Multiple sclerosis
An autoimmune dilemma

Bibiana Bielekova, MD & Joan Ohayon, CRNP

Neuroimmunological Diseases Unit
Neuroimmunology Branch
NIH/NINDS

NIH Demystifying Medicine Series
3/1/2011
Multiple Sclerosis

- Disease of "young adults" causing lesions in the brain and spinal cord that are called "plaques" or "sclerosis"
- These lesions range from inflammation, demyelination, axonal damage and gliosis
- **Cause is not known**, but traditionally it is believed that immune system of MS patients attacks brain by "mistake" (autoimmune disease caused by dysregulated intrathecal immune responses)
- **Genetic predispositions and environmental triggers**
Evolution of Multiple Sclerosis (MS)

Preclinical
Age ???

CIS

Relapsing-Remitting
Age ~10-40

Secondary Progressive
Primary Progressive
Age ~>40

Clinical course
Brain Volume
Lesion Load

Contrast enhancing/new
MS lesions

Inflammation / Response to current Th

Neurodegeneration?
Mitochondrial dysfunction vs compartmentalized IR
MS is a complex disease with multiple contributing pathophysiological mechanisms.

**Inflammation:**
- Cellular cytotoxicity
- C/Ab-mediated damage
- Cytokines/soluble factors

**Demyelination:**
- Result of toxic insult
- Degenerative?

**Axonal damage/gliosis:**
- Result of toxic insult:
  - immune-mediated
  - excitotoxicity
- Degenerative?
Pathophysiology of the Inflammatory phase of Multiple Sclerosis
Most of the information we have is derived from the animal model EAE

Active immunization:
- **Ag:** Spinal cord homogenate, Myelin proteins, Myelin peptides, CFA (Adjuvant) +/- Pertussis toxin

Adoptive transfer:
- Encephalitogenic activated CD4+ T cells

- Usually monophasic or chronic disease mostly involving SC
- Necrotic lesions with CD4+ T cells in inflammatory infiltrate

Based on EAE, MS is believed to be mediated by myelin-specific CD4+ T cells
## However, is EAE a relevant model of MS? (or is it a model of ADEM?)

<table>
<thead>
<tr>
<th>EAE</th>
<th>differences</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induced</td>
<td></td>
<td>Spontaneous disease</td>
</tr>
<tr>
<td>Spontaneous in TCR Tg/RAG KO or BCR Tg</td>
<td></td>
<td>Most often relapsing-remitting course</td>
</tr>
<tr>
<td>Mostly monophasic or chronic course</td>
<td></td>
<td>Inflammatory demyelination in the brain and SC; relative preservation of axons</td>
</tr>
<tr>
<td>Necrotic changes of the spinal cord, with equivalent loss of both myelin and axons</td>
<td></td>
<td>CD8+ T cells predominate</td>
</tr>
<tr>
<td>CD4+ T cells predominate</td>
<td></td>
<td>Only low affinity Ab against myelin Ag</td>
</tr>
<tr>
<td>High affinity Ab against myelin Ag</td>
<td></td>
<td>Poorly amenable to Th; discrepancies between therapeutic interventions defined in EAE (IFN-g, TNF-a inhibitors, rolipram, daclizumab, rituximab)</td>
</tr>
<tr>
<td>Amenable to many different therapeutic interventions</td>
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<table>
<thead>
<tr>
<th>EAE</th>
<th>similarities</th>
<th>MS</th>
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<tbody>
<tr>
<td>Need for susceptible genetic background</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Need for environmental factors in disease induction (pertussis toxin, CFA or “dirty” facility in the case of TCR Tg models)</td>
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</tbody>
</table>
1. T cell priming
   (molecular mimicry?)
2. Diapedesis through
   the endothelium/BBB
   (effector/memory T cells)
3. Antigen presentation
   in the CNS
   (Virchow-Robin space)
4. T cell effector function
   (production of cytokines, B
   cell help, cytotoxicity)
5. Recruitment of non-
   specific immune cells
   Cytokine/chemokine gradient
6. Demyelination
   (TNF-α, NO, O₂ radicals,
   Ab/C/cell-mediated lysis,
   metabolic exhaustion)
7. Axonal damage
8. Suppression of inflam-
   mation/remission
   (apoptosis of activated T cells
   regulatory/suppressor T cells)
9. Remyelination
Myelin specific CD4+ T cells and MS
What is the role of myelin-specific CD4+ T cells in untreated MS patients?

Bielekova et al, JI 2004
Myelin epitope-specific CD4+ T cells isolated from MS patients have high Ag-avidity...
...more pro-inflammatory (Th1) phenotype...

