Lessons from the Bench and Bedside

March 2010

Stephen L. Hauser, MD

Conflicts of Interest:
Pfizer, Wyeth, Roche, Receptos
Multiple Sclerosis
The State of Knowledge 2010

Chronic, multifocal CNS disorder
Immune-mediated myelin loss
Secondary neurodegeneration
Genetic predisposition
Influence of environment
Prevalence has increased
Gender dimorphism
Empirically based therapies
Pathology of Multiple Sclerosis

MOG

CD68
Experimental Allergic Encephalomyelitis (EAE)

Myelin Antigen/CFA

2-3 weeks

Susceptibility Genes:
- MHC class II most important locus
- Complex trait
- Females more susceptible
- Microenvironment (or imprinting) critical

Weight Loss
Paralysis
Experimental Allergic Encephalomyelitis

Myelin Antigen/CFA

Immunized Donor

Harvest Lymph Node Cells

10 Days

T cell Clones

T cell lines

Myelin Antigen

Encephalitogenic T cell Clone (Myelin)

Control T cell Clone (Ovalbumin)

Syngeneic (MHC) Naïve Recipients

Weight Loss

Paralysis
Lesson #1
Conventional wisdom may be wrong

“Especially in the larger laboratory animals, it is possible to reproduce a disease which is indistinguishable from acute multiple sclerosis... (however) the relationship between experimental allergic encephalomyelitis and chronic relapsing multiple sclerosis is obscure.”

Raymond D. Adams, 1959
Acute EAE in Rodents

- Prominent leptomeningitis, panencephalitis
- Sparse demyelination only
- Peripheral nervous system affected
- Th1 cells are necessary and sufficient
Lesson #2
In science, good friends are as important as good ideas

Norman Letvin, M.D.
Professor of Medicine, Harvard Medical School
Chief of the Division of Viral Pathogenesis, Beth Israel Deaconess Medical Center
Director of the Non-Human Primate Research Program, NIH Vaccine Research Center
Lesson #3
Great young investigators drive the success of established laboratories

Luca Massacesi, M.D.
Professor of Neurology
University of Florence, Italy
EAE in *C. jacchus*: Spinal Cord

Claude Genain, M.D.
CPMC Research Institute, San Francisco
MOG-induced EAE  EAE  Human MS
The different EAE phenotypes in *C. jacchus*
Non-demyelinating EAE

Active Immunization  T cell transfer

MBP
(Myelin basic Protein)
The different EAE phenotypes in *C. jacchus*

*Passive transfer of pathogenic antibody restores the demyelinating phenotype*

Lesson #4

Know the Literature in Depth!

“The failure to transfer EAE in vivo with large amounts of serum is a serious objection to the etiological significance of circulating demyelinating antibodies, but need not invalidate an antibody mechanism. Small amounts of a highly specific antibody may not achieve sufficient concentration at the myelin sheath and glial membrane both because of serum dilution and more significantly because of an intact “blood-brain” barrier which prevents direct access to the antigenic site….Alteration of blood-brain permeability does in fact occur in EAE, and may represent the means by which a significant amount of specific antibody or antibody-producing cells gain access to the tissue.”

The application of tissue culture to the study of experimental allergic encephalomyelitis
II. Serum factors responsible for demyelination

Bornstein and Appel
J. Exp. Med. 119:303, 1964
MOG Specific IgG Antibodies Are Deposited Within The Vesiculated Myelin Sheath

*C. jacchus* Marmoset EAE  
Human MS

The Pathogenesis of Multiple Sclerosis: 
Antibody Facilitated Demyelination

Myelin-reactive T-cells (MBP, MOG, peptide) 
→ Blood-brain barrier breakdown
  → Inflammation

Demyelinating antibody (MOG) 
→ Demyelination
  → Gliosis

MOG: Myelin/oligodendrocyte glycoprotein
MBP: Myelin basic protein
MOG Autoantibodies Are Present in MS Lesions

Other myelin proteins are also likely targeted in human MS plaques

H.-Christian von Büdingen, M.D.
Rachleff Assistant Professor of Neurology, UCSF
Targeting MS Autoantibodies

Antibody fragments [F(ab)2 or monovalent F(ab)] as therapeutic tools in EAE and MS.

