JOHN WHITAKER MEMORIAL LECTURE

May 29, 2015

CHARLESTON, SC AUGUST, 1999

ATLANTA,GA NOVEMBER, 2000

ANNE CROSS, MD

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.

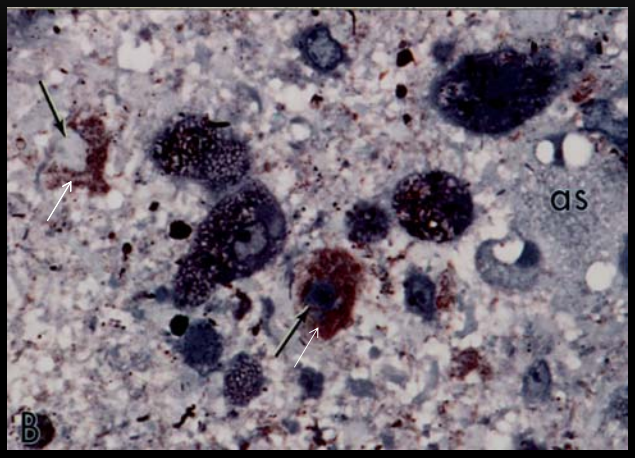
JOHN N. WHITAKER, MD

- 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[1]
- 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

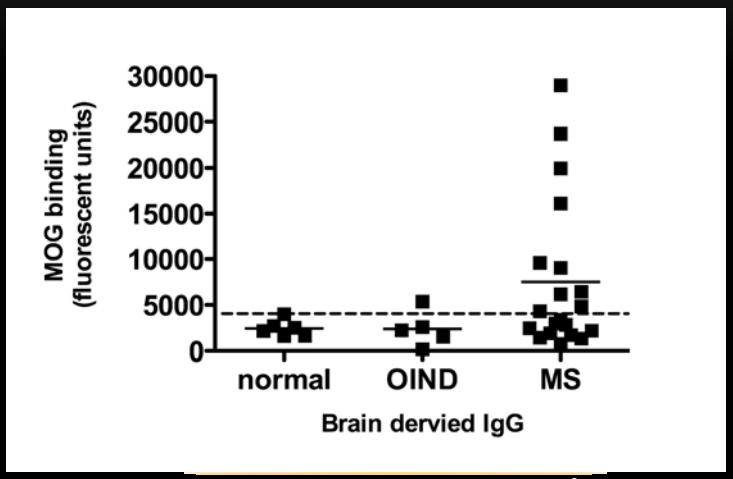


Human Acute MS

MOG=myelin oligodendrocyte glycoprotein

Reprinted with permission. Genain CP et al. *Nature Med*. 1999;5:170.

Autoantibodies to MOG in native conformation extracted from 50% of MS lesions[1]



Also anti-MBP (83-97) in other studies [2]

Graph courtesy of Kevin O'Connor (Yale Univ)

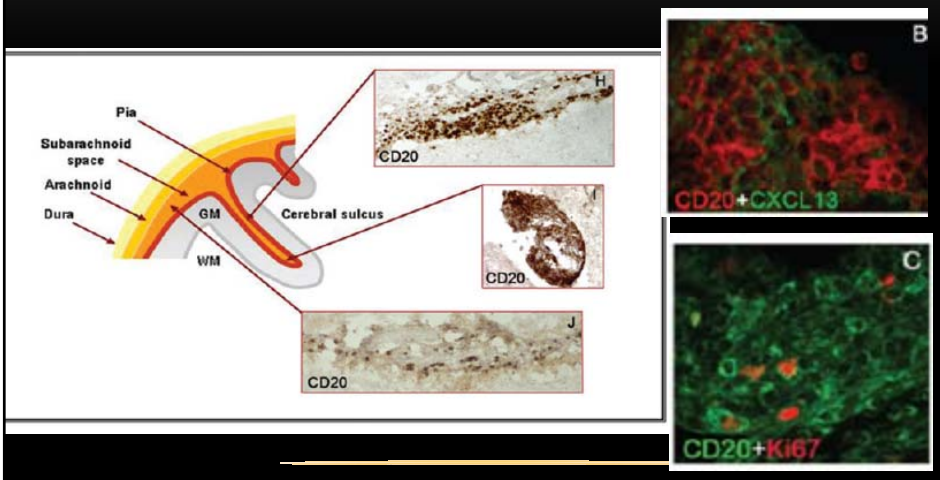
1. O'Connor KC et al. *J Immunol.* 2005;175:1974-1982. 2. Warren KG. *J Neurol Sci.* 1993;115:169-176.

