Autoimmune Aquaporin-4 Myopathy in Neuromyelitis Optica Spectrum

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Neuromyelitis optica (NMO) is currently recognized as an inflammatory central nervous system (CNS) disorder that preferentially affects optic nerves and the spinal cord. It is triggered by binding of pathogenic, complement-activating IgG autoantibodies to the ectodomain of aquaporin-4 (AQP4), which is highly expressed in the plasma membrane of astrocytic foot processes and is the major CNS water channel.

The detection of AQP4 IgG unifies diverse neurological presentations known collectively as NMO spectrum disorders. The presently recognized spectrum includes optic neuritis, transverse myelitis, AQP4-IgG seropositivity, and recurrent myalgias with hyperCKemia. A muscle biopsy revealed scattered myofibers with internal nuclei, atrophy, and regeneration but no necrosis. Mild inflammatory exudates, in endomysial and perivascular spaces, consisted of lymphocytes, histiocytes, and scattered eosinophils. The sarcolemma exhibited loss of AQP4 and deposition of IgG and complement activation products, characteristics not seen in control biopsy samples of healthy muscle and immune-mediated myopathies.

CONCLUSIONS AND RELEVANCE Recurrent hyperCKemia accompanying AQP4-IgG seropositivity reflects targeting of skeletal muscle AQP4 by pathogenic IgG. The entity of autoimmune AQP4 myopathy extends the neuromyelitis optica spectrum beyond the central nervous system.

Report of a Case

In 2001, a 40-year-old woman had an episode of ataxia and left-sided hemiparesis and facial droop that resolved following treatment with intravenous adrenocorticosteroids. In 2004, she experienced pain in her right arm and had hyperCKemia (serum creatine kinase [CK] level, >63 000 U/L [to convert to microkatal per liter, multiply by 0.0167]). In 2005, she presented with pain and vision loss in her right eye. Her visual acuity was 20/400 OD, with right afferent pupillary defect. The results of motor and sensory examinations were normal. Magnetic resonance imaging of her brain demonstrated right optic nerve enhancement. The results of CSF testing were normal. The results of serological testing were negative for antinuclear antibody, rheumatoid factor, and Lyme disease; her rapid plasma reagin test results and erythrocyte sedimentation rate and complement were normal. Her visual symptoms improved after treatment for 3 days with intravenous methylprednisolone.

During the period from 2005 to 2011, she experienced multiple recurrences of optic neuritis, transverse myelitis, and plasmalemmal AQP4 in skeletal and perhaps cardiac muscle. Here we describe the presentation and muscle biopsy findings of a patient who had recurrent hyperCKemia, preceding and coinciding with classic opticospinal NMO symptoms.
proximal muscle pain with hyperCKemia (serum CK levels, 3500–24 531 U/L; normal range, 10–205 U/L), which were treated with intravenous adrenocorticosteroids and/or plasmapheresis (Table). In June 2011, AQP4 IgG was detected by use of an enzyme-linked immunosorbent assay and a transfected cell-based assay. The results of chest radiography, electrocardiography, and metabolic studies, including thyroid function, were normal. Electromyographic findings were consistent with myopathy: left deltoid and vastus lateralis biopsy samples (when her serum CK level was 13 867 U/L) were interpreted as “minimal nonspecific changes.” No subsequent relapses or hyperCKemia episodes were reported after treatment with rituximab and plasmapheresis commenced (in September 2013).

Methods

The patient’s muscle biopsy sample was assessed diagnostically in frozen sections. Hematoxylin-eosin staining and avidin-biotin-based immunohistochemistry were performed on formalin-fixed, paraffin-embedded, 5-μm sections. Control biopsy samples of muscle included 4 normal samples and 2 samples of cases of immune-mediated myopathy (dermatomyositis and autoimmune necrotizing myopathy). The primary antibodies were polyclonal rabbit anti-human AQP4 (1:250; Sigma-Aldrich), anti-human IgG (1:1000; Dako), anti-rat C9 neoantigen (1:2000), and monoclonal mouse anti-human C9 neoantigen (1:400; from B. Paul Morgan, MB, PhD, at Cardiff University’s School of Medicine, Cardiff, Wales).

