JOHN WHITAKER MEMORIAL LECTURE

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JOHN WHITAKER MEMORIAL LECTURE:
Déjà vu all over again\(^1\): The revival of interest in the role of B cells in MS

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\(^1\) Attributed to Yogi Berra

DISCLOSURES

- I will discuss use of several medications OFF LABEL in Relapsing MS.
- Dr. Cross has received honoraria or consulting fees from: Biogen, Genentech, Genzyme (Sanofi aventis), Mallinckrodt, Novartis, Roche, Teva
- Dr. Cross has received research support from Biogen and Roche, as well as NIH, NMSS, Dept of Defense, the Conrad N. Hilton Foundation, Barnes-Jewish Foundation.
JOHN N. WHITAKER, MD

- Born Memphis, TN
- MD from Univ Tennessee, Neurology at Albert Einstein, NIH.
- Chair of Univ Tennessee for many years
- Chair of Neurology at UAB from 1985
- Clinician and Researcher
- President of ANA (95-97)
- Over 250 publications
- Early studies of Beta-interferon, Glatiramer acetate
- Outcomes research for trials
- Myelin basic protein in the periphery
- Anti-idiotypic antibodies as immune regulatory elements

Neuropathology supports B cell role in MS

- 1868- Charcot reports clinical-neuropathology of MS
- MS lesions: Plasma cells, antibody, some B cells.
- Myelin-specific antibody in MS lesions (MBP, MOG)
- Ectopic Lymphoid Follicles
Antibodies to MOG co-localize with demyelination in MS lesions

MOG=myelin oligodendrocyte glycoprotein

Autoantibodies to MOG in native conformation extracted from 50% of MS lesions

Ectopic lymphoid B cell follicles

- Lymphoid-like tissue 1st described by John Prineas (1979) in brain and spinal cord
- CSF B cells have characteristics of centroblasts (CD19+, CD28hi, CD77+, Ki67+)
- Data from Francesca Aloisi and colleagues showed Ectopic follicles (proliferating B cells, CD35+ follicular DC, plasma cells, T cells, CXCL13)


Ectopic follicles in SPMS, associated with earlier onset, earlier wheelchair, earlier death


Ectopic lymphoid B cell follicles associated with cortical MS lesions

- Howell and colleagues later extend observations to show ELFs in ~40% of 123 SPMS cases\(^1\)
- ELFs physically associated with cortical demyelination\(^1\)
- ELFs remain controversial. Not seen by all pathologists, and definition of ELF not universal
- May relate to methods of tissue acquisition and preservation

Plasma cells associated with progressive disease

- 67 MS cases (14 RR, 5 benign, remainder SPMS, PPMS) + 28 controls. Over 1,000 lesions – active, inactive, smoldering
- Density of B and T cells, and plasma cells correlated with axonal injury and axon end-bulbs. B cells 10X less common than T cells
- Lymphocytes mainly seen during active disease.
- Plasma cells correlated with progressive MS even when inactive


Spinal Fluid: B cell alterations of MS

- Increased B cells in relapsing MS vs almost none in OND patients
- Memory B cells (CD27+) predominate in CSF
- B cell chemo-attractant CXCL13 ↑ in CSF
- Evidence of B cell clonal expansion, and traffic between parenchyma and CSF
- CSF B cells correlate with clinical and MRI activity

2. Corcione A et al PNAS USA 2004
CSF B cells associated with worse prognosis

- 'B cell dominant' CSF vs. 'Monocyte-dominant CSF' determined by ratio of B cells : monocytes
- 'B cell dominant' CSF pts had faster disability progression.¹

¹ Cepok S, Jacobsen M et al. Patterns of cerebrospinal fluid correlate with disease progression in MS. Brain 2001; 124: 2169-76. 60 MS patients' CSF were profiled.

