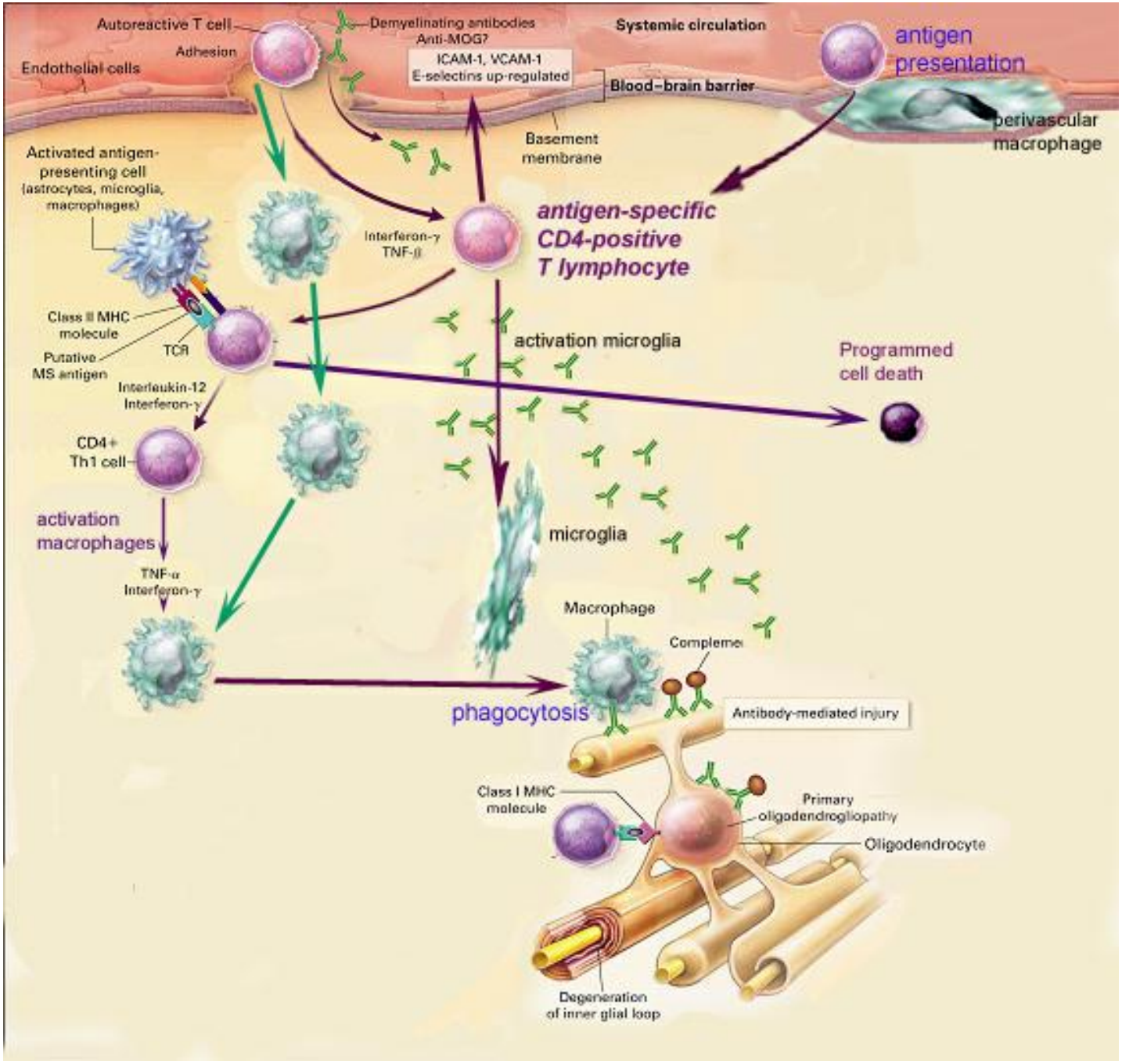
Brain Research Center

Medical University of Vienna

Molecular Pathology of MS: Signposts to new Therapy

Hans Lassmann (MD)



IMMUNOLOGY OF MULTIPLE SCLEROSIS

Noseworthy et al 2000

NEJM

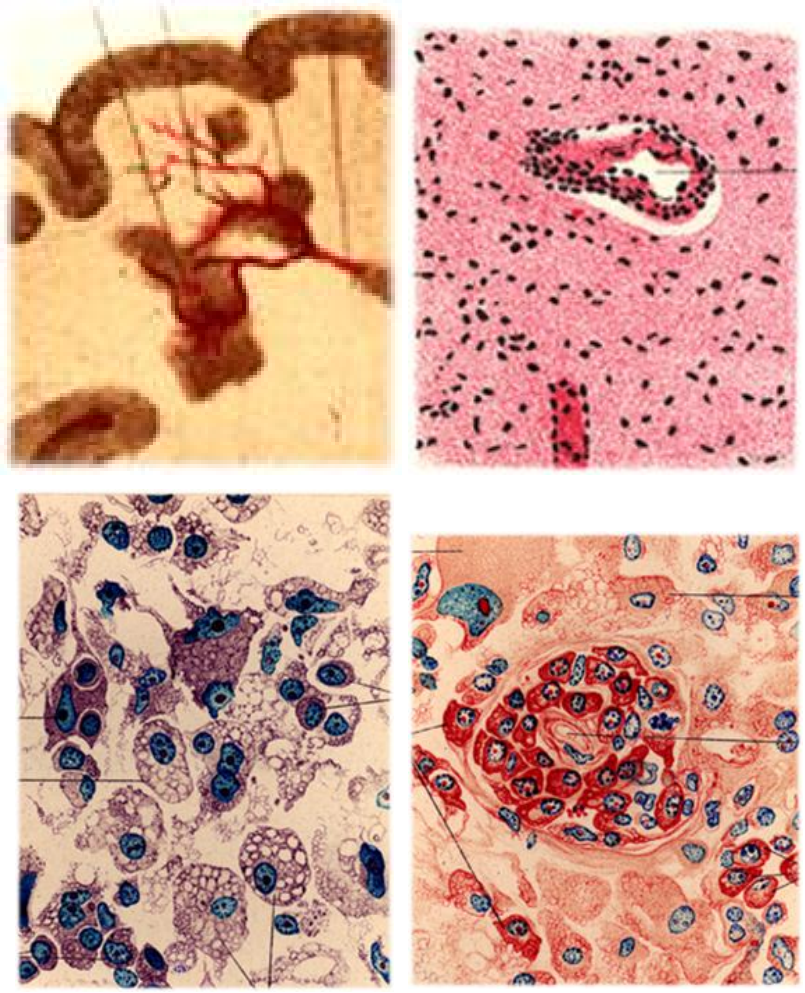
Immunomodulatory Treatment in MS and EAE

Treatment Target	**Effect in EAE**	**Effect in RRMS**	**Effect in Prog. MS**
Anti-Inflammatory: IFN, GA, α-VLA4, α-CD 52, FTY 720, CD20 Immunosuppresion	**++**	**++**	**0**
EAE specific: α-CD4, APLs, Il12/23 p40, γ-IFN; α-TNF	**+++** less disease	**0?** more disease	**n.d.**

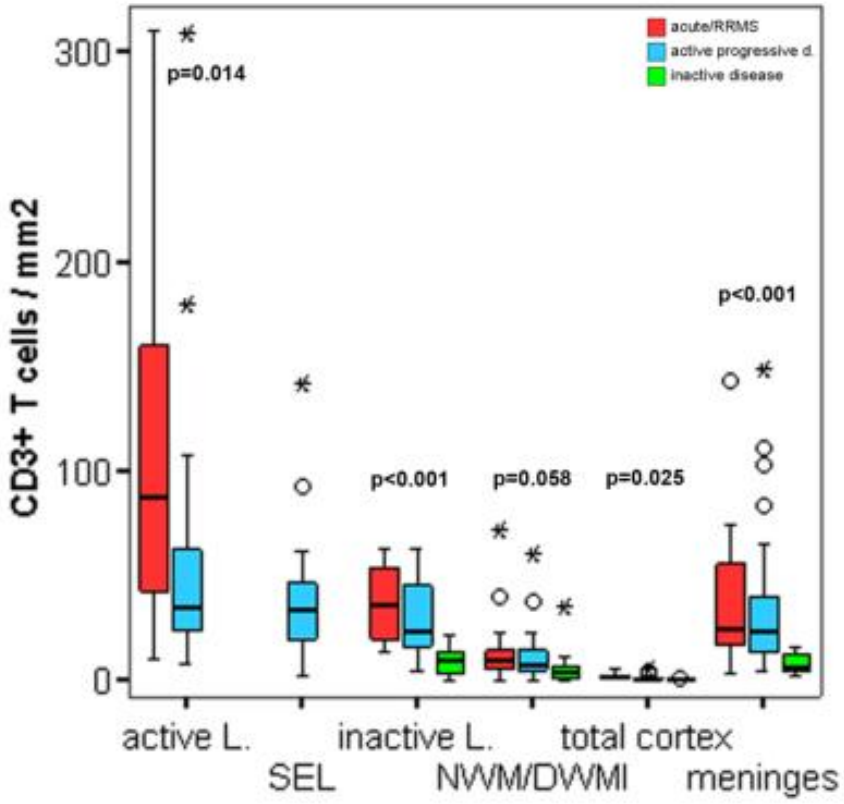
Key Issues to Understand CNS Damage in Multiple Sclerosis