Results

General Muscle Pathology

Numerous myofibers showed internalized nuclei, with scattered atrophic and regenerating myofibers (without necrosis), variable atrophy of type II fibers, and preservation of type I fibers (positive for adenosine triphosphatase). Endomysial and perivasculary spaces contained mild inflammatory exudates (lymphohistiocytic with scattered eosinophils) (Figure 1A-D). No rimmed vacuoles or abnormal mitochondrial accumulations were seen (with modified Gomori trichrome stain). Cytochrome oxidase and acid phosphatase stains were unremarkable.

AQP4 Loss in NMO Skeletal Muscle

Myofibers in the patient’s biopsy samples showed extensive AQP4 loss (Figure 1E and F). By contrast, myofibers surface AQP4 immunoreactivity was abundant in healthy control muscle fibers and detectable, but variably reduced, in the 2 disease control biopsy samples (Figure 1G-J).

Immune Complex Deposition in NMO Muscle

The scattered nonnecrotic myofibers in the NMO biopsy sample exhibited colocalization of sarcolemmal IgG and C9 neoantigen deposits indicative of attack by complement-activating IgG (Figure 2A–C). The healthy control myofibers and the nonnecrotic fibers in control cases with immune-mediated myopathy lacked sarcolemmal immune complexes. However, occasional scattered myofibers and blood vessels in the normal and immune-mediated myopathy biopsy samples showed evidence of endomysial, perimysial, and sarcolemmal IgG deposits or cytoplasmic, but not sarcolemmal, C9 neoantigen immunoreactivity. Necrotic fibers in control immune-mediated myopathy biopsy samples displayed sarcolemmal IgG and cytoplasmic C9 neoantigen immunoreactivity (Figure 2D–I).

Discussion

The case we have documented here, of an AQP4-IgG-seropositive patient with NMO and recurrent hyperCKemia with muscle pathology compatible with complement-activating IgG targeting sarcolemmal AQP4, confirms organ involvement beyond the CNS as a component of NMO spectrum disorders. Restriction of immunoglobulin and complement deposits to linear sarcolemmal regions where AQP4 loss was

### Table. Clinical and Paraclinical Summary of Case Patient

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Clinical Manifestations</th>
<th>Serum Creatine Kinase Level, U/L</th>
<th>Additional Studies</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>02/12/04-02/23/04</td>
<td>Right arm pain</td>
<td>&gt;63 000</td>
<td>EMG</td>
<td>Increased muscle membrane irritability</td>
</tr>
<tr>
<td>06/12/05-06/15/05</td>
<td>Right optic neuritis</td>
<td>131</td>
<td>MRI of the brain</td>
<td>Right optic nerve enhancement</td>
</tr>
<tr>
<td>July 2005</td>
<td>Bilateral thigh pain</td>
<td>3500</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>11/24/05-11/27/05</td>
<td>Left optic neuritis</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>01/18/06-02/01/06</td>
<td>Left optic neuritis, hyperreflexia on left side, lower extremity clonus</td>
<td>34</td>
<td>MRI of the brain</td>
<td>Left optic nerve enhancement</td>
</tr>
<tr>
<td>01/29/11-02/3/11</td>
<td>Right optic neuritis</td>
<td>158</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>03/29/11-04/2/11</td>
<td>Bilateral thigh and arm pain</td>
<td>24 531</td>
<td>EMG</td>
<td>Myopathic changes</td>
</tr>
<tr>
<td>04/28/11-05/06/11</td>
<td>Transverse myelitis</td>
<td>None</td>
<td>MRI</td>
<td>Enhancing midthoracic lesion</td>
</tr>
<tr>
<td>06/07/11-06/16/11</td>
<td>Lower extremity pain and spasms</td>
<td>13 867</td>
<td>Muscle biopsy</td>
<td>Nonspecific</td>
</tr>
<tr>
<td>06/18/11-06/24/11</td>
<td>Transverse myelitis</td>
<td>11 148*</td>
<td>MRI</td>
<td>Enhancing cervical cord lesion with cord expansion</td>
</tr>
</tbody>
</table>

Abbreviations: EMG, electromyography; MRI, magnetic resonance imaging.