Lesson #5
The rubber meets the road at the bedside; this is the ultimate proving ground for medical research

Howard L. Weiner, M.D.
First experimental recipient of plasmapheresis treatment
CD20⁺ B Cells (left) and CD138⁺ Plasma Cells (right) Are Present in MS Lesions

Blue=hematoxylin; brown=anti-CD20
Courtesy of Tonja Kuhlmann, 2008.
Lesson #6
The development of new therapies requires close interactions with industry and the FDA

Emmanuelle Waubant, M.D., Ph.D.
Associate Professor of Neurology, UCSF
B-cell Maturation in Adaptive Immunity

- Naïve B cell
- Activated B cell
- Germinal center B cell
- Memory B cell
- Short-lived PC (secrete IgM)
- Long-lived PC

Affinity maturation of B-cell receptor

Ag = antigen
APC = antigen-presenting cells
PC = plasma cell

Ahmed R et al. *Immunology of Infectious Diseases*; 2002:175–189.
Rituximab

Anti-CD20 Monoclonal Antibody

- Rituximab is a genetically engineered chimeric (mouse-human) monoclonal antibody that targets CD20-positive B lymphocytes
- CD20 is present on B and pre-B lymphocytes but not on stem cells or plasma cells
- Long duration of action
The Corporate History of Rituxan

1986
Synthesis

1995
Co-development

2003
Merger

2009
Merger
Rituximab Phase 2 Study in RRMS

Rituximab in Relapsing Remitting MS

**Primary Endpoint: Mean Gadolinium-Enhancing Lesions from Baseline to Week 48**

**Placebo (N=35)**
**Rituximab (N=66)**

**Missing values imputed by average of available data**

Rituximab Phase 1 Study in RRMS

Screening (4 wks)

Treatment Period (72 wks, rituximab)

Study Week
-2 0 2 4 8 12 24 26 36 48 60 72

Primary endpoint
• Safety

Secondary endpoints
• Relapse frequency
• MRI activity (descriptive)

Gd-Enhancing Lesions in Phase 1 Study

Mean Gd Lesions (N)

Study Week

Imputed by average method.

Lesson #7
Even a disappointing result in a clinical trial can provide important clues to pathogenesis

Wendell, I’m not content
Rituximab Phase 2/3 Study in PPMS (OLYMPUS)

Screening (4 wks)

Treatment Period (96 wks, rituximab or placebo)

Follow-up Period (26 wks)

**Study Week -2**

- 0
- 2
- 6
- 24
- 26
- 48
- 50
- 72
- 74
- 96
- 122

**Primary endpoint**
- Time to confirmed EDSS progression (≥12-week confirmation)

**Secondary endpoints**
- T2 lesion volume (change from baseline)
- Brain volume (change from baseline)

Rituximab in Primary Progressive MS

Time to Confirmed Disease Progression

All Intent-to-Treat Patients (N=439)

HR: 0.77 (95% CI: 0.55 – 1.09)
p-value=0.1442

Rituximab in Primary Progressive MS

Time to Confirmed Disease Progression
Subgroup Analysis

Age <51
Gd (-) at Baseline
n=143

HR: 0.63 (95% CI: 0.34-1.18)
p=0.1427

Age <51
Gd (+) at Baseline
n=72

HR: 0.33 (95% CI: 0.14-0.79)
p=0.0088

Proportion of Patients

Time to Confirmed Disease Progression (weeks)

Placebo

Rituximab
Median Change in T2 lesion Volume

Baseline to Week 96

![Graph showing median change in T2 lesion volume from baseline to Week 96. The graph compares RTX and Placebo groups with a P-value of 0.0008.](image-url)
Two Populations of MS Patients

Relapsing and Progressive

Disability (EDSS)

Percentage of Patients

Relapsing Remitting MS
Minimal Disability
Gait Disturbance
Cane
Crutches
Nonambulatory

Benign Symptoms
Minimal Disability
Gait Disturbance
Cane
Crutches
Nonambulatory

0 1.5 2.5 3.5 4.5 5.5 6.5 7.5 8.5
How Does B-cell Depletion Work in MS?