- Inflammation
  - Inflamatory reaction in different disease stages
  - Inflammation: driver or reaction to tissue injury
  - Nature of inflammatory response at different disease stages
- Mechanisms of tissue injury
  - Multiple potential mechanisms / what is relevant or dominant at different disease stages

MS is a Chronic Inflammatory Disease in all Stages



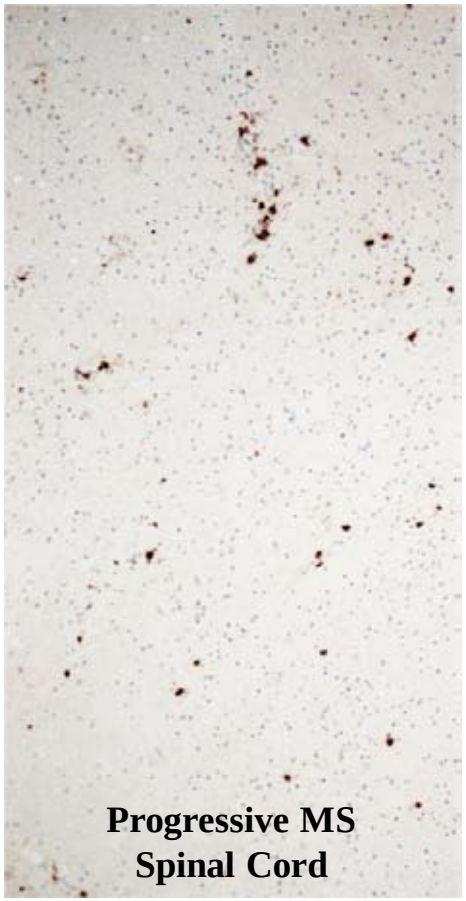
1880 - 1915

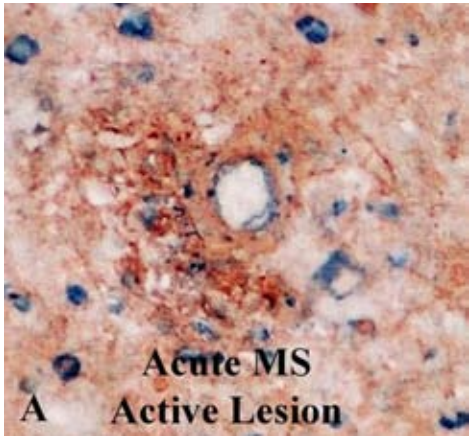


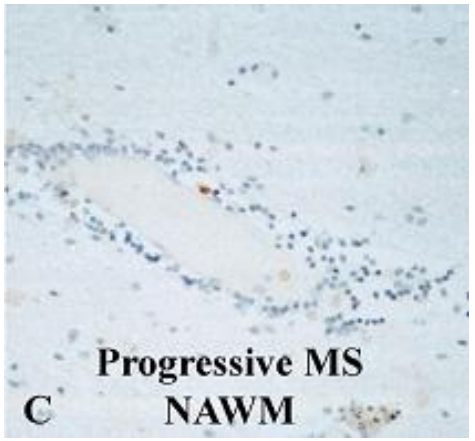
Frischer et al Brain 2009

Compartmentalized Inflammation in Progressive MS

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

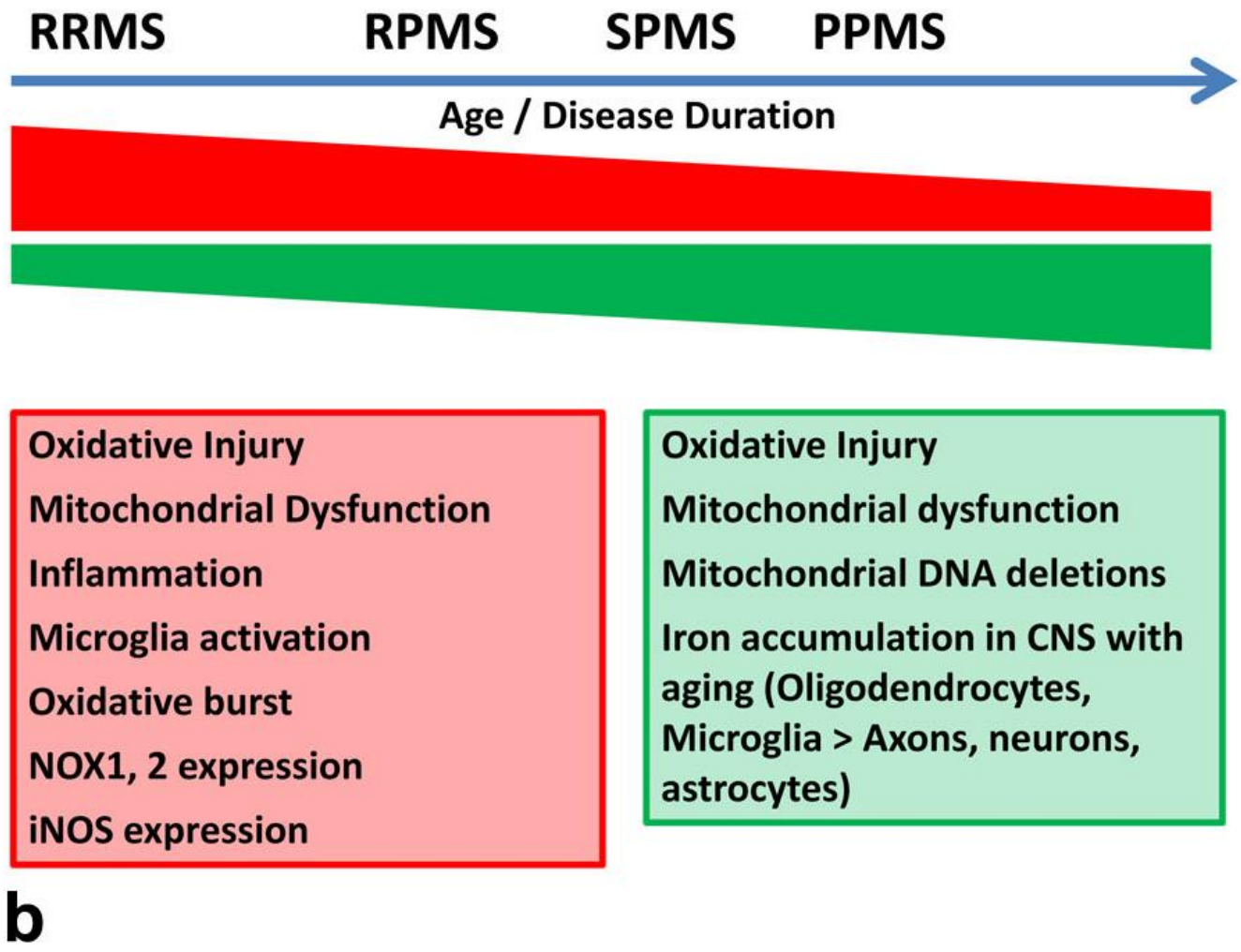






Hochmeister et al 2006

Differences in Tissue Injury between RRMS and SPMS



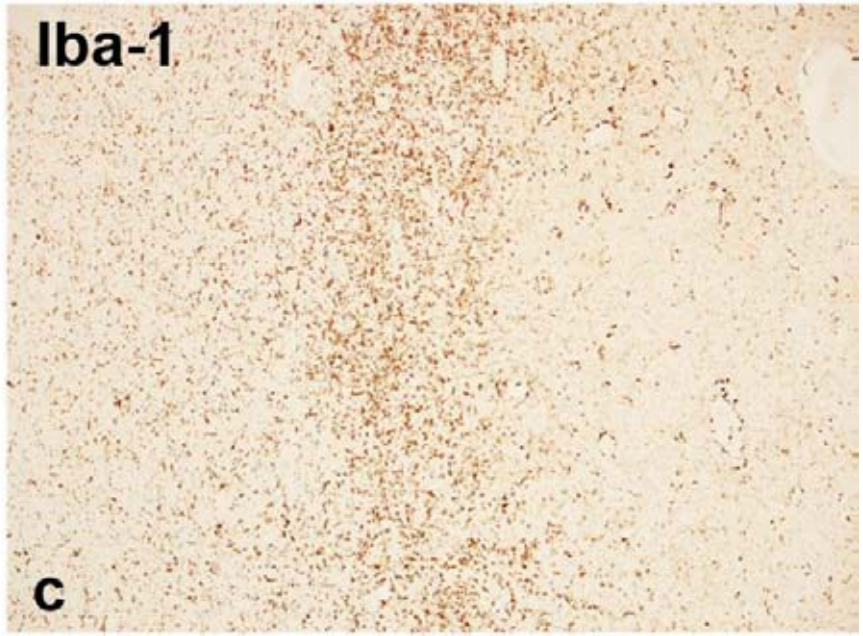
Multiple Sclerosis Therapy Potential Effects in CNS

Drug	Mechanisms of Action	Effect on Inflammation	BBB Penetration
Fingolimod	T-cell retention in LN, CNS effects	+++	Yes
BG12	T-cell Apoptosis Anti-oxidant	+++	Yes
Laquinimod	T_{reg} induction NFkB activation	++	Yes
Lamotrigine	Na^+ Cannel Blocker	++	Yes
Amiloride	Acid sensing Na+ Channel Blocker	?	Yes
Riluzole	Blocks TTX sensitive Na+ Cannels and glutamate receptors	?	Yes