Ectopic lymphoid B cell follicles

- Lymphoid-like tissue 1st described by John Prineas (1979) in brain and spinal cord[1]
- CSF B cells have characteristics of centroblasts (CD19+, CD28hi, CD77+, Ki67+)[2]
- Data from Francesca Aloisi and colleagues others showed Ectopic follicles (proliferating B cells, CD35+ follicular DC, plasma cells, T cells, CXCL13)[3]

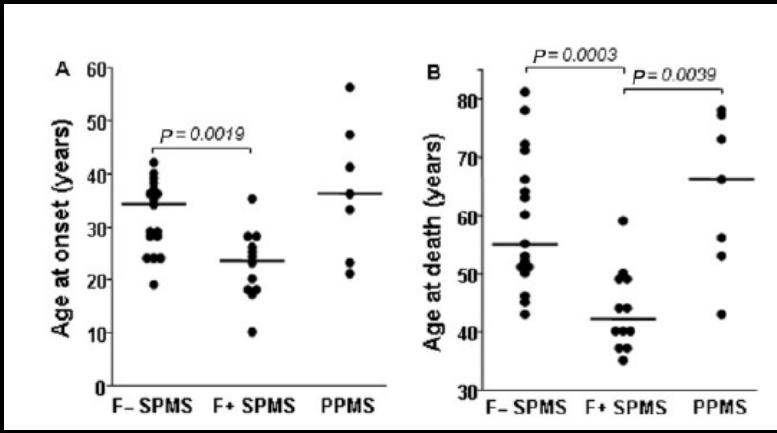
1. Prineas J Science 203: 1123-35, 1979
2. Corcione A, et al. PNAS 101: 11064-11069, 2004.
3. Maggliozzi R et al Brain 2007.

Ectopic Lymphoid Follicles in Meninges



From R. Magliozzi et al. Brain 2007

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



From R. Magliozzi et al. Brain 2007

Ectopic lymphoid B cell follicles associated with cortical MS lesions

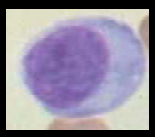
- Howell and colleagues later extend observations to show ELFs in ~ 40% of 123 SPMS cases[1]
- ELFs physically associated with cortical demyelination[1]

- ELFs remain controversial. Not seen by all pathologists, and definition of ELF not universal
- May relate to methods of tissue acquisition and preservation

1. Howell OW et al. Brain 2011; 134:2755-71

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[1]



1. Frischer JM, et al. Brain. 2009;132(Pt 5):1175–89. (Lassman group)

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[1, 2]
- B cell chemo-attractant CXCL13 ↑ in CSF[3]
- Evidence of B cell clonal expansion, and traffic between parenchyma and CSF[4]
- CSF B cells correlate with clinical and MRI activity[5]

1. Cepok S. Brain 2005 & J Neuroimmunol 2006
2. Corcione A et al PNAS USA 2004
3. Kuenz B PLOS ONE 2008
4. Lovato L et al. (K. O'Connor, D. Hafler)l. Brain 2011
5. Krumbholz M et al. Brain 2006

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.

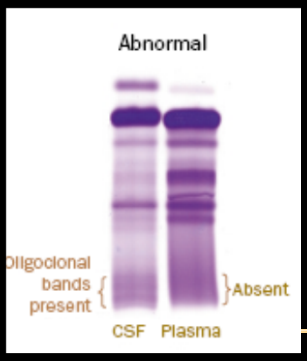
.

Immunoglobulin abnormalities in MS CSF

- 1912 Lange colloidal gold method –globulin fraction of proteins increased in neurosyphilis, MS[1]
- 1942 Kabat - γ-globulins ↑'d in CSF of MS patients[2]
- 1966 Tourtellotte – γ-globulins in CSF reflected γ-globulins in brains[3]
- 1980 Tourtellotte - formula to estimate intrathecal IgG synthesis rate using one sample of CSF and serum

1. Lange, C.
2. Kabat EA et al. J Clin Invest 21: 571ff 1942.
3. Tourtellotte WW & Parker JA. Science 154: 1044, 1966
4. Tourttellotte WW et al. Neurology 30: 240-244, 1980

Oligoclonal bands in MS CSF

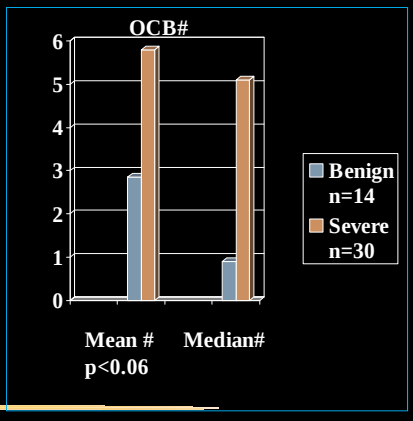


- 1966 Laterre - oligoclonal bands in >85% of MS patients [1,2]
- Oligoclonal bands are CSF-restricted
- Targets remain unsolved mystery

