edema are thought to co-exist in meningitis (8, 9), their relative contributions to brain swelling are not known.

Recently, the bidirectional water channel aquaporin-4 (AQP4) has been found to play an important role in brain-water homeostasis (11–16). AQP4 protein is expressed strongly in astroglia at the BBB and CSF-brain interfaces (17, 18), suggesting involvement in water movement between fluid compartments (blood and CSF) and brain parenchyma. AQP4 deletion markedly reduced brain swelling in mouse models of cytotoxic brain edema, including water intoxication and focal cerebral ischemia (16). In contrast, AQP4 deletion in mice significantly worsened outcome in mouse models of vasogenic brain edema, including intraparenchymal fluid infusion, focal cortical freeze injury, and brain tumor (19). Thus, AQP4 appears to facilitate water movement into brain astroglia in cytotoxic edema, and water movement out of the brain in vasogenic edema.

To investigate the functional role of AQP4 in meningitis, we created a mouse model of meningitis involving injection of live *S. pneumoniae* into the CSF. Although the severity of infection was similar in wild type and AQP4 null mice as assessed by CSF bacterial and white cell counts, the AQP4 null mice had remarkably lower intracranial pressure, brain water accumulation, astrocyte foot process swelling, and mortality. Based on experimental data and computational modeling, we conclude that reduced permeability of the BBB in AQP4 deficiency accounted for the improved outcome in AQP4 null mice. Our results have specific implications regarding aquaporin-based therapies of bacterial meningitis.

MATERIALS AND METHODS

* Mice—AQP4 null mice were generated by targeted gene disruption as described previously (20). These mice have normal growth, appearance, survival, gross cerebrovascular anatomy, brain water content, intracranial pressure, bulk intracranial compliance, and serum chemistries, except for a mild reduction in maximal urinary concentrating ability after prolonged water deprivation (16, 19, 20) and sensorineural hearing loss (21). Experiments were performed on weight-matched wild type and AQP4 null mice in an outbred (CD1) genetic background. Investigators were blinded to genotype information in all experiments. Protocols were approved by the University of California San Francisco Committee on Animal Research.

* Bacterial Culture—*S. pneumoniae* serotype 14 (clinical isolate) was purchased from ATCC (catalog number 700676) and grown at 37 °C on trypticase soy agar-enriched with defibrinated sheep blood (Remel, Lenexa, KS) with shaking (250 rpm) for 18–24 h. For invasion assays, *S. pneumoniae* was cultured on trypticase soy agar-enriched with defibrinated sheep blood (Remel), supplemented with 1 μg/ml of ampicillin and 10 μg/ml of chloramphenicol.

* Acknowledgements—This work was supported by National Institutes of Health Grants DK35124, EY13574, HL59198, EB00415 and HL73856, and a Research Development Program grant from the Cystic Fibrosis Foundation (to S. A. V.), and by a Wellcome Trust Clinician Scientist Fellowship (to M. C. P. sponsored by Sanjeev Krishna). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1794 solely to indicate this fact.

* To whom correspondence should be addressed: 1246 Health Sciences East Tower, Cardiovascular Research Institute, University of California, San Francisco, CA 94143-0521, Tel.: 415-476-8530; Fax: 415-665-3847; E-mail: verkman@tsa.ucsf.edu.

* The abbreviations used are: BBB, blood-brain barrier; CSF, cerebrospinal fluid; AQP4, aquaporin-4; FITC, fluorescein isothiocyanate; ICP, intracranial pressure.
Physiological Parameters—CSF white cell count was determined by counting cells in CSF smears after Gram stain (Remel, Lenexa, KS). The number of live bacteria in the CSF was quantified by culturing serial dilutions of CSF on tryptic soy broth agar with defibrinated sheep blood. Glucose concentrations were measured with a glucometer (OneTouch Ultra, LifeScan, Milpitas, CA) using 1-μl samples of CSF and serum. Arterial blood pressure was recorded from the carotid artery and central venous pressure from the internal jugular vein using the Biopac system. Blood, obtained from the left cardiac ventricle, was analyzed using a blood gas machine (model 248, Chiron Corp., Norwood, MA) and at the Moffitt Hospital clinical laboratory.