Mechanism of Tissue Injury in MS Lesions

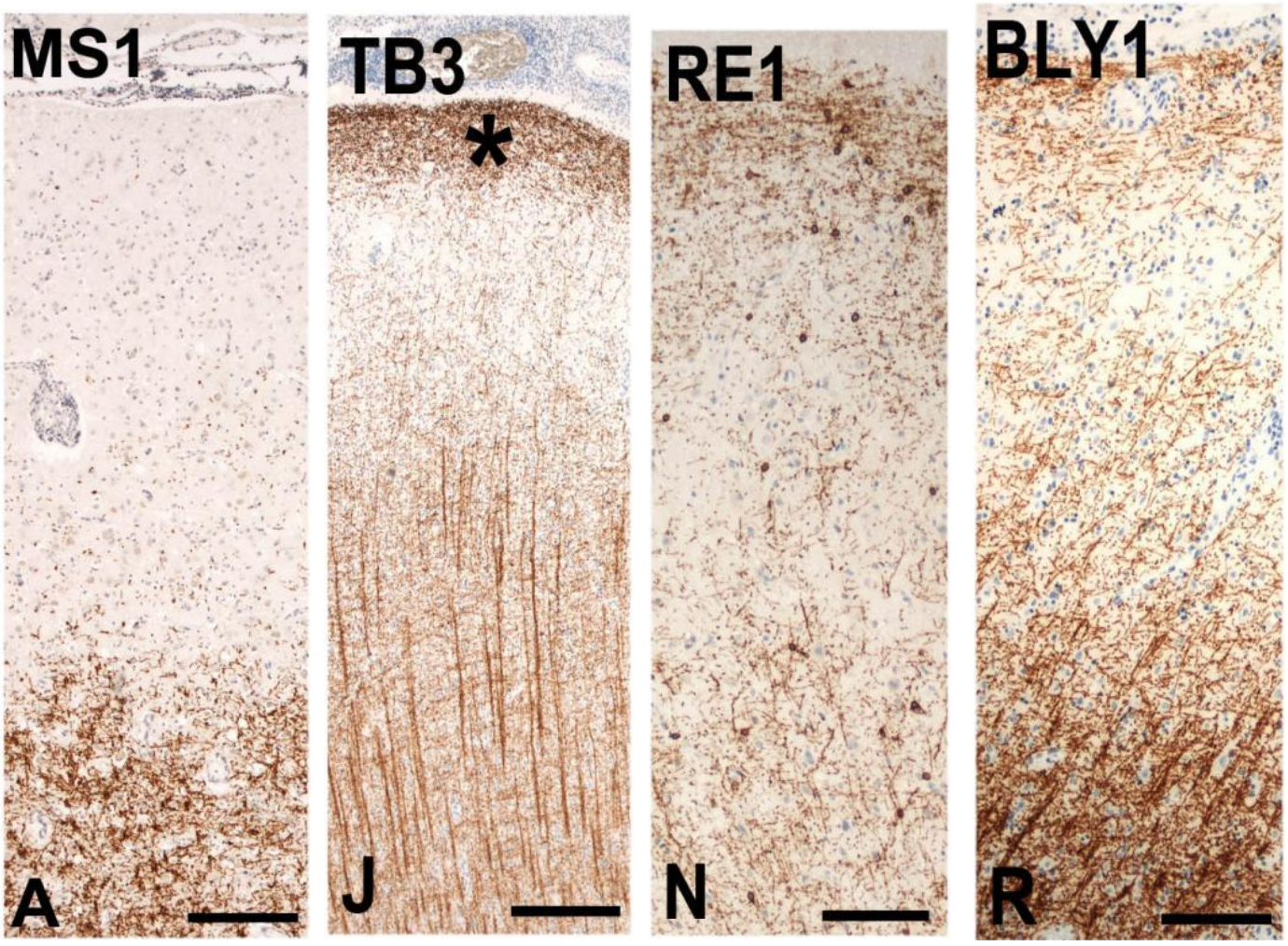
- What type of tissue injury is specific for MS lesions
- What tissue injury pathways discriminate MS lesions from other inflammatory or neurodegenerative CNS diseases
- How can we identify dominant pathways of tissue injury in MS

The Architecture of Active MS Lesions



NAWM (PPWM): Up-regulation of neuroprotective molecules
Initial Lesion: Initial tissue destruction
Early active: Myelin liberation, phagocytosis of debris, axonal injury
Late active: Myelin digestion, Gliosis, Recruitment of OPCs, early remyelnation
Grey matter lesion: additional neuronal injury and plasticity

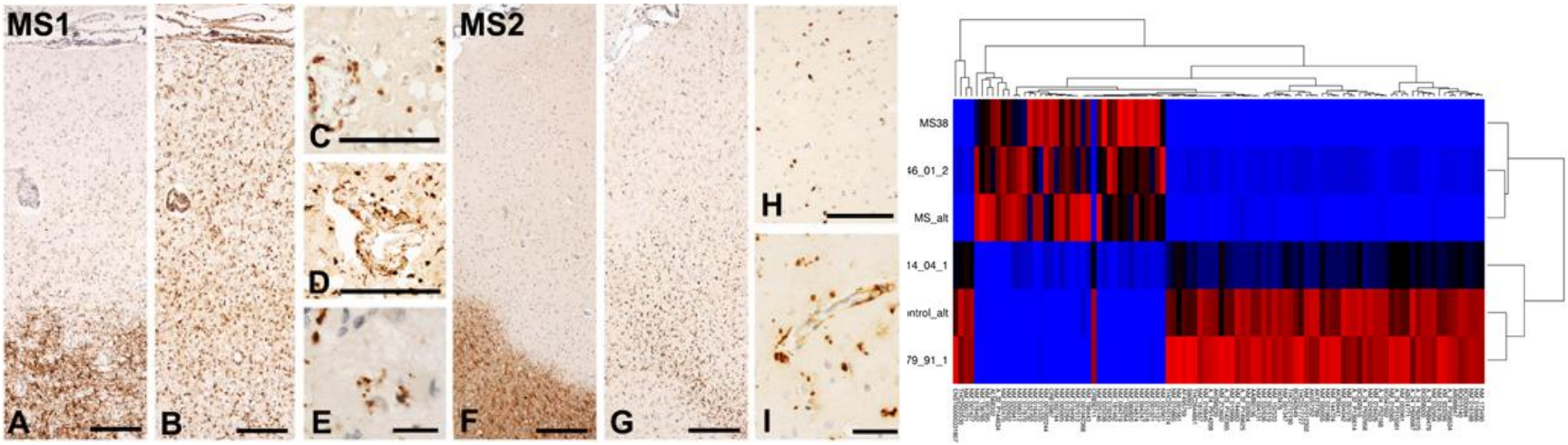
Demyelination is the Pathological Hallmark of all MS Lesions



Subpial Demyelination is the Pathological Hallmark of all MS Lesions

- Extensive subpial demyelination in the MS cortex
- No subpial demyelination is seen in any other neuropathological condition:
  - Rasmussen‘s encephalitis, Paraneoplastic encephalitis, virus encephalitis, NMO
  - Chronic bacterial meningoencephalitis, TB meningitis, Lues
  - SSPE, PML, HIV-encephalitis
  - Leukodystrophies (adrenoleukodystrophy)
  - Neopasms (including B-cell lymphoma)

Gene Expression in Active Cortical MS Lesions



Gene expression in microdissected active cortical lesions from progressive MS (n=3) in comparison to inflammatory controls (TB meningitis, n=3), neurodegenerative controls (Alzheimer's disease; n=3) and normal controls (n=3).

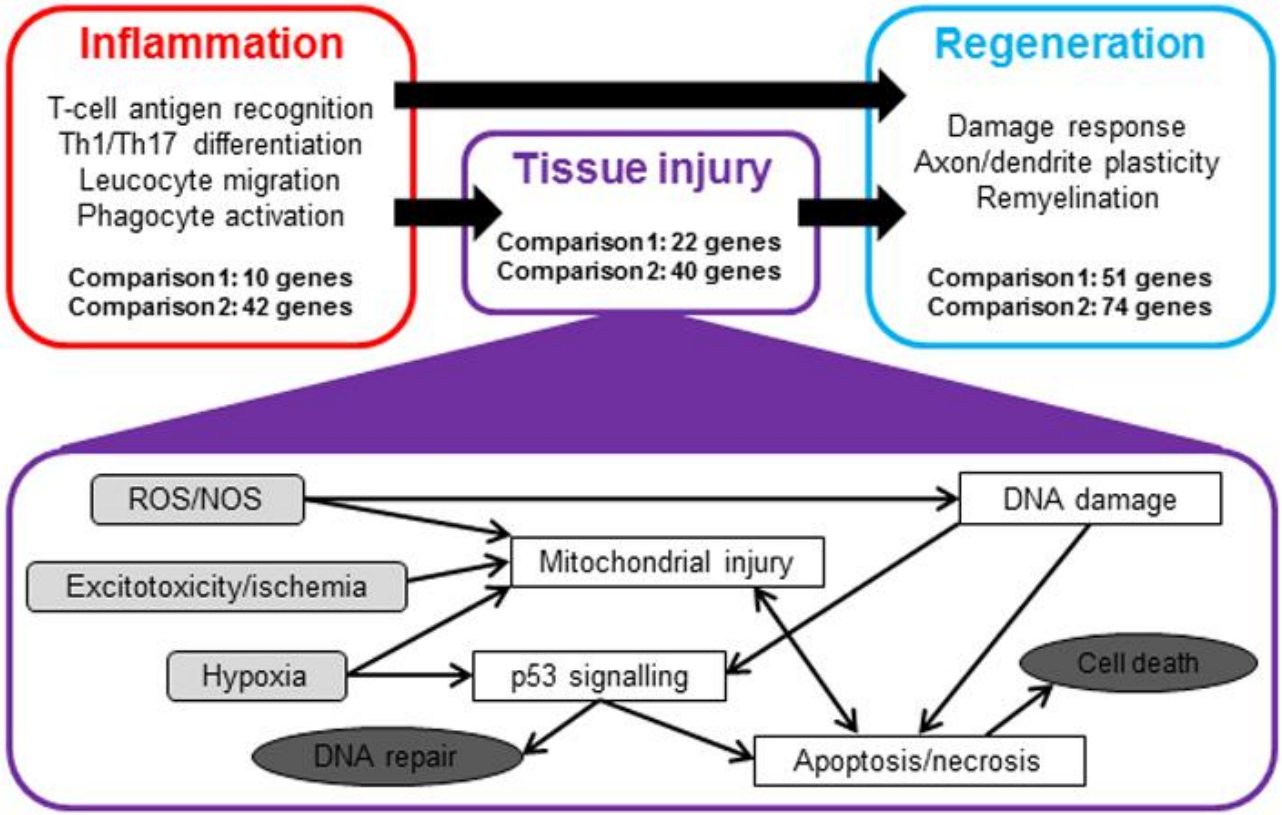
Use of stringent cut-off values (> 4x log 2; $p<0.01$)

