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Aquaporin-4 antibodies, CNS acidosis and neuromyelitis optica: A potential link

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ABSTRACT

Background: Neuromyelitis optica (NMO, Devic's syndrome) is a severely disabling disorder of the central nervous system characterized by optic neuritis and longitudinally extensive myelitis. In around 80% of cases, NMO is caused by autoantibodies to astrocytic aquaporin-4 (AQP4), the most abundant water channel in the CNS. Acute NMO attacks are frequently accompanied by elevated levels of lactate in the cerebrospinal fluid (CSF). As a strongly dissociated anion ($pK' = 3.7$) directly changing the strong ion difference, lactate causes a reduction in the dependent anion [HCO_3^-] and a rise in [H^+], resulting in "metabolic" acidosis in the CSF. CSF acidosis also develops during respiratory failure due to brainstem or high cervical spinal cord lesions, the most common cause of death in NMO. However, lactic acid and more generally, a decrease in pH, has been shown to increase the membrane expression of AQP4 in astrocytes. An increase in AQP4 membrane expression during acute NMO attacks could potentially enhance the complement-mediated humoral immune reaction against AQP4-expressing astrocytes characteristic for NMO and, thus, result in more severe astrocytic damage. Moreover, lactate and acidosis have been shown to cause astrocytic swelling and to affect astrocytic viability, potentially rendering astrocytes more susceptible to AQP4-Ab-mediated damage. Finally, increased AQP4 expression could be an independent risk factor in NMO and other forms of CNS inflammation, as indicated by the finding of grossly attenuated experimental autoimmune encephalomyelitis in AQP4-null mice. Therefore, we hypothesize that CSF acidosis might play a role in the pathophysiology of AQP4-Ab-positive NMO and that alterations in CSF pH might possibly influence the outcome of acute attacks in this condition. In addition, we discuss potential clinical implications and make proposals on how to test the hypothesis. Finally, other factors that influence astrocytic AQP4 membrane expression and might play a role in NMO are discussed.

Neuromyelitis optica (NMO, Devic's syndrome) is a rare disorder of the central nervous system characterized by optic neuritis and (mostly longitudinally extensive transverse) myelitis [1,2]. In 2004, it was reported that NMO is associated with specific serum autoantibodies (termed NMO-IgG) binding to structures adjacent to the pia mater, microvasculature and Virchow–Robin spaces in the majority of cases [3]. Shortly thereafter, aquaporin-4 (AQP4), the most abundant water channel in the central nervous system (CNS), was identified as the target antigen of NMO-IgG. NMO-IgG/AQP4-Ab was later shown to be present in up to 80% of NMO patients [4–9]. Significant epidemiological, clinical, and paraclinical differences were observed between seropositive and seronegative patients, suggesting aetiopathogenetical heterogeneity [1,10]. Over the last few years, overwhelming evidence for a pathogenic role of these antibodies (Ab) has been found both *in vitro* and *in vivo* [11–13]. Within the CNS, AQP4 is mainly expressed by astrocytes. Accordingly, the pathophysiological cascade in NMO was shown to start with astrocytic cell damage and cell loss [14,15]. AQP4 is also thought to play an important role in the pathogenesis of CNS oedema [16–18].

Recently, Morishima et al. reported that lactic acid – and more general, a decrease in pH – increases AQP4 expression in cultured astrocytes [19]. After incubating rat astrocytes in culture media containing 15–35 mM lactic acid (corresponding to pH 7.4–7.1) for 24 h, a dose-dependent, ~1.5- to 2.2-fold increase in AQP4 expression was noted compared to control cells. Incubation for 48 h in medium containing 35 mM lactic acid resulted in a ~3-fold increase as measured densitometrically. Similar effects were achieved with hydrochloric acid and acetic acid (employed at identical pH), suggesting that a decrease in pH rather than specifically lactic acid was responsible for the observed rise in AQP4 expression. In addition, AQP4 expression on the cell surface was increased

Abbreviations: Ab, antibodies; AQP4, aquaporin-4; BBB, blood-brain barrier; CSF, cerebrospinal fluid; CNS, central nervous system; COX, cyclooxygenase; EAE, experimental autoimmune encephalomyelitis; IgG, immunoglobulin G; NMO, neuromyelitis optica; SLE, systemic lupus erythematosus.

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(~3-fold after 24 h) and patchy after incubation with lactic acid as determined by a cell surface biotinylation assay and by immunocytochemical examination. While the authors discussed their finding mainly in the context of the supposed role of AQP4 in the formation of brain oedema, this finding could also potentially be of interest for the pathophysiology of NMO:

1. Astrocytic, membrane-bound AQP4 is the main target of the humoral immune response in NMO.
2. The findings of Morishima et al. suggest that CNS acidosis might result in increased AQP4 expression and altered distribution in astrocytes.
3. An increase in AQP4 membrane expression during acute attacks could potentially enhance the complement-mediated humoral immune reaction against AQP4-expressing astrocytes characteristic for NMO and, thus, result in more severe astrocytic damage.
4. Similarly, changes in the distribution of AQP4 could affect AQP4-Ab binding and AQP4-Ab-mediated cell damage. In fact, complement-dependent cytotoxicity in neuromyelitis optica was shown to be enhanced when aquaporin-4 tetramers assembled as orthogonal arrays in the astrocytic plasma membrane [20,21].
5. Finally, glial cell aquaporin-4 overexpression has been reported to accelerate cytotoxic brain swelling [22].
6. Various conditions can cause CNS acidosis and subsequent AQP4 upregulation in patients with NMO:

