Vorlesung ECN/MolMed Regensburg 09.05.2025

Multiple sclerosis
Inflammation and glial cells

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1 Case and clinical aspects

2 Pathophysiology

- Immune cells
- Glial cells
- Neuroaxonal damage

3 Animal models

4 Etiology

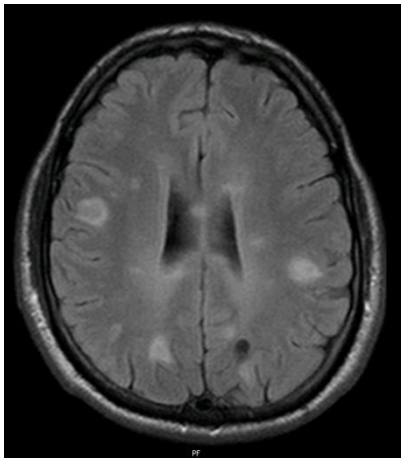
5 Treatment

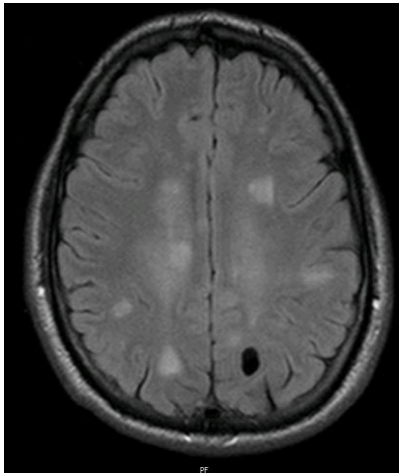
Case Report

- Student Stefanie X., 22 years old, healthy, no previous medical history
- 2024: blurred vision right eye for some weeks, no doctor seen
- 2025: Visual disturbance left eye, sensory deficits right arm

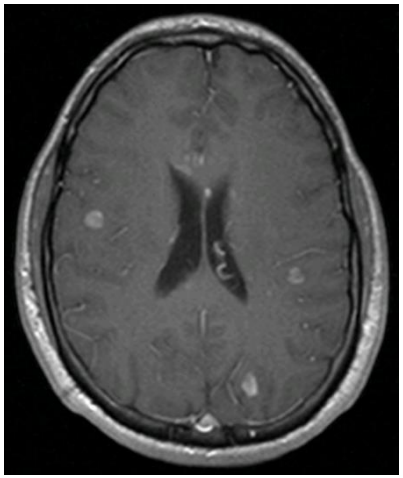
MRI

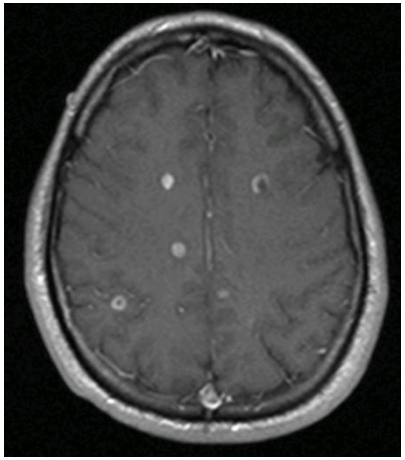
FLAIR



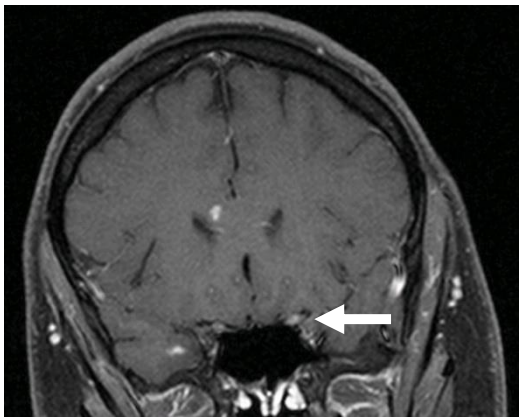


Gadolinium

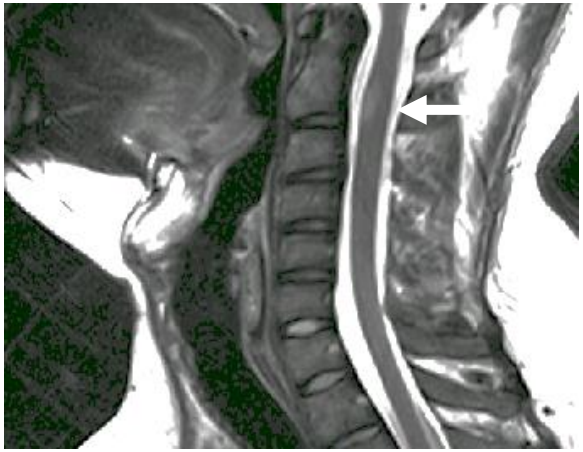




Optikusdarstellung



Rückenmark



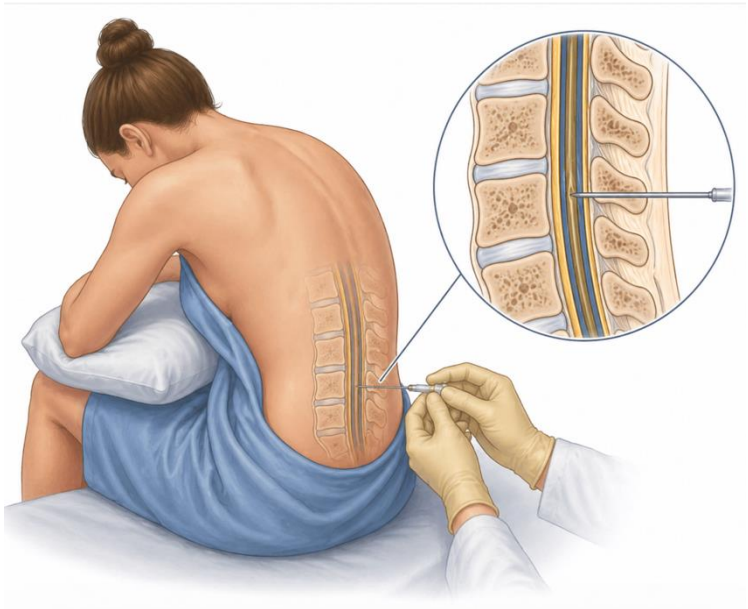
…and now?

Case Report

- Cerebrospinal fluid (CSF):

3 cells/µl, protein normal,

positive oligoclonal bands



- Electrophysiology: Evoked potential with decreased conduction velocities (VEP, SEP)
- Blood tests: unremarkable
- Diagnosis: relapsing-remitting Multiple sclerosis

MULTIPLE SCLEROSIS: Facts

- ~ 277 MS patients in 100.000 habitants (general risk: 1:360 → 420 MS patients in Regensburg)
- Most common neurologic disease in young adults
- more common in women than men (2:1)
- Onset typically at age of 20-40 years
- If not adequately treated: > 50% with irreversible disability (50% in wheelchair after 15-20 years)
- ~ 65% lose job without significant physical impairment due to cognitive deficits, fatigue, depression

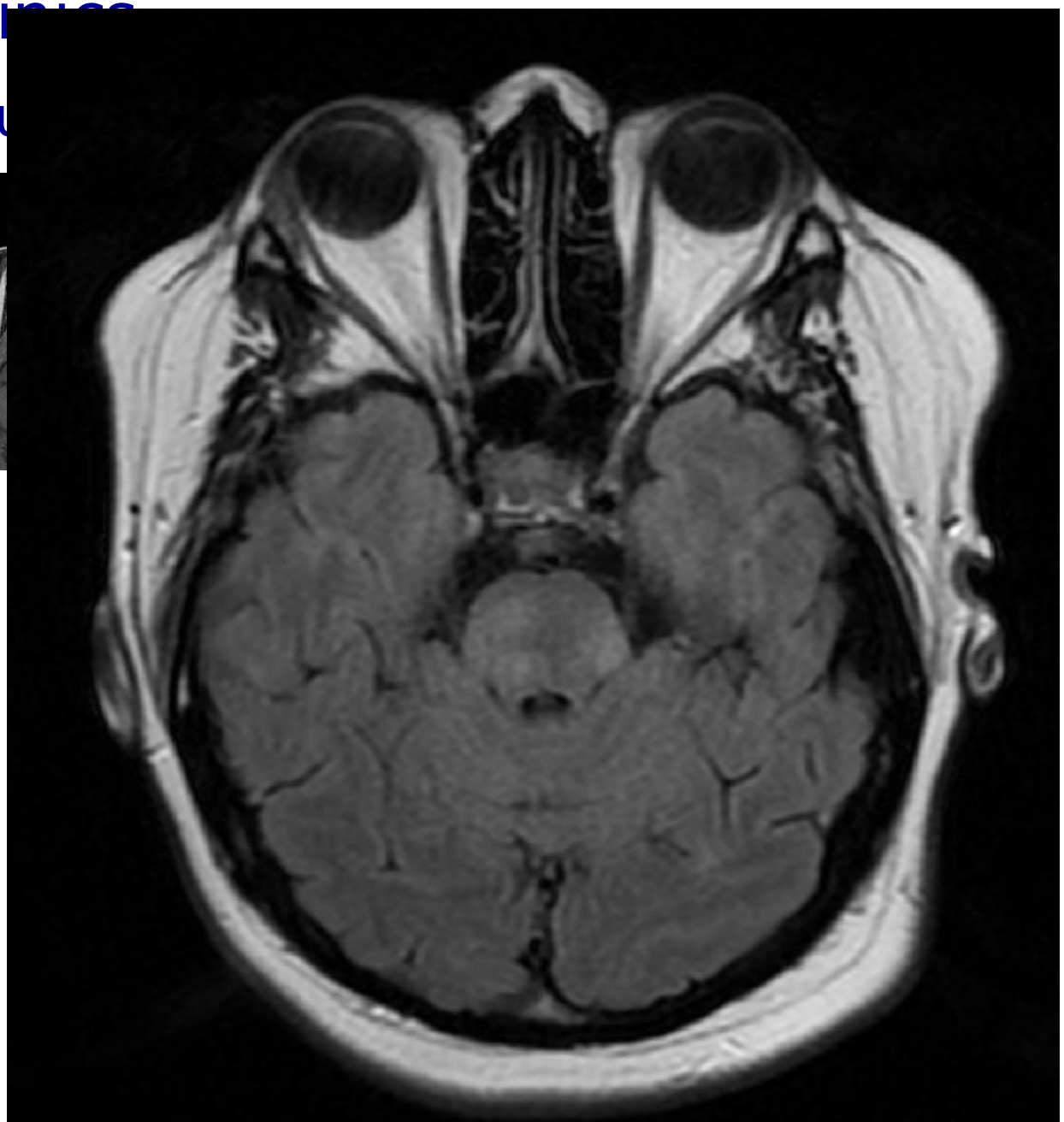
Definition:

Multiple sclerosis is a chronic inflammatory, demyelinating and neurodegenerative disease of the central nervous system

MS: Clinics

MS: Cli

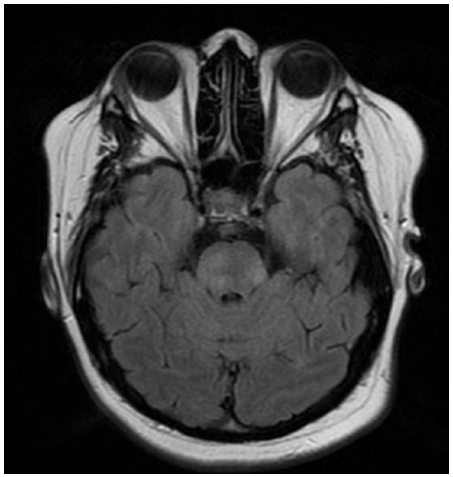
Optic neu

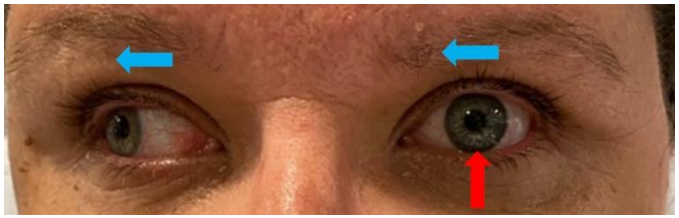


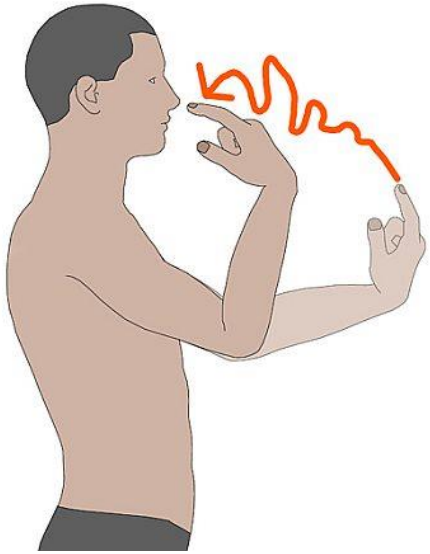
MS: Clinics

Optic nerve

Brain stem, Cerebellum

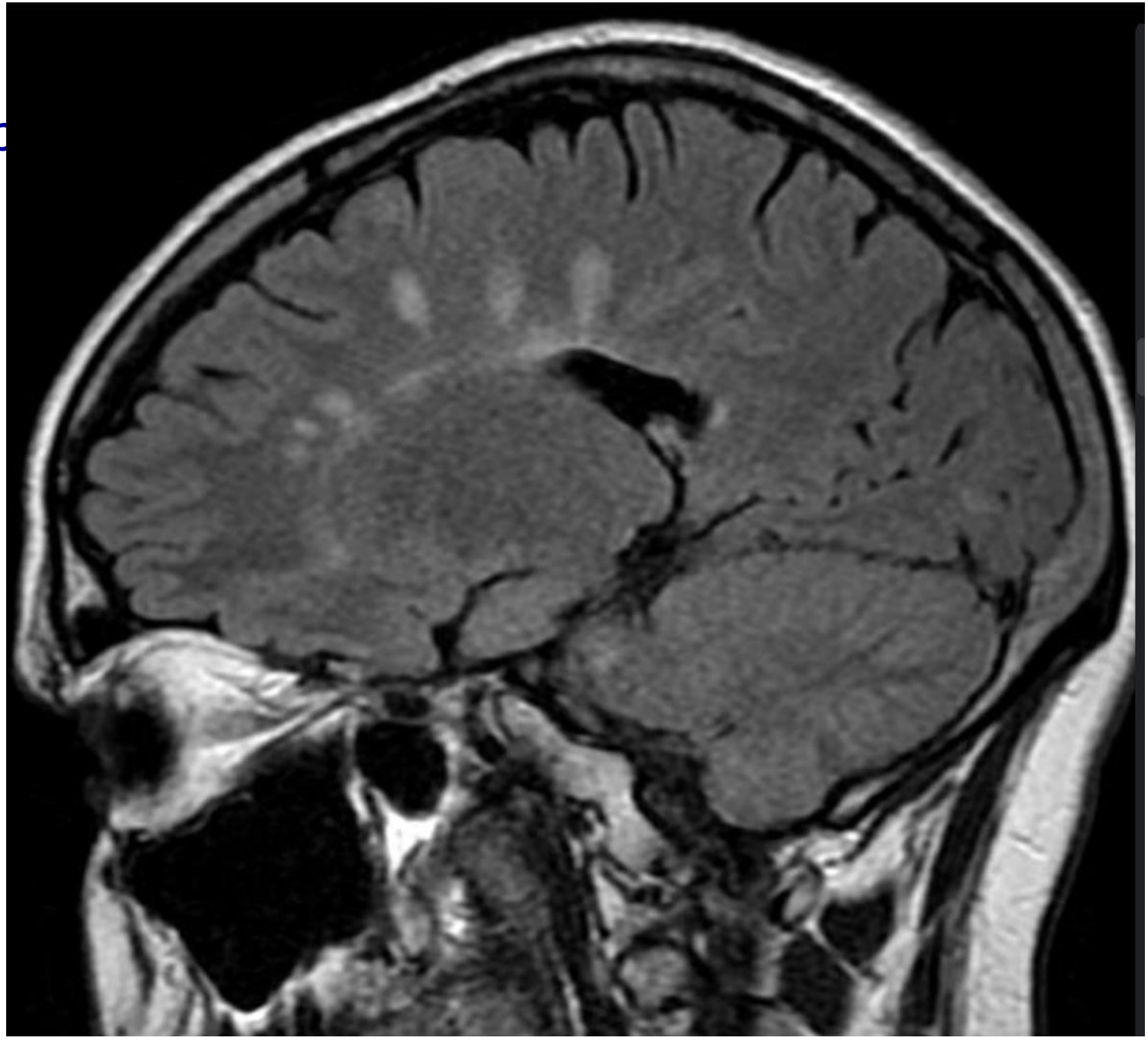






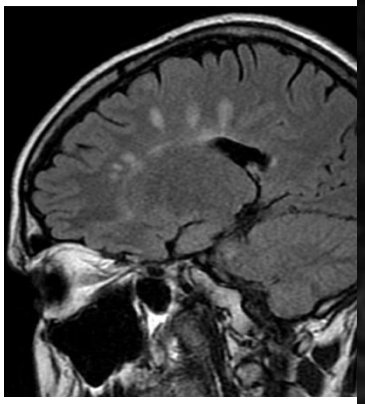
MS:

Cereb



MS: Clini

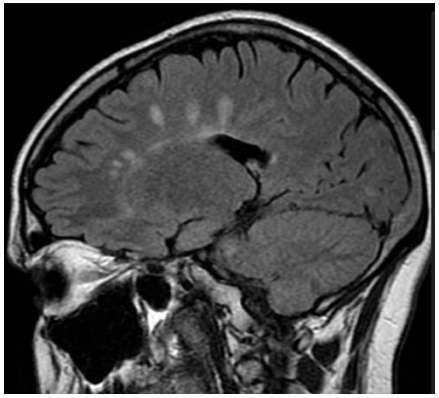
Cerebrum



sion

MS: Clinics

Cerebrum



Fatigue

Cognitive deficits

Depression

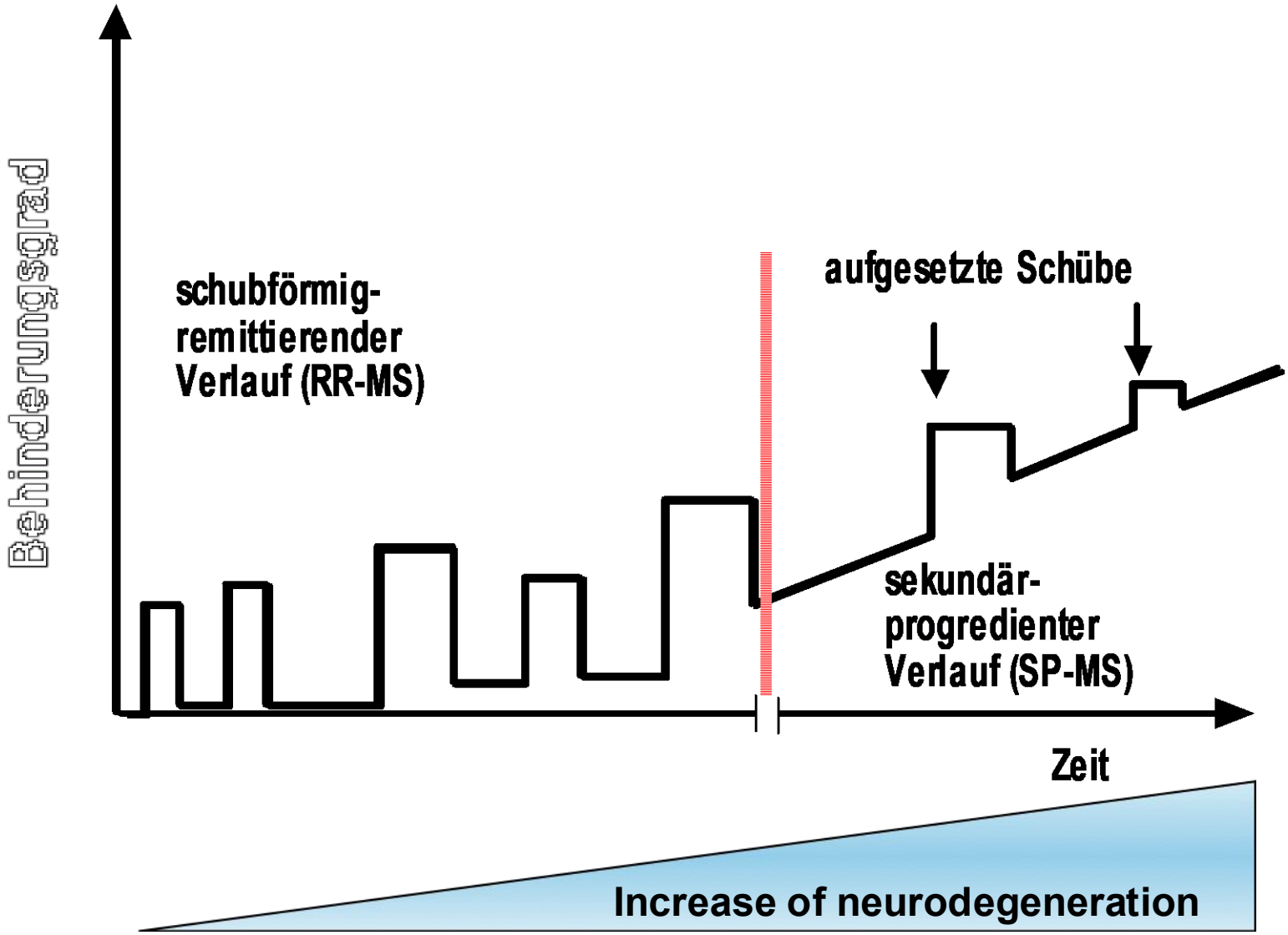
Spinal cord

Sensory deficits

Bowel/Bladder dysfunction

Sexual dysfunction

Multiple Sklerose - Courses



How can we investigate the pathophysiology of multiple sclerosis and identify/evaluate therapeutic approaches?

