Autoimmune Gliopathies: Real Targets in Demyelinating Diseases of the CNS

Sean J Pittock, MB, MRCPI, MMed Sci, MD,
Professor of Neurology
Mayo Clinic
Rochester, MN

DISCLOSURE
Dr. Pittock receives no royalties from the sale of tests performed in the Neuroimmunology Laboratory at Mayo Clinic; however, Mayo Collaborative Services Inc. does receive revenue for conducting these.

Mayo Clinic and Dr. Pittock have a financial interest in technology associated with:
1. "Aquaporin-4 AutoAntibody as a Cancer Marker."
2. "Aquaporin-4 Binding Autoantibodies in Patients with Neuromyelitis Optica Impair Glutamate Transport by Down-Regulating EAAT2."
3. "Peripherin-Specific Autoantibodies as a Marker for Neurological and Endocrinological Disease."

Dr. Pittock receives research support from Alexion Pharmaceutical, Inc., the Guthy-Jackson Charitable Foundation, and the National Institutes of Health (NS065829).

Dr. Pittock has provided consultation to Alexion Pharmaceutical, MedImmune LLC, and Chugai Pharma, but has received no personal fees or compensation for these consulting activities. All compensation for consulting activities is paid directly to Mayo Clinic.

Off Label Usage
- I will mention use of a variety of immunotherapies.
Outline

• Autoimmune AQP4 chanelopathy
• CRMP5 autoimmunity
• Autoimmune MOG-opathy

NMO: Devic’s Disease
Traditional definition (1894)

- Bilateral optic neuritis and myelitis
- Occurred simultaneously or in quick succession
- Severe course
- Extremely rare!
Core Definition

- Optic neuritis
- Myelitis—longitudinally extensive; >3 segments
- Brain spared early

In the past decade NMO is recognized as:

- A severe relapsing autoimmune inflammatory CNS demyelinating disorder
- Commonly misdiagnosed as MS
- Not responsive to MS therapies (may worsen)
- Affecting optic nerves and spinal cord preferentially, but not exclusively
- A disease-specific serum biomarker, AQP4-IgG, unifies an “NMO spectrum”
NMO-IgG Autoantibody (IF)

NMO-IgG yields a unique staining pattern

NMO-IgG colocalizes with AQP4

**NMO-IgG targets AQP4**

*Where in the brain is AQP4?*

- Astrocytic endfeet surrounding intraparenchymal vessels
- Ependymal cells and subependymal layers lining the ventricles
- Hypothalamus

*AQP4 Localization*

- Found on all surfaces of astrocytes.
- Highest concentration in polarized plasma membrane of:
  - CNS perivascular and peripial end-feet that are in direct contact with basal lamina of the endothelium and pia mater. (Nielsen et al, 1997)
  - Ventricular ependyma
  - Interneuronal synaptic junctions
- Not found in neurons, oligodendrocytes, or choroidal epithelial cells.
NMO-IgG initiated modulation of GFP-AQP4 is rapid and reversible

Serum removed, fresh medium added

Immunopathology of NMO

<table>
<thead>
<tr>
<th></th>
<th>NMO</th>
<th>MS</th>
<th>ADEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OS</td>
<td>ST</td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td>+++</td>
<td>+++</td>
<td>+/-</td>
</tr>
<tr>
<td>C9neo Rosettes</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>AQP-4</td>
<td>-</td>
<td>-</td>
<td>↑↑</td>
</tr>
<tr>
<td>NMO-IgG</td>
<td>POS</td>
<td>POS</td>
<td>NEG</td>
</tr>
</tbody>
</table>

Lucchinetti et al. Brain Pathology 2014
Binding of AQP4-IgG to AQP4 M23 isoform results in more effective activation of complement than binding to the M1 isoform

Hinson et al., PNAS 2012
in interest of time, suggest delete this slide
not really the topic of the presentation
Camille, 10/7/2012
Eculizumab in AQP4-IgG-positive relapsing neuromyelitis optica spectrum disorders: an open-label pilot study

Attack frequency before, during, and after eculizumab treatment

No change in AQP4-IgG titers

The Lancet Neurology Volume 12, Issue 6 2013 554 - 562
AQP4 & glutamate transport are related

- EAAT2 is restricted to astrocytes
- EAAT2 removes 90% of neuron-released glutamate
- EAAT2 expression is reduced in AQP4 null astrocytes (Zheng et al, 2007)
- EAAT2 is coupled to AQP4 physically & functionally (Hinson et al, 2008)

