B-cells in MS:
Déjà vu all over again¹

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¹ Attributed to Yogi Berra (1925-2015, b. St. Louis MO)
Disclosures

• Discussions of studies using RITUXIMAB, OCRELIZUMAB, and OFATUMUMAB off-label in MS will be part of this talk. These drugs are not approved for use in MS.

• Dr. Cross has done consulting for: AbbVie, Bayer, Biogen, EMD-Serono, Novartis, Genzyme, Genentech / Roche, Teva

• Dr. Cross is site PI for 2 studies funded by Genentech
OUTLINE

• MS and MS pathology
• B cells and auto-antibodies in MS
• B cell depletion with anti-CD20 monoclonal Abs in MS
• How do B cells foster MS activity and what might that say about relapses?
HOW MANY PEOPLE WITH MS IN THE WORLD?

~2.5 MILLION PEOPLE WITH MS WORLDWIDE
What causes MS?

Multifactorial disease involving the immune system, and genetic and environmental factors

GENETIC SUSCEPTIBILITY

Genes (over 140 identified, almost all relate to the immune system)

ENVIRONMENTAL FACTORS

Infections/EBV
Vitamin D levels
Obesity/diet
Smoking

Autoimmune reaction
0.1% of the US population has MS
Neuropathology is a major key to its understanding
1868- Charcot reports clinical-neuropathology of MS
Neuropathology supports B lymphocyte role in MS

MS lesions: plasma cells (Esiri, Prineas), Ab, some B cells.

MS lesions: CD20+ B cells (left) and CD138+ plasma cells (right)

Blue=hematoxylin; brown=anti-CD20
Courtesy of Tonja Kuhlmann, 2008.
B cell products in MS CSF

- Oligoclonal bands are CSF-restricted Ig (usually IgG).
- 1966 Laterre - oligoclonal bands in >85% of MS patients 1,2
- IEF + Immunoblot most sensitive (>90%)

Enhanced CSF B cell response associated with worse MS prognosis

- Normal IgG Index - more likely benign
- High IgG Index (>1.0) associated with rapid progression
- Lack of OCBs - more likely benign (EDSS 3.0 after 14 yrs)
- LEFT: “Benign” EDSS <3.5 vs “Severe” EDSS >7.5
- Mean FU >15yrs for both “benign” and “severe”
  Higher numbers of OCBs in baseline CSF → severe MS

4. Avasarala J 2001
WHAT IS THE TARGET OF THESE ANTIBODIES? (IS THERE A TARGET?)
Antibodies to MOG co-localize with demyelination in MS lesions

MOG=myelin oligodendrocyte glycoprotein
MS Spinal Fluid: B cell alterations

- Increased B cells in relapsing MS vs almost no B cells in OND patients
- Memory B cells (CD27+) and plasmablasts (CD19+CD138+) predominate in MS CSF
- Evidence of B cell clonal expansion
- B cell (and T cell) chemo-attractant CXCL13↑ in CSF
- CSF B cells correlate with clinical & MRI activity, and with intrathecal CXCL13

2. Corcione A et al PNAS USA 2004
B cells, T cells and plasma cells associated with axon destruction and progressive MS

- Autopsies from 67 MS cases (14RR, 5 benign, remainder SPMS, PPMS) + 28 controls. Over 1,000 lesions – active, inactive, smoldering

- T & B lymphocytes mainly seen during active disease.

- B cells 10X fewer than T cells.

- Plasma cells correlated with progressive MS, present even when inactive

- Density of B and T cells, and plasma cells correlated with axonal injury and axon end-bulbs.