Pathology, human material

- Easy to obtain: Blood, CSF, but only limited information on pathophysiology
- Restricted access to brain/spinal cord tissue, especially from young adults

Animal models

- Easy to obtain: Brain, spinal cord, blood
- BUT: there is no multiple sclerosis in animals

→ Necessity of (more or less) valid disease models

MS: Macropathology

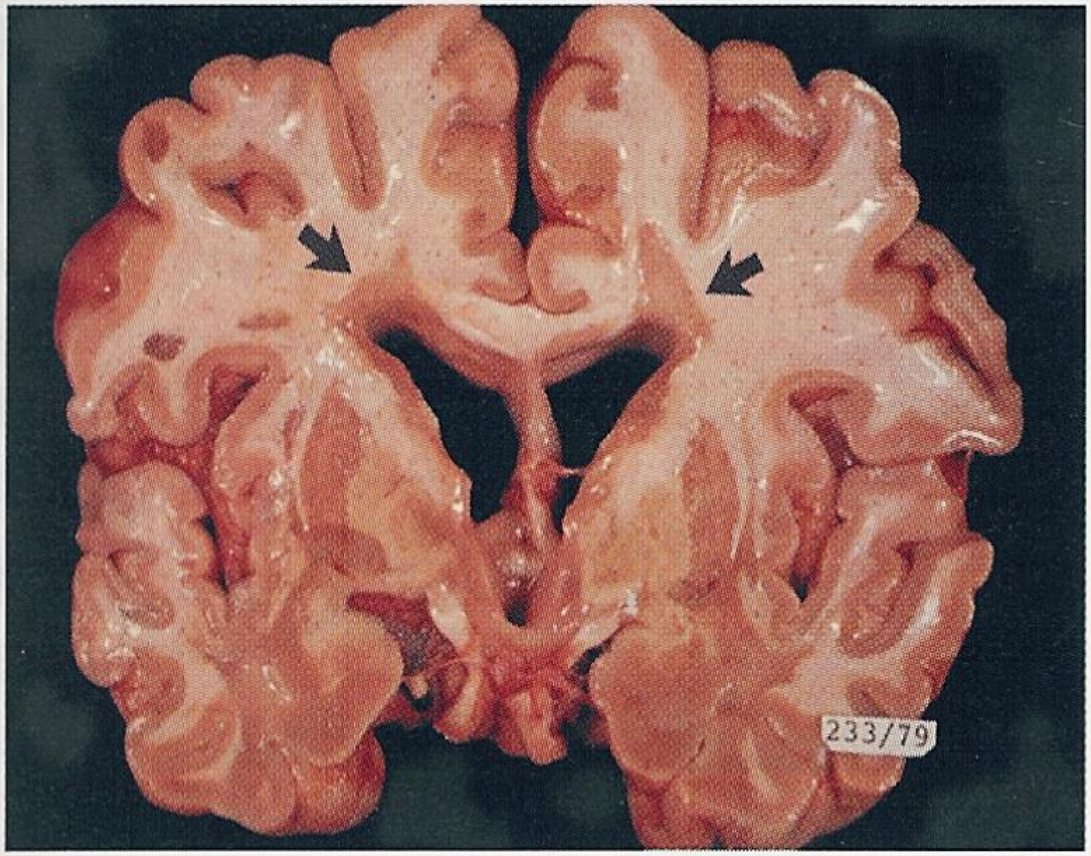
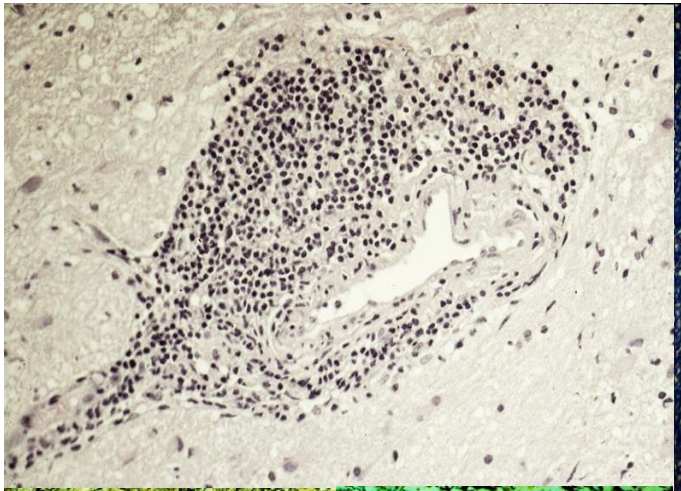


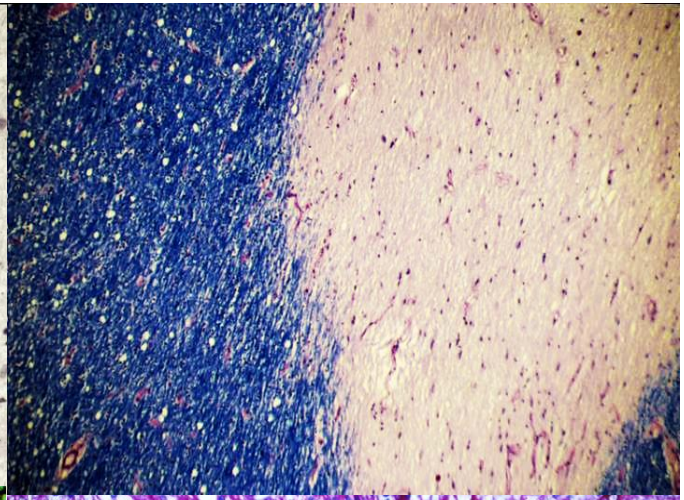
Abb. 3.2: Sektionspräparat des Gehirnes eines MS-Patienten. Die periventrikulären Entmarkungsherde (Pfeile) stellen sich als bräunlichfarbene Läsionen dar.

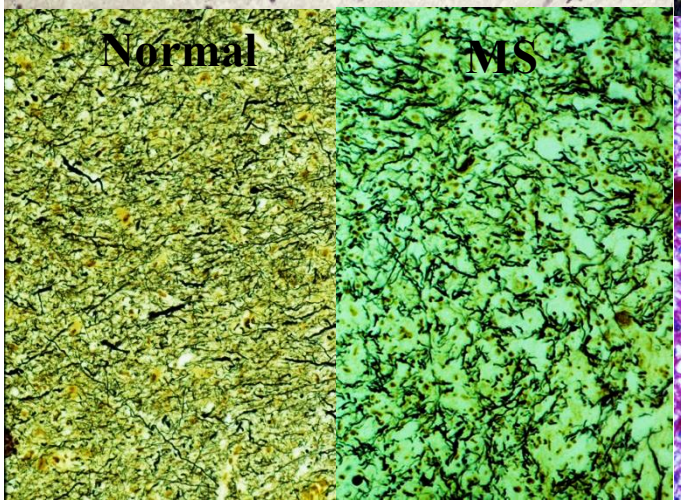
MS: Histopathology

Inflammation

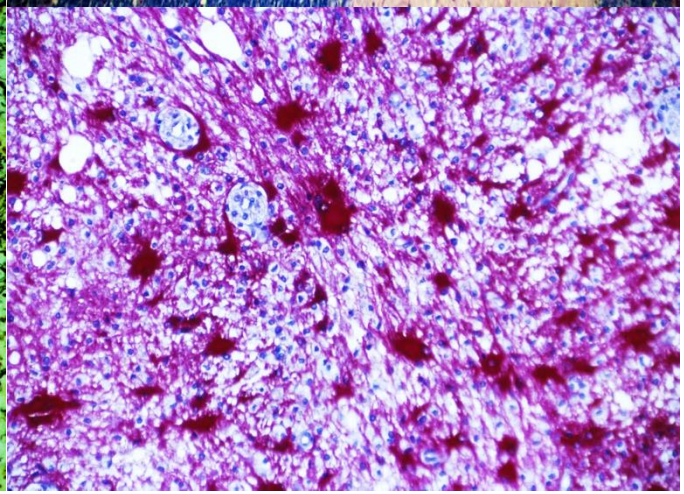


Demyelination

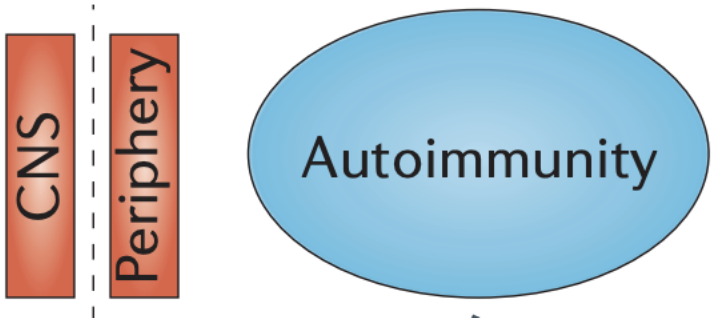




Axonal Injury

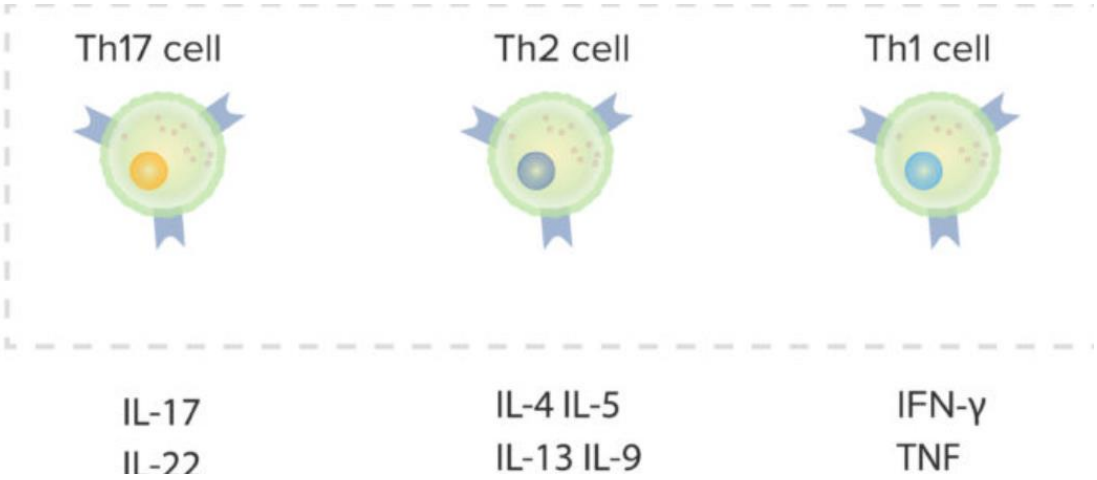


Sclerosis (Gliosis)

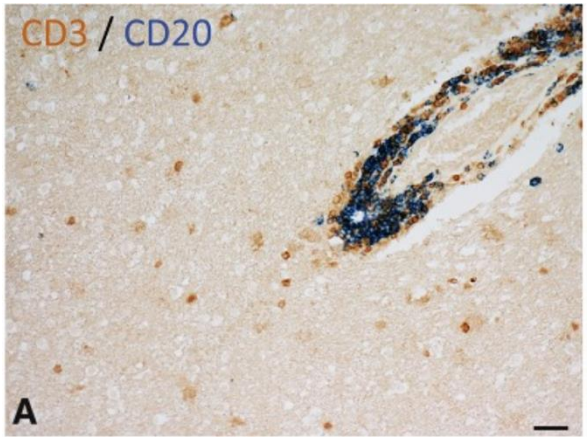

CNS
Periphery
Autoimmunity

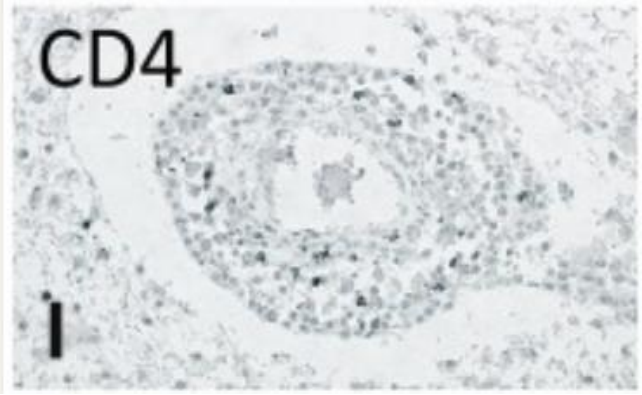
Immunopathology of MS

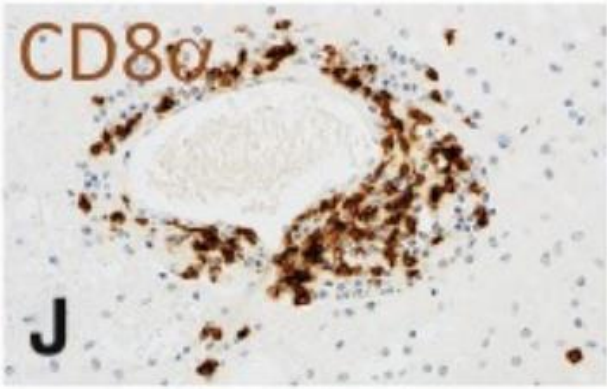
- Lymphocytes
  - T cells
    - CD4+
      - Th1
      - Th2
      - Th17
      - Treg
      - Teff
    - CD8+
  - B cells
    - Plasma c.
- Myeloid cells
  - Granulo
  - Monocytes
    - DC
    - Macroph.



Lymphocyte infiltration in MS lesions







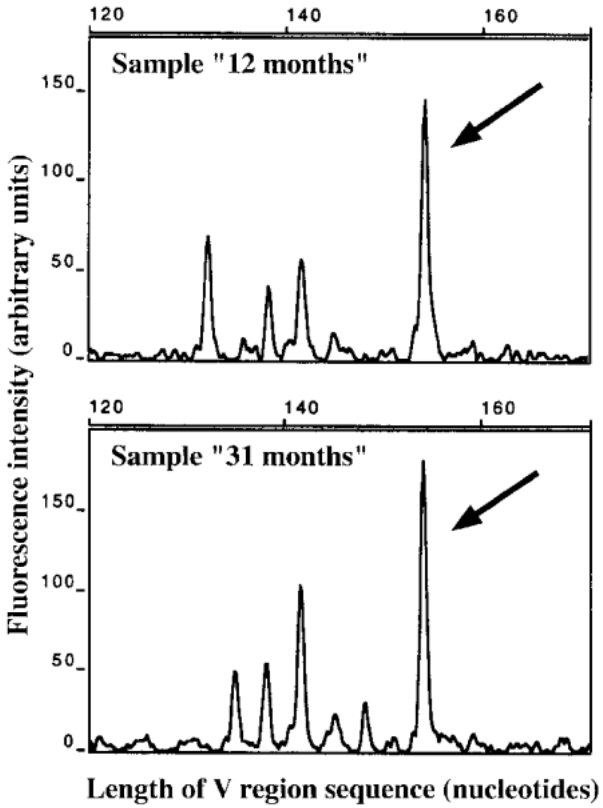
Machado-Santos et al. Brain 2018

Role of CD8 positive T cells in MS

Clonal Expansions of CD8$^+$ T Cells Dominate the T Cell Infiltrate in Active Multiple Sclerosis Lesions as Shown by Micromanipulation and Single Cell Polymerase Chain Reaction

By Holger Babbe,* Axel Roers,* Ari Waisman,* Hans Lassmann,§

	Single parenchymal CD8$^+$ T cells	
Lesion	Clone no.	Frequency
No. 1	1	14/29 (48%)
	9	3/29
	3, 4, 7, 15, 21	1/29
	Sum:	22/29 (76%)
No. 2	1	6/28 (21%)
	2	3/28
	9	2/28
	3, 16, 18, 19	1/28
	Sum:	15/28 (54%)
No. 3	1	8/24 (33%)
	16, 18	2/24
	4, 14, 15, 17	1/24
	Sum:	16/24 (67%)



