Antibodies: Role in MS and Relationship to Triggers

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B Cells and Antibody in MS Lesions

Type II: Mixed T and B cell infiltrate
- ~50% Lesions of RRMS lesions

Ig-Complement deposition
- Hallmark of active pathology

Acute MS Plaque: IgG Deposition
Brain Biopsy

Acute ON Nerve: IgG Deposition
Optic Nerve Biopsy
Oligoclonal IgG in MS CSF

- Kabat et al. (1942)
  • Elevated amount of antibodies in MS CSF
- Yahr et al. (1954)
  • Intrathecal synthesis
- Link (1967)
  • Oligoclonal bands (OCB)

Oligoclonal IgG
- Sensitive marker of disease
  - 95% of MS patients
  - OCBs do not disappear
  - Present in all stages of disease

Oligoclonal Bands in Neurologic Disease

<table>
<thead>
<tr>
<th>Disease</th>
<th>Oligoclonal IgG Directed Against</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subacute sclerosing panencephalitis</td>
<td>Measles virus</td>
<td>Vandvik et al., 1976</td>
</tr>
<tr>
<td>Cryptococcal meningitis</td>
<td>Cryptococcus</td>
<td>Porter et al., 1977</td>
</tr>
<tr>
<td>Mumps meningitis</td>
<td>Mumps virus</td>
<td>Vandvik et al., 1978</td>
</tr>
<tr>
<td>Chronic rubella panencephalitis</td>
<td>Rubella virus</td>
<td>Coyle et al., 1981</td>
</tr>
<tr>
<td>(HSV) encephalitis</td>
<td>HSV glycoprotein B</td>
<td>Grimaldi et al., 1988</td>
</tr>
<tr>
<td>Progressive multifocal leukoencephalopathy</td>
<td>JC virus</td>
<td>Sindic et al., 1997</td>
</tr>
<tr>
<td>Neurosyphilis</td>
<td>Treponema pallidum</td>
<td>Vartdal et al., 1981</td>
</tr>
<tr>
<td>VZV vasculopathy</td>
<td>VZV</td>
<td>Burgoon et al., 2003</td>
</tr>
<tr>
<td>Theiler's virus-induced demyelination</td>
<td>Theiler’s virus</td>
<td>Roos et al., 1987</td>
</tr>
<tr>
<td>Theiler’s virus</td>
<td>Theiler’s virus</td>
<td>Pachner et al., 2007</td>
</tr>
<tr>
<td>Coronavirus-induced demyelination</td>
<td>JHM coronavirus</td>
<td>Dorries et al., 1987</td>
</tr>
<tr>
<td>Multiple Sclerosis</td>
<td>Negative for major myelin proteins and common viruses</td>
<td></td>
</tr>
</tbody>
</table>
What is the Nosology of CNS Demyelinating Disorders?

A Candidate Approach?

Myelin Proteins
- MOG
- MBP
- PLP
- αB-crystallin

Cell Adhesion Molecules
- neurofascin
- contactin

Glycolipids
- galactocerebrosides
- sulfatides

Foreign Antigens

God, Collings, I hate to start a Monday with a case like this."