Women are more likely to be AQP-IgG + than men
Difference more striking in adults than in children

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>F:M</th>
<th>Absolute Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pediatric (1-18)</td>
<td>144</td>
<td>3087</td>
</tr>
<tr>
<td>Young adult (19-39)</td>
<td>774</td>
<td>18551</td>
</tr>
<tr>
<td>Middle-aged (40-64)</td>
<td>1435</td>
<td>26049</td>
</tr>
<tr>
<td>Elderly (65-89)</td>
<td>321</td>
<td>3166</td>
</tr>
</tbody>
</table>

Seropositive Seronegative
Of 2743 AQP4/IgG+ patients

• 146 (5.3%) pediatric
• 333 (12.1%) elderly

A Secondary Progressive Course is Uncommon in NMO

Wingerchuk D M et al.
Neurology 2007;68:603-605
Kaplan-Meier estimates of time to motor and visual disability by:

- AQP4-IgG serostatus (A-D)
- Onset type (E,F)


NMO and SLE/Sjogren’s: Co-associated autoimmune syndromes

NMO
(NMO-IgG +)

Lupus/Sjogren’s
(ANA/ENA +)

NMO-IgG+
NMO-IgG-
## Neural autoantibodies found in 177 NMO Patients

<table>
<thead>
<tr>
<th>Autoantibody (Ab) detected</th>
<th>N positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMO-IgG</td>
<td>148 (83)</td>
</tr>
<tr>
<td>Acetylcholine receptor Ab</td>
<td></td>
</tr>
<tr>
<td>Muscle-type</td>
<td>19 (11)</td>
</tr>
<tr>
<td>Ganglionic-type</td>
<td>11 (6)</td>
</tr>
<tr>
<td>Glutamic acid decarboxylase Ab,</td>
<td>26 (15)</td>
</tr>
<tr>
<td>Collapsin response- mediator protein (CRMP)-5 IgG (by Western blot).</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Voltage-gated potassium channel Ab</td>
<td>9 (5)</td>
</tr>
<tr>
<td>Voltage-gated calcium channel Ab,</td>
<td></td>
</tr>
<tr>
<td>P/Q-type</td>
<td>3 (2)</td>
</tr>
<tr>
<td>N-type</td>
<td>7 (4)</td>
</tr>
<tr>
<td>≥ 1 autoantibody detected (excluding NMO-IgG)</td>
<td>61 (34)</td>
</tr>
</tbody>
</table>
NMO and the BRAIN

- 60% of NMO pts have MRI brain lesions
  Pittock; Arch Neurol 2006
  60% non-specific WM lesions
  15% typical for MS
  10% unique for NMO

MS vs NMO

<table>
<thead>
<tr>
<th></th>
<th>MS</th>
<th>NMO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset</td>
<td>29</td>
<td>39</td>
</tr>
<tr>
<td>Sex (F:M)</td>
<td>2:1</td>
<td>9:1</td>
</tr>
<tr>
<td>Course</td>
<td>85%RR; 15%PP not monophasic</td>
<td>80-90% relapsing 10-20% monophasic</td>
</tr>
<tr>
<td>Attack severity</td>
<td>Usually Mild</td>
<td>Usually Severe</td>
</tr>
<tr>
<td>SP course</td>
<td>Common</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Autoimmune dzs</td>
<td>Uncommon</td>
<td>15-30%</td>
</tr>
<tr>
<td>CSF cells</td>
<td>Rarely &gt; 25 cells; never PMNs</td>
<td>Occ &gt; 50 cells PMNs</td>
</tr>
<tr>
<td>CSF OCBs</td>
<td>85%</td>
<td>15-30%</td>
</tr>
<tr>
<td>MRI spine</td>
<td>Short segment peripheral lesions</td>
<td>Longitudinally extensive</td>
</tr>
<tr>
<td>MRI brain</td>
<td>PVWM lesions</td>
<td>60% Nonspecific 10% unique lesions</td>
</tr>
</tbody>
</table>
NMO and the BRAIN

“NMO-typical” Brain Lesions are in AQP4-rich Sites

Arch Neurol. 2006;63(7):964-8

7 children-brainstem, periaqueductal, and peri-IVth ventricular and spinal cord involvement

McKeon A et al. Neurology 2008;71:93-100
3 children—optic chiasm/diencephalic/peri IIIrd ventricular involvement

McKeon A et al. Neurology 2008;71:93-100

AQP4-rich Area Postrema
First Point of Attack in NMO
(Annals Neurology, 2010)

• Intractable vomiting: initial presenting symptom in 12% of all Mayo Clinic NMO patients

• Initial evaluation in 75% was gastroenterologic.