• Reduction of pathogenic autoantibodies unlikely MOA
• In CNS, somatic hypermutation of B-cell Ig transcripts indicates an Ag driven T-dependent process (Baranzini et al., *J Immunol* 163(9):5133, 1999; Smith-Jensen et al *Neurology*. 54(6):1227, 2001)
• Similar clonotypes in different plaques from the same individual (Owens et al *Ann Neurol* 43:236, 1998)
• B-cell rich lymphoid follicle-like structures present (Serafini et al *Brain Pathol* 4:164, 2004; Maglioizzi et al *Brain* 130:1089, 2007)
• CSF B-cells are the apparent source of oligoclonal Ig (Obermeier et al *Nat Med* May 18, 2008 epub)
• Site of action could occur in peripheral lymphoid tissue (Phan et al *Nat Immunol* 9:992, 2007)
• B-cells are also the reservoir of latent Epstein-Barr virus (Serafini et al *J Exp Med* 204:2899, 2007)
The B-cell Roadblock Hypothesis

Modified from Silverman and Boyle *Immunol Rev* 223:175, 2008

Stem Cells  
Precursors  
Transitional (naïve)  
Naïve Mature  
Activated (GC?)  
Memory  
Plasma Cells

CD20 Expression
Lesson #8

Well-designed clinical trials provide otherwise unobtainable insights into the biology of disease, often sending us back to the laboratory armed with new hypotheses.

Scott Zamvil, M.D., Ph.D.

Associate Professor of Neurology, UCSF
Anti-CD20 prevents or reverses EAE induced by MOG protein.

Weber and Zamvil, submitted 2010
Lesson #9

Technological change moves faster than the pace of clinical experiments

Ocrelizumab

Overlapping epitope

Chimeric vs humanised VH/VL

2 mutations in RTX reduce affinity for NK cells

Ocrelizumab binds more strongly both forms of the FcγRIIIa on NK cells

NK: natural Killer cell
Ocrelizumab Phase 2 Study in RRMS

<table>
<thead>
<tr>
<th>Screening (4 wks)</th>
<th>Treatment Period (24 wks)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Ocrelizumab 2000 mg / MePDN q24 wks</td>
</tr>
<tr>
<td></td>
<td>Ocrelizumab 600 mg / MePDN q24 wks</td>
</tr>
<tr>
<td></td>
<td>Avonex q wk x 6 mos; then open label ocrelizumab</td>
</tr>
<tr>
<td></td>
<td>Placebo infusion X 24 wk; then open label ocrelizumab</td>
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</tbody>
</table>

Primary endpoint
- Total Gd-enhancing lesions

Secondary endpoints
- Annualized relapse rate
- Proportion of relapse-free patients
- Change in total volume of T2 lesions

Exploratory endpoints

MRI for safety evaluation

Study Week
-4 0 2 4 12 16 20 24 48 72 96 144

Study days

Treatment days
Lessons From the B-Cell Experience in MS

• The anti-CD20 trials have demonstrated that B-cells are central players in the pathogenesis of focal inflammatory lesions in MS
• The MOA of other therapies need to be reconsidered in the light of this new data
• Ocrelizumab is likely to be a blockbuster by 2015, if rare adverse events do not emerge in the phase 3 trials
• These trials also set the stage for testing more selective therapies that target subsets of B-cells, B-cell growth/survival factors, or germinal center interactions
• The time required from recognition that humoral immunity is pathogenic in MS to launch of the first B-cell based therapy will be more than 20 years!
Lesson #10
More effective integration of clinical, translational, and basic sciences will accelerate progress against neurological diseases