Confirmation of key findings by immunocytochemistry in large sample of active cortical MS lesions

MS Specific Gene Expression

(Cortical Lesions in MS)

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



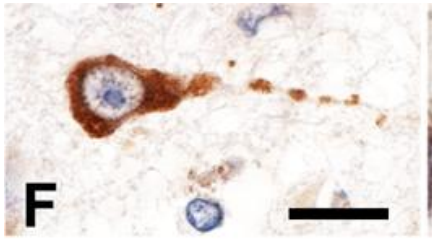
Oxidative Injury is a major mechanism of demyelination and neurodegeneration in MS lesions

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

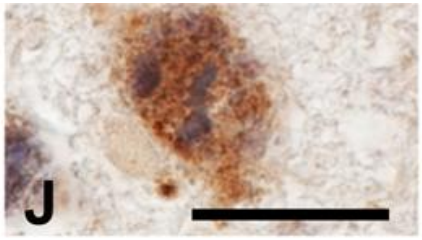
Fischer et al Brain 2013

Mechanisms of Neuronal Injury in Active Cortical MS Lesions

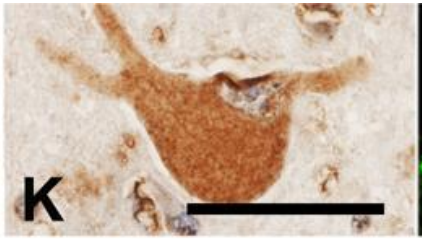
Oxidized Phospholipids (Oxidative Injury)