A/

All Ag-specific TCL:

Autoreactive:
MS: N=436
HD: N=53

Flu-HA-specific:
MS: N=92
HD: N=15

*P=0.00044
*P=0.026

B/

High-avidity TCL:

Autoreactive:
MS: N=283
HD: N=23

Flu-HA-specific:
MS: N=84
HD: N=15

*P=0.017
*P=0.014
...and originate more likely from in-vivo activated (i.e. effector or memory) T cell pool
However, is that a good evidence for their pathogenicity in MS?

i.e.

can we correlate precursor frequency of myelin-specific T cells or their activation status to the clinical or paraclinical markers of disease activity in MS?
Myelin-specific CD4+ T cells may have different role in different MS patients.
In contrast to ADEM (&EAE), MS patients do not have in serum or CSF high-affinity Ab against myelin antigens

Low affinity Ab
Solid phase assay: ELISA

High affinity Ab
Solution phase assay: RIA

O'Connor et al, J Neuroimmunol 2003
T-cell reactivities to CNS-antigens are seen in children with inflammatory demyelination as well as those with remote CNS insult, but only rarely in healthy children.
There is one known Ag target identified in one demyelinating CNS disorder: NMO

...and it is not myelin Ag, not even CNS restricted

⇒ Aquaporin 4 is the target of NMO-IgG

Lennon et al, JEM 2005
### Pathophysiological differences between EAE & MS

<table>
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<th>EAE</th>
<th>MS</th>
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<tr>
<td><strong>Target of the immune response</strong> (i.e. antigen) known: different myelin proteins</td>
<td><strong>Target of the immune response</strong> (i.e. antigen) unknown</td>
</tr>
<tr>
<td>Autoimmune disease</td>
<td>Because Ag is not known, and it can be potentially foreign Ag, we cannot say with certainty that MS is an autoimmune disease, RR-MS is immune mediated disease</td>
</tr>
<tr>
<td><strong>Pathogenic cell population known:</strong> pro-inflammatory (Th1/Th17) CD4+ T cells</td>
<td><strong>Pathogenic cell population unknown (CD8 cells predominate in MS lesions)</strong></td>
</tr>
<tr>
<td>Pathogenic role for Ab in some EAE models, but no clear pathogenic role for B cells</td>
<td>Strong therapeutic response of early RR-MS to immunomodulatory therapies that do not affect OCB or Ab argues against pathogenic role of Ab in early RR-MS, but indicate some pathogenic role for B cells</td>
</tr>
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</table>
New concepts regarding immunopathogenesis of MS
Emerging concept of the neural-immune interactions and “Beneficial autoimmunity”

- Immune cells contribute to the maintenance of neurogenesis and spatial learning in adult animals and their deficiency leads to cognitive dysfunction (Ziv, Nature Neurosci 2006, Kipnis, PNAS 2004)

- During TBI, naturally-occurring autoimmune T cell responses significantly improve recovery of the neurological function (Simon, J Neurotrauma 2006)

- Immune responses enhance proliferation and differentiation of oligodendroglial precursors, leading to remyelination (Bieber, Ann Neurol 2003, Kipnis, Eur J Neurosci 2004)
### Disease heterogeneity in MS

Different pathophysiological mechanisms appear to mediate acute demyelination in different MS patients.

<table>
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<th>Pattern I</th>
<th>Pattern II</th>
<th>Pattern III</th>
<th>Pattern IV</th>
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<tr>
<td>Macrophage associated</td>
<td>Ab/C-mediated</td>
<td>Distal oligodendro</td>
<td>OG degeneration in</td>
</tr>
<tr>
<td>Good remyelination</td>
<td>Good remyelination</td>
<td>Gliopathy (DOD) - ↓MAG</td>
<td>PPWM</td>
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</tbody>
</table>

- **Pattern I**: Macrophage associated Good remyelination
- **Pattern II**: Ab/C-mediated Good remyelination
- **Pattern III**: Distal oligodendro Gliopathy (DOD) - ↓MAG
- **Pattern IV**: OG degeneration in PPWM

*Developed based on Luchinetti et al Annals of Neurology 2000*
Relapsing and Remitting Multiple Sclerosis: Pathology of the Newly Forming Lesion

Michael H. Barnett, MBBS and John W. Prineas, MBBS

Ann Neurol 2004;55:458–468

Homogeneity of Active Demyelinating Lesions in Established Multiple Sclerosis

Esther C. W. Breij, PhD,1 Bianca P. Brink, BSc,2 Rob Veerhuis, PhD,2,3 Christa van den Berg, BSc,2 Rianka Vloet, BSc,1 Riqiang Yan, PhD,4 Christine D. Dijkstra, MD, PhD,1 Paul van der Valk, MD, PhD,2 and Lars Bö, MD, PhD2,5