1. Laterre EC et al. Clin Chim Acta 1964 10: 197-209
2. Laterre EC. Acta Neurol Psych Belg. 1966 66:289-304

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[1]
- High IgG Index (>1.0) associated with rapid progression[2]
- Lack of OCBs -more likely benign (n=12, EDSS 3.0 after 14 yrs)[3]
- 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)[4]
- 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)[4]

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)[1]
- ~50% worsened on one of these: EDSS, AI, 9HPT, Box & Block Test
- κLC >75th%-tile (1.53ug/ml) was associated w disease progression. Hazard Ratio 3.78 by EDSS, 10.8 by 9HPT[1]

1. Rudick RA et al. Mult Scler. 1995; 1:150-155;

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)

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

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

Partial list of autoAbs reported in MS

Antigen	Tissue examined	Investigator
Myelin basic protein (MBP)	CSF, CNS plaque	Warren, Catz; Panitch, K.Johnson
Proteolipid protein (PLP)	CSF, CNS plaque	Warren, Catz
Myelin oligodendroglial glycoprotein (MOG)	CSF; serum; plaques	Xiao; Berger; Raine & Genain, O'Connor
KIR4.1 K+ channels	blood	Hemmer
Oligodendrocyte specific protein (MOBP)	CSF	Bronstein
CNPase	Serum, CSF	Walsh, Murray
Transaldolase	Serum, CSF	Banki; Colombo; Esposito
β-Arrestin (heat shock protein)	Serum (not in CSF)	Ohguro
GD1a ganglioside	Serum, CSF	Mata
Neurofilament heavy chain	CSF	Kuhle
Neurofilament light chain	CSF	Berger; Sharief

1980's and 1990's:

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

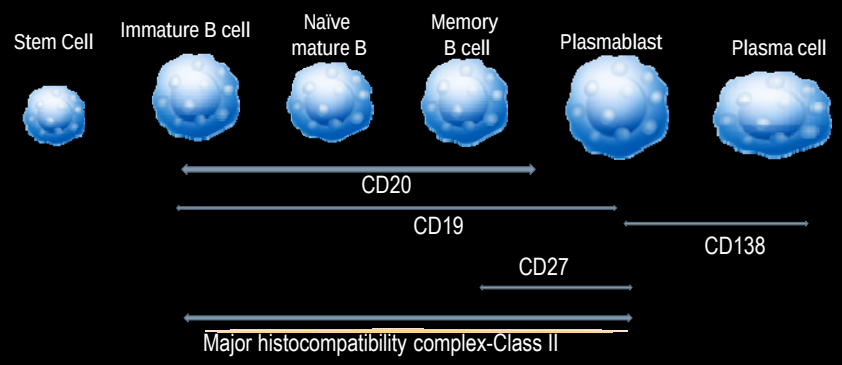
T cells were able to transfer the MS animal model, EAE (B cells & antibody did not)

Monoclonal antibodies

- 1984 Nobel Prize to Milstein and Kohler for their work creating monoclonal antibodies (mAbs) mAbs increasingly used for therapeutics
- 1997 - Rituximab approved for non-Hodgkin's lymphoma. Depletes circulating B cells by lysing cells expressing CD20
- 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]

1. Hauser S et al. NEJM 2008, 2. Naismith et al 2010, 3. Kappos et al. 2011, 4. Sorensen et al. 2014

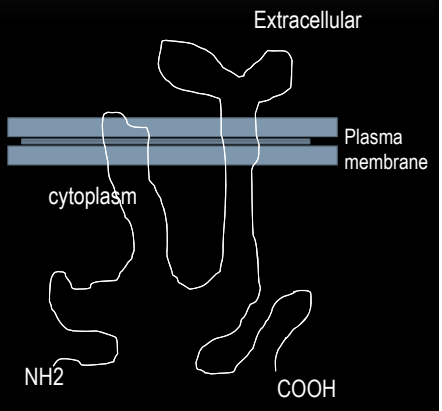
Surface markers during B cell development



Based on Mei HE, et al. Rationale of anti-CD19 immunotherapy: an option to target autoreactive plasma cells in autoimmunity , *Arthritis Research & Therapy* 2012, 14(Suppl 5):S1.