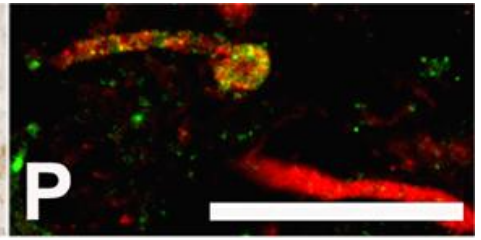
Dendrite Fragments



Apoptotic Neurons

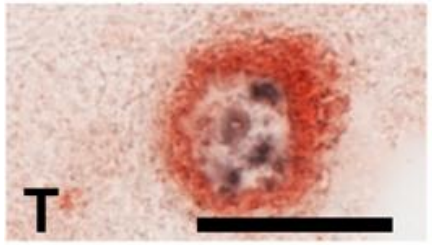


Central Chromtolysis

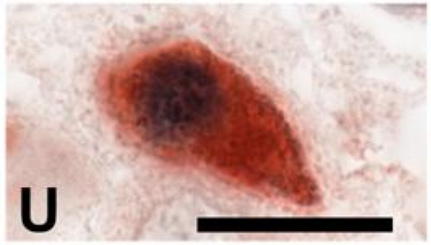


Dystrophic Axons

DNA Damage

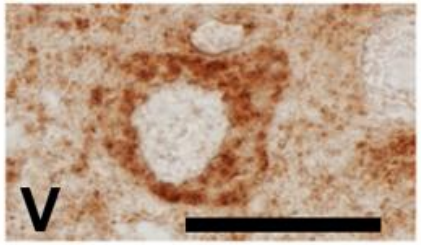


DNA Injury

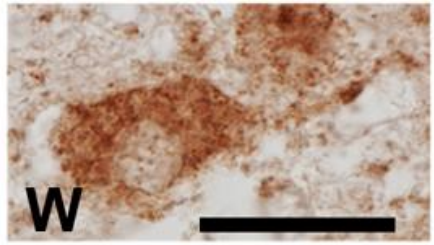


Apoptosis

AIF Liberation

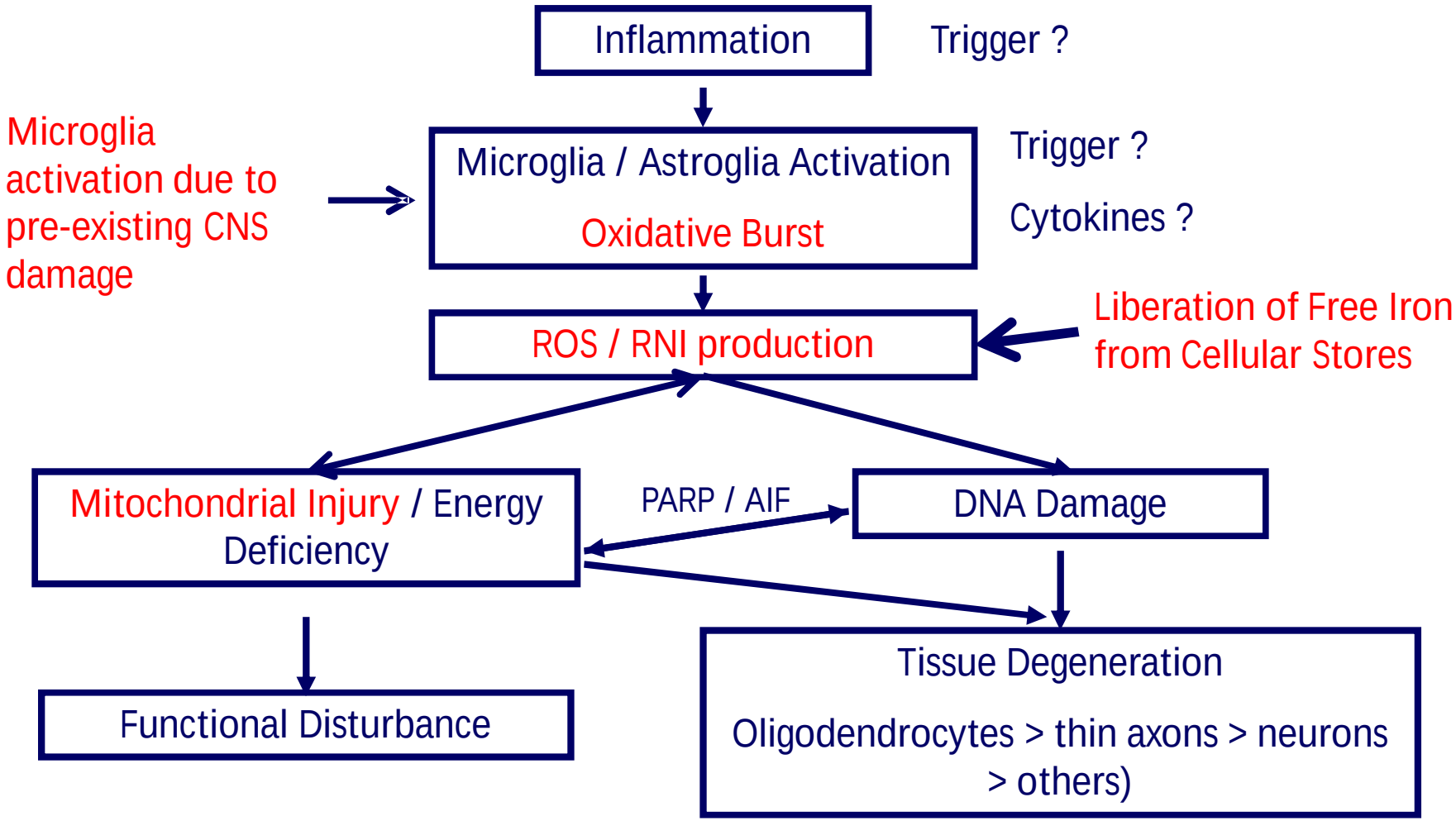


AIF normal

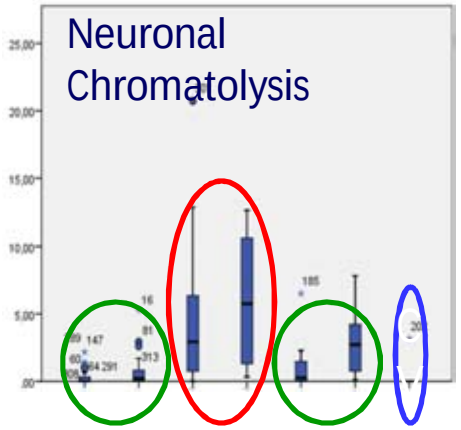


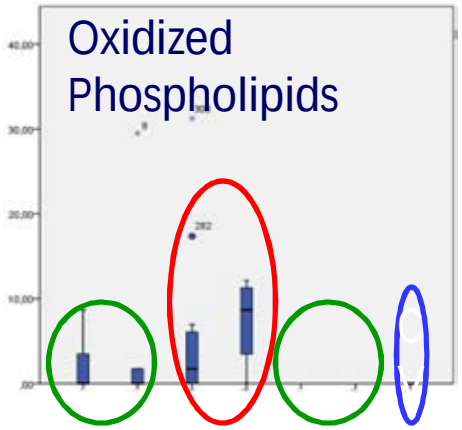
AIF Apoptosis

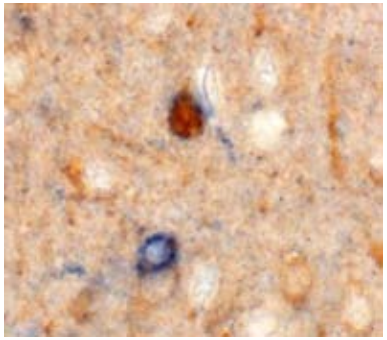
Neurodegeneration in Multiple Sclerosis

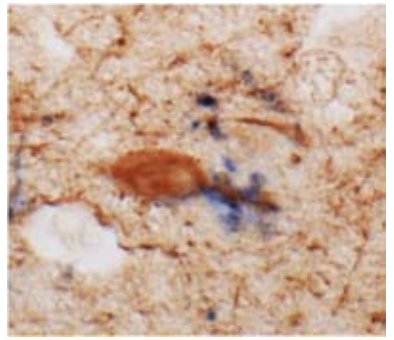


Secondary Degeneration may Precipitate Cortical Lesions in MS

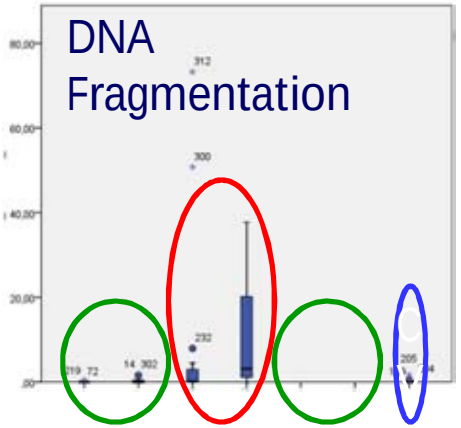








NADPH Oxidase / p. Neurofilament



Cortical Area with Retrograde Neurodegeneration

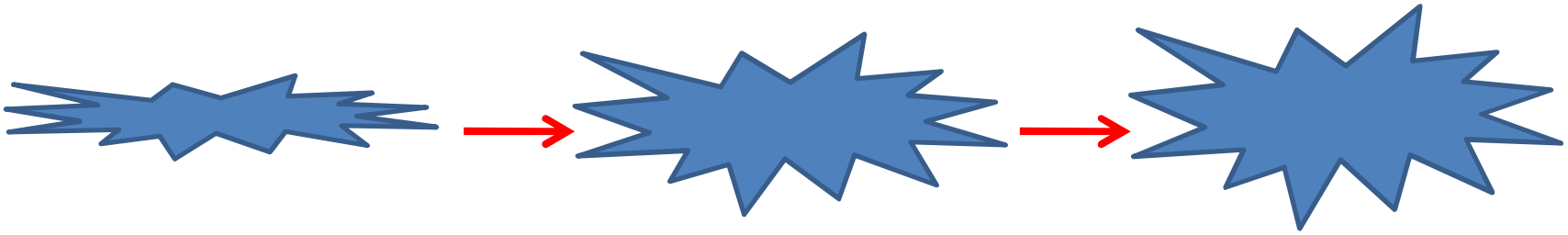
Other cortical lesions

Normal cortex

Cortical areas with secondary neurodegeneration and microglia activation preferentially show oxidative injury and neuronal destruction in the presence of meningeal inflammation

Microglia Activation and Neurodegeneration

Resting MG | Activated MG | Cytotoxic MG



Neurodegeneration
Distant MS lesions
Wallerian Degeneration

Pro-inflammatory
Cytokines
Meningeal
Inflammation

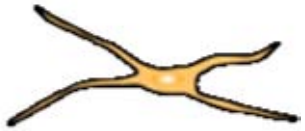
Surveillance

Neuroprotection
Neurotrophins
Anti-inflammatory
Cytokines

Destruction
Pro-inflammatory
Cytokines
ROS, RNI

Perry et al Nature Rev Neurol2010

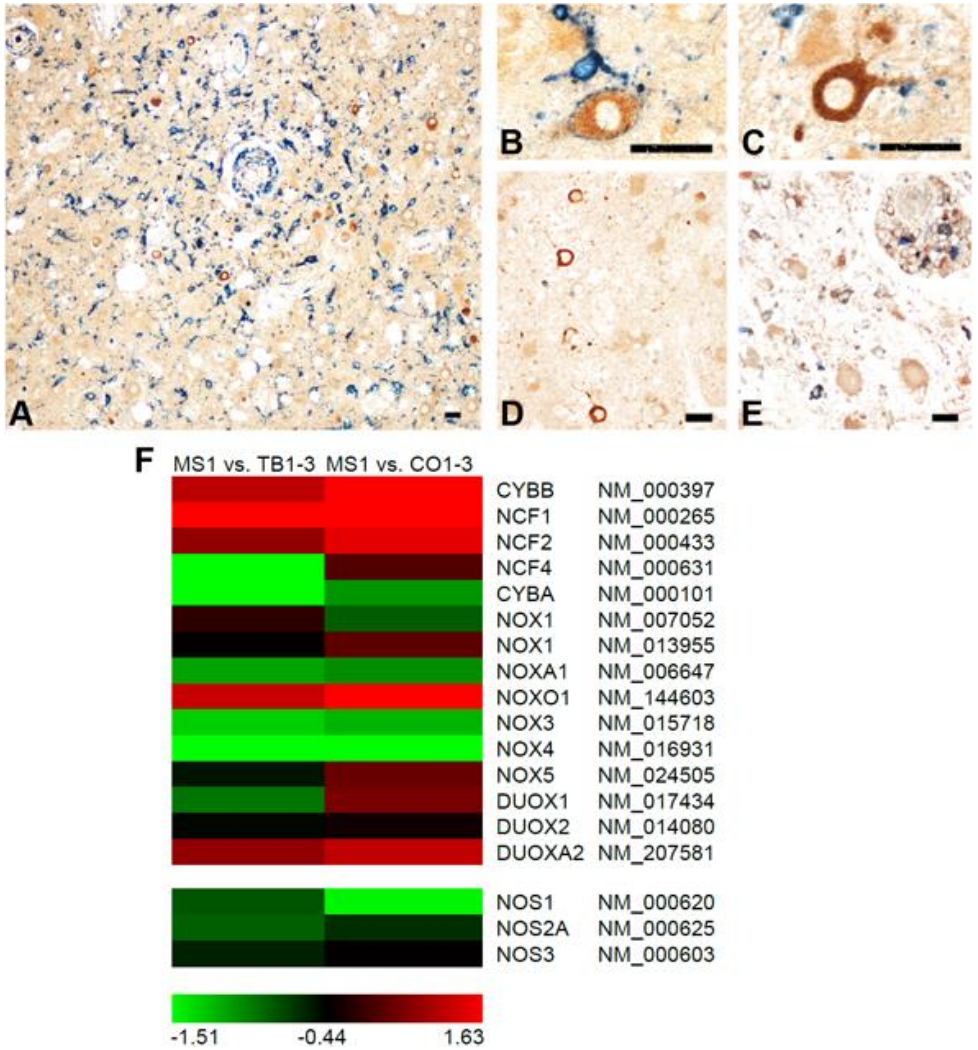
Function of S1P in the Central Nervous System

Neurons		Neurite Extension; Synaptic Transmission; Neurogenesis
Astrozytes		Proliferation; Astrogliosis, Production of trophic factors; cell migration;
Microglia		Regulation of microglia Function (proinflammatory cytokines / radicals)
Oligodendrozytes		Regulation of oligodendroglia function (migration, process extension)

MS, Multiple Sclerosis; modified from Herr YP, Chun J. *Current Drug Targets* 2007; Jaillard C *et al. J Neurosci* 2005; Kimura A *et al. Stem Cells* 2007; Miron VE *et al. Ann Neurol* 2008; Mizugishi K *et al. Mol Cell Biol* 2005; Toman RE *et al. J Cell Biol* 2004; Yamagata K *et al. Glia* 2003; Wu DR *et al. Hum Mol Genet* 2008; Sorensen SD *et al. Mol Pharmacol* 2003; Miron VE *et al. J Neurol Sci* 2008; Chun J, Hartung HP. *Clin Neuropharm* 2010; Nayak D *et al. Neuroscience* 2010

Oxidative Burst 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

How can we target neuroinflammation ?