• Vomiting lasted a median of 4 weeks (range, 2 days – 80 weeks).

• 11 of 12 developed ON or TM after vomiting onset (median interval, 11 weeks; range, 1-156).

• At last follow-up (median, 48 months) 7 fulfilled NMO criteria.
Clinico-Pathological Correlate

- **AQP4 loss** in inflammatory, non-demyelinated foci
  (4th ventricle floor & area postrema)
  Popescu et al. Neurology 2011

- **Reversible medullary lesion**
  Pittock. Arch Neurol, 2006

- **Nausea & intractable hiccups**
  Misu. Neurology, 2005
NMO and Beyond

Myopathy in NMOSD

A-D: Mild endomysial/perivascular inflammation

C: lymphocytes

E and F: AQP4 lost

Dermatomyositis: AQP4

G: preserved in some sarcolemmal regions

H: enhanced in scattered myofibers.

I and J: Normal skeletal muscle: AQP4 abundant in the sarcolemma

Paraneoplastic Neuromyelitis Optica Spectrum Disorder Associated With Metastatic Carcinoid Expressing Aquaporin-4

Aquaporin-4 Autoantibodies in a Paraneoplastic Context
Arch Neurol. 2008;65(5):629-632

AQP4 immunoreactivity in carcinoid tumor cells
A: Sharp border between the hypercellular tumor and normal liver parenchyma
AQP4-immunoreactivity appears to be predominantly cytoplasmic (arrows).

Aquaporin-4 (AQP4) and regulators of complement activation (RCAs) in normal brain and neuromyelitis optica (NMO) lesion.

Samira Saadoun, and Marios C Papadopoulos Mult Scler 2015;1352458515571446
Aquaporin-4 (AQP4) and regulators of complement activation (RCAs) in normal peripheral organs.

FINDING N(e)MO - AQP4 - IgG detection assays - Which test is best?
**FACS>CBA>ELISA**

<table>
<thead>
<tr>
<th></th>
<th>NMO n=35</th>
<th>NMOSD n=25</th>
<th>Total n=60</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>ROC AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIF</td>
<td>17</td>
<td>12</td>
<td>29</td>
<td><strong>48.3</strong></td>
<td>100.0</td>
<td>0.742</td>
</tr>
<tr>
<td>FACS</td>
<td>25</td>
<td>21</td>
<td>46</td>
<td><strong>76.7</strong></td>
<td>100.0</td>
<td>0.883</td>
</tr>
<tr>
<td>CBA-O</td>
<td>24</td>
<td>20</td>
<td>44</td>
<td><strong>73.3</strong></td>
<td>100.0</td>
<td>0.867</td>
</tr>
<tr>
<td>ELISA-R</td>
<td>18</td>
<td>18</td>
<td>36</td>
<td><strong>60.0</strong></td>
<td>100.0</td>
<td>0.800</td>
</tr>
<tr>
<td>FIPA (O)</td>
<td>16</td>
<td>16</td>
<td>32</td>
<td><strong>53.3</strong></td>
<td>100.0</td>
<td>0.767</td>
</tr>
<tr>
<td>FIPA (M)</td>
<td>16</td>
<td>16</td>
<td>32</td>
<td><strong>53.3</strong></td>
<td>97.7</td>
<td>0.755</td>
</tr>
</tbody>
</table>


**NMO-IgG evaluation: CBA**

AQP4 M1 transfected
Non-transfected
FACS Assay Comparison  
M1 vs. M23 AQP4

Healthy and miscellaneous inflammatory disease controls (n = 338)

<table>
<thead>
<tr>
<th></th>
<th>M1</th>
<th>M23</th>
</tr>
</thead>
<tbody>
<tr>
<td># of patients positive</td>
<td>1</td>
<td>23</td>
</tr>
</tbody>
</table>

Characteristics of AQP4

- Two major isoforms (M1 and M23)
- M23 forms large orthogonal arrays
- M1 limits the size of arrays
Outcome prediction

**NMO-IgG detection at initial event**
**Predicts TM relapse or future ON**

11 of 29 patients were **NMO-IgG+ (38%)**

Weinshenker et al, Ann Neurol, 2006
Recurrent Optic Neuritis
NMO-IgG predicts visual outcome, myelitis and death