Evans Blue Extravasation—In some experiments, Evans blue dye (Sigma) (4% in saline, 160 mg/kg) was injected into a femoral or jugular vein at 30 h after bacterial infection. Fifteen minutes later, the left cardiac ventricle was perfused with 20 ml of phosphate-buffered saline. The brain was removed and divided into right and left hemispheres and cerebellum. Each part of the brain was immersed in 2 ml of formamide at 55 °C overnight, and the extracted dye was quantified by optical absorbance at 610 nm against Evans blue/formamide standards. For comparison, Evans blue dye extravasation was also measured 1 h following focal cortical freeze injury, performed as previously described (19).

Cortical Fluorescence Recovery after Photobleaching—Photobleaching measurements were done as described previously (23). Briefly, after anesthesia the head was immobilized in a stereotactic frame and a craniotomy was fashioned to reveal the intact dura and underlying brain surface. FITC-dextran (70 kDa, 30 mg/ml, Sigma) dissolved in artificial cerebrospinal fluid was applied to cover the exposed dura for 2 h. After loading, the dural surface was washed with dye-free artificial CSF. In some experiments, the dura was then opened and the cortical surface exposed for 1 h after bacteria were injected from control mice or mice with meningitis. The stereotactic frame was then transferred to the stage of an upright epifluorescence microscope. For cortical fluorescence recovery after photobleaching measurements, the first order beam of an argon ion laser (488 nm) was deflected by an acousto-optic modulator focused onto the surface of the mouse brain through a dichroic mirror (510 nm) and objective lens (Nikon ×50 air). Fluorescence recovery was quantified by a photomultiplier. Bleaching was accomplished by increasing laser illumination intensity ~4000-fold for 1–2 ms. Apparent diffusion coefficients in brain versus saline (D/D_s) were computed from fluorescence recovery curves.

Electron Microscopy—Brain ultrastructure was evaluated at 30 h after bacterial infection. Mice were killed and the non-injected hemisphere was immersed overnight in 3% (w/v) Karnovsky fixative. Sections were postfixed in 1% buffered osmium tetroxide, dehydrated in ethanol, and embedded in epoxy resin. Ultrathin sections were stained with aqueous saturated uranyl acetate and Reynold’s lead citrate and examined in a 1200 EX JEOL electron microscope. The astrocytic foot process cross-sectional area was measured in randomly selected transmission electron micrographs by image analysis software as described (16).

Osmotic Water Permeability of the Blood-brain Barrier—The product of apparent osmotic water permeability of the BBB, P, and BBB surface area, S, was estimated experimentally from the kinetics of brain water accumulation in response to intraperitoneal injection of distilled water (200 mIkg), according to the relation,

\[ J_w = P_{fSvw}(\psi_{brain} - \psi_{plasma}) \]  

(Eq. 1)

where \( \psi_{brain} \) is the partial molar volume of water (0.018 cm³/mmol), \( \psi_{plasma} \) is brain osmolality (~310 mOsm, (24)), and \( \psi_{plasma} \) is plasma osmolality after intraperitoneal water infusion (~290 mOsm at 3–6 min) measured using a micro-osmometer (Micro-Osmette, Precision Systems, Inc., Natick, MA). Water flux into the brain \( J_w \) was estimated by wet/dry brain weight measurements at 3 and 6 min after water injection.

Statistical Analysis—Data are presented as the mean ± S.E. Statistical analysis was performed using Student’s t test, analysis of variance, and Log Rank tests. Analyses were done using WinSTAT (version 2001.1, A-Prompt, Lehigh Valley, PA) or XLStat-Pro (version 7.5, Addinsoft, Brooklyn, NY) software.

RESULTS

Mouse Model of Meningitis—As evidenced by using Evans blue dye, fluid injected at the base of the skull rapidly distributed throughout the CSF (Fig. 1B). Histological examination of the needle tract in two wild type and two AQP4 null mice did not reveal brain abscess or significant hemorrhage (not shown).