- Drugs have to be able to enter the CNS through an intact blood brain barrier
  - Oral drugs more suitable than injectable biologicals
- Inhibition of NFkB pathway within the CNS in microglia or astrocytes
  - BG12, laquinimod
- Reduction of oxidative injury
  - Stimulation of Nrf2 pathway

Laquinimod: Neuroprotective Effects

- Laquinimod can pass the normal blood brain barrier (8% of blood concentration in CSF, mainly non protein bound)
- Laquinimod inhibits demyelination and neurodegeneration in cuprizone model
  - Brück et al 2012
- Laquinimod inhibits NFkB activation in astrocytes
  - Inhibition of production of pro-inflammatory cytokine
  - Secondary inhibition of microglia activation

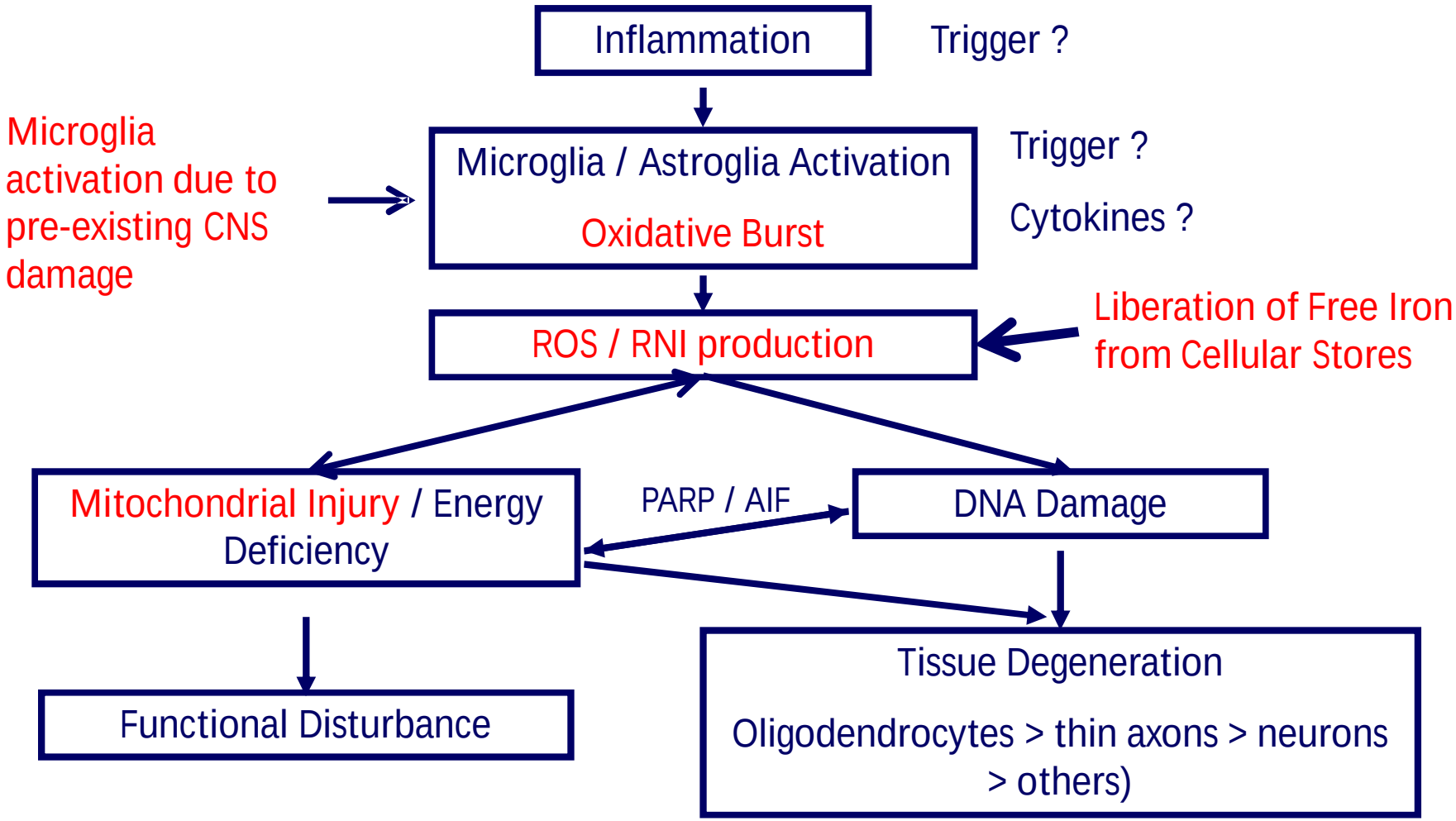
Fumarates (BG12) in MS Therapy

- Anti-Inflammatory Effects
  - Inhibits NFkB activation
  - Inhibition of pro-inflammatory cytokine production (Th1,17 / Th2, Th3 shift)
  - Inhibits adhesion molecule expression (E-selectin, VCAM-1, ICAM-1)
  - Inhibits dendritic cell differentiation and induced dendritic cell apoptosis (high dose)
  - Induces T-cell apoptosis

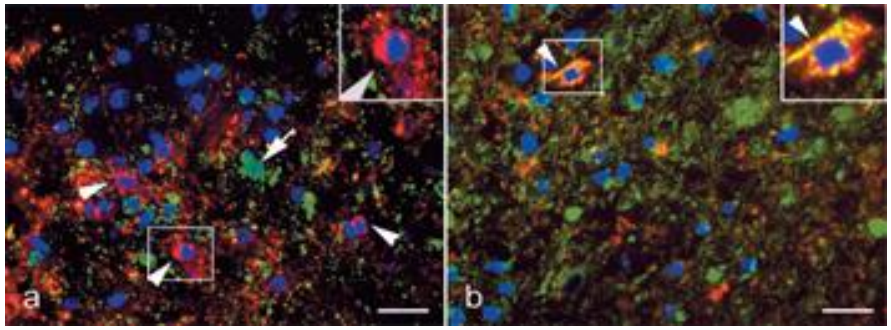
Fumarates (BG12) in MS Therapy

- Anti-oxidant effects
  - Stimulates Nrf-2 pathway
    - Increases production of anti-oxidant and neuroprotective molecules (HO-1, Glutathione, others)
  - Stimulation of Nrf-2 pathway occurs via cell stress
    - Effect only in conditions of moderate oxidative injury ?
    - Potential danger to increase damage in conditions of severe oxidative stress ?
  - Is it possible to further activate Nrf-2 pathway in tissues under full blown oxidative stress ???

