Multiple Sclerosis
The Pathologists Perspective: Where are we?

Hans Lassmann (MD)
MS is a Disease that affects the Entire Central Nervous System

Besides white matter demyelination there is extensive demyelination in the cortex and basal ganglia and diffuse injury in the normal appearing white and grey matter (Kutzelnigg et al Brain 2005)

DISEASE PROGRESSION IN MULTIPLE SCLEROSIS

Confavreux et al. NEJM 2000

No Gd-enhancement Anti-inflammatory therapies ineffective
THE PATHOLOGY OF MS

Types of MS Lesions

- Classical Active
  - Acute
- Slowly Expanding
- Inactive
- Shadow Plaque
- Remyelination
Demyelination in MS is induced by Different Immunological Mechanisms

**INFLAMMATION**

**DEMYELINATION PATTERN I**
- CTL + Macrophages
- Antibodies
- Aquaporin 4
- Others?
- DEVIC

**DEMYELINATION PATTERN II**
- Histotoxic
- Hypoxia
- ROS / RNI
- Increased Susceptibility of CNS Tissue

**DEMYELINATION PATTERN III**
- BALO

**DEMYELINATION PATTERN IV**

Lucchinetti et al Ann Neurol 2000
MS is a Chronic Inflammatory Disease in all Stages

Compartmentalized Inflammation in Progressive MS

- Inflammation behind a closed (repaired) blood brain barrier
  - Anti inflammatory drugs that can pass the normal BBB
  - Intrathecal anti-inflammation therapy

Hochmeister et al 2006
Differences in Pathology between RRMS and SPMS

Pathology of SPMS and PPMS

Kangariu et al AJNR 2007

8T MRI
MS Specific Gene Expression
(Cortical Lesions in MS)

301 Genes identified; > 80% of the genes belong to the pathway shown below.

Oxidative Injury is a major mechanism of demyelination and neurodegeneration in MS lesions.

Similar degree of oxidative injury is not present in other human inflammatory brain diseases or in EAE.

Fischer et al Brain 2013

Mechanisms of Neuronal Injury in Active Cortical MS Lesions

Oxidized Phospholipids (Oxidative Injury)

Dendrite Fragments Apoptotic Neurons Central Chromtolysis Dystrophic Axons

DNA Damage AIF Liberation

DNA Injury Apoptosis AIF normal AIF Apoptosis
Malone Dialdehyde in MS Lesions

Oligodendrocytes & Myelin >> Astrocytes >> Other Cells

Oxidized Phospholipids (E06) in MS Lesions

Neurons & Axons >> Oligodendrocytes & Myelin >> Astrocytes

DNA damage (8-Hydroxy-d-Guanosine) in MS Lesions

Oligodendrocytes >> Astrocytes >> Other Cells

Potential Source of Radicals in Multiple Sclerosis Lesions

• Oxidative Burst (Inflammation, Macrophage / Microglia Activation, Astrocytes)
  – NOS 1-3, NOX 1-5 Complexes, Myeloperoxidase
• Dyscoupling of the Mitochondrial Respiratory Chain
  – Complex I Defect
• Iron Liberation within the Lesions
  – Fenton Reaction
Microglia Activation in MS Lesions:

Driven by Inflammation

Oxidative burst in MS lesions is mainly driven by oxygen radical production through NADPH oxidases (Nox1 and Nox 2)

No up-regulation of NOS molecules in active MS lesions in comparison to controls

Fischer et al 2013

Anterograde or Retrograde Neurodegeneration may Precipitate New Cortical Lesions

Kolasinski et al Brain 2012:
Cortical Lesions in MS are in part topographically related to projections from white matter and deep grey matter lesions

Microglia Activation due to Neurodegeneration

Meningeal Inflammation

Precipitation of New Cortical Lesions
Mitochondrial Injury in MS

COX1 < COX4 < Complex I or II < Porin

Clonal expansion of mitochondrial defects

ROS

Initial ROS injury

normal

increased vulnerability

spontaneous death

Mahad et al, Brain 2008
Mitochondrial Injury in MS

- Mitochondrial injury in MS is driven inflammation and oxidative burst
- Mitochondrial injury leads to energy deficiency
- Mitochondrial damage accumulates with disease duration (RRMS < progressive MS)
- Injured mitochondria are an additional source of reactive oxygen species
- Genetic defects in mitochondrial genes potentiate MS lesions (Harding’s Disease)