SI conversion: To convert serum creatine kinase to microkatal per liter, multiply by 0.0167.

* Aquaporin-4-IgG seropositivity.
Evident was seen exclusively in the NMO index case. This observation supports a pathophysiology mediated by AQP4-specific IgG, as does the lack of an alternative primary or secondary cause to explain the elevations in CK level. Eosinophilic leukocytes are a consistent pathological finding in inflammatory lesions of AQP4 autoimmunity, both in the CNS and in skeletal muscle. Although type II fiber atrophy is generally considered nonspecific, its occurrence in an NMO spectrum disorder is plausibly attributable to the selective localization of AQP4 on type II fiber membranes. Recent data implicating IgG targeting of placental syncytiotrophoblast AQP4 as a cause of fetal loss for women with NMO is further evidence of NMO spectrum pathophysiology extending beyond the CNS.9

Mild endomysial and perivascular inflammation in the skeletal muscle (A-D) is evident in the case patient with neuromyelitis optica (NMO). The inflammation consists of lymphocytes (C) and eosinophils (D [arrows]), and scattered internalized nuclei can be seen as well (D [arrowheads]). Aquaporin-4 (AQP4) immunoreactivity is totally lost in the muscle lesions of the case patient with NMO (E and F), including regions of focal endomysial inflammation (E and F [arrowheads]). Dermatomyositis biopsy samples reveal perifascicular muscle atrophy with variable AQP4 immunoreactivity (G and H); AQP4 immunoreactivity is preserved in some sarcolemmal regions (G [arrowheads]), and cytoplasmic immunoreactivity is enhanced in scattered myofibers. In normal skeletal muscle, AQP4 immunoreactivity is abundant in the sarcolemma of most myofibers (I and J).
Although cardiac muscle is a potential target of NMO IgG, and a potential source of elevated serum CK levels, our patient lacked evidence of cardiac disease and had no alternative cause to explain the elevations of CK level other than the incriminating finding of sarcoplemmal immunocomplex deposition. Sarcoplemmal lesioning by AQP4-specific IgG and complement plausibly explains other reports of hyperCKemia in NMO. It remains to be determined how commonly hyperCKemia occurs in NMO. Episodes may be insufficiently severe to produce myalgic symptoms or prompt serum CK testing.

How might AQP4 loss lead to hyperCKemia in the absence of fiber necrosis? The major CK isoform of muscle is located in the cytoplasm and is largely not bound to the cytoskeleton. Aquaporin-4 is anchored in the sarcoplemma as a component of the dystrophin-associated protein complex linking the cytoskeleton to the extracellular matrix. A breach or structural disorganization in the sarcoplemmal membrane could allow for the leakage of CK into serum. Sarcolemmal damage and an elevated serum CK level are characteristic of conditions such as dysferlinopathy, in which membranous AQP4 is reduced, and Duchenne and Becker muscular dystrophies, in which the dystrophin-associated protein complex is disorganized.

The role of AQP4 in bioenergetic pathways and in the intracellular calcium dynamics of muscles suggests another potential mechanism for the hyperCKemia observed in NMO. Several muscle proteins related to metabolism are reduced in AQP4-null mice, without compromising sarcoplemmal integrity. Calcium homeostasis is particularly perturbed in fast-twitch muscle fibers, which express AQP4 most abundantly. Thus, IgG-induced loss of sarcoplemmal AQP4 in NMO could lead to metabolic and structural myofiber damage with resultant CK leakage.