EAE

Table 1
Antigens to myelin and other CNS autoantigens in MS

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Frequency of Anti-Myelin Antibodies Are Assay Dependent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-1 crystallin</td>
<td>Antibodies present in sera and CSF of MS patients</td>
</tr>
<tr>
<td>Alc repeats</td>
<td>Oligodendrocyte-preferential B cell epitope; antibodies found in sera and CSF of MS patients</td>
</tr>
<tr>
<td>AN-2 (NG-2)</td>
<td>Surface glycoprotein expressed on oligodendrocyte precursor cells. Antibodies present in CSF of MS patients</td>
</tr>
<tr>
<td>Anaplastic T lymphocytes</td>
<td>Antibody present in sera of neuromyelitis optica patients.</td>
</tr>
<tr>
<td>DNA</td>
<td>Antibodies present in CSF of MS patients</td>
</tr>
<tr>
<td>Heat shock protein (HSP) ~60 and ~90</td>
<td>Antigens expressed on oligodendrocyte precursor cells. Antibodies could inhibit myelination</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td>Antibodies to CSF1/4(1a) and Glc(alpha1→3Glc(alpha1 present in sera and of MS patients</td>
</tr>
<tr>
<td>Neurofilaments (NF)</td>
<td>Antibodies present in serum and CSF of MS patients; antibodies correlate with disease progression and MRI activity</td>
</tr>
<tr>
<td>Nogo-A</td>
<td>Neurite outgrowth inhibitor; anti-Nogo antibodies are frequent in serum and CSF of MS patients and other acute neurological diseases</td>
</tr>
<tr>
<td>Oligodendrocyte-specific protein (OSP)</td>
<td>Antibodies present in CSF of MS patients</td>
</tr>
<tr>
<td>Proteins</td>
<td>Proteins complex involved in processing and chaperone function; antibodies present in serum and CSF of MS patients</td>
</tr>
<tr>
<td>Transaldolase (TAL)</td>
<td>Antibodies present in serum and CSF of MS patients</td>
</tr>
</tbody>
</table>


Frequency of Anti-Myelin Antibodies Are Assay Dependent

<table>
<thead>
<tr>
<th>Affinity:</th>
<th>low</th>
<th>high</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency:</td>
<td>high</td>
<td>low</td>
</tr>
<tr>
<td>Isootype:</td>
<td>IgM</td>
<td>IgG</td>
</tr>
<tr>
<td>Antigen:</td>
<td>synthetic MOG peptides</td>
<td></td>
</tr>
<tr>
<td></td>
<td>recombinant human MOG immunoglobulin-like domain expressed in E.coli</td>
<td></td>
</tr>
<tr>
<td></td>
<td>recombinant human MOG expressed in vitro</td>
<td></td>
</tr>
<tr>
<td></td>
<td>recombinant human MOG expressed in mammalian cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MOG purified from human brain tissue</td>
<td></td>
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<tr>
<td>Immunoassay:</td>
<td>Immunoblot</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ELISA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>surface staining (FACS)</td>
<td></td>
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<tr>
<td></td>
<td>fluid-phase assays</td>
<td></td>
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</tbody>
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Myelin Antigen Arrays: High Throughput Antigen Screening

Enhanced Dynamic Range

Epitope Spreading in EAE


Myelin Antigen Arrays: Application in Multiple Sclerosis

- 334 myelin and inflammation related CNS Ags
- CSF: MS vs. OND (non-inflammatory controls)
- 115 Ags > 3SD over OND mean; 29 with ↑ freq

- Antibody Index: Ag (CSF/Ser) / Albumin (CSF/Ser)
- Suggestive of intrathecal synthesis

**Potassium Channel KIR4.1 as an Immune Target in Multiple Sclerosis**

*Immunoprecipitation*
- Serum Ab; 2D PAGE
- Proteomics

*Kir 4.1*
- inwardly rectifying K(+) channel
- Astrocytes and oligodendrocytes
  - α-Kir4.1 Ab
    - 47% of MS patients
    - 0.9% of OND

*Antibody Index (AI)*
- AI > 2 considered intrathecal synthesis
- 3/30 positive

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**FACS Sort of Normal CSF**

(G1: R1 & R2) PRESORT CONTROL SAMPLE

CD19+

CD3+

B Cells (CD19)

T Cells (CD3)
FACS: CSF B and Plasma Cells in ON, MS & NMO

Light Scattering

CD19+ B Cell Population

CD19

CD3

CD138+ Plasma Cell Population

CD138

CD3

CD19

CD3

CSF as a Surrogate to Study Humoral Immunity in CNS Demyelinating Disease

**Strategy**

- Recover Ab-secreting B cells (plasma cells) from CSF
- Clone and produce rAbs that duplicate their Ag specificity

B cells cultured from MS CSF produce oligoclonal bands


Clonally expanded plasma cells in the cerebrospinal fluid of patients with central nervous system autoimmune demyelination produce “oligoclonal bands”
Isolation of Single Plasma or B Cells by FACS