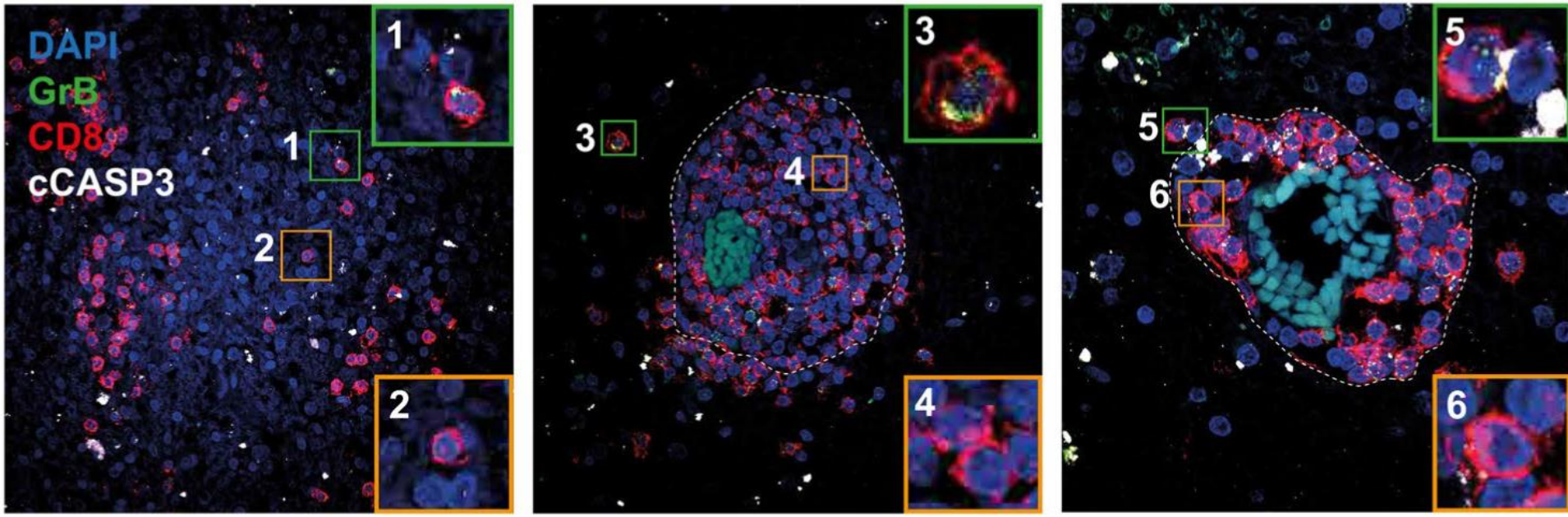
Role of CD8 positive T cells in MS

Acta Neuropathol (2017) 134:383–401
DOI 10.1007/s00401-017-1744-4

ORIGINAL PAPER

Phenotypic and functional characterization of T cells in white matter lesions of multiple sclerosis patients

Gijsbert P. van Nierop[1,2,4] · Marvin M. van Luijn[3,4] · Samira S. Michels[1] · Marie-Jose Melief[3,4] · Malou Janssen[2,3,4] · Anton W. Langerak[3] · Werner J. D. Ouwendijk[1] · Rogier Q. Hintzen[2,3,4] · Georges M. G. M. Verjans[1,5]



Role of Th1 cells and myelin-autoreactivity

The Journal of Immunology

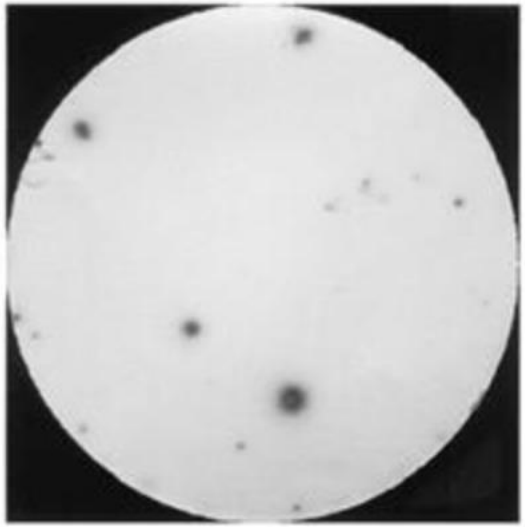
RESEARCH ARTICLE | AUGUST 01 2000

Quantification of Self-Recognition in Multiple Sclerosis by Single-Cell Analysis of Cytokine Production[1] FREE

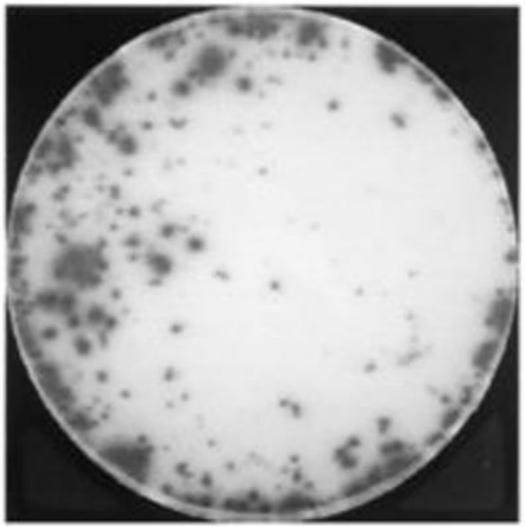
Clara M. Pelfrey; ... et. al

PLP Sequence:	M	G	L	L	E	C	C	A	R	C	L	V	G	A	...
Peptide 1-9:	M	G	L	L	E	C	C	A	R						
Peptide 2-10:		G	L	L	E	C	C	A	R	C					
Peptide 3-11:			L	L	E	C	C	A	R	C	L				
Peptide 4-12:				L	E	C	C	A	R	C	L	V			
Peptide 5-13:					E	C	C	A	R	C	L	V	G		
Peptide 6-14:						C	C	A	R	C	L	L	G	A	

22 MS patients vs
22 healthy controls

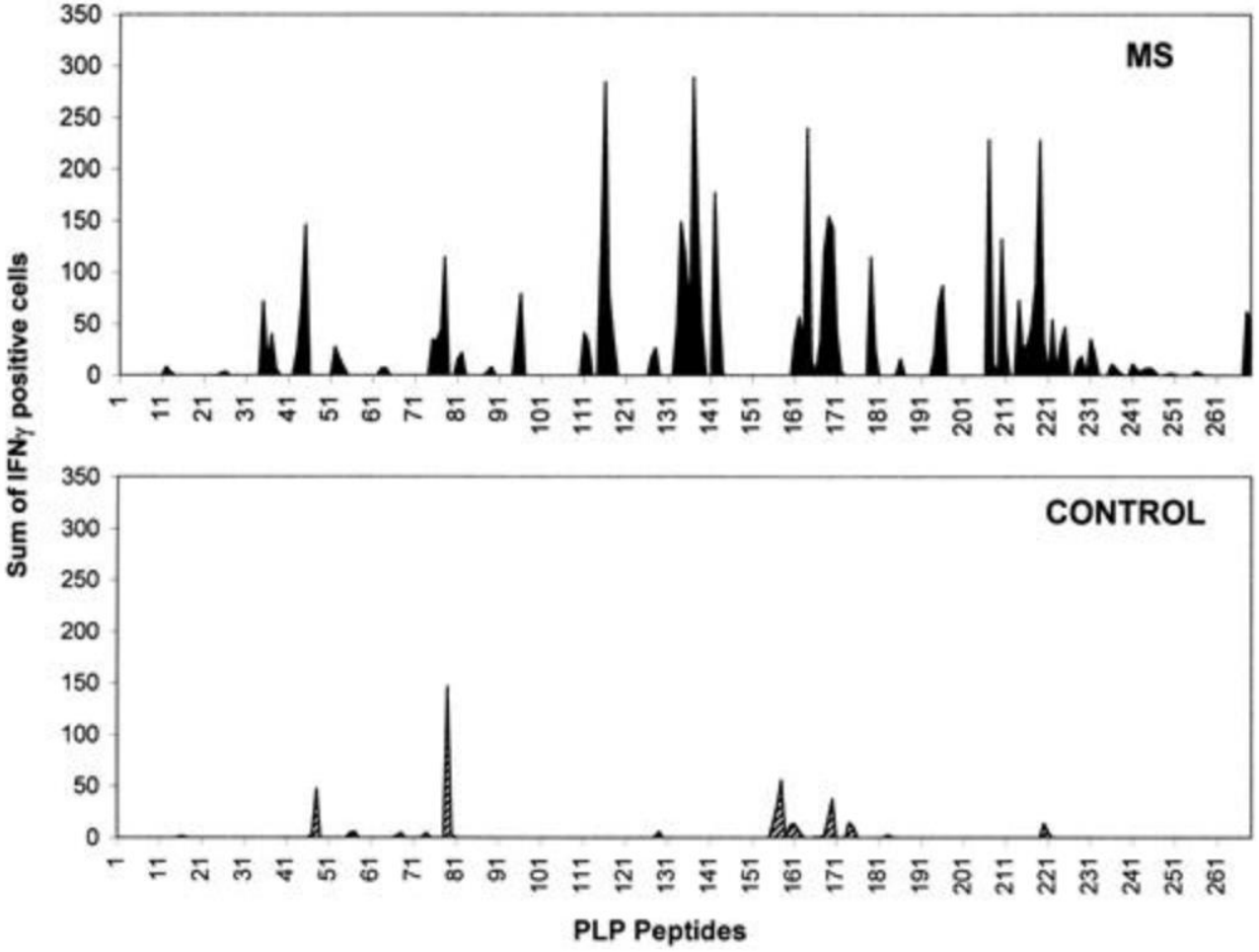


Media



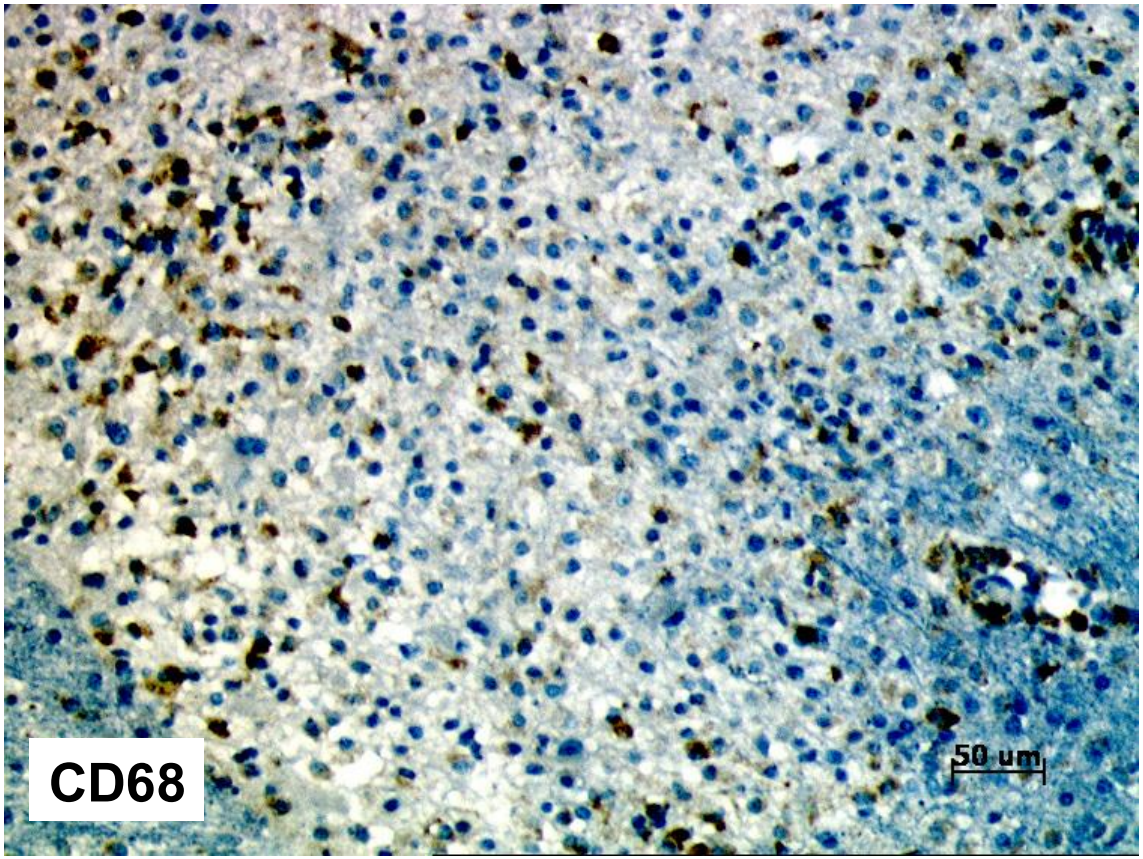
PLP 45-53

Role of Th1 cells and myelin-autoreactivity

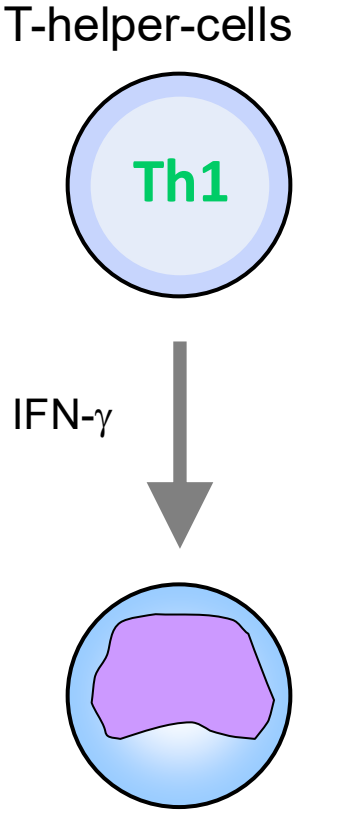


Immunopathology of Multiple Sclerosis: Role of CD4 T-cells and Macrophages

Histopathology of active MS Lesions:

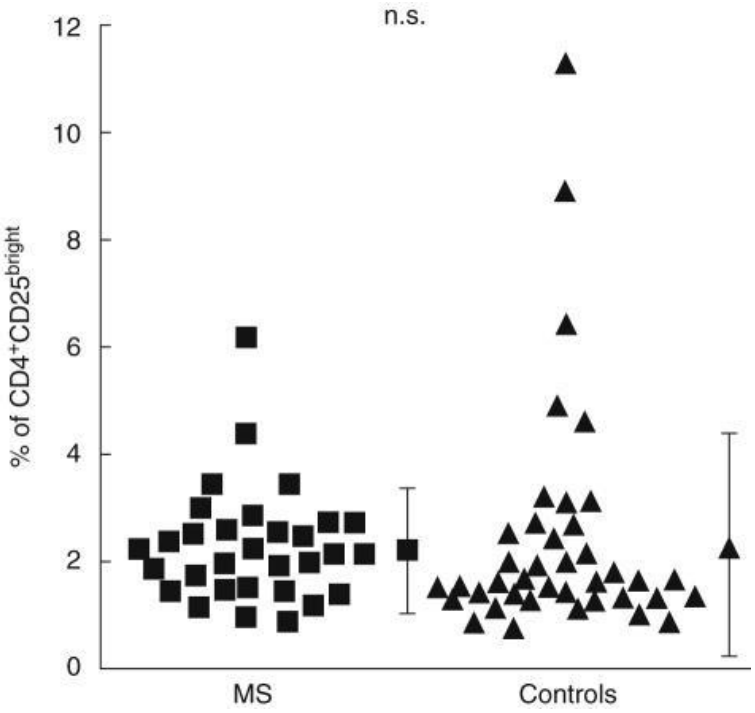


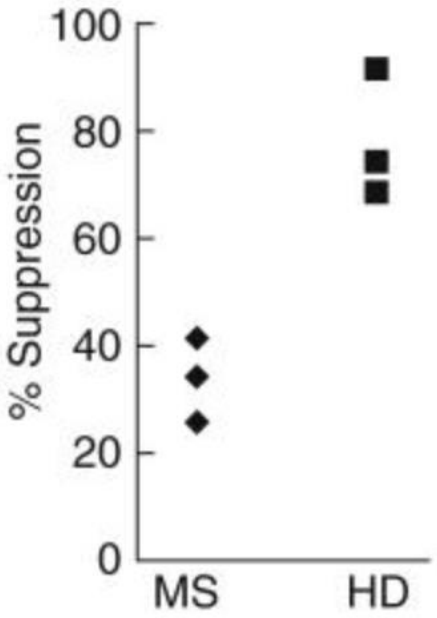
CD68 = Marker for Macrophages



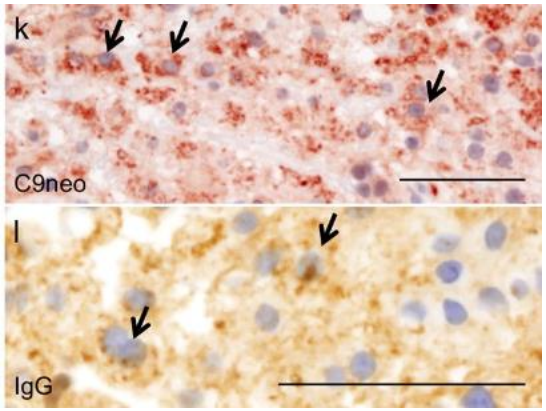
Regulatory T cell – deficiency in MS

- Treg frequency in peripheral blood is similar in MS and HC
- Suppressive capacity of Tregs in MS patients is markedly reduced

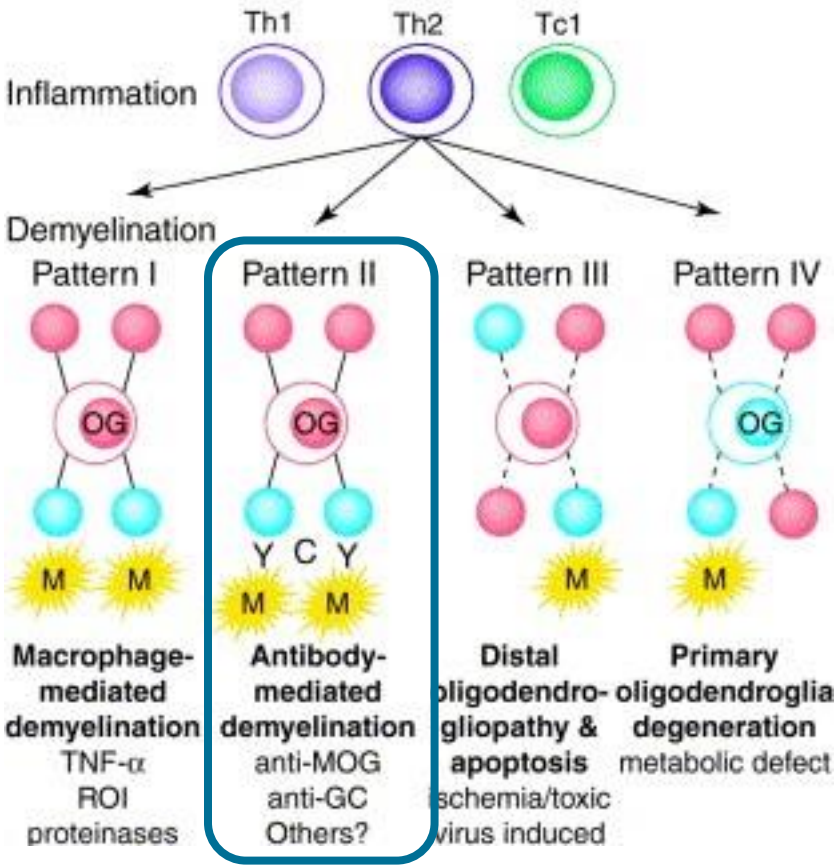


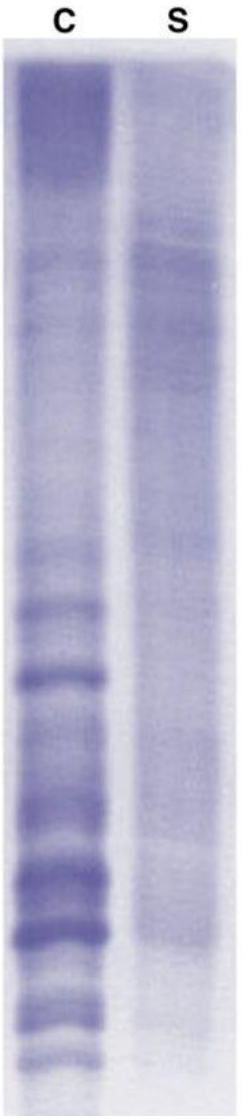


Relevance of B-cells in MS



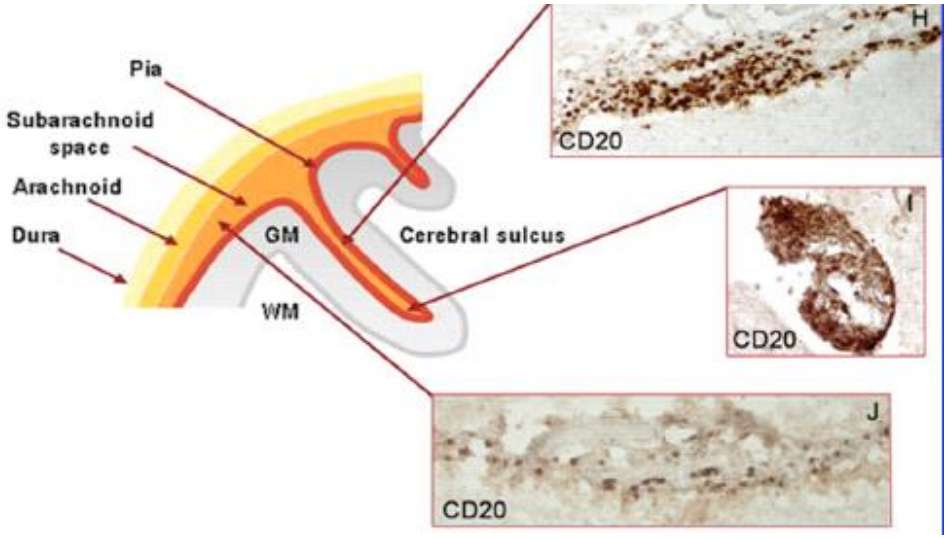
IgG deposition leads to complement activation