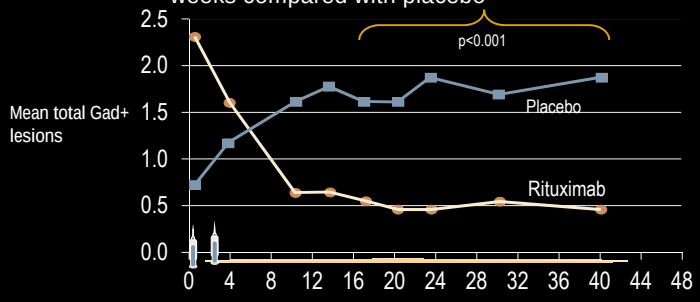
CD20: a target specific to B cells

- 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



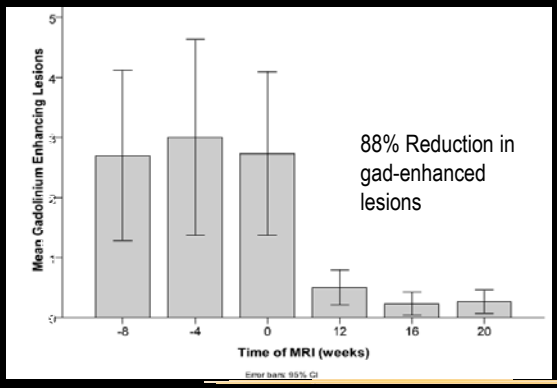
B-cell targeting in RRMS

- 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[1]
    - Reduced the proportion of patients with relapse at 24 and 48 weeks compared with placebo



1. Hauser SL, *et al. N Engl J Med*. 2008;358(7):676–88.. This slide is from a slide reproduced with permission, Copyright 2008, Massachusetts Medical Society.)

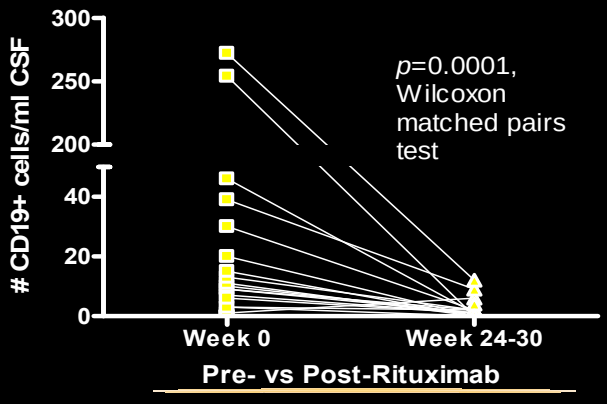
Phase 2 combination trial in 30 relapsing subjects failing β-IFN or GA Open-label with MRI blinding[1,2]



1. Cross AH, et al.. J. Neuroimmunol. 2006; 180:63-70.
2. Naismith RT et al. Neurology 2010; 74: 1860-1867

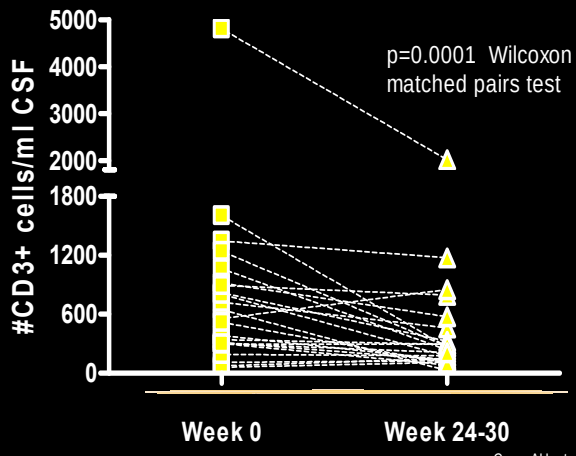
CSF AFTER RITUXIMAB

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



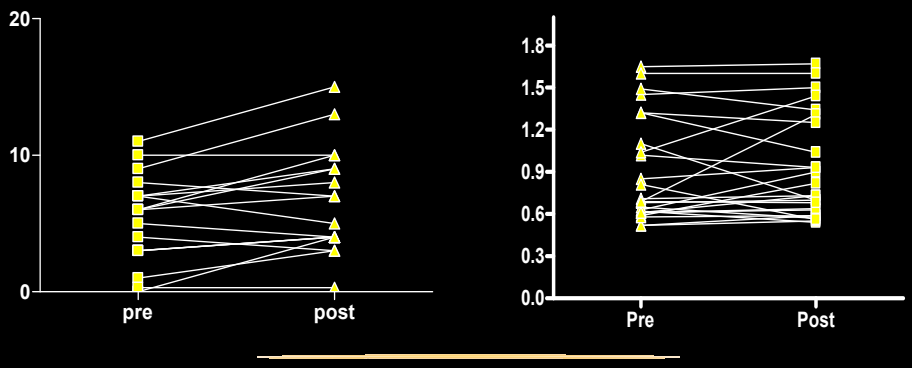
Cross AH, et al.. J. Neuroimmunol. 2006; 180:63-70.
Piccio L et al. Arch. Neurol. 2010; 67: 707-714

