Endogenous Remyelination in Multiple Sclerosis

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Outline

- MS pathology and pathogenesis
- Inducing developmental myelination pathways; success in the lab but not in the clinic
- Animal models recapitulate certain aspects of the human disease
- Modeling the inhibitory effects of inflammation on endogenous remyelination in vitro and in vivo
MS is a Heterogenous Disease
Over 200 Immune gene variants implicated in risk of MS: HLA-DRB*1501, IL-2ra, IL-7ra

- Viruses (EBV, HERV?)
- Vitamin D and sunlight
- Smoking
- Obesity (childhood)
- Diet
- Microbiome

Innate immune response (macrophages and glia)

Adaptive immune response (T and B lymphocytes)
MS is a complex disease with multiple contributing pathophysiological mechanisms.

<table>
<thead>
<tr>
<th>Inflammation</th>
<th>Demyelination: Remyelination:</th>
<th>Axonal damage/gliosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular cytotoxicity</td>
<td>Result of toxic insult</td>
<td>Result of toxic insult:</td>
</tr>
<tr>
<td>C/Ab-mediated damage</td>
<td>Degenerative?</td>
<td>- immune-mediated</td>
</tr>
<tr>
<td>Cytokines/soluble factors</td>
<td></td>
<td>- excitotoxicity</td>
</tr>
<tr>
<td>Neurotrophic factors</td>
<td></td>
<td>- Degenerative?</td>
</tr>
</tbody>
</table>
Remyelination occurs in ~20% of people with MS

- Restores axonal function & health
  - saltatory conduction of action potentials
  - trophic support to underlying axon
  - prevents neurodegeneration
  - protects against glial scarring
- Spontaneous remyelination frequently fails: why?

Factors affecting remyelination

- Age
  - Number of oligodendrocyte precursor cells (OPCs)
  - Macrophage recruitment (clearance of debris)
  - Epigenetic control of differentiation
- Ability to recruit OPCs
- Microenvironment unfavorable to oligodendrocyte differentiation (immune cell activation and glial scarring etc.)
- Axonal health (Neuroregulins? Probably other signals)
https://en.wikipedia.org/wiki/Neuroglia
Oligodendrocyte Progenitor Cells (OPCs): Abundant proliferative glia in adult CNS
Premyelinating Oligodendrocytes present in chronic MS lesions

Inflammatory cells and OPCs in MS CNS

Kutzelnigg and Lassmann 2014 (Handbook of Clinical Neurology)
The Yin and Yang of the immune system and remyelination

- M2 monocytes (TGFβ, IGF-1, Activin, and endothelin) important for clearance of myelin debris and promoting remyelination
- IFNγ, IL-17a and TNF all shown to inhibit OPC differentiation

McMurran, Jones, Fitzgerald, and Franklin; *Front Cell Dev Biol*. 2016

→ Gallo; Popko; Vartanian
→ Ransohoff and X. Li
Factors affecting remyelination

Fig. 3. Schematic of the oligodendrocyte lineage showing some of the intrinsic and extrinsic factors that influence oligodendrocyte differentiation and the myelination of individual axons. Oligodendrocyte differentiation requires the integration of multiple extracellular signals through coordination of multiple intrinsic pathways. Myelination is regulated both at the level of oligodendrocyte differentiation and more subtly at the level of individual axons.
Laboratory approaches:
Murine & human OPCs are differentiated into OLs via T3 or GC1 in vitro

Mouse OPC:
NG2-DS red
PLP-eGFP

Human OPC:
isolated from biopsy using A2B5

Baxi et al; Glia
How do we test compounds *in vivo*?

- EAE, may depend on the model
  - MOG peptide mice have extensive axonal damage
  - Full length rMOG1-125 in rats have more primary demyelination
- Cuprizone
  - Can compounds promote remyelination of corpus callosum or hippocampus?
  - Endogenous remyelination occurs too readily
- Lysophosphatidyl choline stereotactica injections
Pathology & DTI of mouse EAE spinal cord

A) control                 B) EAE

LFB                       Beta-APP

Toluidine Blue Thin Plastic Sections

Number of axons quantified
In medial dorsal column
Cuprizone model of demyelination

Black gold staining of corpus callosum
Can we test drugs in the Cuprizone model of demyelination?

- No Cup CTRL
- Vehicle

4 wks  5 wks  6 wks

Corpus Callosum

% of CTRL

- 4 wk Veh
- 5 wk Veh
- 5 wk GC1
- 6 wk Veh
- 6 wk GC1
- 6 wk T3

GC1  T3
Anti-LINGO (opicinumab): “Releasing the developmental brakes”

• Enhances OPC → Oligodendrocyte in vitro (Sha Mi Nature Medicine 2005)

• Enhances remyelination in whole MOG rat EAE and cuprizone models (Mi, Nature Medicine 2007 and Annals of Neurology)

• Phase 1 trial of anti-LINGO Mab completed

• Phase 2 trials:
  • Treat Acute Optic Neuritis (OCT and VEP)
    • 30% greater recovery of latency w/ anti-LINGO
  • RRMS patients (Improvement in function in some)

• Caveats: Will release of this brake be enough to overcome all the inhibitory cues?
Opicinumab in Acute Optic Neuritis: RENEW Study Design

- Primary endpoint: improvement in optic nerve conduction latency by full-field (FF) VEP
  - Latency at end of treatment (Week 24), study end (Week 32) for affected vs. unaffected fellow eye at baseline

AON = acute optic neuritis; VEP = visual evoked potential; IV = intravenous.