Immunoglobulin abnormalities in MS CSF

- 1912 Lange colloidal gold method –globulin fraction of proteins increased in neurosyphilis, MS¹
- 1942 Kabat - γ-globulins ↑’d in CSF of MS patients²
- 1966 Tourtellotte – γ-globulins in CSF reflected γ-globulins in brains³
- 1980 Tourtellotte - formula to estimate intrathecal IgG synthesis rate using one sample of CSF and serum

¹ Lange, C.
³ Tourtellotte WW & Parker JA. Science 154: 1044, 1966
⁴ Tourtellotte WW et al. Neurology 30: 240-244, 1980
Oligoclonal bands in MS CSF

- 1966 Laterre - oligoclonal bands in >85% of MS patients 1,2
- Oligoclonal bands are CSF-restricted
- Targets remain unsolved mystery


Oligoclonal band number and prognosis

- 44 MS patients followed at WUSM >10yrs with preserved gels with IEF and silver stain
- Blinded band counting
- “Benign” EDSS <3.5
- “Severe” EDSS >7.5
- Mean FU 15.8 and 16.2yr for “benign” and “severe”
- All had >2 attacks

Avasarala, Cross & Trotter Arch Neurol 2001
Enhanced CSF humoral response associated with worse MS prognosis

- Normal, non-elevated IgG Index - more likely benign
- High IgG Index (>1.0) associated with rapid progression
- Lack of OCBs - more likely benign (n=12, EDSS 3.0 after 14 yrs)
- Lack of IgM OCBs - lower relapse rate in 2yr prospective study: 3 to 4x more relapses in IgM-OCB+ vs IgM-OCB negative (2.7 vs 0.8; P = 0.0004)
- In the same study, 5 years after disease onset, probability of being relapse-free was about double for patients without IgM-OCBs vs those with IgM-OCBs (32.5%, n=26 without vs. 17.9% , n=22 with IgM-OCBs; P = 0.0004)

1. Stendahl-Brodin 1980
2. Izquierdo G 2002
3. Zeman 1996
4. Villar M 2005

Increased intrathecal kappa light chains correlated with MS progression

- Kappa Light chains – by-product of Ig production
- In CSF, relatively specific for MS (Hans Link). Stable on repeat LP.
- Rudick studied 36 pts (30 MS +6 CIS) prospectively studied. Median FU over 3 years (median FU 38.9 mon)
- ~50% worsened on one of these: EDSS, AI, 9HPT, Box & Block Test
- $\kappa$LC >75th%-tile (1.53ug/ml) was associated w disease progression. Hazard Ratio 3.78 by EDSS, 10.8 by 9HPT

Increased intrathecal kappa light chains correlated with poor prognosis

- 57 pts with κLC followed median 15 years, CSF evaluated 1991-1995 (RIA) at Washington Univ.
- High κLC predicted need for support to ambulate (89% of those in top 25%-tile needed aid over disease course)

<table>
<thead>
<tr>
<th>Progression</th>
<th>Unadjusted Risk</th>
<th>Adjusted for gender, ethnicity, MS subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any ambulatory assistance</td>
<td>2.0 (1.1-3.8)</td>
<td>10.6 (2.2-50.9)</td>
</tr>
<tr>
<td>Cane/unilateral</td>
<td>16.9 (2.7-104)</td>
<td>4.6 (1.8 – 11.7)</td>
</tr>
</tbody>
</table>

Rinker J et al. Neurology 2006; 67:1288-1290

Partial list of autoAbs reported in MS

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Tissue examined</th>
<th>Investigator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myelin basic protein (MBP)</td>
<td>CSF, CNS plaque</td>
<td>Warren, Catz; Panitch, K.Johnson</td>
</tr>
<tr>
<td>Proteolipid protein (PLP)</td>
<td>CSF, CNS plaque</td>
<td>Warren, Catz</td>
</tr>
<tr>
<td>Myelin oligodendroglial glycoprotein (MOG)</td>
<td>CSF; serum; plaques, blood</td>
<td>Xiao; Berger; Raine &amp; Genain, O’Connor</td>
</tr>
<tr>
<td>KIR4.1 K+ channels</td>
<td></td>
<td>Hemmer</td>
</tr>
<tr>
<td>Oligodendrocyte specific protein (MOBP)</td>
<td>CSF</td>
<td>Bronstein</td>
</tr>
<tr>
<td>CNPase</td>
<td>Serum, CSF</td>
<td>Walsh, Murray</td>
</tr>
<tr>
<td>Transaldolase</td>
<td>Serum, CSF</td>
<td>Banki; Colombo; Esposito</td>
</tr>
<tr>
<td>β-Arrrestin (heat shock protein)</td>
<td>Serum (not in CSF)</td>
<td>Ohguro</td>
</tr>
<tr>
<td>GD1a ganglioside</td>
<td>Serum, CSF</td>
<td>Mata</td>
</tr>
<tr>
<td>Neurofilament heavy chain</td>
<td>CSF</td>
<td>Kuhle</td>
</tr>
<tr>
<td>Neurofilament light chain</td>
<td>CSF</td>
<td>Berger; Sharief</td>
</tr>
</tbody>
</table>
1980’s and 1990’s:

T cells were found to be more prevalent than B cells in MS lesions.

T cells were able to transfer the MS animal model, EAE (B cells & antibody did not)

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Monoclonal antibodies

- 1984 Nobel Prize to Milstein and Kohler for their work creating monoclonal antibodies (mAbs) mAbs increasingly used for therapeutics
- 2000 - Studies in MS being developed
- Three different monoclonal antibodies that lyse B cells, rituximab, ocrelizumab, and ofatumumab, have all shown rapid profound ↓ in gad+ enhancing lesions on MRI 1-4

Surface markers during B cell development

- Stem Cell
- Immature B cell
- Naïve mature B
- Memory B cell
- Plasmablast
- Plasma cell

CD20
CD19
CD27
CD138
Major histocompatibility complex-Class II


CD20: a target specific to B cells

- Found on B cells in MS Lesions
- 297 AA (33-37kD)
  - membrane-associated phosphoprotein
  - Not shed or secreted

- Selective expression
  - not on stem cells, plasma cells

- Anti-CD20 binding
  - Expression not rapidly modulated
  - Does not internalize
B-cell targeting in RRMS

- Phase II HERMES Trial
  - A single rituximab course of therapy (1000mg day 0, 15) resulted in rapid, profound and prolonged reduction in Gad+ inflammatory brain lesions in patients on drug
  - Reduced the proportion of patients with relapse at 24 and 48 weeks compared with placebo

Phase 2 combination trial in 30 relapsing subjects failing β-IFN or GA
Open-label with MRI blinding


88% Reduction in gad-enhanced lesions

CSF AFTER RITUXIMAB

CSF B cells pre- vs post-rituximab: Profound drop in B cells in CSF and blood 24 weeks post-treatment

# CD19+ cells/ml CSF

Pre- vs Post-Rituximab

$p=0.0001$, Wilcoxon matched pairs test

CSF T Cells: Unexpected reduction (>50%) in T cells at 24 weeks, despite that rituximab does not target most T cells

$\text{p}=0.0001$ Wilcoxon matched pairs test


OCBs and IgG Index – no change

Median = 6 pre and 6 post

$P = 0.62$ Wilcoxon paired test
Antibodies to recombinant human MOG in CSF pre- vs post-rituximab*

Mean 2.2 (pre) vs 2.5 (post): no significant difference
* - total Ig

CXCL13 levels decline in blood and CSF post-rituximab

CXCL13 is critical to germinal center formation & recruits B cells. CSF CXCL13 levels are ↑ in MS vs HC (20.7pg/ml vs 10.0pg/ml)
CXCL13 is expressed by CD14+ cells in CSF of active MS patients

CCL19 levels decline in CSF post-rituximab
CCL19 levels were decreased in CSF after rituximab

- Also known as “EBI1 ligand chemokine” and macrophage inflammatory protein-3-beta (MIP-3-beta).
- Expressed in thymus and lymph nodes. Produced by DC and Macrophages
- Ligand is CCR7
- Chemoattractant for many cell types expressing CCR7: mature DCs and antigen-engaged B cells, CCR7+ central-memory T-Cells and naïve T cells.