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



Cross AH, et al.. J. Neuroimmunol. 2006; 180:63-70.
Piccio L et al. Arch. Neurol. 2010; 67: 707-714

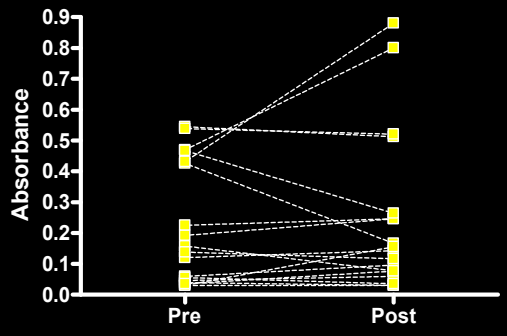
OCBs and IgG Index – no change



Median = 6 pre and 6 post

P = 0.62 Wilcoxon paired test

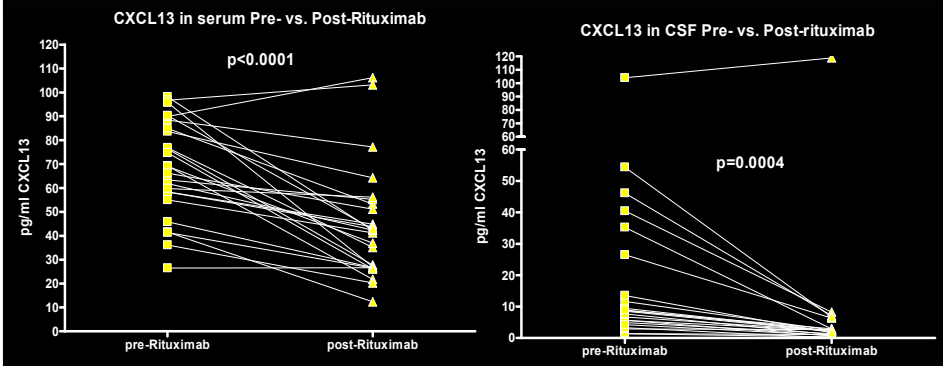
Antibodies to recombinant human MOG in CSF pre- vs post-rituximab*



Mean 2.2 (pre) ~~vs 2.5 (post): no significant~~ difference

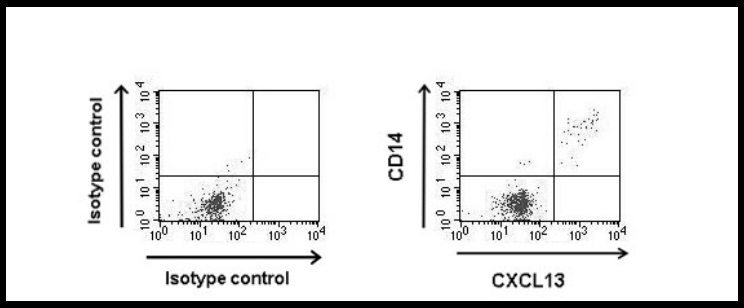
*- total Ig

CXCL13 levels decline in blood and CSF post-rituximab

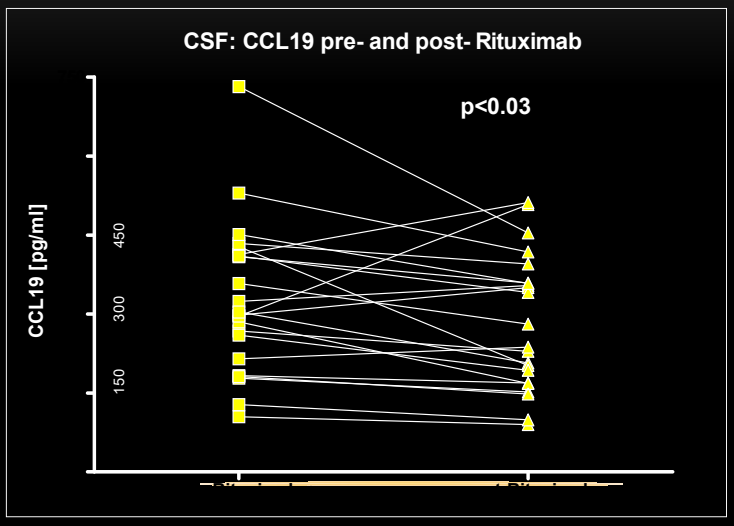


CXCL13 is critical to germinal center formation & recruits B cells. CSF CXCL13 levels are ↑ in MS vs HC (20.7pg/ml vs 10.0pg/ml)