Monoclonal antibodies

- 1984 - Milstein and Kohler win Nobel Prize
- 2000 - Studies in MS 1st in development
Surface markers during B cells Development

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

Surface markers:
- CD19
- CD20
- CD27 (memory)
- CD138

Major histocompatibility complex-Class II

CD20: a good target to eliminate B cells

- Selective expression
  - not on stem cells, plasma cells
  - Rare T cells
- Anti-CD20 binding
  - Expression not rapidly modulated
- Highly anchored into membrane
  - Shed, but not extensively
Rituximab: Phase 2 trial in 30 RRMS subjects failing β-IFN or GA
Open-label add-on, with MRI blinding\textsuperscript{1,2}

Mean Gadolinium Enhancing lesions

88% Reduction in gad-enhanced lesions

No corticosteroids for pre-treatment

Rituximab in RRMS

- Chimeric anti-CD20. Three early studies all highly positive $^{1,2,3}$
- HERMES placebo-controlled trial (104 RRMS 2:1 RTX:PBO); Hermes Jr open-label $^3$
  - A single rituximab course of therapy (1000mg @ day 0, 15) $^1$
  - Our Add-on with 375mg/m$^2$ x 4 doses at beginning$^2$

Switch from Natalizumab to rituximab vs fingolimod. Ritux better in MRI activity & RR

- 256 MS Patients (were given either fingolimod or rituximab after being on natalizumab and all changed therapy for same reason: JCV+. ¹
- Some differences in usage among the 3 centers, but statistically accounted for
- Retrospective, observational. Not randomized. 2% RIT had relapse vs 18% FGL
- Patients switched to RIT fared much better in terms of relapse-free survival, MRI activity, % remaining on drug and AE-free survival.
- Many Swedish centers are using Rit first Line – A retrospective study found no difference in the 2 doses, most are using 500mg q 6months ²

Ocrelizumab

- Ocrelizumab is a fully humanized IgG1 anti-CD20 (lytic). Expected to reduce immunogenicity
- Has a modified Fc portion that potentially reduces side effects related to complement activation (c/w rituximab)
- Greater ADCC and less CDC than rituximab
- Genetic variants of FcγRIIIA in humans may impact
- Overall: potentially improved efficacy/safety profile
OPERA I AND OPERA II: TWO IDENTICAL STUDIES EVALUATING THE EFFICACY AND SAFETY OF OCRELIZUMAB IN RMS

- ACTIVE RMS diagnosis
- 18–55 yrs
- EDSS 0.0–5.5

Double-blind Double-dummy Treatment Period

**Ocrelizumab**
- Dose 1: 300 mg i.v. x 2 (days 1 & 15)
- Doses 2-4: 600 mg i.v. x 1

**IFN β-1a**
- Dosed 44 μg s.c. 3 x per week


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Reduction in relapse rate vs. IFNβ1a in RRMS: OPERA I and II

Primary endpoint:
Significant reduction in ARR compared with IFN β - 1a

**OPERA I**

<table>
<thead>
<tr>
<th>Group</th>
<th>Adjusted ARR at 96 Weeks*</th>
<th>p-value</th>
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<tbody>
<tr>
<td>IFN β-1a 44 µg (n=411)</td>
<td>0.292</td>
<td>&lt;0.0001</td>
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<tr>
<td>Ocrelizumab 600 mg (n=410)</td>
<td>0.156</td>
<td></td>
</tr>
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</table>

**46% ARR reduction vs IFN β -1a**

**OPERA II**

<table>
<thead>
<tr>
<th>Group</th>
<th>Adjusted ARR at 96 Weeks*</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN β-1a 44 µg (n=418)</td>
<td>0.290</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ocrelizumab 600 mg (n=417)</td>
<td>0.155</td>
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</table>

*Adjusted ARR calculated by negative binomial regression and adjusted for baseline EDSS score (<4.0 vs ≥4.0), and geographic region (US vs ROW). ARR, annualised relapse rate; EDSS, Expanded Disability Status Scale; IFN, interferon; ROW, rest of the world.
12 AND 24 WEEK CONFIRMED DISABILITY WORSENING REDUCED 40% IN OPERA I & II

**Secondary endpoints:** Significant reduction in CDP in the pre-specified pooled analysis of OPERA I and OPERA II

**Time to 12-week CDP**

- **IFN-β-1a 44 μg (n=829):** 15.2
- **Ocrelizumab 600 mg (n=827):** 9.8
- Risk reduction: 40%
  - **HR (95% CI):** 0.60 (0.45, 0.81); **p=0.0006**