5/25 (20%) patients evaluated at MC with RON were NMO-IgG+


• 5.8% acute monosymptomatic ON+ for AQP4 Abs
• 50% + cases met criteria for NMO vs 0% of – cases at follow up
NMO Spectrum Defined by AQP4-IgG

- **Myelopathy** (long lesions, relapsing, lupus/ Sjögren-related)
- **Optic neuritis** (relapsing)
- **Encephalopathies** (inflammatory, demyelinating, pediatric>>adult)
- **CVO disorders**: intractable vomiting/nausea, SIAD, endocrinopathies
- **Myopathy**: muscle pain and elevated CK
- **Paraneoplastic NMO**: breast ca, thymoma, lymphoma, lung ca, thyroid ca

*Asian/African ethnics disproportionally affected*
CRMP-5
Autoimmunity

- Neuronal cytoplasmic Ag
- Paraneoplastic associations (70%)
  - lung ca (small-cell)
  - thymoma
  - renal ca
  - thyroid ca

Ann Neurol 54:38 2003 (Yu et al.)
63:531, 2008 (Keegan et al.)
CRMP-5 Myelopathy (n=57)

- Female, 51%
- Age onset, median 63 yrs (38-86)
- Smoking hx, 81%
- Presentation progressive, 77%
- Cancer* detected, 68%
- Coexisting neurological Sxs, 15% (PN>ON)

* lung>> thyroid, endometrial, bladder, renal, melanoma


Leaky Retinal Vessels

Vitreous CD4+ Inflammatory Cells

CRMP-5 Ophthalmopathy

Spectrum of MOG Autoimmunity

MOG-IgG Detection and MOG-IgG+ Cases

- Methods detecting conformation-sensitive myelin-oligodendrocyte glycoprotein (MOG)-antibodies (e.g., cell-based assays)  
  \( (O'\text{Connor}, \text{Nat Med 2007}) \)
- MOG-IgG+ inflammatory CNS diseases, especially pediatric cases  
  \( (\text{Probstel, Neurology 2011}; \text{Reindl, Nat Rev Neurol 2013}) \)
- MOG-IgG present in some anti-AQP4-IgG-seronegative NMOSD  
  \( (\text{Mader, J Neuroinflamm 2011}; \text{Kitley, Neurology 2012}; \text{Sato, Neurology 2014}; \text{Kitley, JAMA Neurol 2014}) \)

\( (\text{Sato et al., Neurology AAN 2014}) \)
MOG: Molecule & Localization

MOG protein
• Outermost lamellae of the myelin sheath
• Single Ig V extracellular domain
• Adhesion molecule?
• Immune function? C1q binding
• MOG peptides are encephalitogenic

(Sato et al., Neurology AAN 2014)
Summary of Clinical Features

<table>
<thead>
<tr>
<th></th>
<th>Anti-AQP4+ (n = 166)</th>
<th>Anti-MOG+ (n = 35)</th>
<th>Seronegative (n = 89)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset Age</td>
<td>36 (4 - 78)</td>
<td>35 (3 - 79)</td>
<td>33 (10 - 80)</td>
<td>0.6770</td>
</tr>
<tr>
<td>Female Sex</td>
<td>88.6% (147)</td>
<td>54.3% (19)</td>
<td>64.0% (57)</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Single Attack</td>
<td>16.3% (27)</td>
<td>42.9% (15)</td>
<td>38.2% (34)</td>
<td>&lt; .0001</td>
</tr>
</tbody>
</table>

**Clinical Phenotype**

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NMO</td>
<td>60.8% (101)</td>
<td>11.4% (4)</td>
<td>21.4% (19)</td>
<td></td>
</tr>
<tr>
<td>LETM</td>
<td>29.5% (49)</td>
<td>25.7% (9)</td>
<td>55.1% (49)</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Recur ON/Bil ON</td>
<td>9.7% (16)</td>
<td>62.9% (22)</td>
<td>23.6% (21)</td>
<td></td>
</tr>
<tr>
<td>EDSS (last visit)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monophasic</td>
<td>6 (2 - 8.5)</td>
<td>2 (0 - 6)</td>
<td>4 (0 - 8.5)</td>
<td>0.0009</td>
</tr>
<tr>
<td>Recurrent</td>
<td>5 (1 - 8.5)</td>
<td>2 (0 - 8)</td>
<td>4 (0 - 7)</td>
<td>&lt; .0001</td>
</tr>
</tbody>
</table>