*For patients with AON who had not already been treated.
Primary Endpoint: Recovery of FF-VEP Latency

Adjusted mean change in FF-VEP latency, ms

**Population analyzed**

<table>
<thead>
<tr>
<th>Average difference</th>
<th>Week 24 a</th>
<th>Week 32 b</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Placebo</strong></td>
<td>20.83 n = 41</td>
<td>14.69 n = 33</td>
</tr>
<tr>
<td><strong>Opincinumab 100 mg/kg IV</strong></td>
<td>17.34 n = 41</td>
<td>15.08 n = 41</td>
</tr>
<tr>
<td><strong>ITT</strong></td>
<td>21.15 n = 41</td>
<td>22.24 n = 36</td>
</tr>
<tr>
<td><strong>PP</strong></td>
<td>22.35 n = 36</td>
<td>13.22 n = 33</td>
</tr>
</tbody>
</table>

**95% CI**

<table>
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<tr>
<th>95% CI</th>
<th>Week 24 a</th>
<th>Week 32 b</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Placebo</strong></td>
<td>−10.61 to 3.65</td>
<td>−15.12 to −2.14</td>
</tr>
<tr>
<td><strong>Opincinumab 100 mg/kg IV</strong></td>
<td>−12.66 to 0.53</td>
<td>−16.11 to −2.14</td>
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</table>

**P-value**

<table>
<thead>
<tr>
<th>P-value</th>
<th>Week 24 a</th>
<th>Week 32 b</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Placebo</strong></td>
<td>0.33</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Opincinumab 100 mg/kg IV</strong></td>
<td>0.07</td>
<td>0.01</td>
</tr>
</tbody>
</table>

CI = confidence interval. a Analysis of covariance (ANCOVA). b Mixed-effect model repeated measure (MMRM).
Opicinumab in MS: SYNERGY Study Design & Primary Endpoint

- Disabled participants (EDSS 2–6)
- 18–58 years
- Active RRMS or SPMS over previous year

• Percentage with improvement in neurophysical and/or cognitive function over 72 weeks
• Multicomponent endpoint

418 participants were randomised but 412 were assessed for efficacy; 6 were excluded due to GCP violations. EDSS = Expanded Disability Status Scale; 9HPT = 9-Hole Peg Test; IFNβ = interferon beta; IM = intramuscular; IV = intravenous; PASAT = 3-Second Paced Auditory Serial Addition Test; T25FW = Timed 25-Foot Walk.
SYNERGY: Results of Primary Endpoint Confirmed Improvement Responders

- Pre-specified primary endpoint using a linear trend test across 5 arms was not met
  - Increased proportion (not statistically significant) of improvement responders at 10 mg/kg and 30 mg/kg opicinumab vs placebo
    - Suggests an inverted U-shaped dose response
- Opicinumab generally well tolerated

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Based on a linear contrast in logistic regression. Nominal $p$-values for pairwise comparison of each opicinumab arm with placebo.
Will manipulating developmental OPC differentiation pathways such as Thyroid Hormone or anti-LINGO be effective in MS and do we have the right models to screen therapeutic agents?
Adoptive Transfer (AT)-Cuprizone Model designed to recapitulate the inhibitory effects of T cells on OPCs

Baxi et al; J. Neurosci, 2015
Fate Tracing in PDGF-Rα_Cre\textsuperscript{ER}; Rosa26_YFP reporter mice following cuprizone mediated demyelination reveals large pool of new OPCs becoming OLs

Baxi et al. Glia, 2017
Adoptive transferred T cells not only inhibit OPC differentiation into OL but reduce the absolute number of OPCs.
IFNγ inhibits myelination in vitro

MBP

SOX10

STAT1

β-Actin

P-STAT1

MBP

T3 + IFNγ

T3 + IL17

MBP/Olig2

T3

NG2

Neurofilament

Merge

0
10
20
30
40
50

0
100
200
300
400
500

0
2,000
4,000
6,000
8,000
10,000
12,000

0
100
200
300
400
500

***
**
*

T3 + IFNγ

T3 + IL17

IFNγ inhibits myelination in vitro
IFNγ Inhibits Remyelination In Vivo
Cuprizone in GFAP/tTA x TRE/IFNγ

A
IFN Expression Level – Doxycycline OFF

<table>
<thead>
<tr>
<th></th>
<th>1W</th>
<th>2W</th>
<th>3W</th>
<th>4W</th>
<th>5W</th>
<th>6W</th>
<th>7W</th>
<th>8W</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN Expression – Doxycycline ON or OFF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuprizone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

B
DOXY ON
DOXY OFF

C
MBP  DAPI

L. Kirby
IFNγ induces significant gene expression changes in OPCs.
OPCs exposed to IFNg upregulate MHC class I and present antigens to CD8s.
**Class I MHC**
- Recognized by TCR of CD8 T cells
- α2 α1 α3 β2μ with bound peptide

**Class II MHC**
- Recognized by TCR of CD4 T cells
- α1 β1 α2 β2 with bound peptide

Class I function is to display fragments of proteins from within the cell to T cells; the pathway of MHC class I presentation is often called the **cytosolic or endogenous pathway**.