AQP4 in Meningitis
Fig. 1. Mouse model of pneumococcal meningitis. A, stereotactic setup for injection into CSF. B, injected fluid (colored with Evans blue dye) distributes throughout the CSF including (top left) intrathecal, (top right) cisterna magna, (bottom left) perimesencephalic, and (bottom right) basal spaces. C, hematoxylin and eosin-stained control brain (left) showing normal leptomeninges (white arrowheads) and brain 30 h after bacterial infusion into the CSF (right) showing marked meningeal inflammation (black arrowheads). D: left, Gram-stained CSF smear showing neutrophils (black arrowheads) and Gram-positive cocci (white arrowheads). Right, bacterial and leukocyte counts in CSF of wild type and AQP4 null mice.

No focal histological abnormality was noted after hematoxylin/eosin staining of brain sections from these mice.

The pneumococcal infection in mice showed multiple similarities to human meningitis. There was marked leukocyte infiltration of the leptomeninges in infected, but not in saline-injected mice (Fig. 1C). Inflammation was prominent in the basal meninges, but was absent from meninges overlying the hemispheric convexities, and was qualitatively similar in wild type and AQP4 null mice. Gram stain of CSF smears revealed that >90% of leukocytes were polymorphonuclear and associated with Gram-positive streptococci (Fig. 1D).

The severity of pneumococcal infection in the CSF and the systemic reaction were evaluated (Fig. 1D and Table I). At 30 h after bacterial infusion, CSF leukocyte and bacterial counts were comparably elevated in wild type and AQP4-deficient mice. There was no significant difference in serum sodium concentration to account for the differential brain swelling between wild type and AQP4 null mice with meningitis (presented below). The CSF:serum glucose ratio was low in all mice with meningitis compared with saline-injected control mice. Pneumococcus-infected wild type and AQP4 null mice became comparably hypothermic, which is a manifestation of systemic sepsis, whereas three wild type and three AQP4 null mice injected with sterile saline remained normothermic. Because hypothermia can attenuate brain swelling, measurements of ICP and brain water content were performed after re-establishing normothermia using a heating lamp for 1 h prior to measurements. Arterial blood gas analysis at 30 h after bacterial infusion revealed metabolic acidosis with compensatory respiratory alkalosis. Hemodynamic parameters that could independently influence ICP, including central venous and arterial blood pressures, were also comparable in wild type and AQP4 null mice.

AQP4 Expression—Because AQP4 expression in brain is reported to be up-regulated in brain edema from multiple causes (12, 25), AQP4 protein expression was determined in normal mice and mice with pneumococcal meningitis. Immunoblot analysis showed AQP4 protein in brain homogenates from wild type mice as bands at ~30 and 34 kDa representing the two AQP4 protein isoforms expressed in brain, which were absent in homogenates from AQP4 null mice (Fig. 2A). Quantitative immunoblot analysis of brain homogenates taken at 30 h after bacterial infusion indicated ~7-fold increased AQP4 protein expression in wild type mice compared with control wild type mice. Immunohistochemistry showed AQP4 expression in wild type mice in pericapillary astrocyte foot processes, external glial limiting membrane, and ependyma (Fig. 2B). Increased AQP4 immunolabeling was found in the external glial limiting membrane, pericapillary regions, and in a fibrillary pattern throughout the brain parenchyma in meningitis (Fig. 2C). AQP4 protein was absent from the brain of an AQP4 null mouse with meningitis (Fig. 2D).

Outcome Measures—After injection of pneumococcus into the CSF and recovery from anesthesia, we noted progressive global neurological deterioration of wild type and AQP4 null mice (Fig. 3A). At 30 h post-infection, AQP4 null mice showed less neurological impairment than wild type mice, as quantified by neurological score. AQP4 null mice also had significantly lower mortality than wild type mice assessed over a one-week period after bacterial infusion (Fig. 3B). Because human subjects with meningitis are likely to receive antibiotics, in the survival studies mice were treated with intraperitoneal cefotaxime (115 mg/kg twice daily for 5 days) starting at 24 h after bacterial infusion. Cefotaxime is the first line treatment for pneumococcal meningitis in humans and has good CSF penetration in meningitis (26).