(a) *Primary "metabolic" CNS (lact)acidosis*. Lactate production accompanies many pathological CNS conditions, including NMO [23]. Lactate, a strongly dissociated anion ($pK' = 3.7$) directly changing the strong ion difference, causes a reduction in the dependent anion [HCO_3^-] and a rise in [H^+] resulting in "metabolic" acidosis in the cerebrospinal fluid (CSF) [24]. While pH has not been measured in the CSF of patients with NMO so far, elevated CSF L-lactate has been found in 43% of these patients ($n = 80$), with L-lactate levels ranging between 2.1 and 6.8 mmol/l (median, 2.9) [23]. Interestingly, L-lactate was exclusively elevated in CSF samples obtained during acute disease attacks ($p = 0.0004$). An association between NMO attacks and lactate levels is further supported by the fact that lactate levels negatively correlated with the period between onset of relapse and lumbar puncture [23]. CSF L-lactate levels were both more frequently elevated and higher in patients with acute myelitis than in patients with acute ON, probably reflecting differences in lesion volume between these two conditions [23]. Accordingly, L-lactate levels also correlated with the length of the spinal cord in patients with acute myelitis in the same study. The blood–brain barrier (BBB) is relatively impermeable to L-lactate – intravenous infusion of lactate does not increase the brain's lactate content [25–28] –, strongly suggesting an intrathecal origin of L-lactate in NMO.
Extracellular lactacidosis was found to cause swelling of cultured rat astrocytes and to affect cell viability [29,30] (for detrimental effects associated with astrocyte swelling see also Ref. [31]), possibly due to osmolyte uptake via various pathways including facilitated influx of lactate [32], Na+/H+ exchange [33], and Cl^- influx [34], and could thus independently increase AQP4-Ab-mediated astrocytic damage.
There are three potential sources of L-lactate in NMO. Firstly, the presence of CSF granulocytes has been shown to induce L-lactate production [35]. In accordance with this finding, a positive correlation between the presence of polymorphonuclear leukocytes and CSF lactate has been demonstrated [36,37]. Moreover, activated granulocytes themselves have been reported to be a source of CSF lactate [38,39]. Acute NMO attacks are in fact associated with the presence of granulocytic CSF pleocytosis in around 50% of cases [23,40]. Secondly, L-lactate is thought to be produced by astrocytes following glutamate stimulation [41,42]. AQP4-Ab has been reported to result in increased extracellular glutamate concentrations due to coupled endocytosis of AQP4 and the excitatory amino acid transporter 2 (EAAT2), which exist in astrocytic membranes as a macromolecular complex [43]. In addition to increasing L-lactate production, such an increase in extracellular glutamate would have multiple, potentially detrimental effects itself, e.g., by overstimulating glutamate receptors in neurons and oligodendrocytes [43]; moreover, it renders oligodendrocytes susceptible to Ig-independent (alternative pathway) complement attack [43,44].
Finally, neurons may switch to glycolysis, in particular if their capacity to metabolize anaerobically the lactate of astrocytic origin is exhausted, which may further increase lactate levels [42].
Primary CNS acidosis is often overlooked since it cannot be detected by determining the blood acid–base status.

(b) *Secondary CSF acidosis following respiratory acidosis*. Both cervical and brainstem lesions are common in NMO and often cause respiratory failure, the most frequent cause of death in NMO [45]. Respiratory insufficiency, however, can cause respiratory acidosis, which is rapidly paralleled by CSF acidosis as CO_2 can easily cross the BBB [46–50]. Respiratory acidosis not only develops in patients with NMO-related central respiratory failure, but can also occur in NMO patients with pulmonary disease and in intensive care patients treated with permissive hypercapnia [51,52].

(c) *CSF acidosis due to CNS hypoxia*. Severe respiratory acidosis is often accompanied by CNS hypoxia. In turn, CNS hypoxia increases the rate of glycolysis, which may aggravate the severity of the CSF acidosis by producing lactic acid [25,53,54] (circulus vitiosus). Moreover, acidosis may cause vasodilatation, increasing CNS blood volume and, in consequence, intracranial/intravertebral pressure, which can decrease perfusion pressure and, thus, increase hypoxia and, in consequence, acidosis (vicious circle) [55,56]. Finally, NMO causes so-called longitudinally extensive spinal cord lesions (median contiguous lesion length at first myelitis: 6 vertebral segments; range, 1–21; $n = 137$; in 21% additional, smaller lesions are present [1]), occasionally affecting the entire spinal cord. These extensive lesions are often associated with marked spinal oedema (as a result of inflammatory – functional and/or structural – damage to astrocytes, which are both key players in CNS water homoeostasis and integral constituents of the BBB, of bystander damage to other constituents of the BBB such as the endothelium and its basal membrane, and, possibly, of hypoxic damage), which could impair perfusion and thus aggravate hypoxia and, in consequence, acidosis.

(d) *CSF acidosis due to systemic metabolic acidosis*. NMO is frequently associated with non-CNS autoimmune diseases such as systemic lupus erythematosus (SLE) [1,57] and may coexist with common diseases such as diabetes mellitus, which in severe cases can cause metabolic (uremic or ketoacidotic) acidosis and, thus, induce or aggravate CSF acidosis (with or without CNS symptoms) [58]. It must be stressed, however, that CNS acid–base regulation is much more efficient in metabolic acidosis than in respiratory acidosis [46,48,59–61].

(e) *Paradoxical CSF acidosis*. Finally, so-called "paradoxical CSF acidosis" may initially develop after treatment with IV sodium hydrogencarbonate for peripheral metabolic acido-