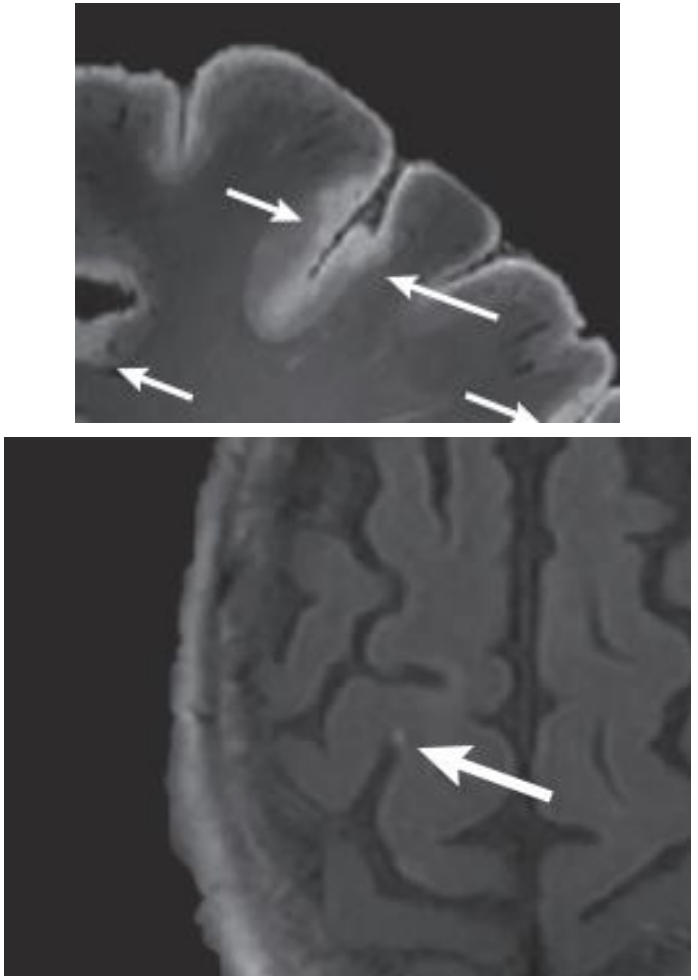
Oligoclonal bands = antibodies in CSF

B cell containing follicle-like structures in the meninges of chronic MS patients



Follicle like structures adjacent to cortical demyelination

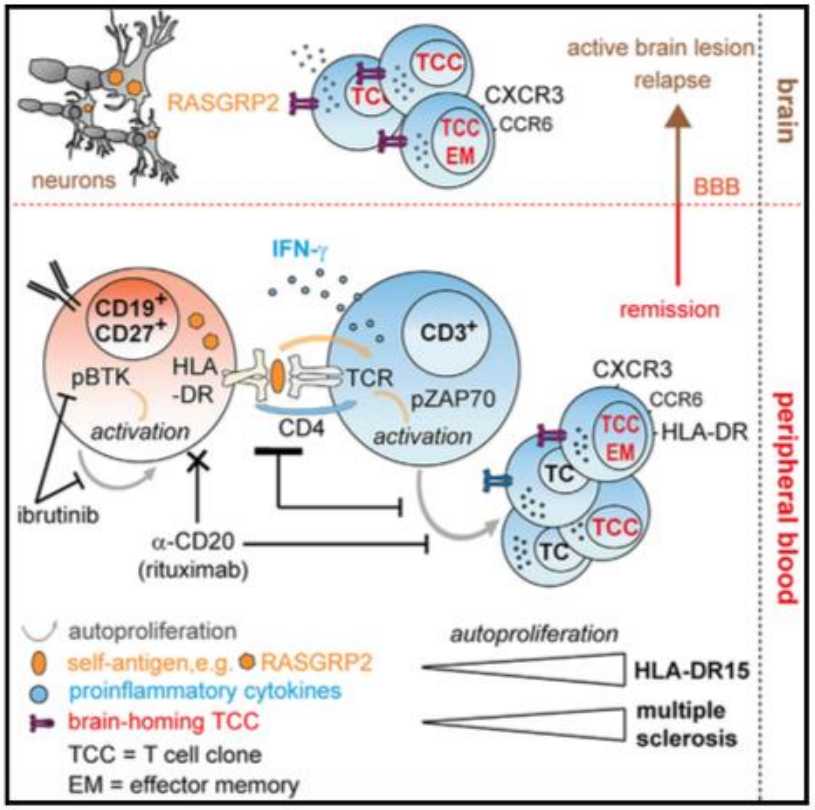
Magliozzi et al., BRAIN, 2007



Absinta et al., Nat Rev Neurol, 2016

Cell

Memory B Cells Activate Brain-Homing, Autoreactive CD4⁺ T Cells in Multiple Sclerosis



Authors

Ivan Jelcic, Faiez Al Nimer, Jian Wang, ..., Fredrik Piehl, Mireia Sospedra, Roland Martin

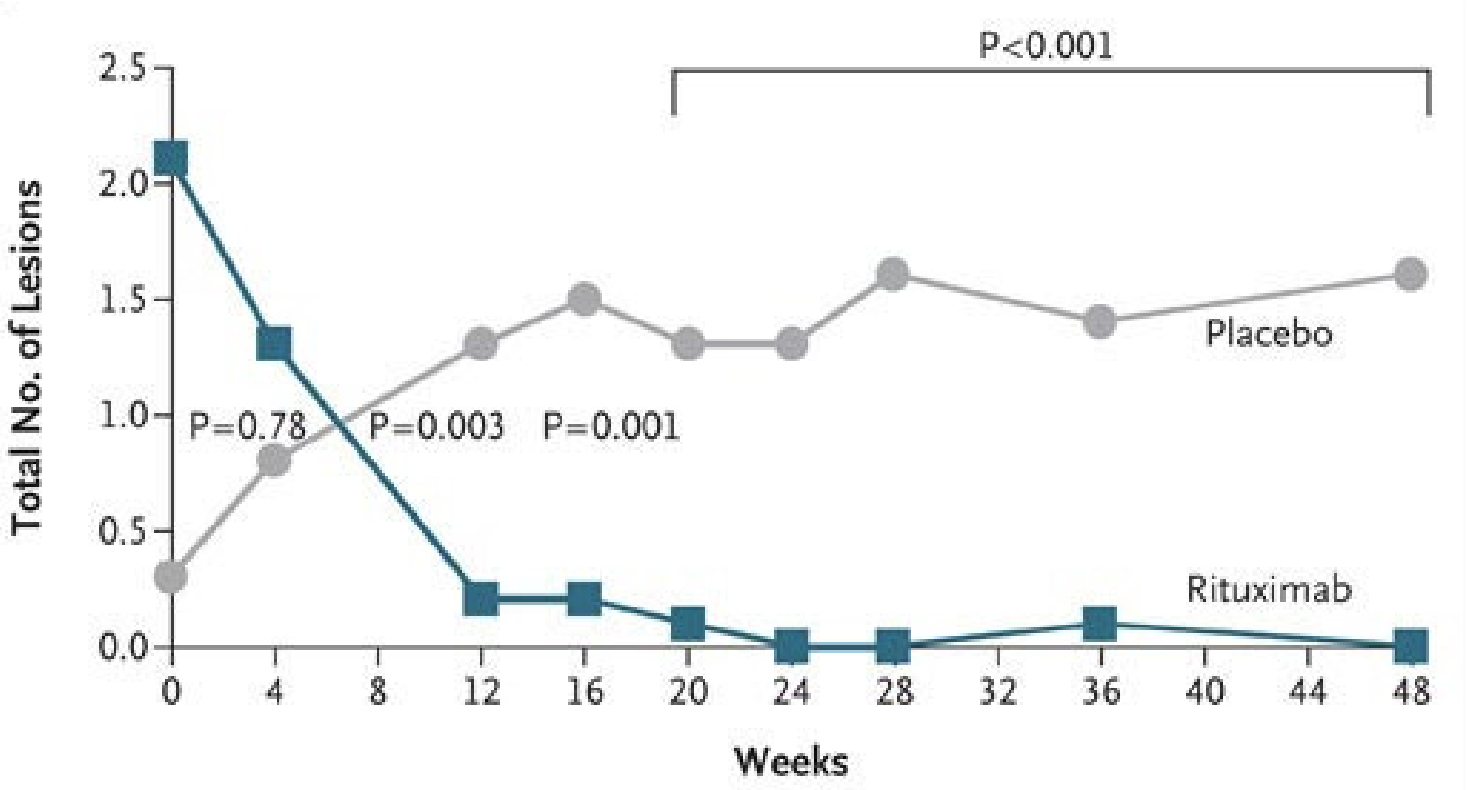
In Brief

Memory B cells drive proliferation of self-reactive brain-homing CD4⁺ T cells, which recognize autoantigens expressed in B cells and in brain lesions with target potential in multiple sclerosis.

B-Cell Depletion with Rituximab in Relapsing–Remitting Multiple Sclerosis

Authors: Stephen L. Hauser, M.D., Emmanuelle Waubant, M.D., Ph.D., Douglas L. Arnold, M.D., Timothy Vollmer, M.D., Jack Antel, M.D., Robert J. Fox, M.D., Amit Bar-Or, M.D., +5 , for the HERMES Trial Group* Author Info & Affiliations

Published February 14, 2008 | N Engl J Med 2008;358:676-688 | DOI: 10.1056/NEJMoa0706383 | VOL. 358 NO. 7

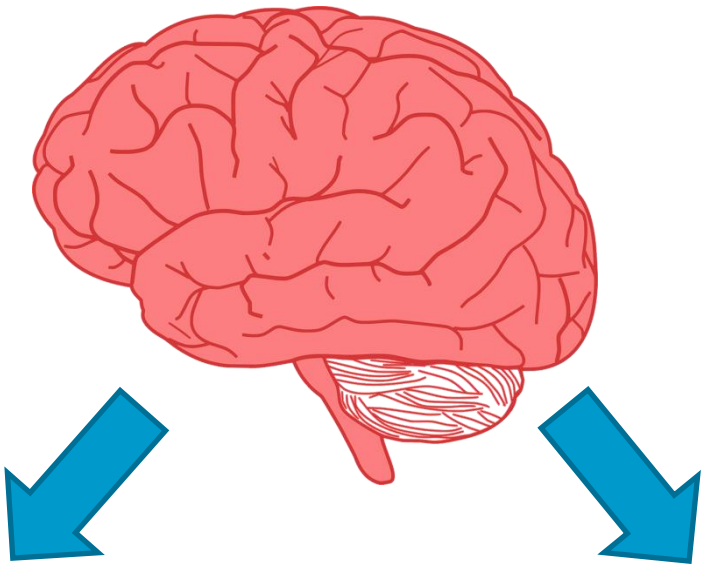


Immune cells in MS

- Pathogenic CD4 positive T-Helper-cells: Th1, Th17
- Macrophages: Effector cells, ROS, cytokine secretion
- Cytotoxic CD8 positive T-cells: dominate active lesions
- B-cells: role in T-cell activation (Antigen presenting cells), Plasma cells (Antibodies)