CXCL13 is expressed by CD14+ cells in CSF of active MS patients



CCL19 levels decline in CSF post-rituximab

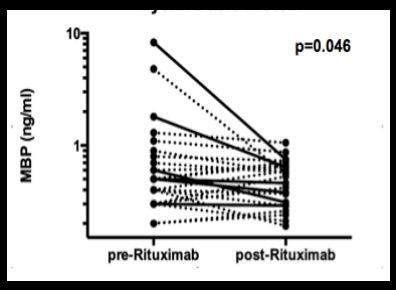


CCL19 levels were decreased in CSF after rituximab

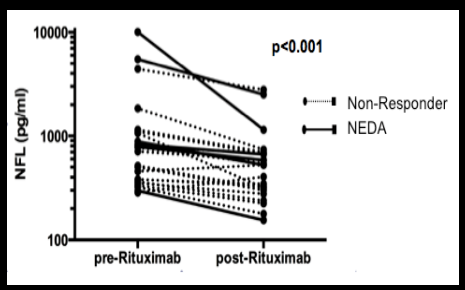
- Also known as “EBI1 ligand chemokine” and macrophage inflammatory protein-3-beta (MIP-3-beta).
- Expressed in thymus and lymph nodes. Produced by DC and Macrophages
- Ligand is CCR7
- Chemoattractant for many cell types expressing CCR7: mature DCs and antigen-engaged B cells, CCR7+ central-memory T-Cells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

Reductions in MBP and NF-L post-rituximab

Myelin basic protein



Neurofilament Light Chain

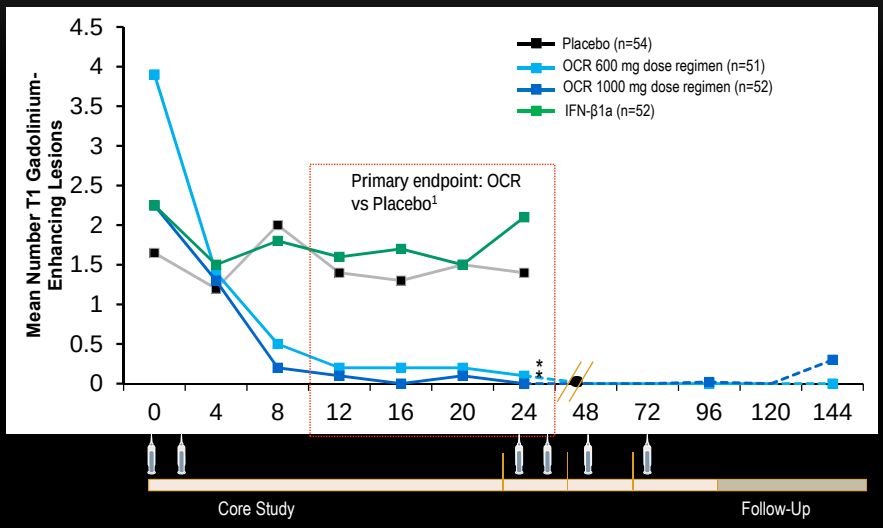


Ocrelizumab and Ofatumumab- two "second generation" anti-CD20 monoclonal antibodies being studied in MS

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

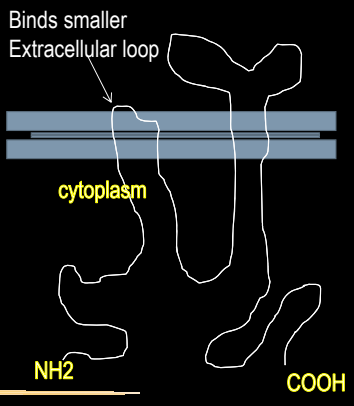
Ocrelizumab Phase II: Primary EP: Mean number of gd-enhancing T1w lesions,
'core study' (0-96 weeks) & 'follow up' (97-144 weeks)



. Kappos L, *et al. Lancet*. 2011;378(9805):1779–87; 2. Kappos L, *et al.* Abstract presented (P362) ECTRIMS 2012 , October 11

Ofatumumab

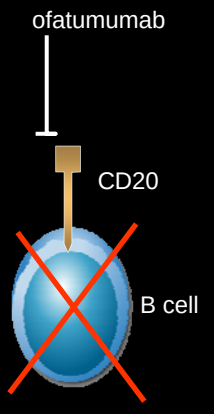
- Human mAb that also targets CD20, but at the smaller extracellular loop, different than rituximab and ocrelizumab.
- In vitro, binds CD20 better than rituximab. Slower dissociation from target
- Approved for refractory CLL in U.S. and Europe[1]