**Time to 24-week CDP**

- **IFN-β-1a 44 μg (n=829):** 12.0
- **Ocrelizumab 600 mg (n=827):** 7.6
- Risk reduction: 40%
  - **HR (95% CI):** 0.60 (0.43, 0.84); **p=0.0025**
>90% REDUCTION IN GAD+ LESIONS IN RRMS: OPERA I AND II

Secondary endpoint: Significant reduction in number of T1 Gd+ lesions compared with IFN β -1a

OPERZA I
- Mean Number of T1 Gd-enhancing Lesions per MRI Scan
- IFN β-1a 44 μg (n=411)
- Ocrelizumab 600 mg (n=410)
- 94% Reduction vs IFN β-1a p<0.0001

OPERZA II
- Mean Number of T1 Gd-enhancing Lesions per MRI Scan
- IFN β-1a 44 μg (n=418)
- Ocrelizumab 600 mg (n=417)
- 95% Reduction vs IFN β-1a p<0.0001

EDSS, Expanded Disability Status Scale; Gd+, gadolinium-enhancing; IFN, interferon; ITT, intent to treat; MRI, magnetic resonance imaging; ROW, rest of the world; US, United States.
POSITIVE PHASE 3 TRIAL: OCRELIZUMAB VS PLACEBO IN PPMS (ORATORIO)

PRIMARY ENDPOINT MET: ↓’d proportion of pts with 12-wk CDP by 24% c/w PBO (P=0.032).

- OCR binds CD20. Depletes B cells
- 732 PPMS pts, mean baseline age 44.6yr
- ↓’d 24-wk CDP by 25% (P=0.036)
- ↓ whole brain volume loss by 17.5% (P=0.02)
- OCR ↓’d worsening on Timed 25’ Walk by 29% c/w PBO (P=0.04)
- AEs: Infusion-related reactions, upper respiratory tract infections, and oral herpes infections more frequent with OCR.
- Neoplasms occurred in 2.3% of OCR patients and 0.8% of PBO group.

Montalban, X. et al. NEJM Dec 2016
Ofatumumab

- Human lytic mAb that targets CD20 at the smaller extracellular loop, different than rituximab and ocrelizumab.
- Lysis -- complement-dependent
- Binds CD20 better than rituximab. Slower dissociation
- Approved for refractory chronic lymphocytic leukemia in U.S. and Europe\(^1\)

Results in Relapsing MS with 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
- ↓Gad+ lesions by > 90% for weeks 4-12 for all except lowest dose
- similarly high efficacy seen w/ 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
HOW LONG CAN HUMANS BE SAFELY B-CELL DEPLETED?

- Long-term Safety of B cell depletion not known, may be especially concerning in younger people.
- Lack of B cells will likely eventually reduce antibodies, perhaps increasing risks of infections and cancers.
- PDR recommends anyone starting rituximab be screened for Hep B and Hep C prior to treatment.
- New or reactivated CMV, HSV, Parvovirus B18, VZV, West Nile, Hep B and Hep C have been seen post-rituximab.
- Hypogammaglobulinemia seen with long-term treatment.
- Loss of regulatory B cells may cause problems.
These excellent results implicate B cells in the pathogenesis of relapsing MS, but how?
Must explain:

- Relapses and Gad+ lesions are *rapidly* inhibited
- Effect on Gad+ lesions same magnitude as with natalizumab
Roles B cells might play in MS pathophysiology

- Cytokines, chemokines (IL-10, TGFβ, IL-6, TNFα and lymphotoxin β, CCL3/MIP1α, CCL4/MIP1β, CCL22).
- Antibody production (opsonization, complement activation).
- Ectopic lymphoid follicles.
- Antigen processing and presentation to T cells, particularly CD4 T cells.

Plasma cell

B

T

Cytokines, chemokines
B cells, including CD27+memory B cells, are potent antigen presenting cells\(^1\)

**Diagram:**
- CD4+ T cell
- B cell
- CD28
- CD80
- CD86
- TcR
- MHCII / peptide
- BcR / Target protein

**Text:**
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.

Does lack of B cells inhibit *relapses because they are required to present low concentration Ag* to T cells of same specificity?