- Beneficial CD4 positive T-cells: Treg, Th2

Role of CNS cells: Culprits and victims



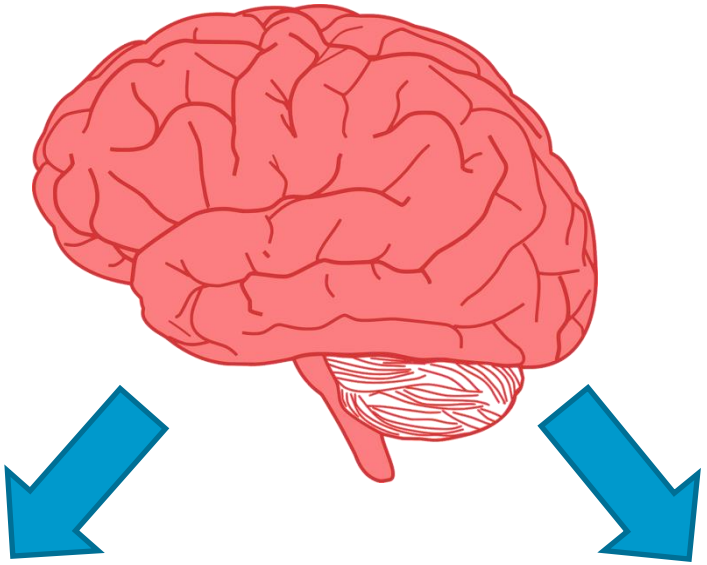
Oligodendrocytes and myelin

Neurons and axons

Astrocytes

Microglia

Role of CNS cells: Culprits and victims



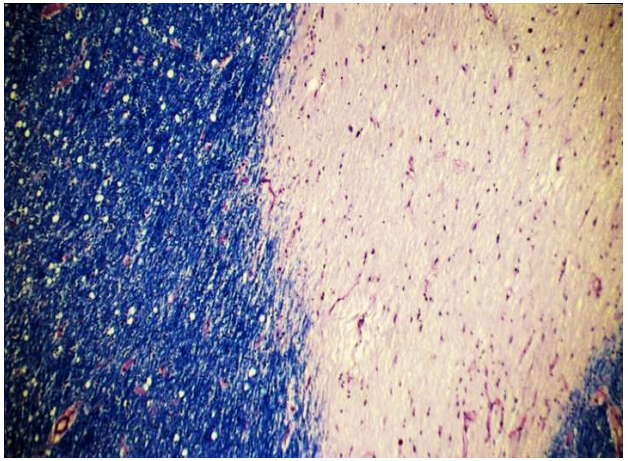
Oligodendrocytes and myelin

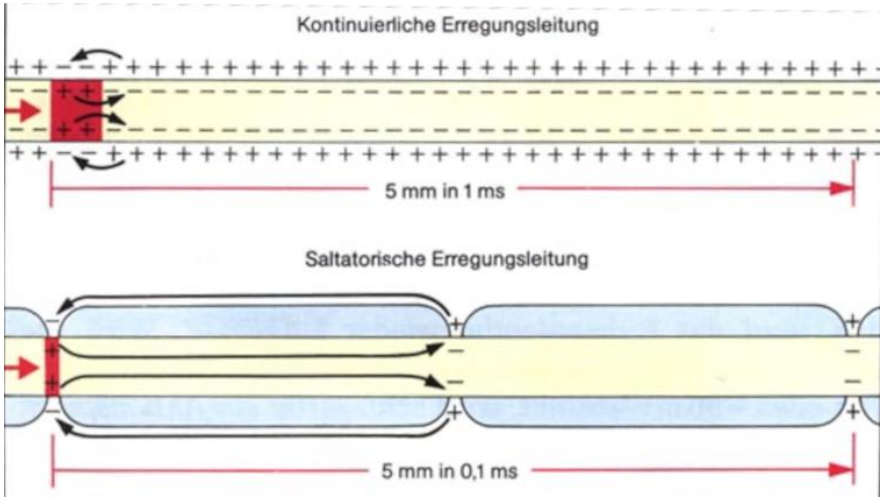
Neurons and axons

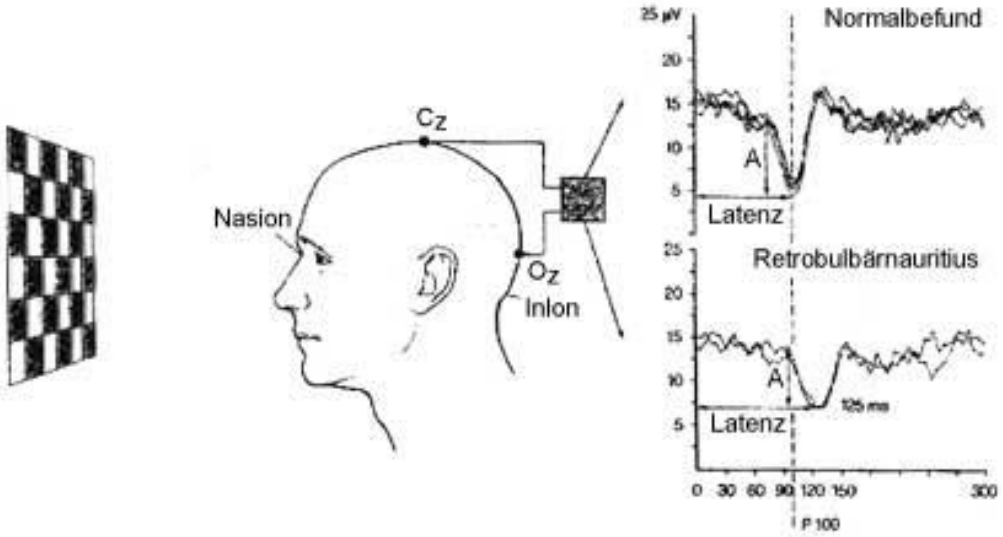
Astrocytes

Microglia

Demyelination as key characteristic in MS





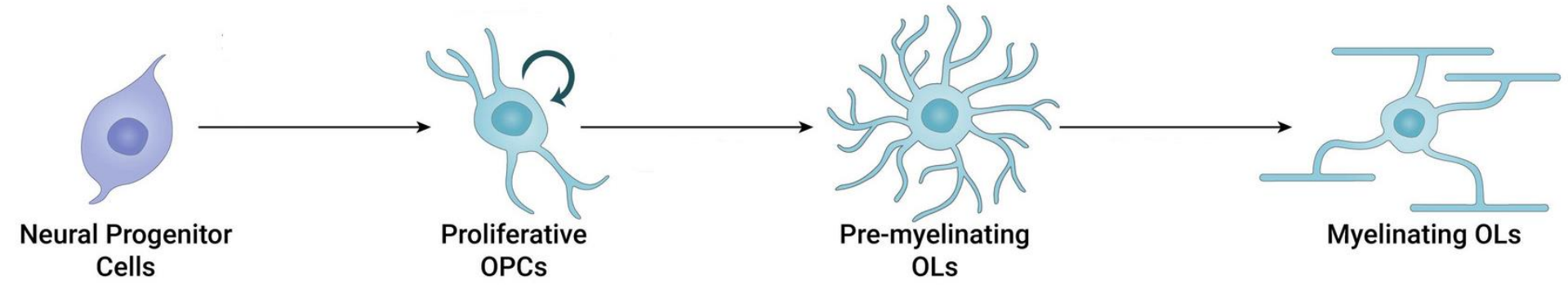


OL and myelin: **insulate axons + provide metabolic support**

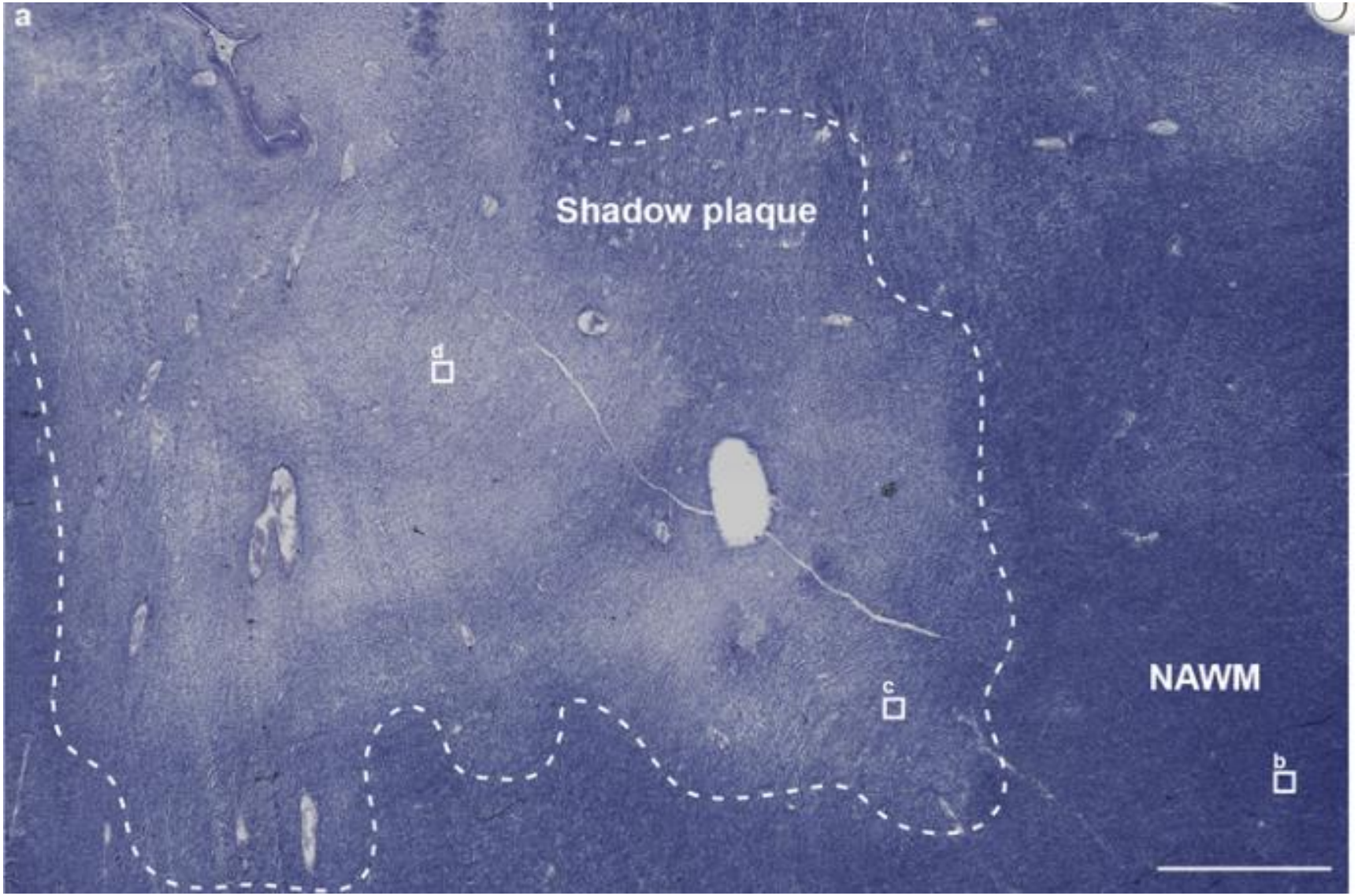
→ without metabolic support axonal degeneration ensues

Oligodendrocyte precursors – hope for remyelination?

- In rodents, oligodendrocytes can regenerate from stem cells, oligodendrocyte precursor cells (OPC)



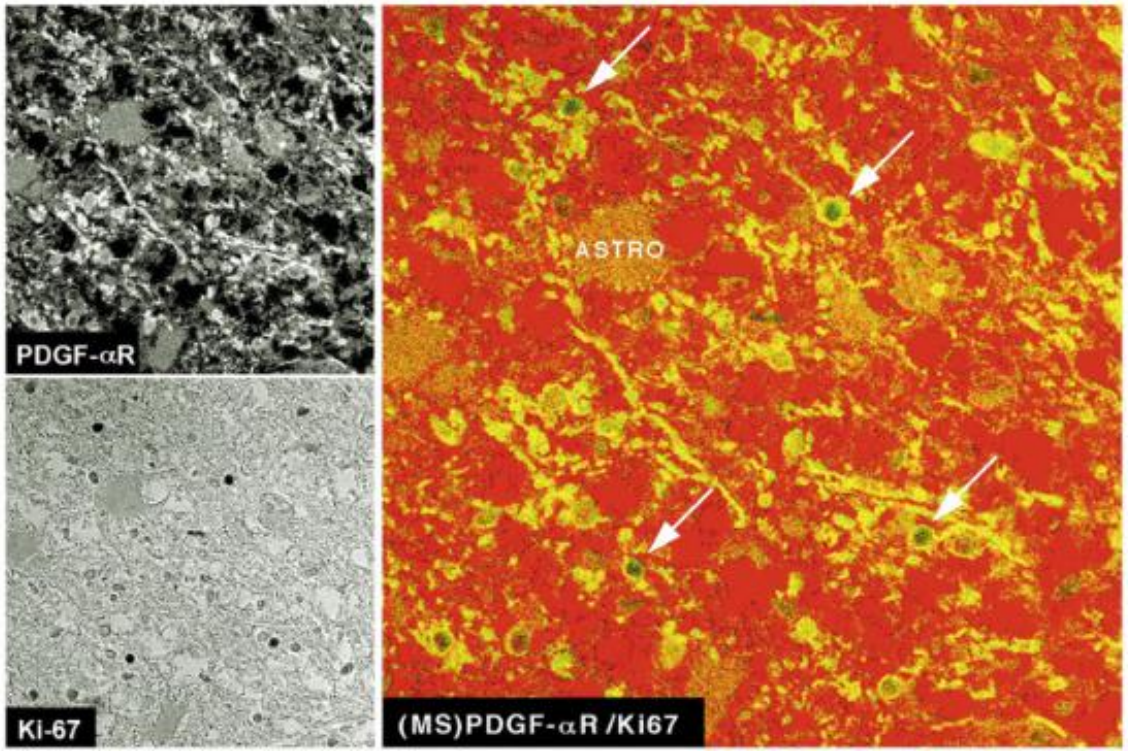
- Up to 40% of MS lesions remyelinate → „shadow plaques“
- Why is remyelination unsuccesful in most lesions?


a
Shadow plaque
d
c
NAWM
b

Oligodendrocyte precursors – hope for remyelination?

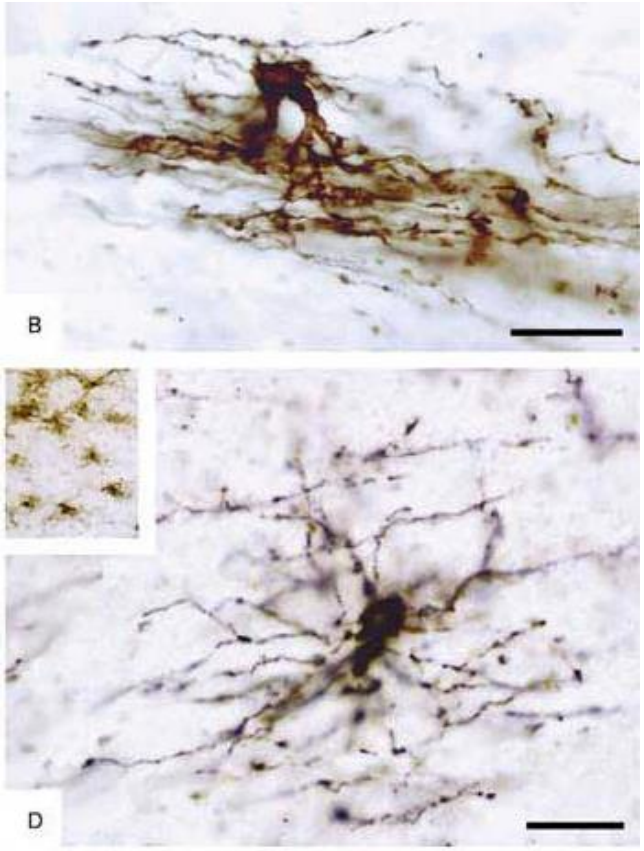
Platelet-Derived Growth Factor-α Receptor-Positive Oligodendroglia Are Frequent in Multiple Sclerosis Lesions

Yasuhiro Maeda, MD, Mukesh Solanky, MD, Joseph Menonna, BS, John Chapin, BS, Weiping Li, MD, and Peter Dowling, MD



Oligodendrocytes (OL) in MS: classical view

NG2 pos OPC in MS lesion



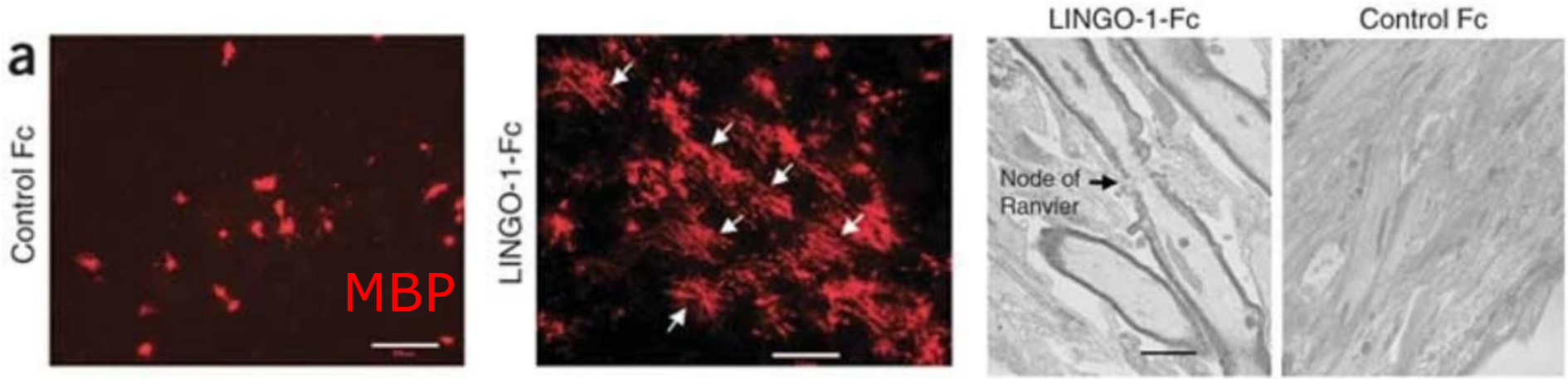
PLP pos pre-myelinating OL in MS lesion

→ Early lesions: 40% remyelinate
→ Premyelinating OL present in chronic MS lesions of multiple sclerosis
→ Remyelination not limited by absence of OL progenitors or failure to generate OL
→ Axons are not receptive for remyelination (e.g. LINGO)

Chang et al., NEJM, 2002

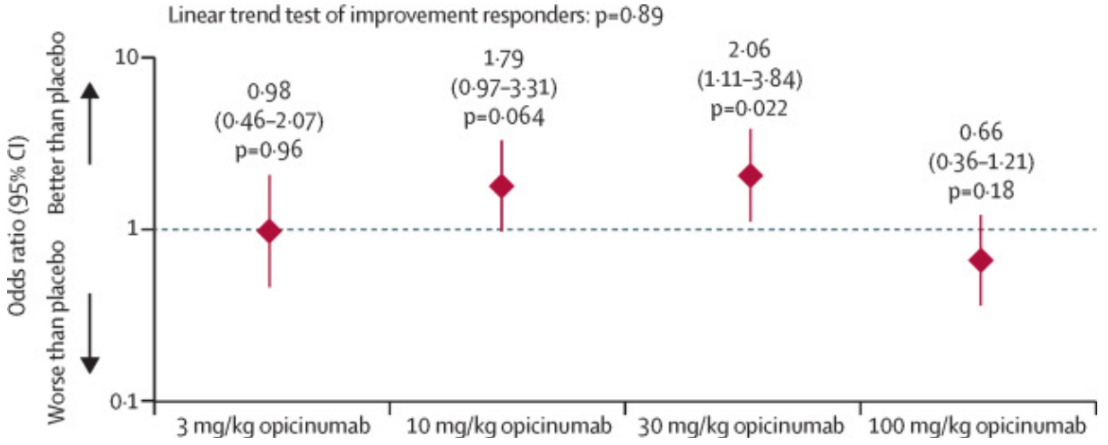
Are unreceptive axons part of the problem?

- LINGO-1 expressed by neurons and OL hampers OL differentiation and myelination



- Opicinumab (LINGO-1-ab) was tested in clinical trial

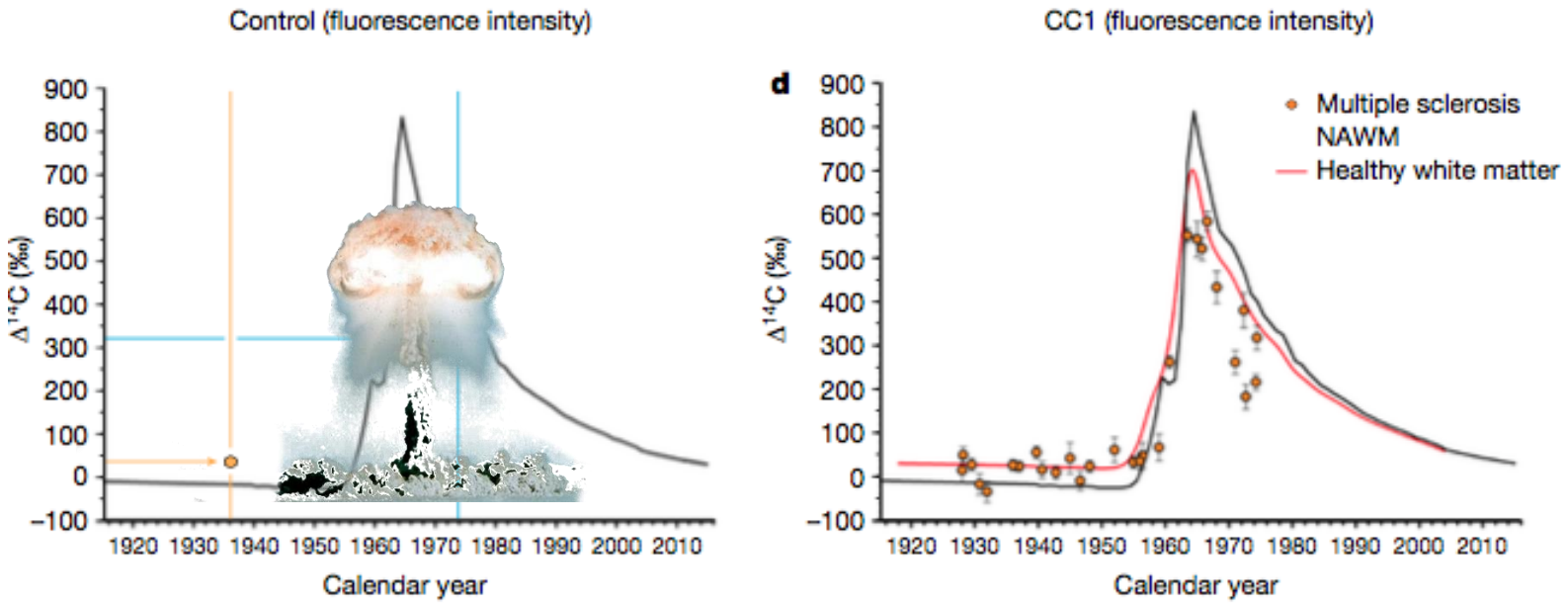
- Negative results



OL in MS 2019:

Dynamics of oligodendrocyte generation in multiple sclerosis

Maggie S. Y. Yeung[1], Mehdi Djelloul[1], Embla Steiner[1], Samuel Bernard[2], Mehran Salehpour[3], Göran Possnert[3], Lou Brundin[4] & Jonas Frisén[1]*



- **Generation of new OL increased in NAWM in some aggressive MS cases**
- **But: Generation not increased in most subjects with the disease**
- **Oligodendrocytes in MS shadow plaques are old and not new**