### Table I

| Parameter                          | Wild type | n   | AQP4 null | n   |
|------------------------------------|-----------|-----|-----------|-----|
| General                            |           |     |           |     |
| Temperature (°C)                   | 34 ± 1    | 16  | 34 ± 1    | 16  |
| Weight loss (%)                    | 12 ± 1    | 14  | 9 ± 2     | 14  |
| Hemodynamics                       |           |     |           |     |
| Mean arterial blood pressure (mm Hg)| 86 ± 3    | 3   | 73 ± 8    | 3   |
| Central venous pressure (mm Hg)    | 1.4 ± 0.5 | 3   | 1.3 ± 0.4 | 3   |
| Arterial blood gases               |           |     |           |     |
| pO2 (mmHg)                         | 94 ± 7    | 4   | 90 ± 10   | 4   |
| pCO2 (mmHg)                        | 24 ± 1    | 4   | 23 ± 2    | 4   |
| pH                                 | 7.25 ± 0.01 | 4 | 7.26 ± 0.01 | 4 |
| Chemistries                        |           |     |           |     |
| Serum [Na+] (mM)                   | 142 ± 3   | 2   | 143 ± 1   | 2   |
| CSF:blood glucose                  | <0.25*    | 3   | <0.25*    | 3   |

* Cerebrospinal fluid glucose level below the detection limit.
AQP4 in Meningitis—Because AQP4 has opposing roles in cytotoxic (16) versus vasogenic (19) edema, we sought to determine the type(s) of edema that occur(s) in the bacterial meningitis model used here. Electron microscopy of cerebral cortex from mice with meningitis revealed swelling of pericapillary astrocyte foot processes, a marker of cytotoxic edema (Fig. 4A). Averaged pericapillary foot process area was ~6-fold greater in brain cortex from wild type compared with AQP4 null mice, which had about the same area as non-infected control mice. No tight junction opening (the hallmark of vasogenic brain edema) was found in 20 randomly chosen capillary endothelial tight junctions from wild type and AQP4 null mice (Fig. 4B). Fig. 4C shows that meningitis produced little increase in extravasation of Evans blue into brain compared with cortical freeze injury, an established model of vasogenic brain edema (10).

Cortical fluorescence recovery after photobleaching was used as an independent method to assess cytotoxic versus vasogenic brain edema. We showed recently that diffusion of FITC-dextran in brain extracellular space is remarkably slowed in cytotoxic brain edema (23), whereas diffusion is accelerated in vasogenic edema (27). The differential response occurs because the extracellular space contracts in cytotoxic (cell swelling) brain edema, but expands in vasogenic brain edema. As shown in Fig. 5A, the diffusion of 70 kDa FITC-dextran was markedly impaired at 30 h after bacterial infusion. Consistent with previous findings (23), the baseline apparent diffusion coefficient was slightly higher in AQP4 null mice. Meningitis produced greater slowing of FITC-dextran diffusion in wild type than AQP4 null mice (0.05) and saline-injected control mice (0.01; **, p < 0.001). The increase in brain water was much greater in wild type mice compared with AQP4 null mice, which had about the same area as non-infected control mice. No tight junction opening (the hallmark of vasogenic brain edema) was found in 20 randomly chosen capillary endothelial tight junctions from wild type and AQP4 null mice (Fig. 4B). Fig. 4C shows that meningitis produced little increase in extravasation of Evans blue into brain compared with cortical freeze injury, an established model of vasogenic brain edema (10).

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Because the severity of CSF infection and the systemic response were similar in wild type and AQP4-deficient mice over the first 30 h, we investigated whether the improved outcome in AQP4 null mice was because of reduced brain swelling. Brain swelling was quantified using ICP and brain water content was determined from measurement of wet and dry brain weights. In wild type mice at 30 h after bacterial infection, ICP was elevated to ~3 times normal (Fig. 3C), and associated with a substantial increase in total brain water content (Fig. 3D). Remarkably, ICP did not rise above baseline in AQP4 null mice with meningitis, and the increase in brain water was much reduced (Fig. 3D). In control studies, there was no rise in ICP or brain water content at 30 h after injecting sterile saline into the CSF.