The peptides presented by class II molecules are derived from extracellular proteins (not cytosolic as in **class I**); hence, the MHC class II-dependent pathway of antigen presentation is called the **endocytic or exogenous pathway**.

**Cross-presentation** is the ability of certain **antigen**-presenting cells to take up, process & present extracellular **antigens** with MHC I molecules to CD8 T cells (cytotoxic T cells).
CD8s in juxtaposition with OPCs
In the AT-Cup Model callosum
MHC Class I Pathway is activated by IFNγ but not IL17
IFNγ stimulated transgenic OT-1 OPCs express peptide loaded MHC I

In vivo expression of IFNγ induces immunoproteasome genes

**Figure Legend**

1. **OVA Specific H2Kb antibody**
2. **OVA257-264**
3. **MHC Class I H2Kb**
4. **IFNγ + OVA**
5. **Merge**
6. **DAPI**
7. **PDGFRα**
8. **H2Kb-OVA257-264**

**Graphs**

- **Fold Induction**
  - IFNγ
  - T-IFNγ
  - PSMB5
  - PSMB6
  - PSMB7
  - PSMB8
  - PSMB10
  - PSMB11
  - PSMB12
  - PSME1
  - PSME2
  - TAP1
  - TAP2
  - TAPBP

- **Cell Percentage**
  - H2Kb
    - Doxy ON
    - Doxy OFF

**Notes**

- **PARENT GATING**
  - CD11B^{+/-}
  - PDGFRα^{+/-}
  - A2B2^{+/-}
Coculture of OT-1 Transgenic OPCs and CD8 T cells

OT1 TCR Transgenic Mice

Dendritic Cell
Ovalbumin

MHC I
CD8
CD3
CD80/CD86
CD28
OVA Specific TCR

Spleen/Lymph Node

CD8 (-) Selection

Proliferation Dye

IFNγ/Ovalbumin Removal → CD8 Application

12HR
20HR
48HR
72HR

OPC Culture
OPC/CD8 Coculture

Ovalbumin
IFNγ

-
OT-1 OPCs engulf antigen and activate CD8 cells

- **Cell Proliferation**
  - CD8α
  - Vβ5

- **IFNγ Stimulation**
  - Non Stimulated
  - IFNγ Stimulated
  - No Peptide
  - Ovalbumin

- **Percent Live**
  - NP
  - OVA

- **Percent IFNγ**
  - NP
  - OVA

- **Percent TNFα**
  - NP
  - OVA

- **Percent Perforin**
  - NP
  - OVA

- **Percent Granzyme B**
  - NP
  - OVA

- **Live/Dead**
  - 2.72
  - 3.48
  - 3.28
  - 53.9

- **Percent OT1⁺**
  - NP
  - OVA
Pharmacological inhibitors of antigen processing and cross-presentation decrease CD8 activation by ovalbumin but not peptide, which can be presented through the vacuolar pathway.
Activated CD8s promote cytotoxicity in OPCs

- Non Stimulated
  - 0HR
  - 24HR
  - 48HR

- IFNγ Stimulated
  - 0HR
  - 24HR
  - 48HR

- Stained OPC
  - Caspase 3/7 Active
  - Overlap Mask

- FMO
  - Non Stimulated
  - IFNγ Stimulated

- Percent FAS
  - Non
  - IFNγ

- Caspase3/7 Active OPCs (Number/Image)
  - OPC Only
  - Ovalbumin
  - No Peptide
  - IFNγ Stimulated

- FMO
  - No Peptide
  - IFNγ Stimulated

- H2Kb
  - Count
  - 0 10 20 30 40 50

- FAS
  - Count
  - 0 10 20 30 40 50 60
Detection of antigen presenting OPCs in vivo using C3HeB/Fej mice in which epitope spreading occurs

OPCs cross present and undergo apoptosis *in vivo*
Immunoproteasome subunit PSMB8 is highly upregulated on Sox-10 lineage cells in MS plaques.
Summary

• Inflammation (IFNg) suppresses OPC differentiation and induces antigen presentation (immunoproteasome) in OPC
• OPC express MCH I and can cross-present exogenous antigen to CD8 T cells, which become CTL
• Cytotoxic T cells then kill OPCs
• Immunoproteasome subunits are highly expressed on OL lineage cells in MS WM plaques
• Perhaps this explains presence of CD8s in MS CNS and is a targetable mechanism of remyelination failure
• Understanding this pathway could be important for designing remyelinating strategies
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