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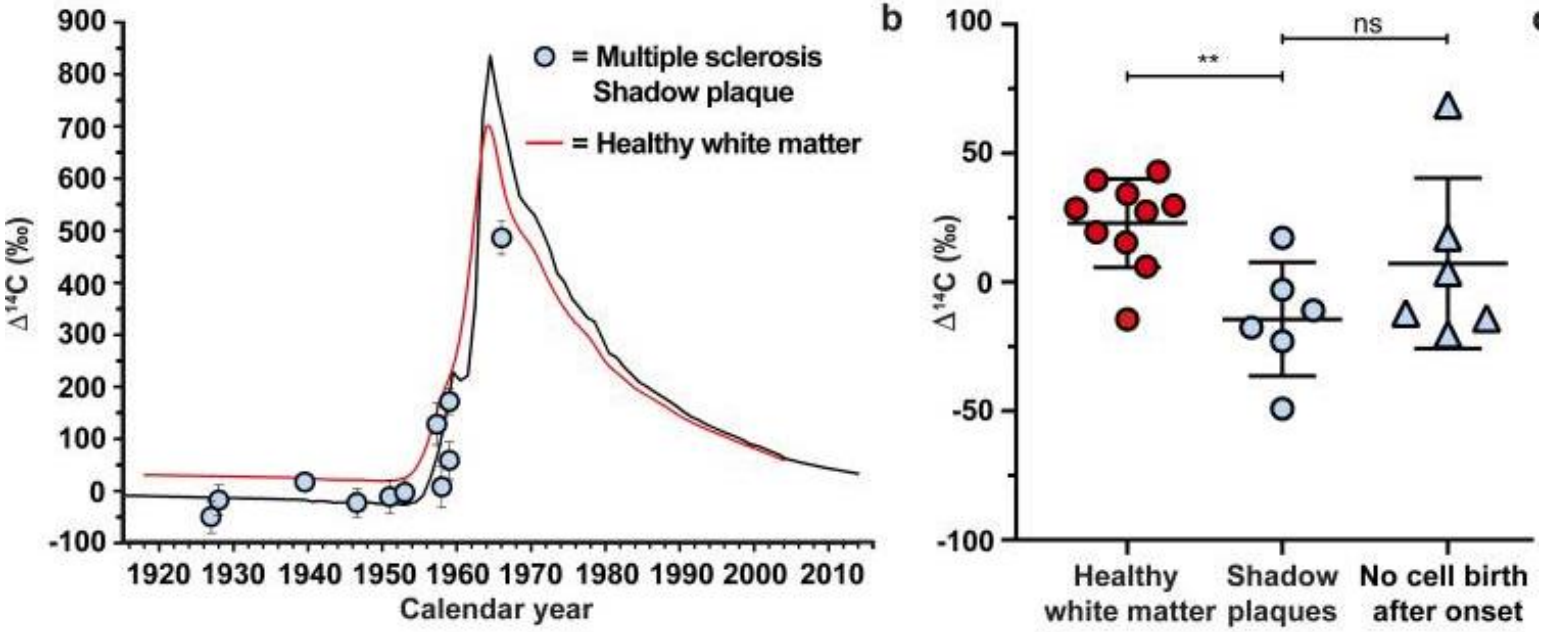
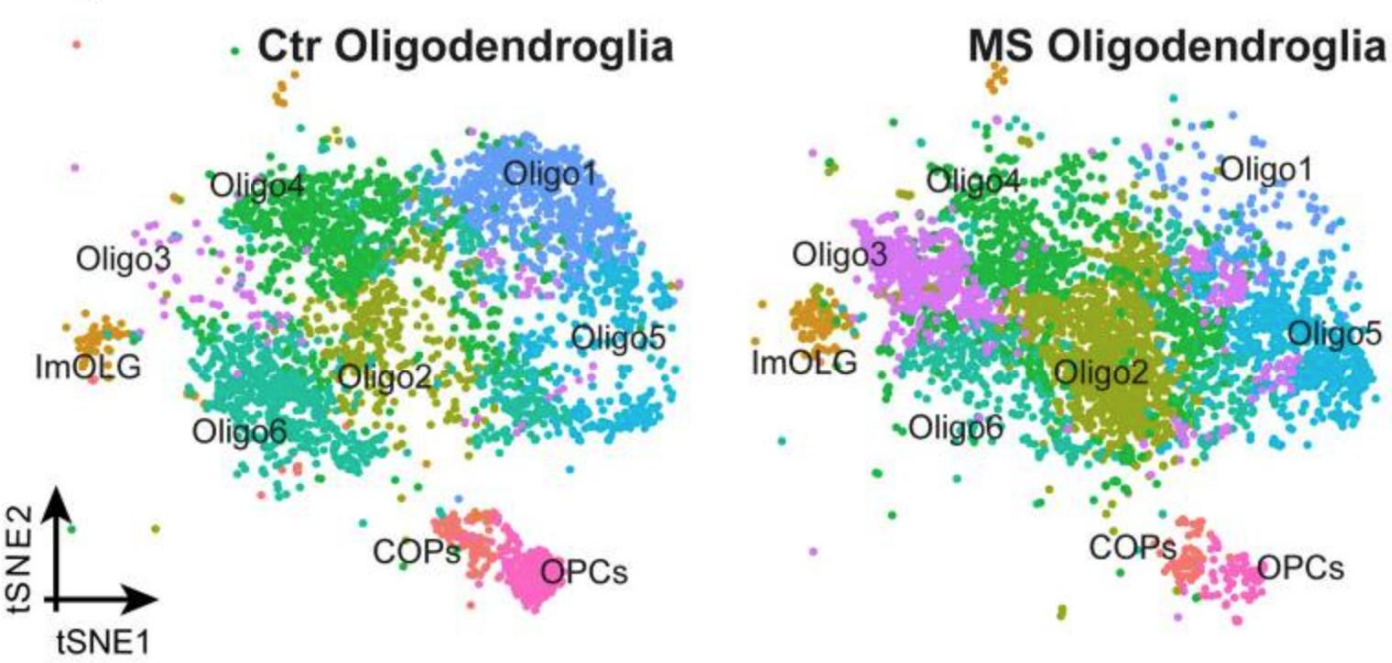


- **Generation of new OL increased in NAWM in some aggressive MS cases**
- **But: Generation not increased in most subjects with the disease**
- **Oligodendrocytes in MS shadow plaques are old and not new**

OL in MS 2020:

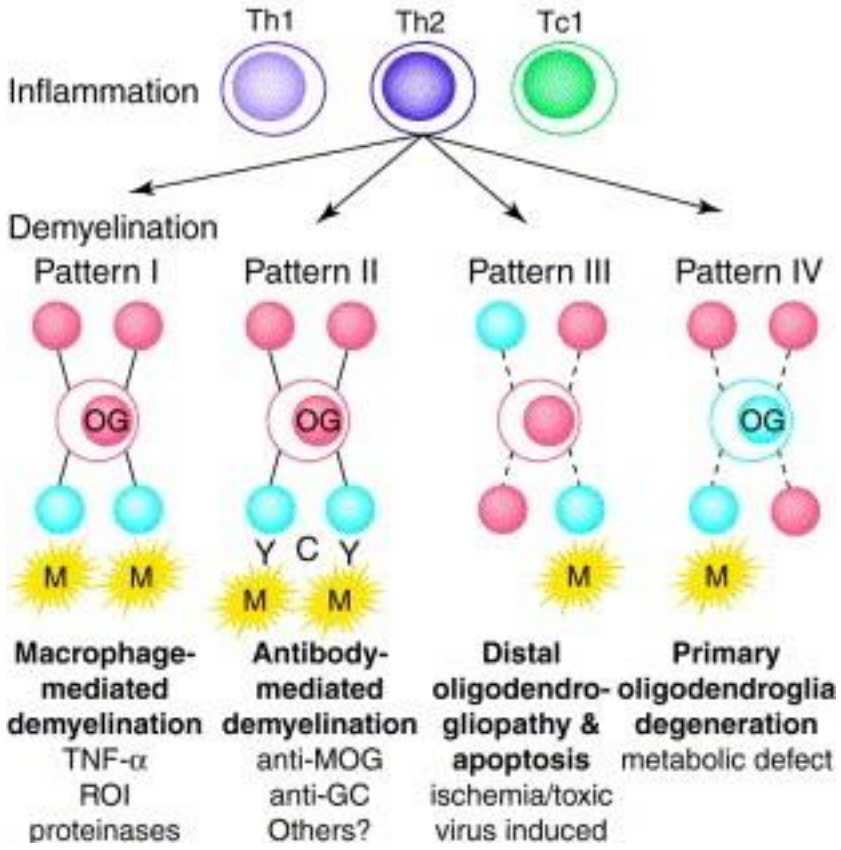
Altered human oligodendrocyte heterogeneity in multiple sclerosis

Sarah Jäkel[1,5], Eneritz Agirre[2,5], Ana Mendanha Falcão[2], David van Bruggen[2], Ka Wai Lee[2], Irene Knuesel[3], Dheeraj Malhotra[3,6], Charles ffrench-Constant[1,6*], Anna Williams[1,6*] & Gonçalo Castelo-Branco[2,4,6*]



Myelin, Oligodendrocytes (OL) and Precursors (OPC)

- Demyelination is a key characteristic in MS

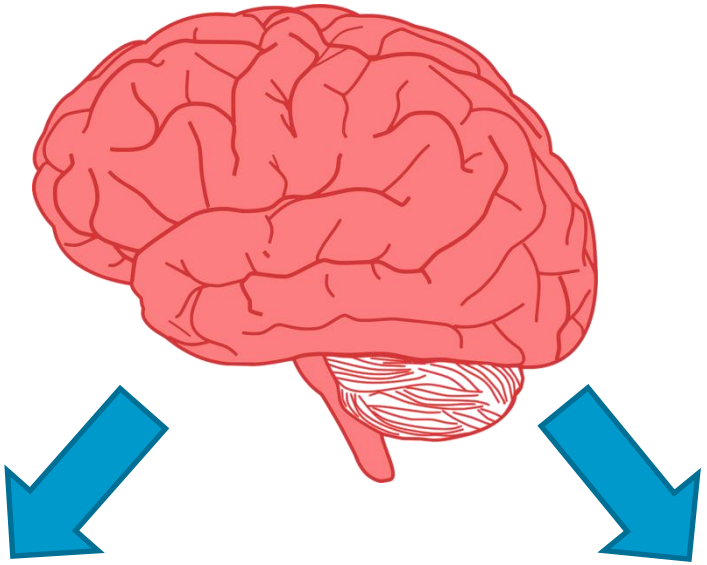


Myelin, Oligodendrocytes (OL) and Precursors (OPC)

- 40-50% of lesions do initially remyelinate (shadow plaques)
- Over the course: incomplete repair
- Reasons:

- Depletion of OL; OPC (TNF-mediated apoptosis)
- Myelination is blocked, while Migration, Proliferation and Differentiation not affected
- Unreceptive Axons (LINGO-1)
- Altered OL heterogeneity in MS patients

Role of CNS cells: Culprits and victims



Oligodendrocytes and myelin

Neurons and axons

Astrocytes

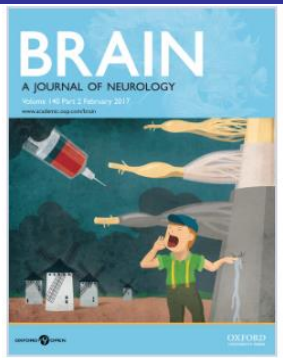
Microglia

Astrocyte physiology and pathology

- Architectural structure in the CNS: blood-brain-barrier, stabilization of synpases
- Metabolic support to neurons: glycogen, lactate
- Uptake of neurotransmitters (glutamate) to prevent excitotoxicity, regulation of extracellular K+
- Influence myelination by signaling to OL

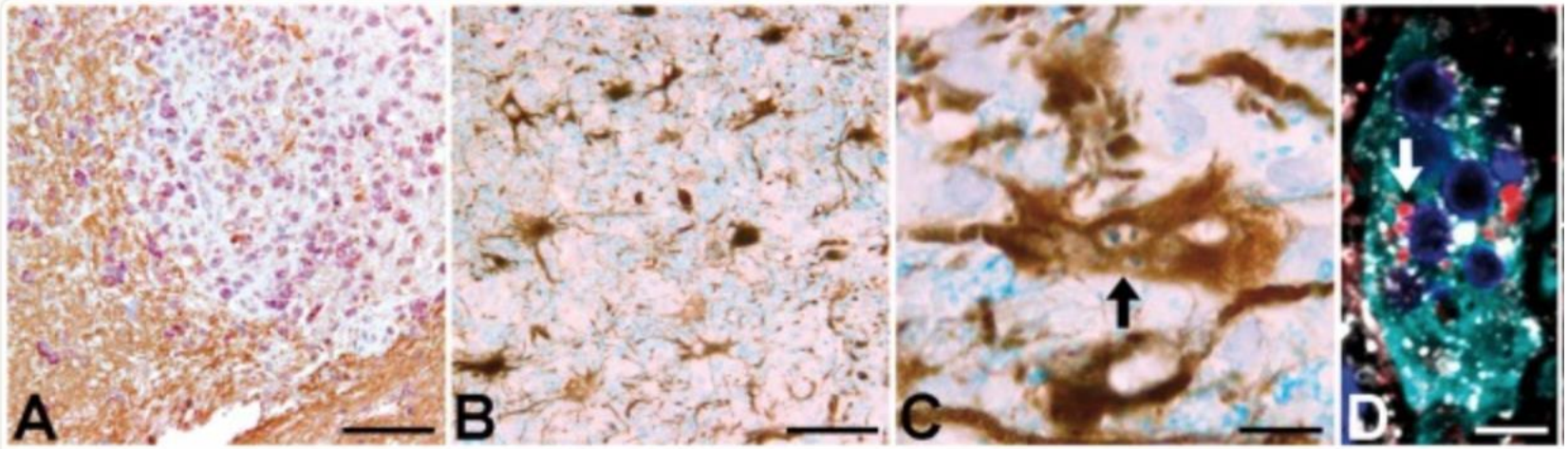
In disease:

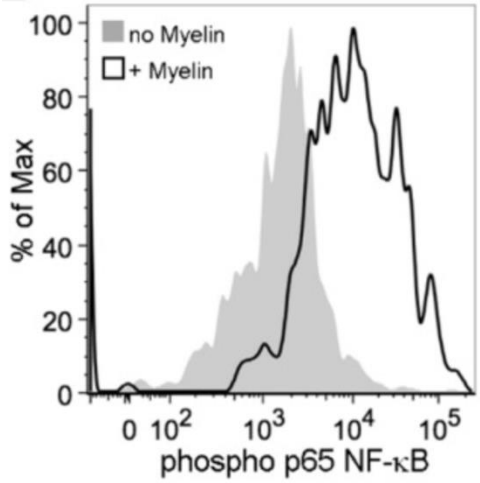
- Secretion of growth factors and physiologic functions vs.
- Inflamm. Cytokines (IL-1ß, TNFa), glial scars (sclerosis), ROS

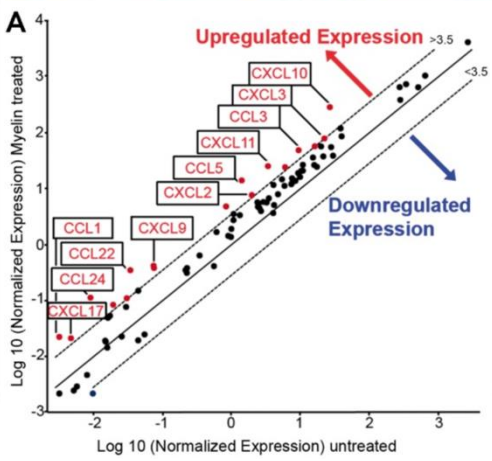


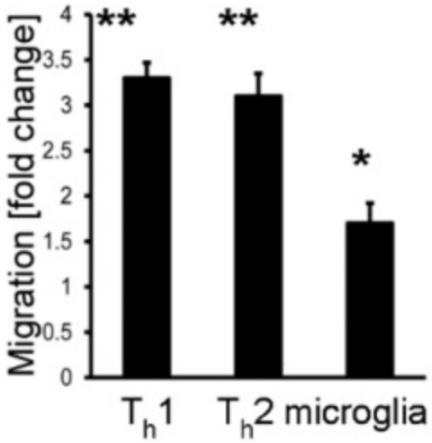
Myelin phagocytosis by astrocytes after myelin damage promotes lesion pathology FREE

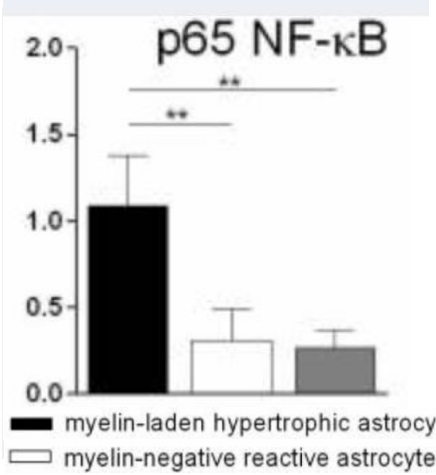
Gerald Ponath ✉ , Sriram Ramanan , Mayyan Mubarak , William Housley , Seunghoon Lee , F. Rezan Sahinkaya , Alexander Vortmeyer , Cedric S. Raine , David Pitt

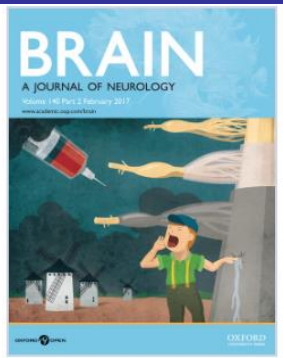












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- Astrocytes are activated in MS lesions, uptake of myelin debris
- In vitro: Myelin uptake leads to NFkB activation + nuclear translocation

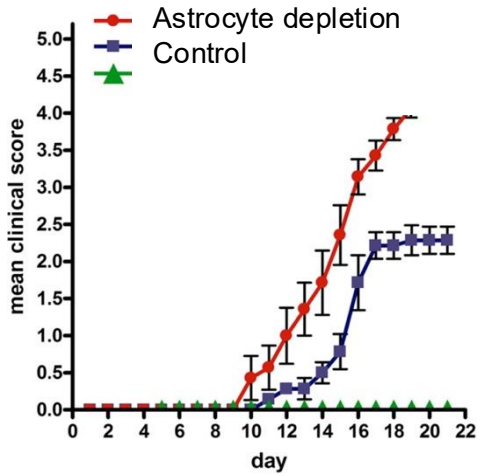
→ cytokine and chemokine secretion

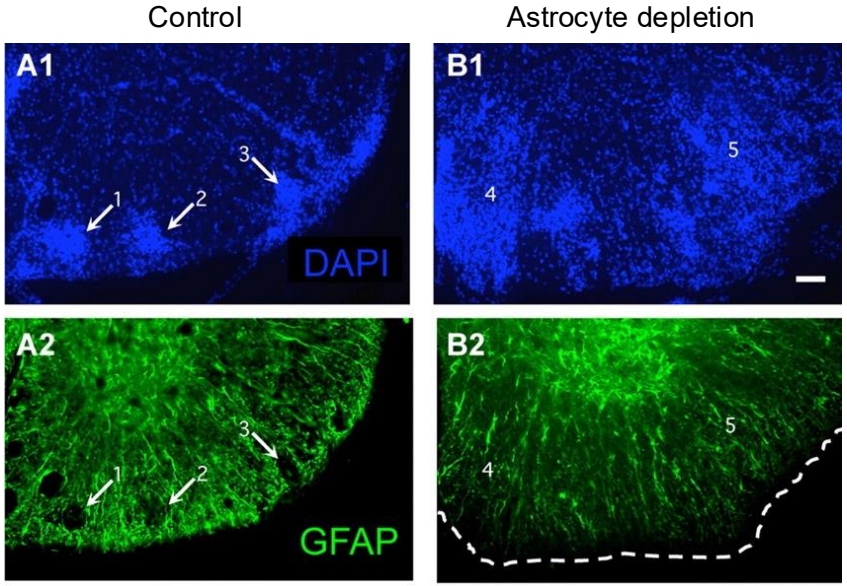
→ migration of lymphocytes

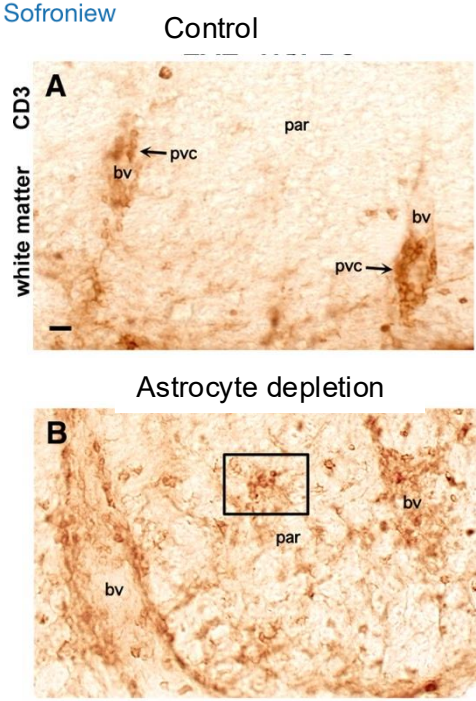
- Ex vivo: Nuclear NFkB is increased in myelin laden astrocytes in MS lesions, compared to myelin negative reactive astrocytes

Reactive Astrocytes Form Scar-Like Perivascular Barriers to Leukocytes during Adaptive Immune Inflammation of the CNS

Rhonda R. Voskuhl, R. Scott Peterson, Bingbing Song, Yan Ao, Laurie Beth J. Morales, Seema Tiwari-Woodruff, and Michael V. Sofroniew