Cytotoxic Versus Vasogenic Brain Edema in Meningitis—Because AQP4 has opposing roles in cytotoxic (16) versus vasogenic (19) edema, we sought to determine the type(s) of edema that occur(s) in the bacterial meningitis model used here. Electron microscopy of cerebral cortex from mice with meningitis revealed swelling of pericapillary astrocyte foot processes, a marker of cytotoxic edema (Fig. 4A). Averaged pericapillary foot process area was ~6-fold greater in brain cortex from wild type compared with AQP4 null mice, which had about the same area as non-infected control mice. No tight junction opening (the hallmark of vasogenic brain edema) was found in 20 randomly chosen capillary endothelial tight junctions from wild type and AQP4 null mice (Fig. 4B). Fig. 4C shows that meningitis produced little increase in extravasation of Evans blue into brain compared with cortical freeze injury, an established model of vasogenic brain edema (10).

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Mechanism by Which AQP4 Deficiency Limits Brain Edema—Two possible mechanisms were investigated to explain the reduced cytotoxic brain edema in meningitis in wild type versus AQP4 null mice: (a) greater intrinsic brain cell swelling in response to meningitis in wild type versus AQP4 null mice, and (b) reduced BBB water permeability in AQP4 null mice. To investigate mechanism a, we measured FITC-dextran diffusion in the extracellular space in brains of wild type versus AQP4 null mice after exposure to meningetic CSF. Meningetic CSF applied directly to brain cortex after removal of the dura produced marked cell swelling within 10 min (Fig. 5B). However, the magnitude of slowing of FITC-dextran diffusion (60–64% reduction in D/D0 compared with baseline) was independent of the genotypic type of receiving the meningetic CSF. Therefore, mechanism a is unlikely to explain the reduced cytotoxic brain edema in AQP4 null mice.

Mechanism b predicts reduced BBB osmotic water permeability in AQP4 null mice compared with wild type mice with meningitis. BBB water permeability was estimated by intraperitoneal injection of a water bolus (0.2 ml/g body weight), which reduced serum osmolality from 310 ± 5 to 290 ± 5 mOsm at 3–6 min after infusion. Fig. 5C shows a ~9-fold greater rate of water entry into the brain in control (non-meningitis) wild type mice compared with AQP4 null mice. After 30 h of meningitis, water entry into the brain was greatly increased in wild type mice (~5-fold compared with control wild type mice), which is consistent with the up-regulation of AQP4 protein in meningitis shown in Fig. 2.

Mathematical Model of Brain Swelling in Meningitis—A quantitative model of brain swelling was developed to examine whether the reduced water permeability of the BBB in AQP4 null mice could account for their reduced ICP and brain water gain in meningitis. As diagrammed in Fig. 6A, model parameters include the rates of CSF secretion (CSF out) and outflow (CSF out), brain compliance (C_B), osmotic water permeability of the BBB (P(t)), and a time-dependent term describing menin-
**Fig. 4.** Cytotoxic brain edema in mouse model of meningitis. A: left, electron micrographs showing marked swelling of pericapillary astrocyte foot processes (arrowheads) in AQP4+/- mice, but not AQP4-/- mice. L, capillary lumen; N, endothelial cell nucleus. Right, astrocyte foot process areas in control mice (5 mic- crographs per mouse, 1 mouse each) and mice with meningitis (10 micrographs per mouse, 2 mice each), *, p < 0.05. B, electron micrographs of capillary endothelial tight junctions (arrowheads) from brains of AQP4+/+ and AQP4-/- mice with meningitis. Each tight junction is representative of 20 examined. BM, endothelial cell basement membrane; L, capillary lumen. C, Evans blue dye extravasation into brain parenchyma (μg of dye per g dry hemisphere weight) of control mice, mice with meningitis, and mice after cortical freeze injury (5 mice per group). There was no significant difference between AQP4+/- versus AQP4-/- mice.

**Fig. 5.** FITC-dextran diffusion in brain extracellular space measured by cortical surface photobleaching. A, representative fluorescence recovery curves (left), and calculated apparent diffusion coefficient (D/D0) (right), for 70 kDa FITC-dextran in brain extracellular space of normal mice (4 per group) and mice with meningitis (5 per group, p < 0.01 comparing AQP4+/+ versus AQP4-/- meningitis mice). B, FITC-dextran diffusion in brain extracellular space after exposing brains of AQP4+/- and AQP4-/- mice to CSF collected from control mice (control CSF) or mice after 30 h of meningitis (meningitis CSF) (5 mice per group, p < 0.001 comparing normal versus meningitis CSF). C, water accumulation in brains of control mice and mice with meningitis induced by intraperitoneal injection of water (200 μL/kg).