1. Cheson BD J Clin Oncol. 2010 Jul 20;28(21):3525-30.

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 increased[2]

1. Krumbholz, M., et al. . 2005; J Exp Med 201: 195-200.
2. Hartung HP and Kieseier BC. 2010.

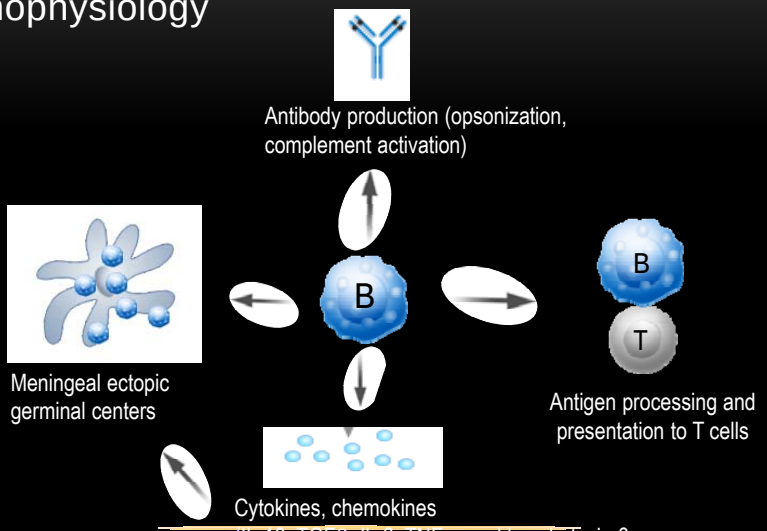
Atacicept

- 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

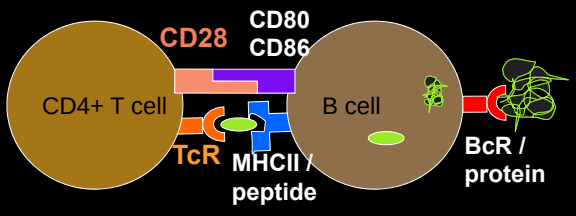
Hartung HP and Kieseier BC. 2010.

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

Roles B cells might play in MS pathophysiology



B cells, including CD27+memory B cells, are potent antigen presenting cells[1]



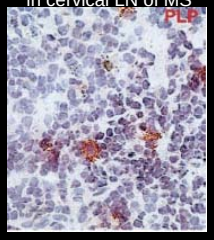
CD4+ T cell activated by processed peptides presented on MHC Class II.
B cells constitutively express MHC II, and are optimal APCs for antigens *in low abundance*, such as myelin antigens.

1. Morbach H et al. Activated memory B cells may function as antigen-presenting cells in the joints of children with juvenile idiopathic arthritis. Arthritis Rheum. 2011;63:3458-66

How might eliminating B cells inhibit *relapses*?

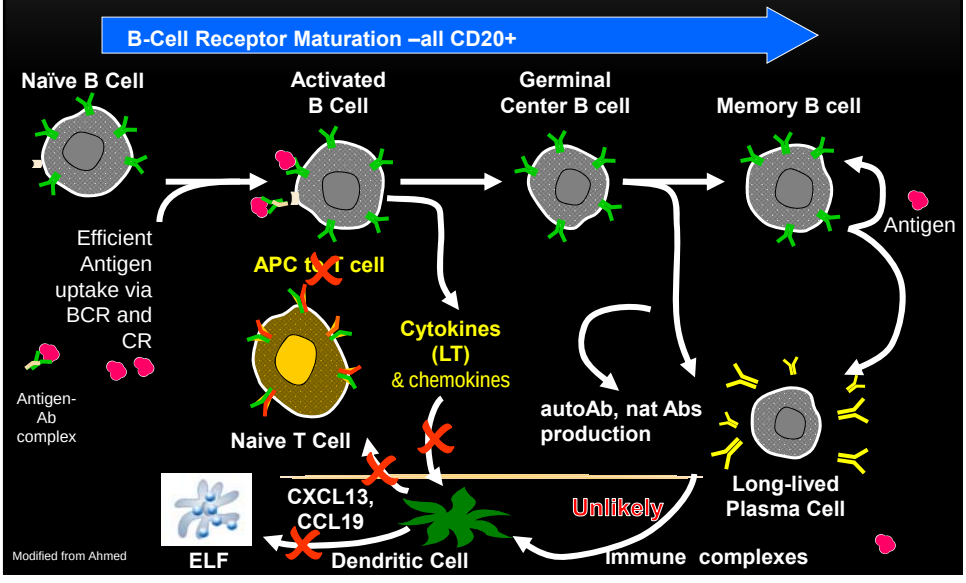
- Rapid inhibition of MRI and clinical relapse activity is c/w ability of B cells to present autoantigen (via BcR &/or capture antigen-Ab-complement complexes).
- Most likely occures cervical lymph nodes[1,2]
- (would removal of these nodes alter the process?)