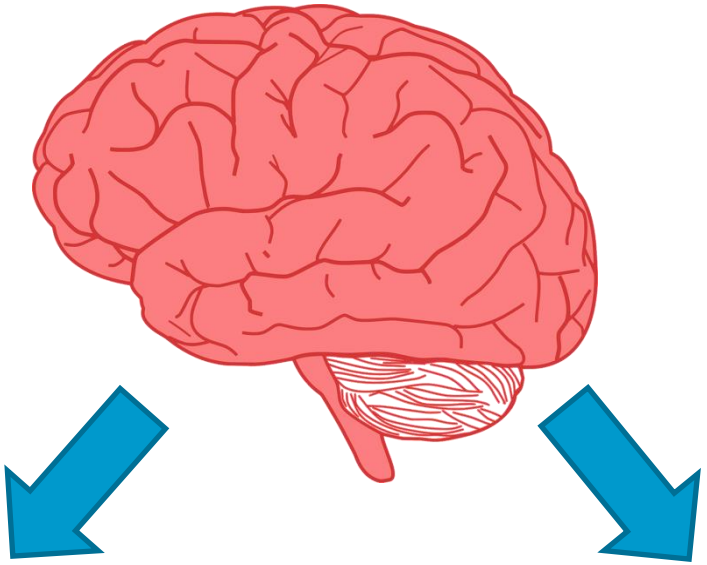


- Astrogliosis secludes inflammation
- In a MS disease model, mice with depleted astrocytes show greater disability
- Depletion of astrocytes leads to diffuse lymphocytic infiltration of CNS

Astrocytes: Janus-Faced Players in MS

- Not only passive players
- Actively involved in inflammation (Production of cytokines, microglia recruitment)
- Limit toxicity (Glutamate transporter) and possibly inflammation → Glial scar
- Gliosis limits long-term regeneration

Role of CNS cells: Culprits and victims



Oligodendrocytes and myelin

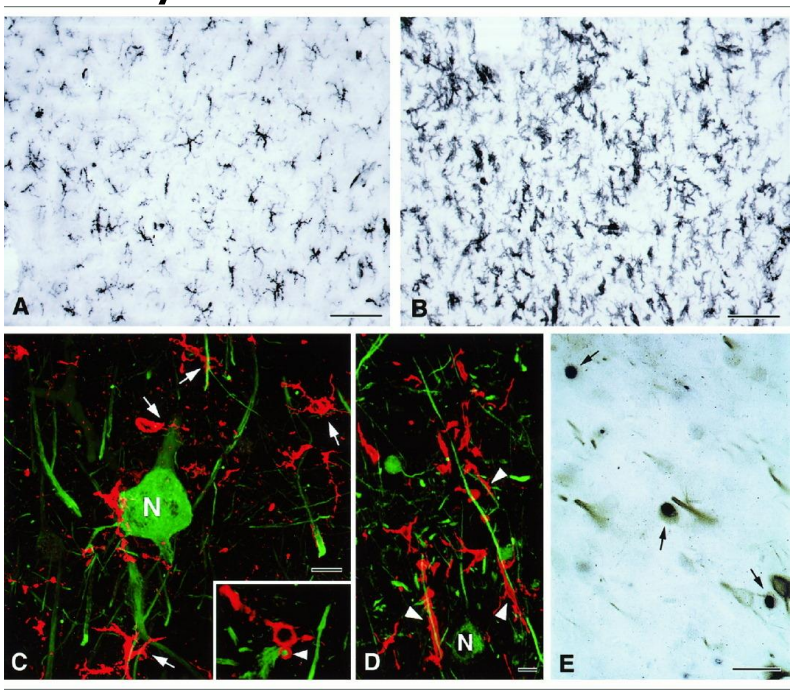
Neurons and axons

Astrocytes

Microglia

MS: Role of microglia

- CNS resident, local differentiation and proliferation
- Depending on subtype beneficial or deleterious effects on MS lesions
- Effects via Phagozytosis, NO, Cytokine production, proinflammatory milieu



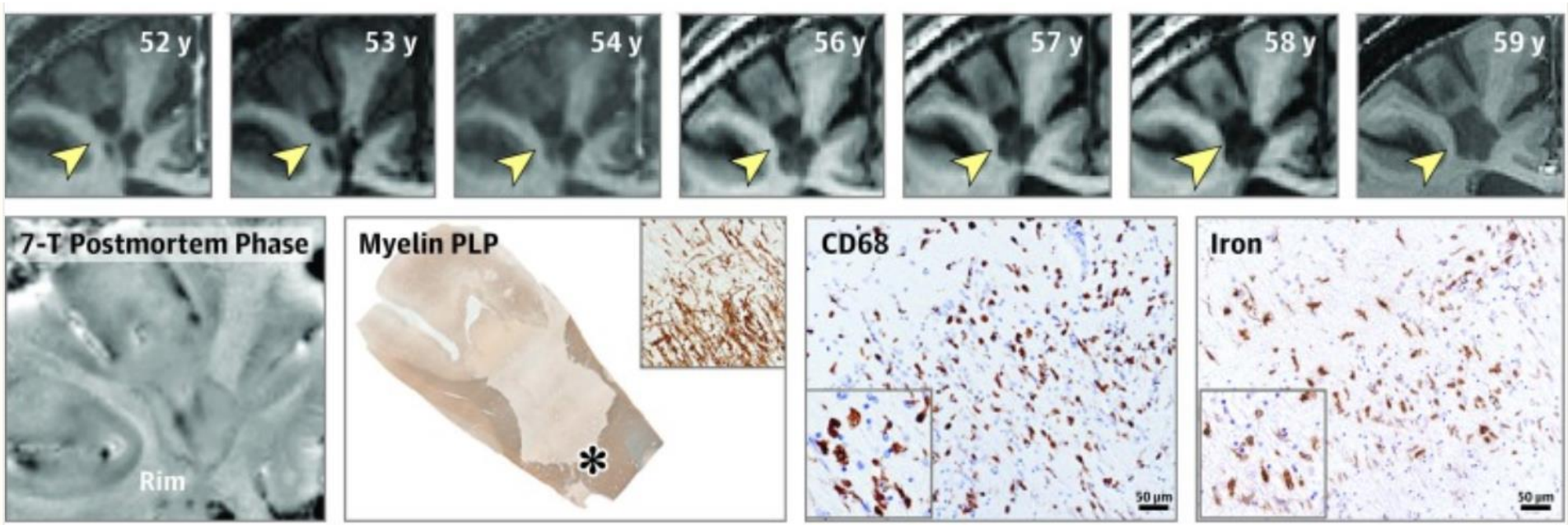
MS: Role of microglia

- Accumulation in chronic MS-stages
- Correlation with Neurodegeneration
- Not amenable to peripheral immunmodulation!

→ **Compartimentalization** of inflammation

→ „smoldering" MS lesions

Absinta et al. 2019, JAMA Neurol



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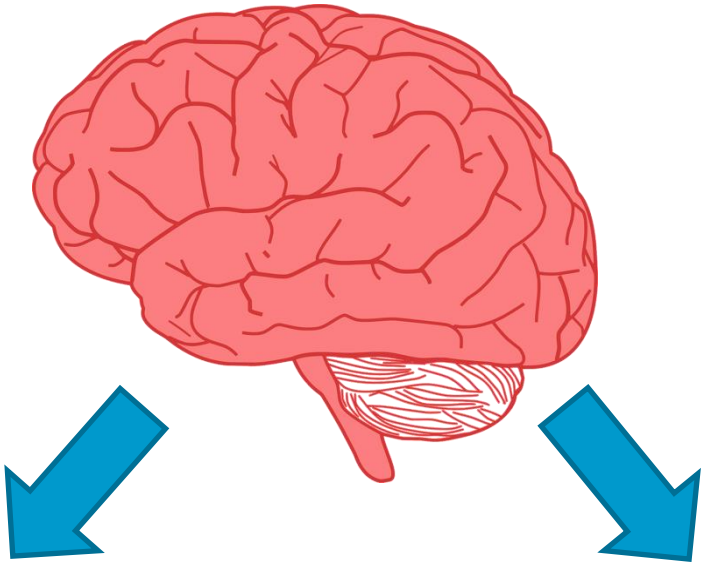


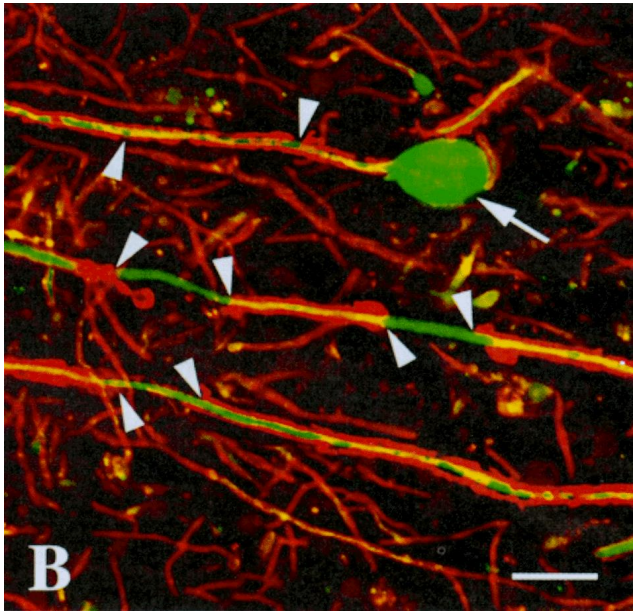
Oligodendrocytes and myelin

Neurons and axons

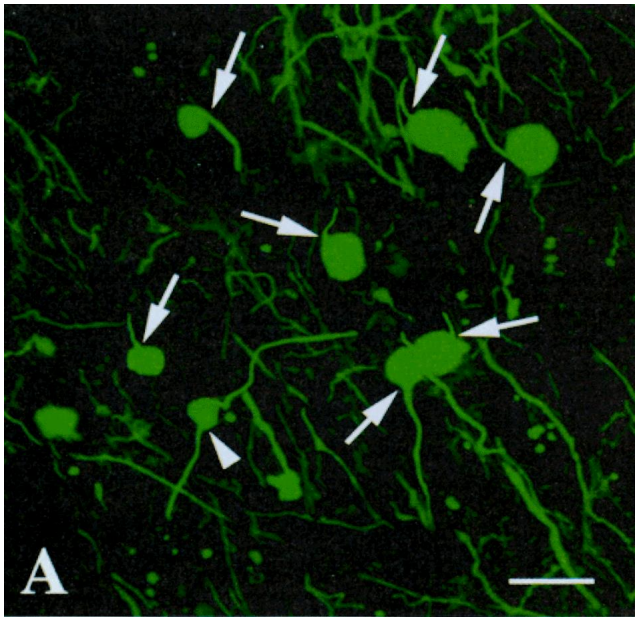
Astrocytes

Microglia

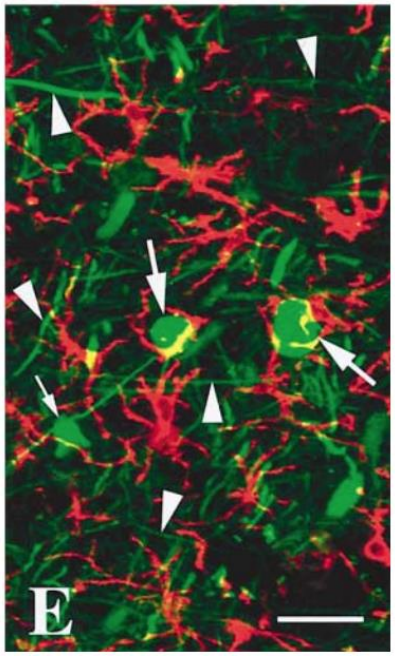
Neurodegeneration: Axonal Degeneration



Red: Myelin (CNPase)



Green: Axon marker (Neurofilament)

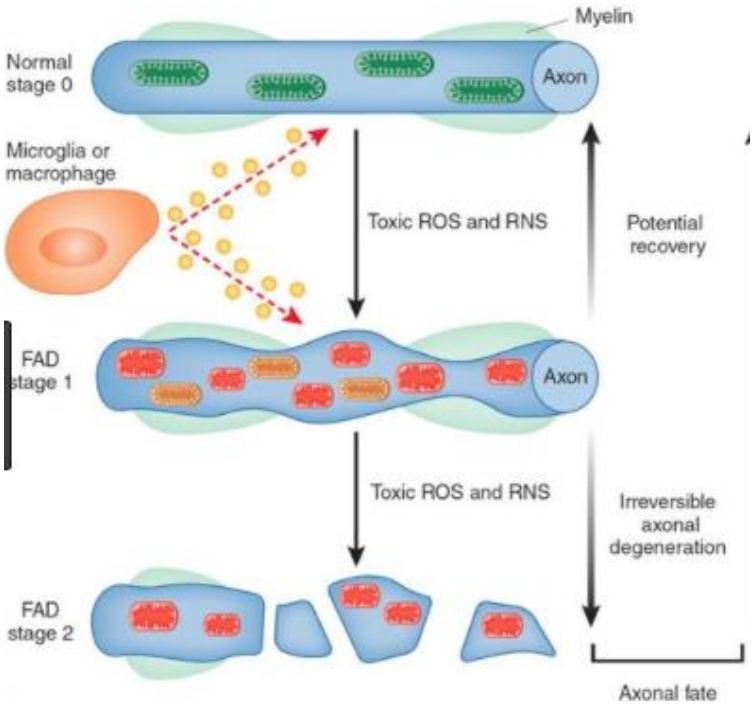


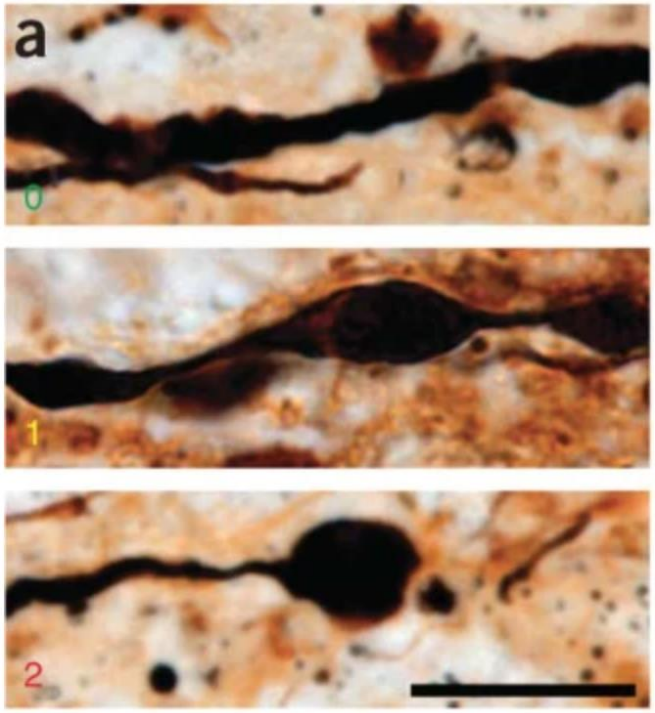
Red: Microglia

- Transection of axons as correlate of irreversible impairment
- Transected axons end in ovoids, which are engulfed by microglia

Trapp et al., NEJM 338, 278 (1998)

Focal axonal degeneration (FAD) in EAE and MS





- In EAE, early stages of FAD appear in spite of intact myelin
- FAD is induced by intra-axonal mitochondriopathy, which is induced by ROS from macrophages
- Similar axonal changes in MS lesion compared to FAD in EAE

Nikic et al., Nat Med, 2011

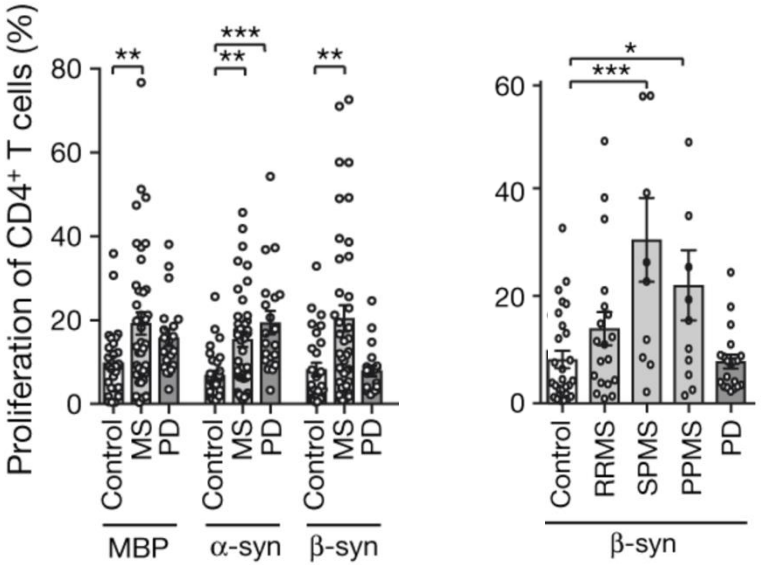
Grey Matter Damage in MS: Different targets?

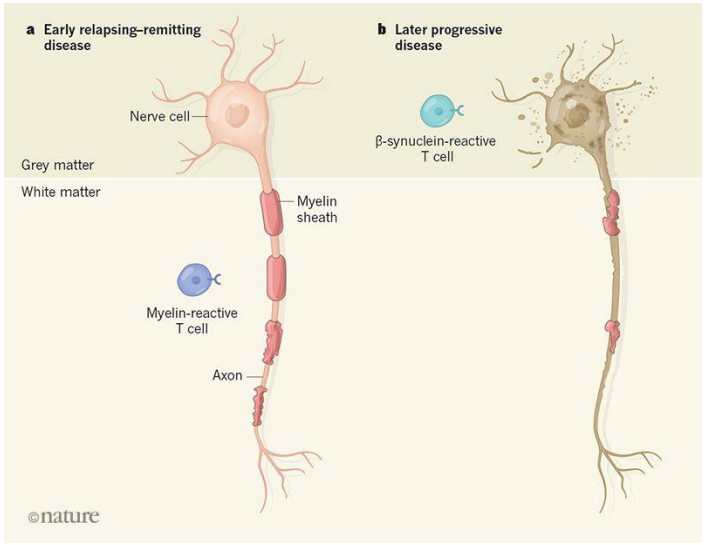
ARTICLE

https://doi.org/10.1038/s41586-019-0964-2

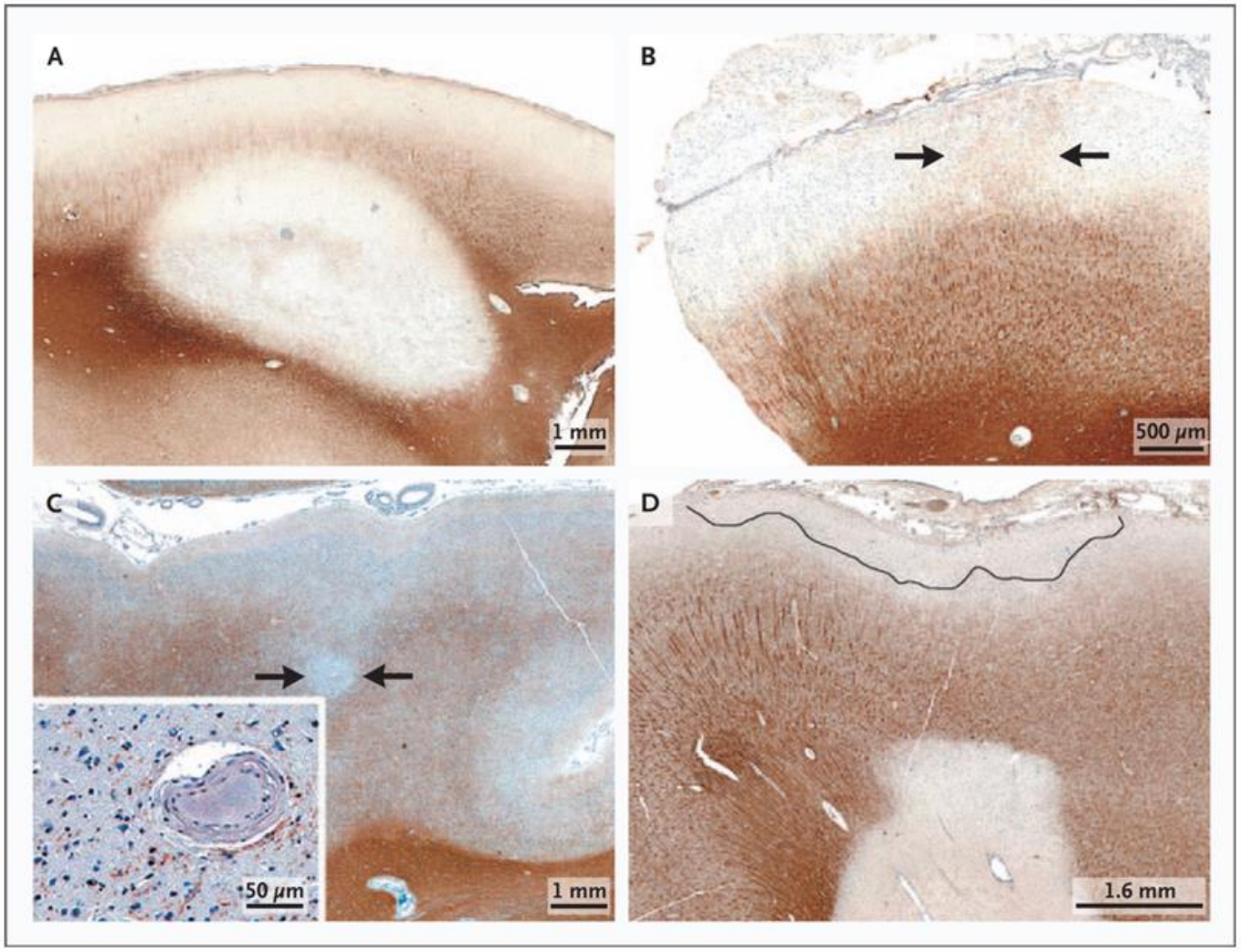
β-Synuclein-reactive T cells induce autoimmune CNS grey matter degeneration