Iron: promoter of free radical damage

1. Physiologic function of iron: necessary cofactor in
   neurogenesis, myelin synthesis, neurotransmitter production
   oxygen transport

2. Iron and oxidative stress:
   - Fe^{2+}: low toxicity
   - H_{2}O_{2}: low toxicity
   - Fenton reaction: Fe^{2+} + H_{2}O_{2} \rightarrow Fe^{3+} + OH^{-} + OH^{-}
Iron in Human MS Brains

Accumulation of Iron with Age in MS Brain

Iron Export Proteins in MS

Iron Related Proteins in Lesions of Progressive MS

- NAWM:
  - Ferritin and iron mainly in oligodendrocytes
- Active MS Lesion:
  - Destruction of oligodendrocytes
  - Uptake of iron in microglia and macrophages
  - Ferritin positive macrophages and microglia degenerate
  - Liberation of iron from degenerating cells may lead to radical production
Demyelinated Lesions in the MS Brain

Superimposed image of 19 MS cases

Higher lesion density in interconnected areas and in areas of hypoperfusion (watershed areas)

Haider & Zrzavy unpublished

Grey Matter Neurodegeneration in MS

Superimposed image of 19 MS cases

Accumulation of neurons with cytoplasmic accumulation of phosphorylated neurofilament as a sign of neuronal dystrophy in basal ganglia and watershed areas

Haider & Zrzavy unpublished

Neurons / Case / Area: <50; 10-50; 0-10
Grey Matter Atrophy in MS
Lansley et al Neuroscience and Behavioural Review 2013

Meta Analysis of 16 MRI studies on grey matter atrophy in MS by Signed Differential Mapping

Most profound atrophy in deep grey matter nuclei and in watershed areas

Disease Mechanisms in Early MS

Inflammation

<table>
<thead>
<tr>
<th>Inflammation (T-cells; B-cells)</th>
<th>Microglia Activation</th>
<th>Histotoxic hypoxia</th>
<th>Genuine Hypoxia</th>
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</thead>
<tbody>
<tr>
<td>Direct immune mediated injury</td>
<td>Oxidative burst</td>
<td>Ionic imbalance</td>
<td>Energy deficiency</td>
</tr>
<tr>
<td>Cytotoxic T-cells</td>
<td>Mitochondrial injury</td>
<td>Demyelination</td>
<td>due to mitochondrial injury</td>
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<tr>
<td>Antibodies</td>
<td>Energy failure</td>
<td>Axonal injury</td>
<td>Accumulation of lesions and neurodegeneration in areas of low vascular perfusion (Watershed areas)</td>
</tr>
</tbody>
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Amplification

Age and Disease Duration
Disease Mechanisms in Late MS

<table>
<thead>
<tr>
<th>Inflammation</th>
<th>Amplification</th>
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<tbody>
<tr>
<td>Accumulation of Lesion Burden</td>
<td>Age dependent Iron accumulation in myelin and oligodendrocytes</td>
</tr>
<tr>
<td>Retrograde and anterograde degeneration</td>
<td>Burnt out disease</td>
</tr>
<tr>
<td>Amplification of microglia activation</td>
<td>Progression of age related neurodegeneration</td>
</tr>
<tr>
<td></td>
<td>Exhaustion of functional reserve capacity</td>
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<tr>
<td>Mt DNA Deletion</td>
<td>Increased energy deficiency</td>
</tr>
<tr>
<td>Clonal Expansion of defective mitochondria</td>
<td>Iron liberation in demyelinating lesions</td>
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<tr>
<td></td>
<td>Amplification of oxidative stress</td>
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<tr>
<td>ROS production by mitochondria</td>
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Age and Disease Duration

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Funded by: FWF Austria