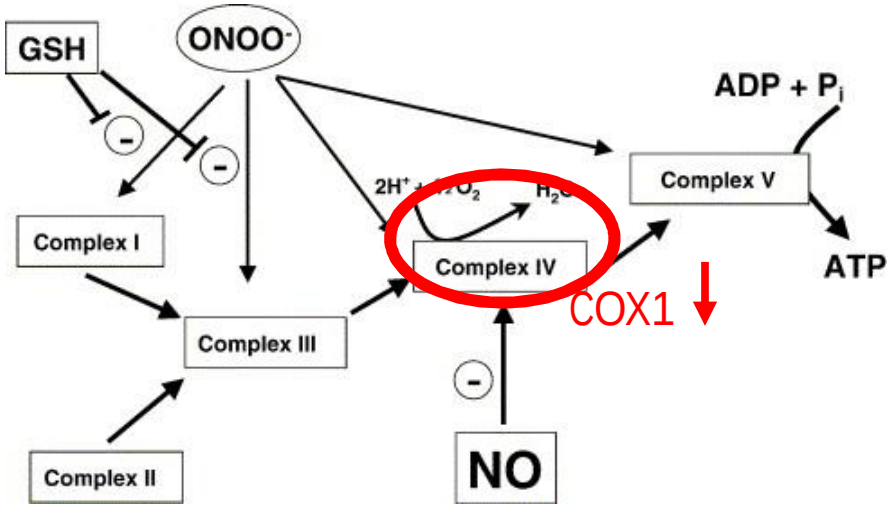
Neurodegeneration in Multiple Sclerosis

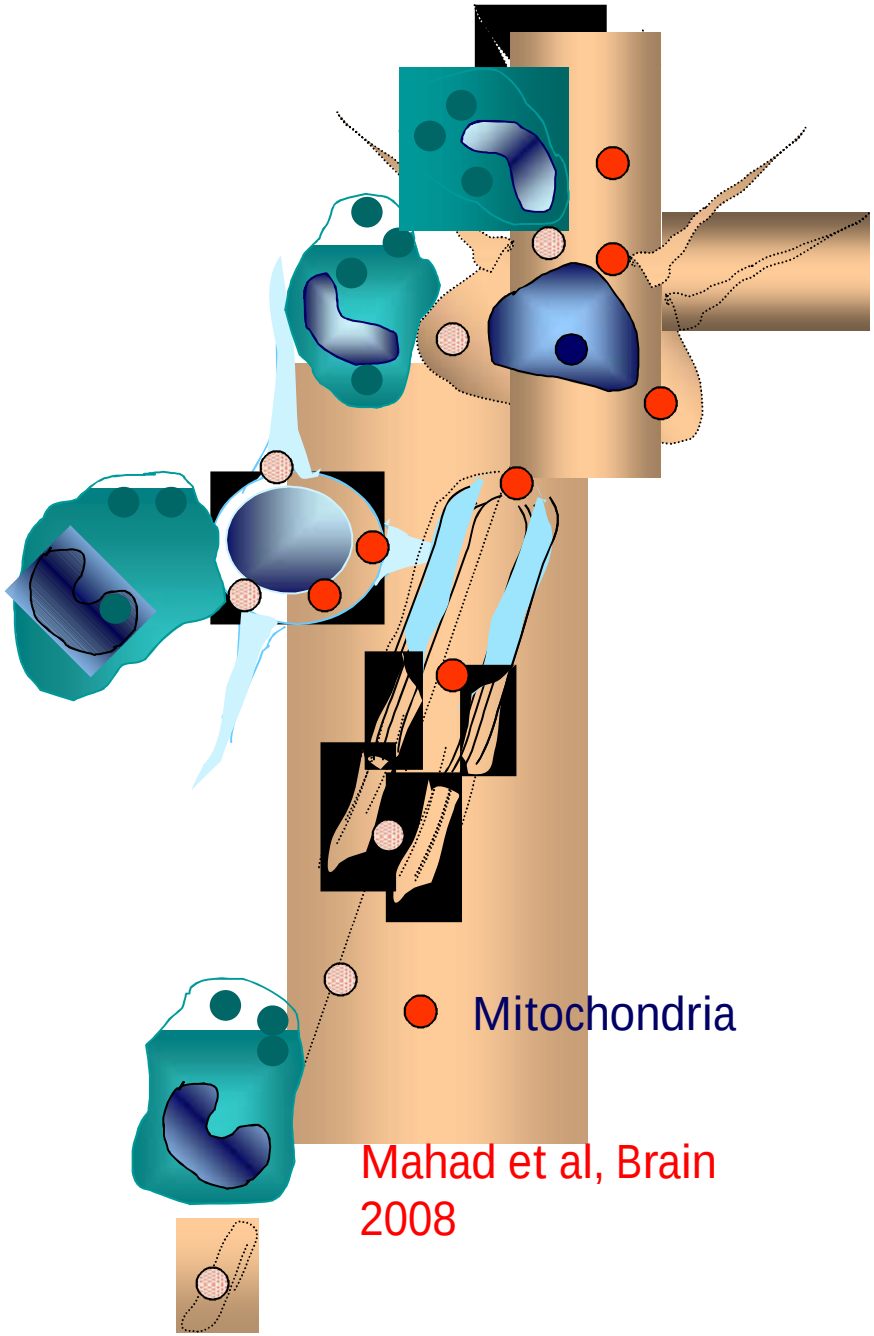


Mitochondrial Injury in MS



COX1 < COX4 < Complex I or II < Porin





Mitochondria

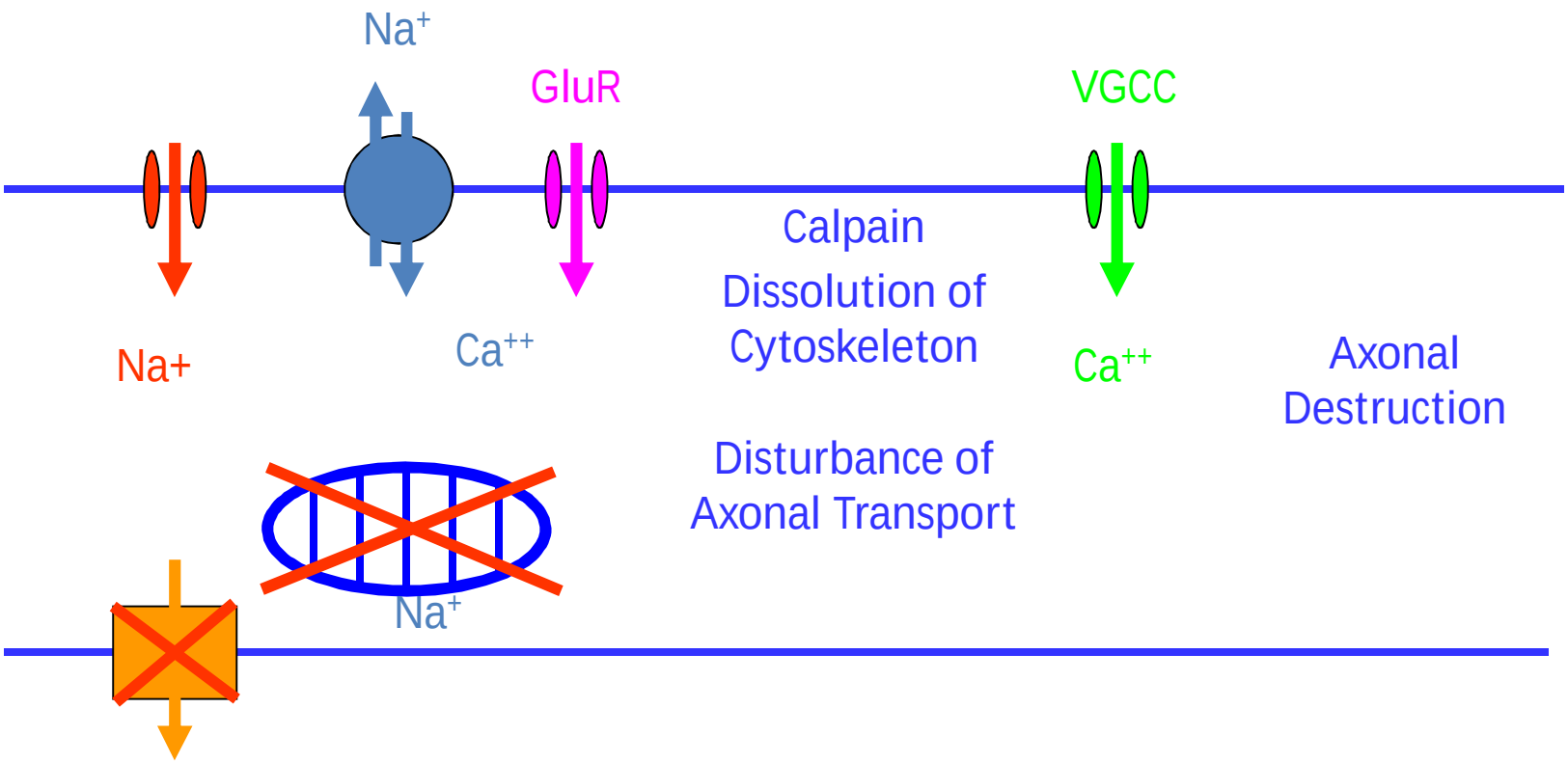
Mahad et al, Brain 2008

Mitochondrial Injury in MS: Potential Therapeutic Targets

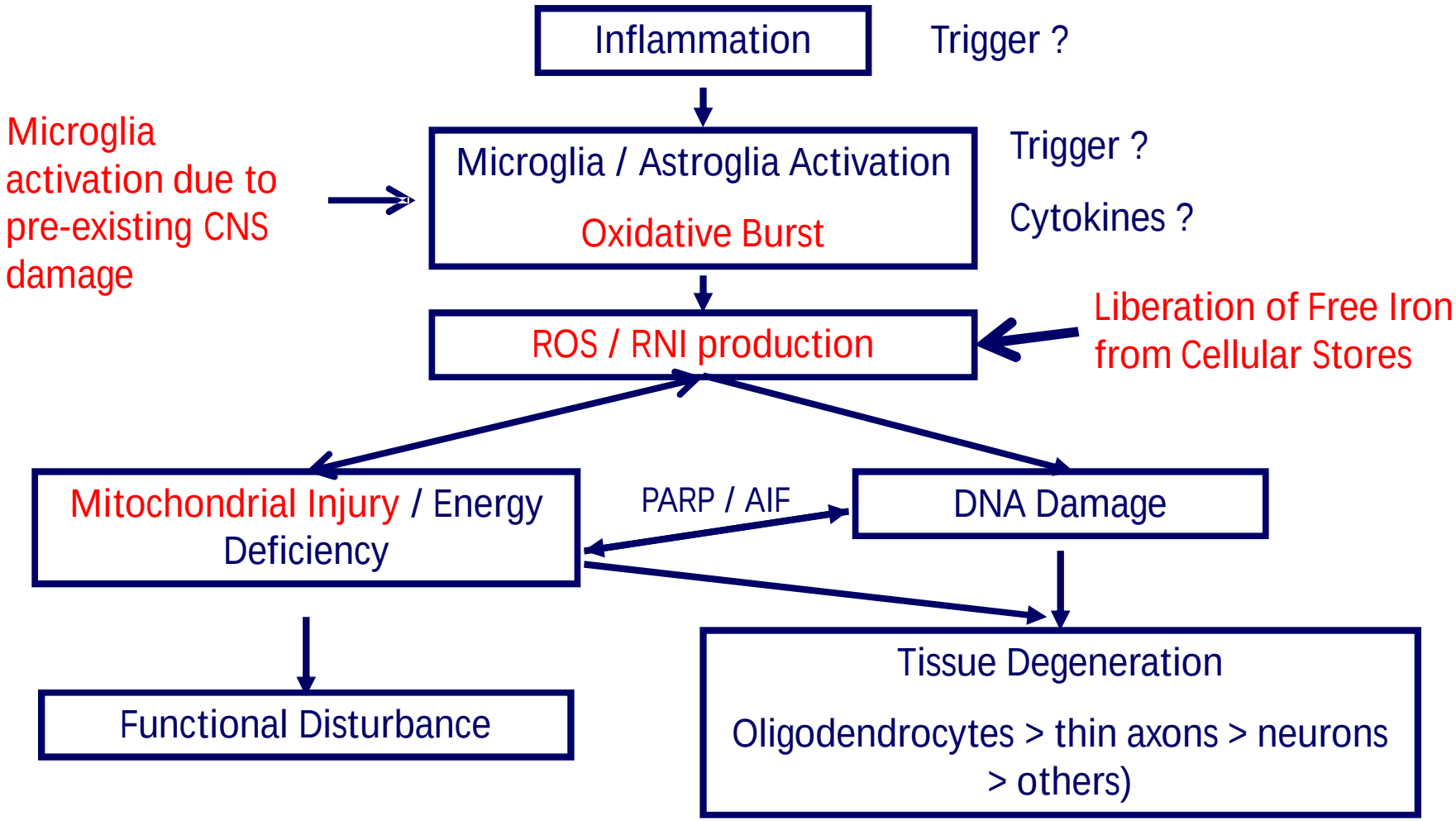
- Mitochondrial redox enzyme p66ShcA
  - Involved in radical production by damaged mitochondria – apoptosis induction
  - Blockade reduces neurodegeneration
- Sirtuins
  - Mitochondrial proteins
  - Released in conditions of energy failure
  - Stimulate production of proteins involved in energy metabolism
  - SIRT1 activators are neuroprotective