Reductions in MBP and NF-L post-rituximab
Ocrelizumab and Ofatumumab- two “second generation” anti-CD20 monoclonal antibodies being studied in MS

Ocrelizumab

- Ocrelizumab has a modified Fc portion that potentially reduces side effects related to complement activation (c/w rituximab)
- Fully humanized IgG1 expected to reduce immunogenicity
- Greater ADCC and less CDC
- Overall: improved potency and potentially improved efficacy/safety profile
Ocrelizumab Phase II: Primary EP: Mean number of gd-enhancing T1w lesions, ‘core study’ (0-96 weeks) & ‘follow up’ (97-144 weeks)

![Graph showing mean number of T1 Gadolinium-enhancing lesions](image)


Ofatumumab

- Human mAb that also targets CD20, but at the smaller extracellular loop, different than rituximab and ocrelizumab.
- In vitro, binds CD20 better than rituximab. Slower dissociation from target
- Approved for refractory CLL in U.S. and Europe

B cell depletion in relapsing MS with subcutaneous ofatumumab

- Dose-finding 12 week Phase 2 study (n=232) vs placebo, then additional 12 weeks
- 3mg or 30mg or 60mg q12wks or 60mg q4wks vs placebo
- Dramatic reduction of Gad+ lesions by > 90% for weeks 4-12 for all except lowest dose
- Similarly high efficacy seen at dose regimen that only partially depleted circulating B cells (may not need to achieve ‘complete’ peripheral depletion to have substantial efficacy)
- Injection site reactions common

Bar-Or A et al. The MIRROR Study. ACTRIMS/ECTRIMS Abstract S23.0062014

Atacicept

- BAFF and APRIL are two factors that normally enhance B-cell maturation, function and survival.
- Atacicept is a human recombinant fusion protein with the receptor binding site for both.
- BAFF is upregulated in brain of MS patients, and astrocytes may be a main source.¹
- Selectively acts on mature B cells, blocks plasma cells and late stages of B-cell development.²
- Somewhat spares memory B cells; In a Phase 1 trial, CD27+CD19+ B cells were increased²

Atacicept

• Investigated in active relapsing MS (ATAMS)
• **Increased disease** activity occurred
• Development for MS abandoned
• An ongoing study (ATON) in acute unilateral optic neuritis -CIS was halted.
• Double % moved on to Clinically Definite MS in Atacicept arm vs placebo arm in post-hoc analysis
• The failed trials may implicate Memory B cells in the development of MS disease activity


The data presented implicate B cells in the pathogenesis of relapsing MS, but how?
Roles B cells might play in MS pathophysiology

- Antibody production (opsonization, complement activation)
- Antigen processing and presentation to T cells
- Meningeal ectopic germinal centers
- Cytokines, chemokines (IL-10, TGFβ, IL-6, TNFα and lymphotoxin β, CCL3/MIP1α, CCL4/MIP1β, CCL22)

B cells, including CD27+memory B cells, are potent antigen presenting cells

CD4+ T cell activated by processed peptides presented on MHC Class II. B cells constitutively express MHC II, and are optimal APCs for antigens in low abundance, such as myelin antigens.

How might eliminating B cells inhibit relapses?

• Rapid inhibition of MRI and clinical relapse activity is c/w ability of B cells to present autoantigen (via BcR &/or capture antigen-Ab-complement complexes).

• Most likely occurs cervical lymph nodes\(^1,2\)
• (would removal of these nodes alter the process?)

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Proposed mechanism of MS activity Reduction with CD20 cell depletion

Modified from Ahmed
What about the initiation of MS itself?

• Hypothesis:
  • T and B cells collaborate to initiate MS.
  • The TcR is redundant; the autoreactive T cell may have been primed by a cross-reactive environmental trigger, e.g. EBV
  • B cells capture, process and present antigens of low abundance, such as CNS antigens, to T cells with the same target.
  • Activation of T and B cells in periphery allows them to cross the BBB, where they may find target, and recruit other cells into CNS

Safety of long-term B cell-depleting monoclonal antibody therapies

• Immune reactions
  • HACA=human anti-chimeric antibodies; HAHAs=human anti-human antibodies
  • Reactivation of Hepatitis B (up to 24 mon after Tx). Screen prior to RTX (PDR) with HBsAg and anti-HBc before initiation
  • Infections (bacteria, viruses, and fungi) and PML are concerns.
  • Hypogammaglobulinemia often ensues with chronic RTX treatment
Acknowledgements

- John Trotter MD (1943-2001)
- Laura Piccio MD PhD
- Robert Naismith MD
- Greg Wu MD PhD
- Cedric Raine PhD
- Michael Ramsbottom
- Bob Mikesell
- Neville Rapp PhD