(Sato et al., AAN 2014)

MOG-IgG+ Optic Neuritis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Anti-AQP4+</th>
<th>Anti-MOG+</th>
<th>Negative</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe ON (VA &lt; 20/200)</td>
<td>73.5% (86/117)</td>
<td>34.6% (9/26)</td>
<td>42.5% (17/40)</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>No light perception</td>
<td>26.5% (31/117)</td>
<td>3.9% (1/26)</td>
<td>12.5% (5/40)</td>
<td>0.0106</td>
</tr>
<tr>
<td>Bilateral ON</td>
<td>25.6% (30/117)</td>
<td>46.2% (12/26)</td>
<td>37.5% (15/40)</td>
<td>NS</td>
</tr>
</tbody>
</table>

28y, male
Bilateral ON
MOG-IgG+ (1:4,096x)

STIR T1Gd+

(Image: Sato et al., Neurology 2014)
MOG-IgG+ Myelitis

<table>
<thead>
<tr>
<th></th>
<th>Anti-AQP4+</th>
<th>Anti-MOG+</th>
<th>Negative</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical</td>
<td>72.5%</td>
<td>46.2%</td>
<td>63.2%</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(108/149)</td>
<td>(6/13)</td>
<td>(43/68)</td>
<td></td>
</tr>
<tr>
<td>Thoracic</td>
<td>67.8%</td>
<td>76.9%</td>
<td>63.2%</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(101/149)</td>
<td>(10/13)</td>
<td>(43/68)</td>
<td></td>
</tr>
<tr>
<td>Lumbar</td>
<td>3.4%</td>
<td>46.2%</td>
<td>14.7%</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>(5/149)</td>
<td>(6/13)</td>
<td>(10/68)</td>
<td></td>
</tr>
<tr>
<td>Lesion length (VS)</td>
<td>6 (1 – 19)</td>
<td>6 (3 – 14)</td>
<td>4 (3 – 19)</td>
<td>0.0272</td>
</tr>
<tr>
<td>Painful tonic spasm</td>
<td>40.7%</td>
<td>7.7%</td>
<td>26.5%</td>
<td>0.0152</td>
</tr>
<tr>
<td></td>
<td>(55/135)</td>
<td>(1/13)</td>
<td>(18/68)</td>
<td></td>
</tr>
</tbody>
</table>

6y, female, 3x LETM MOG-IgG+ (1:1,024x)

Summary of Results

AQP4-Ab+ patients = 60.0% (156/260)
MOG-Ab+ patients = 10.4% (27/260)
No NMOSD patients were double-positive

Features of MOG-Ab+ patients (vs. AQP4-Ab+ and seronegative)
No female predominance
ON (simultaneous bilateral) – common
Caudal myelitis – relatively common
Fewer attacks and better recovery

(Sato et al., Neurology 2014)
MOG antibody binding and titers


(Kitley et al., JAMA Neurol 2014)
Severe Astrocytic Damage in NMO

1) Pathogenicity of AQP4-Ab *in vitro* and *in vivo*
2) Low myo-inositol/creatine value on ¹H-MRS
3) Astrocyte pathology (AQP4 loss, etc.) in the lesions
4) Remarkable elevation of CSF-GFAP in relapse

GFAP: glial fibrillary acidic protein
(an astrocytic protein)

Astrocytic Damage is Far More Severe Than Demyelination in NMOSD

Remarkably Elevated CSF-GFAP Levels in NMOSD

(Takano et al., Neurology 2011)
No Elevation of CSF-GFAP in AQP4-IgG-negative LETM

(LETM: longitudinally extensive transverse myelitis)

(Hyun et al., Mult Scler 2014)

Severe Demyelination But No Astrocytopathy in MOG-IgG-positive Definite NMO

31 yo man
Rt ON (VA 20/200)
Rapid recovery after IVMP

Acute myelitis (2 wks later)
T2 sensory level, dysuria
T2 lesions in C3-5, C6-T5 (central)
AQP4-IgG-neg, MOG-IgG-pos
Good recovery after IVMP

CSF-MBP 1190 pg/ml
(normal < 102)
CSF-GFAP < 0.004 ng/ml

(Ikeda et al., Mult Scler 2015)
MRI in MOG Ab positive patients. Note resolution!