Human cervical lymph nodes \(^1,2\)

Location? Secondary Lymphoid organs draining CNS?

- DeVos examined myelin antigens in cervical nodes in Chronic EAE in marmoset monkeys (MOG).
- Myelin degradation – ID’d by ORO, & IHC for myelin proteins
- T cells from the Cerv LNss proliferated to MOG

Brain (A&B) and cerv LN (C&D) from marmoset MOG-induced EAE. A &C -ORO, B &D – PLP containing cells

Lymphatics in the CNS?

- CNS has been thought to lack conventional lymphatic vessels
- John Prineas reported lymphatic-like thin-walled channels in 1979, containing lymphocytes in PV spaces

Lymphatic drainage of CNS

- Various markers injected into brain substance ends up in the cervical lymphatic vessels and cervical lymph nodes.
- Labeled albumin injected on one side of brain is found at greater levels in ipsilateral lymph nodes.
- Foldi extensively ligated cervical lymphatics in several mammalian species. Edema in intracerebral vessels, & swelling of associated projections of astrocytes. Termed “hemangio-lymphatic pathway”
- Recently, M. Nedergard and “glymphatics”

1. Foldi M. The brain and the lymphatic system. Lymphology 29: 1-9, 1996 * feels that Virchow-Robin space terminology is invalid
2. Nedergaard M.
Ectopic lymphoid tissue in MS CNS: site of B & T cells cross education?

- Antonio Uccelli Lab: CSF B cells similar to centroblasts (CD19+, CD38hi, CD77+, Ki67+) Normally, centroblasts found restricted to secondary lymphoid organs.¹

- Francesca Aloisi: Ectopic follicles in SPMS CNS (identified by proliferating B cells, CD35+ follicular DC, plasma cells, T cells, CXCL13)²

“WHAT HAS BEEN DONE WILL BE DONE AGAIN; THERE IS NOTHING NEW UNDER THE SUN.” -----ECCLESIASTES 1:9
In some old MS plaques, perivascular lymphoid tissue was found organized similarly to the antibody-producing medullary region of LN’s in brain and spinal cord (1979)¹
• CXCL13, essential for lymphoid tissue development,
• CSF CXCL13 levels are ↑ in MS vs HC (20.7pg/ml vs 10.0pg/ml)
• ↑CXCL13 levels assoc’d with worse outcomes

Ectopic follicles in ~40% SPMS, assoc’d with earlier onset, time to wheelchair, death

From R. Magliozzi et al. (Aloisi lab) Brain 2007
Changes in B- and T-Lymphocyte and Chemokine Levels With Rituximab Treatment in Multiple Sclerosis

Laura Piccio, MD, PhD; Robert T. Naismith, MD; Kathryn Trinkaus, PhD; Robyn S. Klein, MD, PhD; Becky J. Parks, MD; Jeri A. Lyons, PhD; Anne H. Cross, MD

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

$p=0.0001$, 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.

Week 0

Week 24-30

p=0.0001 Wilcoxon matched pairs test

#CD3+ cells/ml CSF

OCBs and antibodies to recombinant Human MOG

→ no change

Median = 6 pre and 6 post

Mean 2.2 (pre) vs 2.5 (post): no signif. Difference; *- total Ig
CXCL13 is not made by B cells, but levels declined post-rituximab

CXCL13: ELF and germinal center formation, recruits B cells & activated T cells.
CD20 cell role in MS immune cascade

B-Cell Receptor Maturation – all CD20+

Naïve B Cell
- Efficient Antigen uptake via BCR and CR
- CD40

Activated B Cell
- APC to T cell
- CD40L

Germinal Center B cell
- Cytokines (LT) & chemokines
- autoAb, nat Abs production