As measured in mice by intraventricular perfusion and dye dilution, CSF_{in} = 0.4 μL/min and CSF_{out} = 0.14 (ICP(t) - 0.87) μL/min (28). From data in Fig. 5C, the P_{S} product for wild type (P_{S} + (t)/S) and AQP4 null (P_{S} + (t)/S) control mice were 1.7 × 10^{-5} and 0.18 × 10^{-2} cm³/s, respectively, and 9.3 × 10^{-3} and 1.3 × 10^{-5} cm³/s at 30 h after bacterial infusion. For computations we assumed constant P_{S} for the first 240 min followed by a linear increase thereafter (Fig. 6A). The functional form of Ω_{in} was chosen to reflect bacterial growth in CSF: Ω_{in}(t) = Ω_{in}(0) e^{t/\tau} - 1), where Ω_{in}(0) = 0.04 m³/cm³ and α = 1.4 × 10^{-3} were specified to give the measured ICP of 29 cm H₂O at 30 h after bacterial infusion in wild type mice.

Fig. 6B shows the predicted V_{brain}(t) and ICP(t) from numerical integration of Equations 2 and 3. ICP and brain water increased progressively in the wild type mice. As found experimentally (Fig. 3, C and D), ICP and brain water accumulation in AQP4 null mice were remarkably reduced, which was simulated by replacing P_{S} + (t)/S by P_{S} + (t). A third simulation, labeled “constant P_{S},” was done in which (P_{S} + (t)/S) was fixed at (P_{S} + (t)/S) as measured in control wild type mice, rather than allowed to increase as a consequence of meningitis-induced regulation. Interestingly, maintaining a constant P_{S} produced little increase in ICP and brain water over 30 h. These computations support the idea that reduced water permeability of the BBB in AQP4 deficiency accounts for the improved outcome in meningitis, and predict substantially reduced brain swelling in meningitis after either inhibition of AQP4 water permeability or its up-regulation.

**DISCUSSION**

Our data implicate AQP4 as the major determinant of BBB water permeability. In wild type and AQP4 null mice with meningitis, inflammatory and bacterial products in the CSF induced water movement from the vascular compartment into the brain. About 80% of the excess water in meningitis entered the brain parenchyma through AQP4 water channels. AQP4 null mice with meningitis had a much better outcome than wild type mice, with remarkably lower intracranial pressure and brain water accumulation, and improved neurological status and survival.
Pneumococcal infection caused a 7-fold up-regulation of AQP4 protein expression in brain, which further increased BBB water permeability and hence the accumulation of excess brain water. It is not impossible to study experimentally whether the increase in AQP4 expression is key to meningitis-induced brain edema because inhibitors of AQP4 up-regulation are not available. To assess the role of AQP4 up-regulation, we constructed a mathematical model taking into account the different pathways for water brain water entry/exit. The model predicted that the majority of the excess water in meningitis entered the brain through up-regulated AQP4 water channels. AQP4 up-regulation in meningitis is thus a maladaptive response that accelerates brain swelling. AQP4 up-regulation has also been found in the brain of a human patient with brain edema secondary to acute bacterial meningitis (29), suggesting that similar mechanisms might occur in humans. Increased astrocyte AQP4 expression is not restricted to meningitis, but occurs in several pathologies associated with cytotoxic brain edema, including traumatic brain injury in humans (29) and rats (30), cerebral ischemia in humans (31) and rats (32), and hyponatremia in rats (33). Direct measurement of BBB water permeability \((P_f \cdot S_f)\), as was done here for meningitis, will be required to determine whether AQP4 up-regulation also accelerates brain water accumulation in these conditions. In general, brain edema is associated with increased AQP4 transcript expression in brain water accumulation (25, 30, 32, 34), suggesting that up-regulation of AQP4 protein involves increased mRNA synthesis or decreased degradation. Altered AQP4 expression in astroglia might be mediated by changes in osmolality through a p38 mitogen-activated protein kinase-dependent pathway (34). It is not known whether cytokines or bacterial products can also influence AQP4 expression.