Douglas Goodin, M.D.
Professor of Neurology, UCSF

Jorge Oksenberg, Ph.D.
Professor of Neurology, UCSF

Daniel Pelletier, M.D.
Associate Professor of Neurology, UCSF
Lesson #10
More effective integration of clinical, translational, and basic sciences will accelerate progress against neurological diseases

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Emmanuelle Waubant, M.D., Ph.D.
Assistant Professor of Neurology, UCSF
7T Imaging of Cortical MS Lesions
### Clinical and MRI measures

<table>
<thead>
<tr>
<th>MRI Date</th>
<th>Visit</th>
<th>Dis. Course</th>
<th>EDSS</th>
<th>MSSS</th>
<th>T2 LV (ml)</th>
<th># of new T2</th>
<th># of CE lesion</th>
<th>annual PBVC*</th>
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<tr>
<td>3/30/05</td>
<td>Baseline</td>
<td>RR</td>
<td>3.5</td>
<td>2.05</td>
<td>3.68</td>
<td>n/a</td>
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<td>n/a</td>
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<td>3/28/06</td>
<td>F/U Yr 1</td>
<td>RR</td>
<td>3.5</td>
<td>2.08</td>
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<td>0</td>
<td>0.001</td>
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<td>3/15/07</td>
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<td>RR</td>
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<td>0</td>
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<td>-0.235%</td>
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<td>6/12/08</td>
<td>F/U Yr 3</td>
<td>RR</td>
<td>3.0</td>
<td>1.52</td>
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<td>0</td>
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<td>-0.796%</td>
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<td>5/28/09</td>
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<td>RR</td>
<td>3.0</td>
<td>1.52</td>
<td>3.68</td>
<td>0</td>
<td>0</td>
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### Genetic Profile

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<tr>
<th>Gene Name</th>
<th>Chrom.</th>
<th>B. Pair Copies - Allele</th>
<th>OR-Risk Allele</th>
<th>Gene Name</th>
<th>Chrom.</th>
<th>B. Pair Copies - Allele</th>
<th>OR-Risk Allele</th>
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<tr>
<td>CD58</td>
<td>1 G/A</td>
<td>1</td>
<td>1.29</td>
<td>GPCS</td>
<td>13 A/A</td>
<td>2</td>
<td>1.10</td>
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<tr>
<td>IL7R</td>
<td>5 T/T</td>
<td>0</td>
<td>(1.12)</td>
<td>CLEC16A</td>
<td>16 G/A</td>
<td>1</td>
<td>1.16</td>
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<tr>
<td>DRB1</td>
<td>6 G/G</td>
<td>0</td>
<td>(2.77)</td>
<td>IRF8</td>
<td>16 G/G</td>
<td>2</td>
<td>1.24</td>
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<td>IL2RA</td>
<td>10 C/T</td>
<td>1</td>
<td>1.16</td>
<td>CD226</td>
<td>18 G/A</td>
<td>1</td>
<td>1.13</td>
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<td>CD6</td>
<td>11 C/C</td>
<td>0</td>
<td>(1.16)</td>
<td>TYK2</td>
<td>19 T/C</td>
<td>1</td>
<td>1.16</td>
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<tr>
<td>TNFRSF1A</td>
<td>12 T/T</td>
<td>0</td>
<td>(1.22)</td>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>

### Clinical and MRI Graphs

- **Annual Brain Volume Change (PBVC)**
- **T2 Lesion Volume (ml)**
- **PASAT3 - Score**

*Percent Brain Volume Change (PBVC)*
The Clinical Neurosciences Initiative at UCSF

• Focuses on prevention, treatment and repair of the patient
• Brings outstanding scientists and clinicians from multiple disciplines under one roof
• Trains a cadre of young, talented neuroscience investigators to be future leaders in academia and industry
• Provides collaboration and technology transfer to clinical medicine and industry
• Increases extramural research funding involving multi-disciplinary collaborations