Memory B cell
- Antigen

Dendritic Cell
- ELF
- CXCL13, CCL19

Modified from Ahmed
MS activity Reduction with CD20 cell depletion

B-Cell Receptor Maturation – all CD20+

Efficient Antigen uptake via BCR and CR

Antigen-Ab complex

Naïve B Cell

Activated B Cell

Germinal Center B cell

Memory B cell

APC to T cell

Cytokines (LT) & chemokines

autoAb, nat Abs production

ELF

CXCL13, CCL19

Dendritic Cell

Long-lived Plasma Cell

Immune complexes
Washington University MS Team

- Back row, L to R: Cathie Martinez LPN, Megan Orchard PA, Anne Cross MD, Laura Piccio, MD/PhD
- Front row: Rob Naismith, MD and Gregory Wu MD/PhD
- ….and John L Trotter MD (1943-2001)
<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of Action related to B-cells</th>
<th>Effect on Circulating B-cells</th>
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<tbody>
<tr>
<td><strong>Interferon-Beta</strong></td>
<td>Increased BAFF</td>
<td>Increased total numbers</td>
</tr>
<tr>
<td></td>
<td>Decreased expression of co-stimulatory molecules</td>
<td>Relative ↑ transitional</td>
</tr>
<tr>
<td></td>
<td>Impaired antigen presentation</td>
<td>Relative ↓ class-switched memory B</td>
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<tr>
<td></td>
<td>Inhibits pro-inflammatory cytokines (e.g. IL-1β, IL-23)</td>
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<tr>
<td></td>
<td>Increased anti-inflammatory cytokines (IL-10)</td>
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<tr>
<td><strong>Glatiramer acetate</strong></td>
<td>Decreased BAFF</td>
<td>Decreased total numbers</td>
</tr>
<tr>
<td></td>
<td>Impaired antigen presentation</td>
<td>Relative ↑ naïve</td>
</tr>
<tr>
<td></td>
<td>↓ pro-inflammatory cytokines (e.g. IL-17, IL-6, TNFα, LT)</td>
<td>Relative ↓ plasmablast, memory B</td>
</tr>
<tr>
<td></td>
<td>Increased anti-inflammatory cytokines (IL-4, IL-10)</td>
<td></td>
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<tr>
<td><strong>Fingolimod</strong></td>
<td>Sequesters B-cells in lymphoid tissue;</td>
<td>Decreased total numbers</td>
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<tr>
<td></td>
<td>↓ expression of co-stimulatory molecules and pro-inflammatory cytokines (e.g. TNF-α)</td>
<td>Relative ↑ naïve</td>
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<tr>
<td></td>
<td>Increased anti-inflammatory cytokines (IL-10)</td>
<td>Relative ↓ newly produced B-cells, memory</td>
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<tr>
<td><strong>Dimethyl fumarate</strong></td>
<td>↑ B1 B-cells (secrete IL-10)</td>
<td>Slight ↓ in total numbers</td>
</tr>
<tr>
<td></td>
<td>↑ CD24highCD38high T2-MZP (secrete IL-10)</td>
<td></td>
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<tr>
<td><strong>Teriflunomide</strong></td>
<td>↓ pyrimidine synthesis enzyme required for T &amp; B cell proliferation</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>↓ multiple transcription factors and enzymes (COX-2, iNOS)</td>
<td></td>
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<tr>
<td><strong>Natalizumab</strong></td>
<td>Impairs transmigration into CNS, as well as other tissues</td>
<td>Increased total circulating numbers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ precursors, regulatory, marginal-zone like, and memory cells</td>
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<tr>
<td></td>
<td></td>
<td>Relative ↓ naïve B-cells</td>
</tr>
<tr>
<td><strong>Alemtuzumab</strong></td>
<td>Increased BAFF</td>
<td>↑ newly produced cells (soon after infusions); mature naïve cells</td>
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<td></td>
<td>Temporary depletion of B-cells with subsequent reconstitution; long term depletion of T-cells</td>
<td>Relative ↓ memory B cells</td>
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<td></td>
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<tr>
<td><strong>Mitoxantrone</strong></td>
<td>Cytotoxic for rapidly dividing cells</td>
<td>↓ total numbers and memory B after infusions</td>
</tr>
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