Although it has been suggested that both cytotoxic and vasogenic edema types might co-exist in acute bacterial meningitis, several studies have shown that astroglia are primarily damaged, suggesting that cytotoxic edema predominates. In vitro, lipopolysaccharides cause ultrastructural changes (35) and selectively depolarize (36) rodent astroglia, whereas pneumococcal cell walls selectively kill human astroglia (37). In rodents in vivo, the invading neutrophils become intimately associated with astroglia (38) and Haemophilus influenzae causes massive astroglial swelling (39). Our data also suggest that excess brain water in meningitis primarily accumulates in the intracellular compartment (cytotoxic brain edema). This is supported by morphological evidence of diffuse astroglial foot process swelling and biophysical evidence of reduced macromolecular diffusion in brain extracellular space. Pneumococcal meningitis produced only little opening of the BBB as demonstrated by electron microscopy of brain capillary endothelial tight junctions and measurement of Evans blue dye extravasation, suggesting little vasogenic brain edema. This agrees with the observation that in humans (40), dogs (41), and rats (42) non-complicated bacterial meningitis does not on magnetic resonance scans cause parenchymal enhancement (which would suggest disruption of the BBB), but only meningeal enhancement.

We hypothesize that, during meningitis, astroglia become unable to regulate ionic gradients across the plasma membrane. This causes accumulation of intracellular sodium (with accompanying chloride and water), resulting in cell swelling. The mechanisms of cytotoxic brain edema have been extensively investigated in early focal cerebral ischemia in cats (43), rats (44–46), and gerbils (47), which manifest increased salt and water accumulation from the blood into the brain. Many factors present in CSF of human subjects or animals with acute bacterial meningitis have been proposed to impair the ability of cells to regulate their ionic gradients, including cytokines, free radicals, excitatory amino acids, interleukin-1β, tumor necrosis factor-α, and \(\text{H}_2\text{O}_2\) (48–54).

When the BBB is intact, water flows from the blood into the brain through AQP4, which are abundant at the capillary-facing plasma membranes of astroglial foot processes (Fig. 6C). The excess water enters astroglia and increases intracellular space volume at the expense of extracellular space volume, as found in Fig. 5. Not all parts of the astroglial cell swell equally: foot processes swell more because they are adjacent to compressible capillaries (Fig. 4), but cell bodies expand less because they are next to poorly compressible swollen brain cells.

For these studies, a mouse model of acute bacterial meningitis was developed that reproduced many features of acute bacterial meningitis in humans including leptomeningeal inflammation, CSF leukocytosis, low CSF glucose, impaired neurologic function, and fulminant course. Several approaches were evaluated to introduce bacteria into the CSF, including cisternal, intraparenchymal, and transparentchymal basal infusions at different rates and using different needle types. We
found that slow infusion of a bacterial suspension through brain parenchyma into the basal CSF space with a blunted 27-gauge needle avoided problems of fluid leakage and abscess formation, and gave a highly reproducible course of meningitis. The presence of pneumococcus in the CSF produced marked tracraneal pressure and neurological function in saline-injected control mice. We chose S. pneumoniae, because it is a major cause of meningitis in humans whose incidence is increasing compared with Neisseria meningitidis and H. influenzae (1–5).

Because increased AQP4 expression accounts for the majority of edema predominates as in brain tumor or late stroke (11). Decreased expression of water channels is associated with glioblastoma multiforme (55), and increase brain swelling where vasogenic brain edema predominates as in brain tumor or late stroke (11). Because increased AQP4 expression accounts for the majority of the excess brain water in meningitis, we propose that inhibitors of AQP4 up-regulation may be better drugs than AQP4 channel blockers. Inhibitors of AQP4 up-regulation would efficiently reduce brain edema in meningitis without the potential side effects of inhibition of AQP4 water permeability.

Acknowledgments—We thank Liman Qian for mouse breeding and care, Vibeke Pedersen for electron microscopy, and Drs. S. Saadoun and S. Krishna for critical review of the manuscript.

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