Ocrelizumab significantly reduced ARR by week 24 (2° EP)

Adjusted ARR* (95% CI)

<table>
<thead>
<tr>
<th>Group</th>
<th>Adjusted ARR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n=54)</td>
<td>0.636</td>
<td>0.364 - 0.733</td>
</tr>
<tr>
<td>OCR 600 (n=55)</td>
<td>0.169</td>
<td>0.076 - 0.263</td>
</tr>
<tr>
<td>OCR 1000 (n=55)</td>
<td>0.125</td>
<td>0.028 - 0.222</td>
</tr>
<tr>
<td>IFN-β1a (n=54)</td>
<td>0.636</td>
<td>0.364 - 0.733</td>
</tr>
</tbody>
</table>

CI: confidence interval
*Adjusted for geographical region.

In the Week 48 database, one additional protocol-defined relapse was reported in the ocrelizumab 1000 arm after the Week 24 database lock. It is not included in the above analysis.
CHEMOATTRACTANTS INFLUENCED BY B CELLS

<table>
<thead>
<tr>
<th>Chemokine</th>
<th>Produced by</th>
<th>Receptor and T-cell subsets targeted</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP-1/CCL2</td>
<td>Induced: Mac, EC, PMN, fibroblasts, astrocytes</td>
<td>CCR2; NK T and Th1 T</td>
<td>no change (P = .17)</td>
</tr>
<tr>
<td>MIP1α/CCL3</td>
<td>Induced: B cells, PMN, CD8, Mo/Macs, Eo, DC</td>
<td>CCR1, NK and effector memory T cells; CCR5</td>
<td>no change (low levels)</td>
</tr>
<tr>
<td>MIP1β/CCL4</td>
<td>See CCL3</td>
<td>CCR5; effector, Th1, and NK T cells</td>
<td>no change</td>
</tr>
<tr>
<td>RANTES/CC1</td>
<td>Induced: EC, T cells, monocytes, NK, DC</td>
<td>CCR1/CCR5 (see CCL3, CCL4)</td>
<td>undetectable in CSF</td>
</tr>
<tr>
<td>SDF-1/CXCL12</td>
<td>Stromal cells, EC, astrocytes</td>
<td>CCR4; most T’s, naive, memory B, macs</td>
<td>no change (P = .11)</td>
</tr>
<tr>
<td>CXCL13</td>
<td>HEV of secondary LNs</td>
<td>CXCR5; mature B and some CD4 &amp; CD8</td>
<td>no change</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T cells, DC</td>
<td></td>
</tr>
<tr>
<td>CCL19/ELC</td>
<td>Constitutive by DC in lymphoid tissue; also by Mφ; role: homing to LN</td>
<td>CCR7+ B cells, naive and central memory T cells, mature DC, myeloid cells, NK cells</td>
<td>*↓35% P = .0004</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3a</td>
<td>Complement activation</td>
<td>C3aR on lymphocytes, other cells</td>
<td>no change (P = .48)</td>
</tr>
<tr>
<td>C5a</td>
<td>Complement activation</td>
<td>C5aR on B, T cells</td>
<td>undetectable in CSF</td>
</tr>
<tr>
<td>SLC/ CCL21,</td>
<td>ED, HEV, DC</td>
<td>CCR7+ naive, central memory T cells,</td>
<td>undetectable in CSF</td>
</tr>
<tr>
<td>CCL22</td>
<td></td>
<td>mature DC</td>
<td></td>
</tr>
<tr>
<td>CXCL10/IP-10</td>
<td>Inducible in EC, astrocytes, microglia, monocytes</td>
<td>CXCR3 on activated T cells, CD8+ and NK cells</td>
<td>no change</td>
</tr>
</tbody>
</table>

ROLE OF B CELLS IN MS: LYMPHOGENESIS

- B-cell cytokines and chemokines contribute to the generation and maintenance of germinal centers in lymphoid follicles, which are essential to adaptive responses.
- In MS, ectopic follicle-like structures may form within the CNS and promote ongoing local immune injury.
- Suggestive of B-cell replication and activation within the region.
- May be associated with a more severe secondary progressive disease course.