PLP-containing cells in cervical LN of MS



1. De Vos AF et al. J.Immuno. 2002, 169: 5415-5423;
2. Knopf PM, Cserr HF et al. 1995, Neuropathol Appl Neurobiol 21: 175ff

Proposed mechanism of MS activity Reduction with CD20 cell depletion



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
  - HACAs (rituximab) and HAHAs (ocrelizumab, ofatumumab)

HACA=human anti-chimeric antibodies; 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

Physicians Desk Reference: rituximab

Acknowledgements

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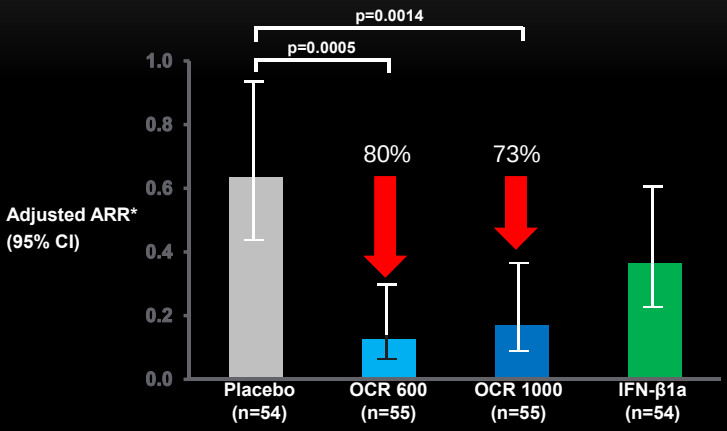
- Greg Wu MD PhD
- Cedric Raine PhD
- Michael Ramsbottom
- Bob Mikesell
- Neville Rapp PhD

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

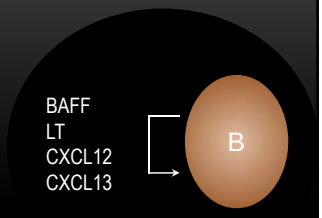


CI : confidence interval
*Adjusted for geographical region.
In the Week 48 database, one additional protocol-defined relapse was reported in the ocrelizumab 1000 arm after the Week 24 database lock. It is not included in the above analysis.

CHEMOATTRACTANTS INFLUENCED BY B CELLS

Chemokine	Produced by	Receptor and T-cell subsets targeted	Result
MCP-1/CCL2	Induced: Mac, EC, PMN, fibroblasts, astrocytes	CCR2; NK T and Th1 T	no change (*P* = .17)
MIP1α /CCL3	Induced: B cells, PMN, CD8, Mo/Macs, Eo, DC	CCR1; NK and effector memory T cells; CCR5	no change (low levels)
MIP1β/CCL4	See CCL3	CCR5; effector, Th1, and NK T cells	no change
RANTES/CCL5	Induced: EC, T cells, monocytes, NK, DC	CCR1/CCR5 (see CCL3, CCL4)	*undetectable in CSF*
SDF-1/CXCL12	Stromal cells,EC,astrocytes	CXCR4; most T's, naïve, memory B, macs	no change (*P*=.11)
CXCL13	HEV of secondary LNs	CXCR5; mature B and some CD4 & CD8 T cells, DC	***↓ 35% P = .0004**
CCL19/ ELC (EBV-induced molecule 1)	Constitutive by DC in lymphoid tissue; also by MΦ; role: homing to LN	CCR7+ B cells, naïve and central memory T cells, mature DC, myeloid cells, NK cells	***↓ P < .03**
C3a	Complement activation	C3aR on lymphocytes, other cells	no change(*P*=.48)
C5a	Complement activation	C5aR on B, T cells	*undetectable in CSF*
SLC/ CCL21, CCL22	ED, HEV, DC	CCR7; naïve, central memory T cells, mature DC	*undetectable in CSF*
CXCL10 /IP-10	Inducible in EC, astrocytes, microglia, monocytes	CXCR3 on activated T cells, CD8+ and NK cells	no change

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[1]
- In MS, ectopic follicle-like structures may form within the CNS and promote ongoing local immune injury[1]
  - Suggestive of B-cell replication and activation within the region[2]
  - May be associated with a more severe secondary progressive disease course[2]

1. Barun B, Bar-Or A. *Clin Immunol.* 2012;142(1):31-37; 2. Lehmann-Horn K et al. *Ther Adv Neurol Disord.* 2013;6(3):161-173.