Ann Neurol 2008;63:16–25
Disease heterogeneity in MS
Different approach

Disease onset

Disability

time

Immune contribution
Inflammation
(CEL – number, V)

CNS contribution
Tissue destruction
(BFV, BHV, BHFr)

Negative/Deleterious
Tissue destruction: Cytotoxicity, Ab/C-mediated demyelination, formation of ROS, NO, TNF-a

Positive/Beneficial
Remyelination and repair: production of growth factors & others

Negative/Deleterious
Primary CNS defect?, Susceptibility to tissue damage

Positive/Beneficial
Resistance to damage, effective remyelination
MRI imaging may identify patients with predominantly inflammatory versus degenerative MS subtypes

<table>
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<tr>
<th>Low inflammatory activity</th>
<th>High inflammatory activity</th>
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<tbody>
<tr>
<td>Low tissue destruction</td>
<td>High tissue destruction</td>
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**MRI measures:**

- **Inflammation:**
  - Average Gd# (3 mo)

- **Diffuse atrophy:**
  - Brain Fractional V
  - Brain parenchymal Fr

- **Focal destruction:**
  - Black hole fraction

Bielekova et al, Neurology 2005
MRI classification has clinical relevance

Differences in the rate of accumulation of clinical disability between MS subgroups

Accumulation of T2LL

Accumulation of T1LL

Accumulation of EDSS disability

Accumulation of Scripps-NRS disability

Exacerbation rate

*B<0.001

PI-EDSS (ΔEDSS/DD)

PI-NRS (ΔNRS/DD)

Bielekova et al, Neurology 2005
Independent/confirmatory cohort:

A/ Differences between MRI markers at untreated baseline and at follow-up 8 years later

B/ development of clinical disability at follow-up
Conclusions I

1. Immune system plays an important role in MS disease process, but heterogeneity of the immune response may determine to what degree it promotes CNS tissue destruction, versus remyelination and repair.

2. Neither the molecular targets, nor the effector cells of the deleterious immune response in MS are fully defined.

3. Important pathophysiological differences exist between EAE and MS and we need to understand them in order to better translate predictions from animal models to human disease.

4. Current research did not exclude the possibility of primary CNS event that underlies development of MS and that induces secondary immune response (especially in PP-MS).

5. Immunomodulatory treatments need to be applied early on to limit development of future disability.
What underlies development of disability in (progressive) MS?
Pathology of progressive MS
SP-MS vs. PP-MS

Progressive MS: the diffuse CNS process characterized by microglial activation and diffuse axonal injury in the white matter and by cortical demyelination and neuronal loss in the gray matter.
A. Intact neuron

B. Structural repair
   (demyelinated axons)

C. Functional repair
   (transected axons)
What causes neurodegeneration/lack of repair in the later (secondary progressive) stages of MS?

- exhaustion of OG precursors?
- factors limiting endogenous remyelination (Notch 1, NoGo, HMW hyaluronan etc)?
- metabolic exhaustion of OG that are trying to remyelinate damaged myelin sheets?
- primary neurodegeneration that remains uncompensated?
- lack of interneurons available for remodeling of axonal connections?

Kerschensteiner/Schwab et al, JEM 2004
On fMRI MS patients show greater areas of activation following simple task than controls – this correlates with the extent of tissue damage.

Activation areas during PASAT: patients with better scores show larger areas of activation.

Mainero et al, Neuroimage 2004
Inflammation versus degeneration?

Disability

time

SP-stage threshold
The axonal loss is not limited to the later stages of the MS disease process, but occurs even in the CIDS stage.

Secondary neurodegeneration caused by early inflammatory process.
Evidence in support of the hypothesis that RR-MS is pure inflammatory disease and inflammation causes secondary neurodegeneration

- Stabilization of disability progression after aggressive immunomodulatory/immunosuppressive therapies:
  - Autologous bone marrow transplantation
  - Camphath 1H (Alemtuzumab)
  - Daclizumab & others

Coles et al, NEJM 2008; 17: 1786-1801
What if the low degree of axonal loss happens irrespective of inflammation due to primary CNS defect?
Continuous, but compartmentalized immune response in SP-MS?
Pathological evidence for continuous inflammation in SP-MS

- LFB
- Demyelination

- CD68
- MΦ
- Microglia

- CD3
- T cells

- APP
- Axonal dysfunction

Frischer et al, Brain 2009
Meningeal lymphoid follicles: Compartmentalized immune response in SP-MS

- Chronic inflammation: development of tertiary lymphoid follicles in affected organ ⇒ priming and differentiation of adaptive immune responses (B and T cells) within affected organ

- Ectopic B cell follicles described in SP-MS patients (Serafini et al. 2004, 2007) in association with cortical demyelinating lesions

Serafini et al. Brain 2007
F+ SP-MS patients have greater number of cortical lesions & greater cortical atrophy, which develops in gradient-specific manner & involves both neurons and OG.