Figure 2. IgG and Complement Deposits in Skeletal Muscle of Case Patient With NMO

Sarcolemmal IgG (A [arrowhead]) and C9 neoantigen (B and C [arrowheads]) deposits are extensive in the skeletal muscle of the case patient with neuromyelitis optica (NMO). Dermatomyositis lesions exhibit cytoplasmic IgG (D [arrowhead]) and occasional sarcolemmal IgG in atrophic myofibers. Some atrophic myofibers in the dermatomyositis biopsy samples show cytoplasmic C9 neoantigen immunoreactivity (E and F [arrowheads]). In normal skeletal myofibers, IgG is confined to blood vessels in the endomysium and perimysium (G), and C9 neoantigen immunoreactivity is in proximity to scattered perimysial vessels (H and I).
Although, to date, experimental models of AQP4 autoimmunity have not reproduced the clinical and histopathological opticospinal manifestations typical of NMO, mild myositis and nephritis have accompanied experimental autoimmune encephalomyelitis-like brain lesions in naive rats that received adoptively transferred AQP4-specific T cells. Those observations demonstrate the potential of an AQP4-specific immune response to target antigen-expressing cells in non-CNS organs.

In conclusion, myopathy can be considered a pathophysiological component of the NMO spectrum disorders defined by AQP4-IgG seropositivity. Recurrent hyperCKemia accompanying AQP4-IgG-seropositivity is evidence for targeting of extra-CNS AQP4 by pathogenic IgG. Lack of evident muscle pathology in previously reported cases of NMO-related hyperCKemia may reflect (1) patchy muscle involvement in NMO, (2) a limited number of muscle biopsy sites, and (3) failure to evaluate biopsy samples immunohistochemically for AQP4, IgG, and activated complement component immunoreactivities. Skeletal muscle involvement in NMO may be underrecognized clinically. Episodic muscle pain may not be reported by patients with NMO, and serum CK testing is not routinely performed. Application of AQP4 immunohistochemistry may be useful for evaluating muscle biopsy samples in the investigation of unexplained hyperCKemia. In addition to skeletal muscle, we predict that the spectrum of NMO will extend to other AQP4-enriched organs, such as the kidney, heart, retina, inner ear, stomach, and bronchioles. Therapies targeting humoral immune responses can be anticipated to limit extra-CNS tissue injury in patients with NMO spectrum disorders as is observed for acute CNS manifestations.

ARTICLE INFORMATION
Accepted for Publication: March 18, 2014.
Published Online: June 9, 2014.

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Author Contributions: Dr Lucchinetti had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Guo, Lennon, Parisi, Lucchinetti. Acquisition, analysis, or interpretation of data: Guo, Lennon, Popescu, Grouse, Topel, Milone, Lassmann, Pittock, Stefoski, Balabanov, Lucchinetti. Drafting of the manuscript: Guo, Lennon, Popescu, Lucchinetti. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: Lucchinetti. Obtained funding: Lucchinetti. Administrative, technical, or material support: Milone, Lassmann, Balabanov, Lucchinetti. Study supervision: Lucchinetti.

Conflict of Interest Disclosures: Dr Lennon is a named inventor on a patent issued to the Mayo Foundation for Medical Education and Research that relates to the NMO antigen and its application to the diagnosis of NMO, and she accrues revenue from royalties regarding AQP4-associated antibodies for diagnosis of NMO. Dr Pittock accrues revenue from royalties regarding AQP4-associated antibodies for diagnosis of NMO and AQP4 autoantibody as a cancer marker, and he has received research support from the National Institutes of Health, Alexion Pharmaceuticals, and the Guthy-Jackson Charitable Foundation. Dr Lucchinetti accrues revenue from royalties regarding AQP4-associated antibodies for diagnosis of NMO and has received research support from Koltan Pharmaceuticals, the National Institutes of Health, the US Department of Defense, and the Guthy-Jackson Charitable Foundation.

Role of the Sponsor: The funding agency had no role in the design and conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: We thank Patricia Ziemer at the Mayo Clinic in Rochester, Minnesota, for her expert technical assistance in immunohistochemistry. She did not receive any financial compensation from the funding sponsor.

REFERENCES