Channel Blockers in MS Therapy

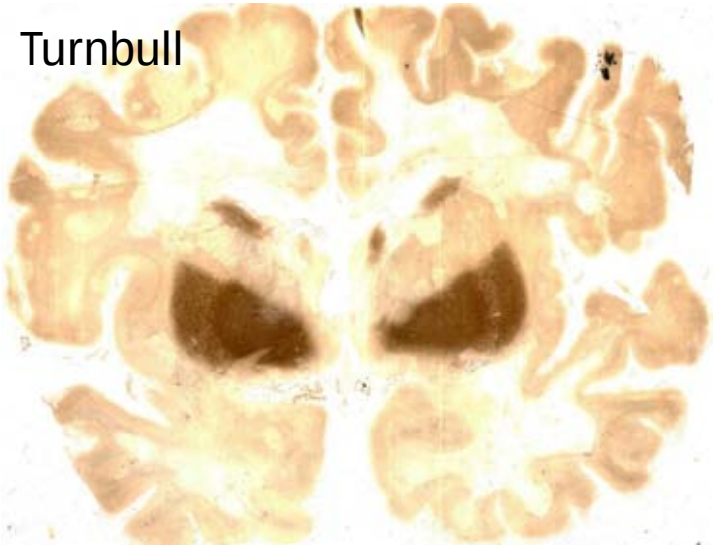
Na+ Channel Blockers: Lamotrigine, Phenytoin
Acid sensing Na+ Channel Blockers: Amiloride
Glutamate receptor / Na+ Channel Blockers: Riluzole



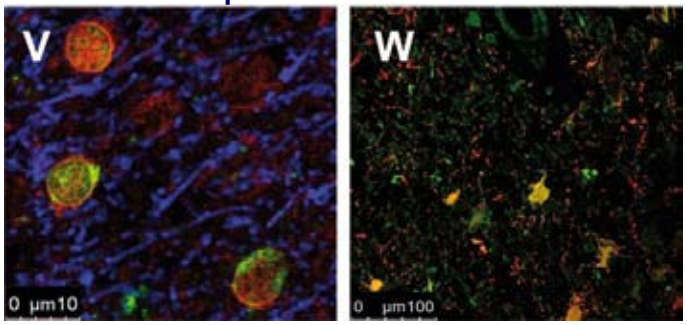
Neurodegeneration in Multiple Sclerosis



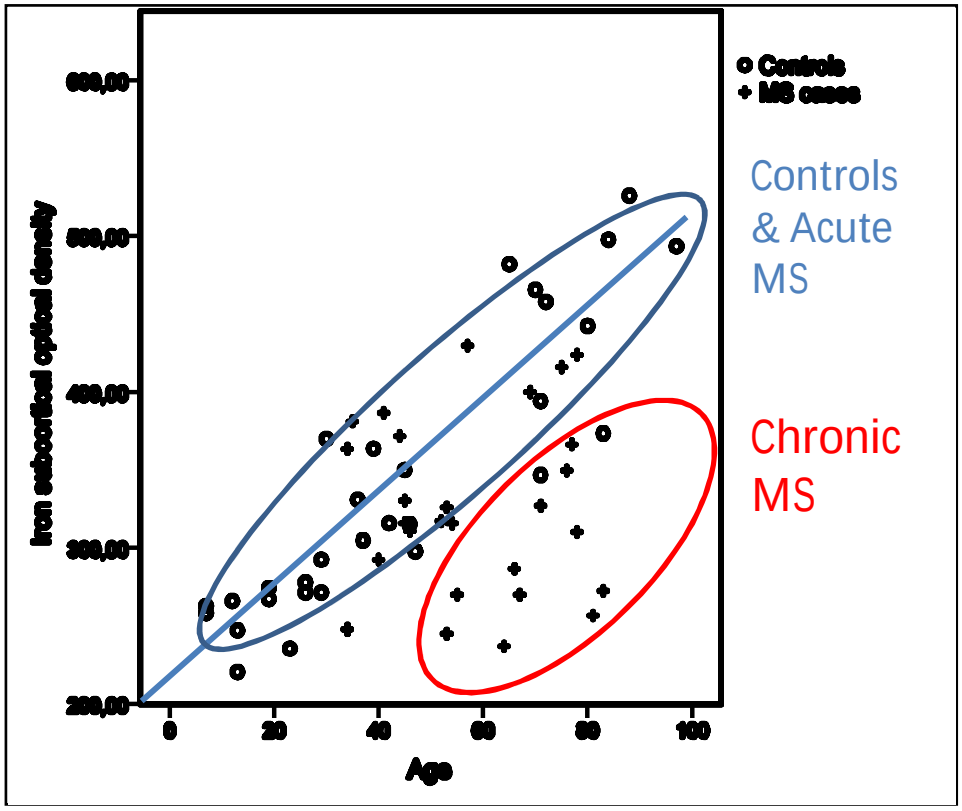
Iron in Human MS Brains



Iron Export Proteins in MS

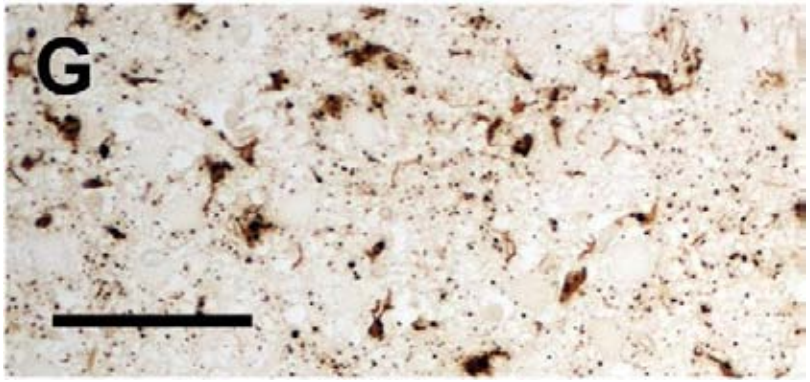


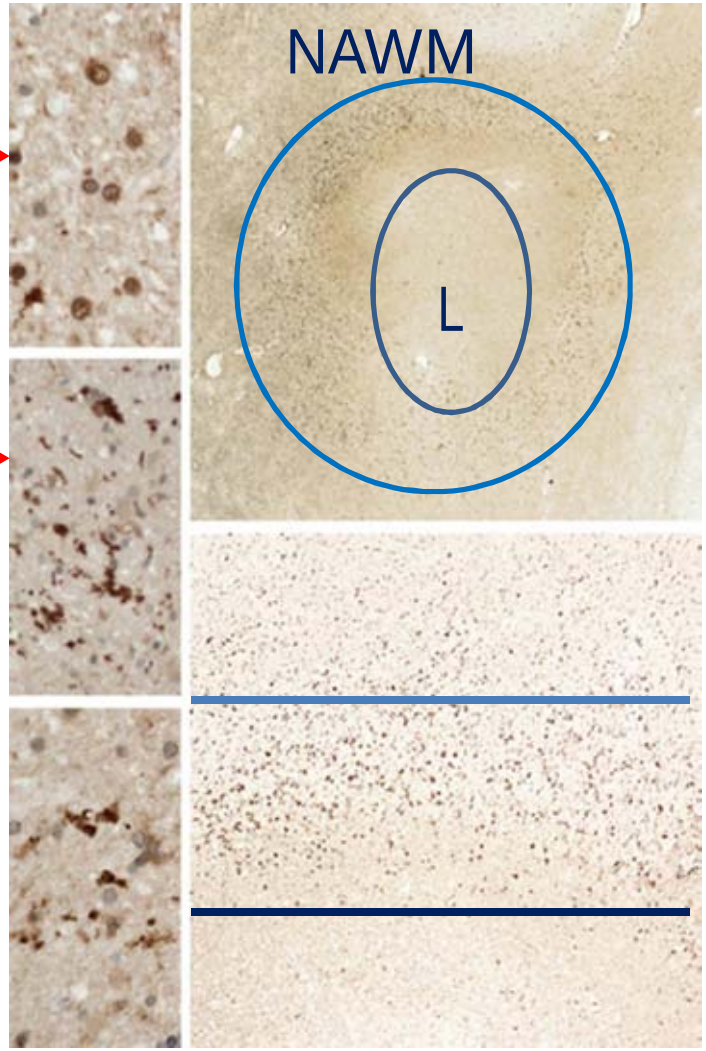
Accumulation of Iron with Age in MS Brain



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





Brain Iron is currently not a Prime Target for MS Therapy

- Physiologic function in neurogenesis, myelin synthesis, neurotransmitter production and mitochondrial function
  - Loss of iron in the course of inflammation and in demyelinated plaques
- Amplification of oxdiative injury, when liberated within active lesions
  - Increased liberation in active MS lesions in patients with increased age
- Mechanisms of iron accumulation in the human brain with aging are so far unknown

Pathogenesis of Progressive MS

Consequences for Therapy

- Drugs, which target progressive MS, have to act within the CNS
- PotentialTargets
  - Intrathecal inflammation (T-cells, B-cells)
  - Microglia activation
  - Oxidative injury / Apoptosis
  - Mitochondrial protection
  - Ion channel blockade
- Therapeutic strategies are currently identified, but their beneficial effect has to be shown in controled clinical trials