In contrast, MHC-II+ microglia is increased in gradient-specific manner in cortex of follicle+, but not follicle- patients.

Magliozzi et al, Ann Neurol 2010
But what about PP-MS?
No exacerbations and low inflammation


- Pathology demonstrates less inflammation in PPMS compared to SPMS (Revesz et al 1994)
  - 4 cases PPMS/5 cases SPMS
  - Increased perivascular and parenchymal inflammation in SPMS

- Less evidence for intrathecal IgG synthesis (Pirttila 1995)

- The diffuse CNS process in PP-MS is characterized by microglial activation and diffuse axonal injury in the white matter and by cortical demyelination/remyelination and neuronal loss in the gray matter (Kutzelnigg, Lucchinetti et al. 2005)
Mitochondrial dysfunction in progressive axonal loss

**Myelinated axon**
- Na channels only at nodes of Ranvier
- Saltatory conduction
- Trophic support of axon by myelin

**Acutely demyelinated axon**
- Conduction block
- Axonal dystrophy 2nd to immune mediators
- Initiation of compensatory changes

**Subacutely demyelinated axon**
- Redistribution of Na channels (Nav 1.2 vs Nav 1.6)
- Restoration of conduction, ↑E demands
- Redistribution & proliferation of mitochondria

**Chronically demyelinated axon**
- Mitochondrial/E failure ⇒ ↑ production of ROS
- ↑ Extramitochondrial glucose metabolism (LA)
- Oxidative damage, Axonal swelling, Neuronal death

Adapted from Andrews, Nichols, Bates, Turnbull; 2005
Increase mitochondrial density/activity in demyelinated axons

LFB
Demyelination

HLA
MΦ/Microglia

Mito complex IV
Cytochrome C oxidase

Mito complex II
Succinate dehydrogenase

Cortex – high mitoch. activity

Mito Complex IV
Phosphoryl. neurofilament
Overlay

Mahad et al, Brain 2009
Conclusions II

- Current immunomodulatory therapies do not alter the progression of disability in PP-MS or SP-MS patients that have no evidence of BBB disruption measured by CEL ⇔ need for novel Th

- In patients with SP-MS, who may have persistent meningeal inflammation and may harbor tertiary lymphoid follicles, therapeutic destruction of lymphoid follicles (LT-β-targeted therapies, IT rituximab) may inhibit progression of disability

- Therapies that enhance mitochondrial function and limit oxidative stress should be investigated for therapeutic effect in progressive MS

- Enhancing remyelination in all stages of MS will likely limit development of progressive axonal loss in later stages of disease
IPPoMS (NCT00950248)
Double blind placebo-controlled Phase I/II clinical trial of Idebenone in patients with Primary Progressive Multiple Sclerosis

RIVITaLISe (NCT01212094)
Double blind combination of Rituximab by Intra Venous and Intra Thecal injection versus placebo in patients with Low-Inflammatory Secondary progressive multiple sclerosis

Legend:
- Clinical and MRI evaluations
  - 3T comprehensive brain MRI
  - 3T spinal cord MRI
  - I.5T limited "safety" MRI
  - CSF
  - OCT and electrophysiological st.
- Collection of biological samples
  - Whole blood (2 SST, 2 EDTA)
  - Spinal tap (CSF)
  - Skin bx: collection of fibroblasts
  - PAXGene whole blood mRNA
- Laboratory evaluations for safety
  - Regular clinic laboratory eval: Urine pregnancy test before each MRI, CBC w diff, chemistry panel, including LFTs
  - Additional CBC w diff

Sample collection schedule:
- Pre-treatment baseline period
  - 1 year
  - Mo 0, 1, 2, 6, 12
- Rituximab treatment period
  - 2 years
  - Mo 12, 14, 18, 24, 30, 36
- rituximab arm
  - IT rituximab 25mg vs placebo
  - IV rituximab 200mg vs placebo
- placebo arm
  - IT rituximab 25mg vs placebo
  - IV rituximab 200mg vs placebo
Acknowledgements

NIH Clinical Center

Neuroimmunology Branch clinical group

Neuroimmunological Diseases Unit

Patients & their caregivers

TNU: Danny Reich
VIS: Steve Jacobson

Our NIH colleagues & collaborators

Irene Cortese
Joan Ohayon
Kaylan Fenton
Helen Griffith
Danny Reich
Henry McFarland
Bibi Bielekova

Simone Wuest
Jayne Martin
Qungang Xu
Justin Perry
SunPil Han
Matt Hermann
Elena Romm
Pradeep Shukla
Azita Kashani
Thank you for your attention!