Patient CSF

Pellet CSF cells and label with conjugate antibodies

CD19-APC
CD138-PE
CD3-FITC
CD14-APC cy7

Deposit single CD138+ CD3- plasma cell

Reverse Transcriptase PCR and PCR Amplification

RT-PCR

cDNA Synthesis

Primary PCR

Nested PCR

Electrophoresis of PCR product

DNA Sequence Positive VH and Vk products
MS and NMO B Cells Are Antigen-Targeted

- Restricted and dynamic population of clonally expanded antibody secreting cells with a phenotype of plasma blasts are consistent with ongoing stimulation
- IgG V regions display somatic hypermutation indicating progression through germinal center reactions

- MS CSF
  - Qin et al., 1998
  - Colombo et al., 2000
  - Owens et al., 2003
  - Ritchie et al., 2004
  - Monson et al., 2005

- CIS
  - Qin et al., 2003
  - Haubold et al., 2004
  - Monson et al., 2005

- MS Tissue
  - Owens et al., 1998
  - Baranzini et al., 1999

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Generation of Monoclonal Human IgG1 Recombinant Antibodies

A

![Diagram of IgG1 C Region](image1)

B

![Diagram of Recombinant IgG1](image2)

C

![Diagram of SDS-PAGE](image3)
Staining of HEK 293 Cells Expressing MOG with MS CSF rAbs


MS CSF rAbs Bind to Glial Cell Antigens

Courtesy of G. Owens
MS04-2#30 binds white matter antigen in CNS

MS05-3#38 binds neuronal targets in the cerebellum

Courtesy of K. Blauth and G. Owens
NMO Pathogenesis: Assays

Complement + Serum NMO-IgG

Control Ab  AQP4 Ab

In vitro

Calcein-AM EthD-1

Live Dead

Tradtrantip et al., Annal Neurol, 2011

NMO

Ex Vivo Spinal Cord Explants

Saadoun et al., Brain, 2010

7 days

24 hr

Spinal Cord Slice Explants

Zhang et al., Annal Neurol, 2011

MS rAbs Cause Complement-Mediated Demyelination

10 µg/ml 50 µg/ml

DAPI MBP

284 M505-3#192 284 M505-3#192

10% human Complement 10% human Complement

DAPI MBP

Spinal Cord Slice Explants

Courtesy of K. Blauth and G. Owens
rAb: Glycolipid binding

<table>
<thead>
<tr>
<th>Source</th>
<th>% Glycolipid Reactive</th>
<th>Specificity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple Sclerosis</td>
<td>27% (5 patients, 20 rAbs)</td>
<td>• Sulfatide&lt;br&gt;• Sulfatide complexes&lt;br&gt;• Cholesterol</td>
</tr>
<tr>
<td>Inflammatory Controls</td>
<td>33% (3 patients, 9 rAbs)</td>
<td>• Sulfatide&lt;br&gt;• Lipid complexes</td>
</tr>
</tbody>
</table>

Brennan et al., J. Neuroimmunol, 238: 87 (2011)

Sulfatide Lipids Inhibit Axon Outgrowth

Sulfatide Specifically Inhibits Axonal Outgrowth

O4 Ab (Anti-Sulfatide) Antibody Restores Neurite Outgrowth

Winzeler et al., J Neurosci, 31:6481 (2011)
**B Cells Are Multifunctional at Neuro-immune Interface**

- **Produce Antibodies**
- **Instruct T Cells**
  - Antigen Presentation
- **Activate Immune Cells**
  - Antigen Presenting Cells
- **Immunoregulation**

Dependent on B cell Receptor


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**B Cells Are Antigen-Presenting Cells**

- **Protein Antigens**
- **Antigen Internalization**
  - BCR (Ag) Dependent
  - Costimulatory Molecule Expression

Secretion of Toxins and Cytokines

Secretory products of multiple sclerosis B cells are cytotoxic to oligodendroglia in vitro


B cell depletion may ameliorate disease through ablation of IL-6–producing B cells


Acknowledgments

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NIH
Guthy Jackson