Dmitri Lodygin[1,10], Moritz Hermann[1,10], Nils Schweingruber[1], Cassandra Flügel-Koch[2], Takashi Watanabe[3], Corinna Schlosser[1], Arianna Merlini[1], Henrike Körner[1], Hsin-Fang Chang[1], Henrike J. Fischer[1], Holger M. Reichardt[4], Marta Zagrebelsky[5], Brit Mollenhauer[6,7], Sebastian Kügler[7], Dirk Fitzner[7], Jens Frahm[3], Christine Stadelmann[8], Michael Haberl[1], Francesca Odoardi[1,9,11]* & Alexander Flügel[1,11]*



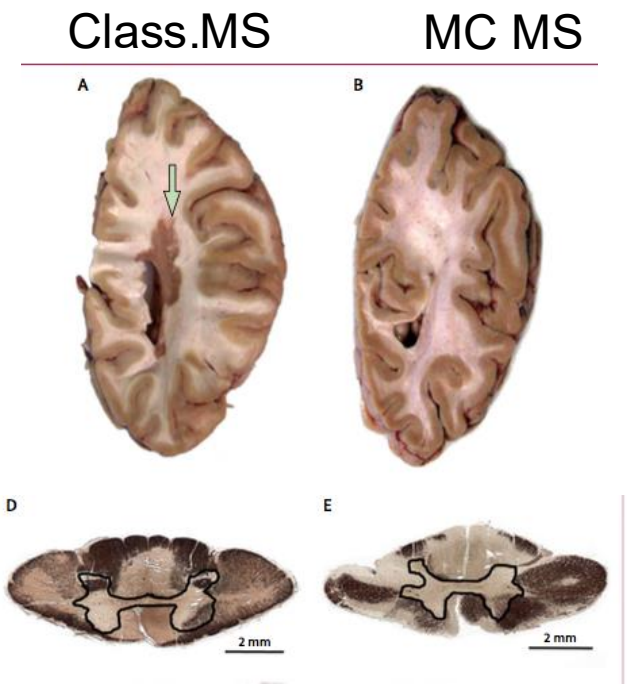


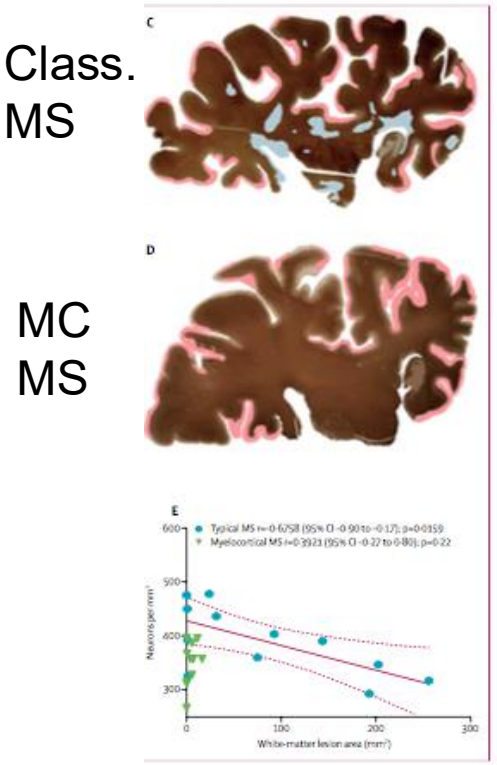
Demyelination takes place in white, but also in grey matter/cortex

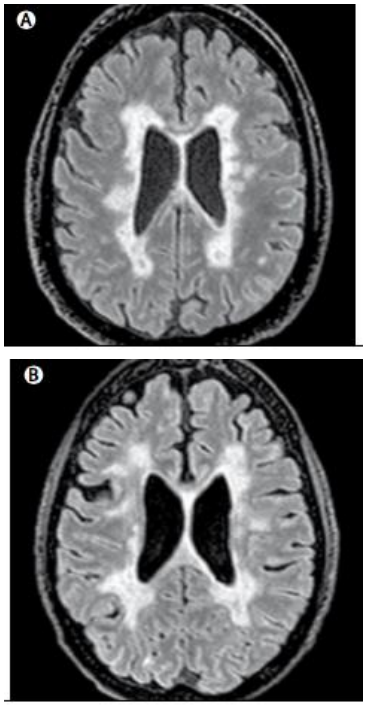


Lucchinetti et al., NEJM, 2011

Myelocortical (MC)-MS: a new entity?







- Correlation of WM demyelinated lesion and neuronal degeneration only in classical MS
- In MC MS, cortical neuronal degeneration appears independent of WM lesions

→ Distinct pathomechanisms?

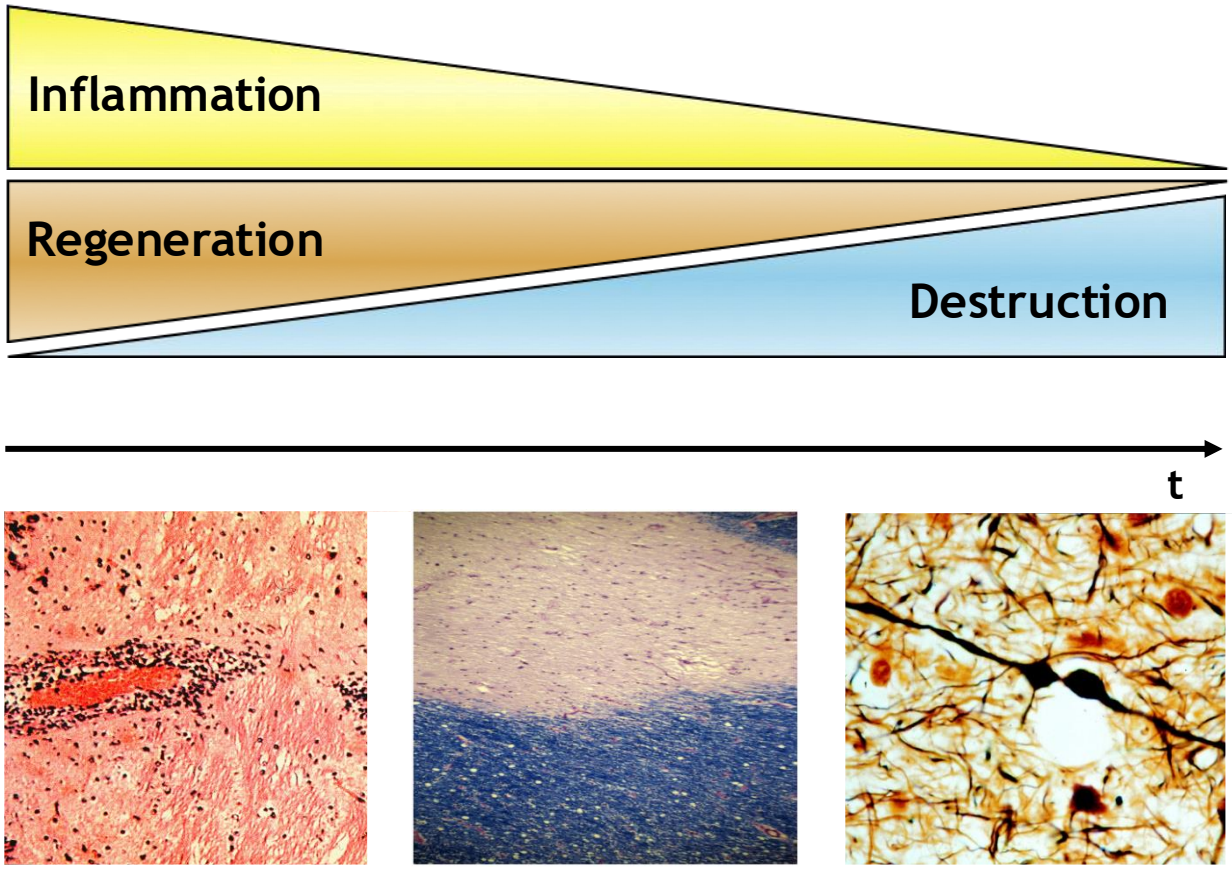
- Grey matter atrophy is present regularly in all MS-patients

Trapp et al., Lancet, 2018

Mechanisms of neuroaxonal injury

- Cytotoxicity (CD8-Cells, GrB, Perforins)
- Phagocytosis (Macrophages, Microglia, TNF)
- Oxidative Stress (ROS/NO)
- Chronic Demyelination:
  - Lack of trophic support (growth factors)
  - Conduction block (electr. silcening)
  - Energy deficiency
- Ionic imbalance

Immunpathogenesis of Multiple Sclerosis



Neuroaxonal Damage = Basis for persistent disability
Degeneration starts early and increases over time
Dissociation from inflammation

How can we investigate the pathophysiology of multiple sclerosis and identify/evaluate therapeutic approaches?

Pathology, human material

- Easy to obtain: Blood, CSF, but only limited information on pathophysiology
- Restricted access to brain/spinal cord tissue, especially from young adults

Animal models

- Easy to obtain: Brain, spinal cord, blood
- BUT: there is no multiple sclerosis in animals

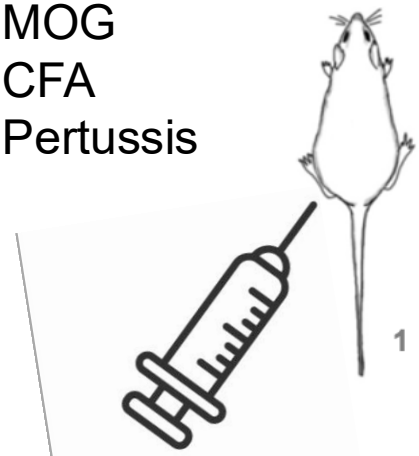
→ Necessity of (more or less) valid disease models

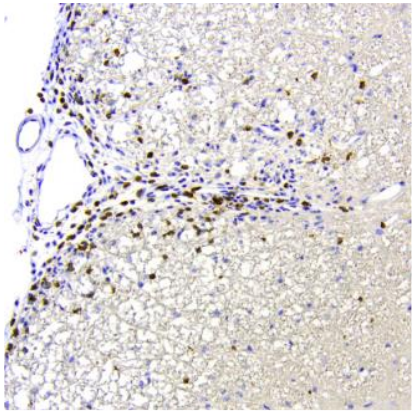
Experimental autoimmune encephalomyelitis

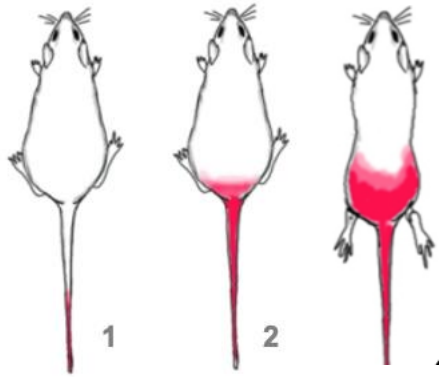
- Rabies vaccine was initially made from rabbit spinal cords
- In some patients, which received the vaccine an encephalomyelitis developed

Experimental autoimmune encephalomyelitis MOG-EAE

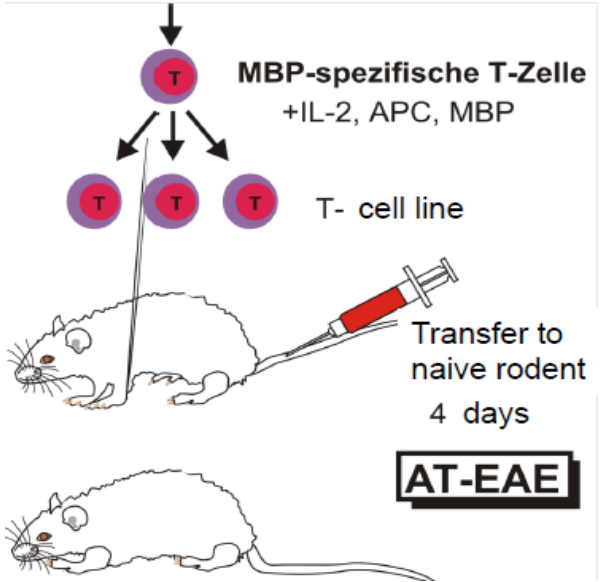
MOG
CFA
Pertussis





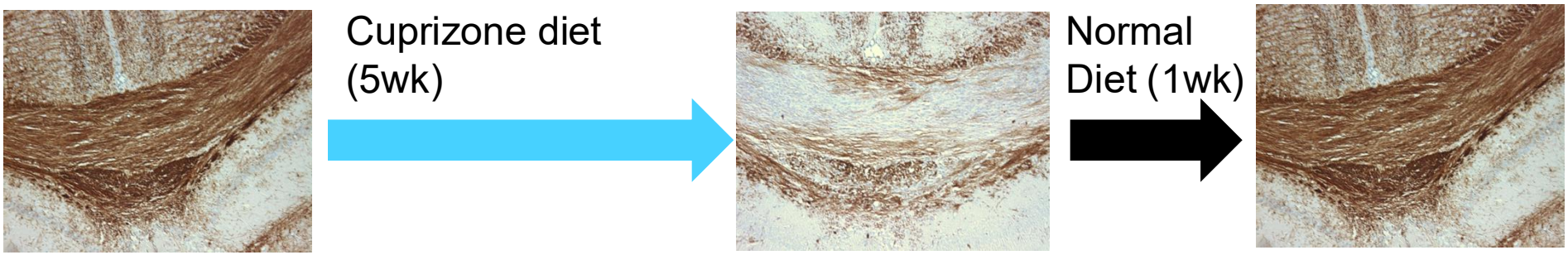


- Generation of MOG-specific T cells
- Migration to CNS
- Demyelination and Gliosis



Cuprizone model of toxic de- and remyelination

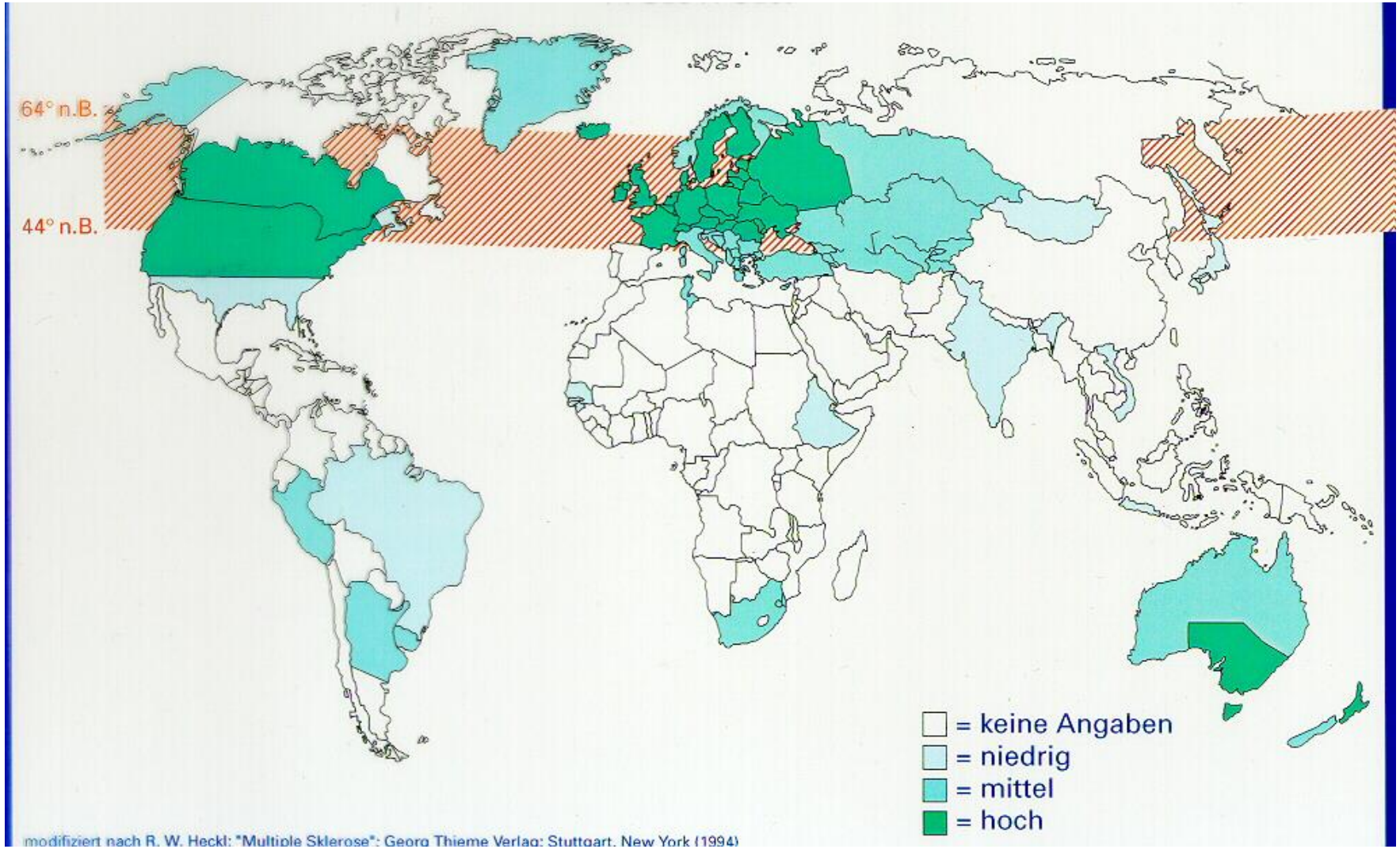
- Cuprizone is a copper-chelator
- induction oligodendrocyte apoptosis by ROS as result of respiratory chain disruption



MS – Animal models

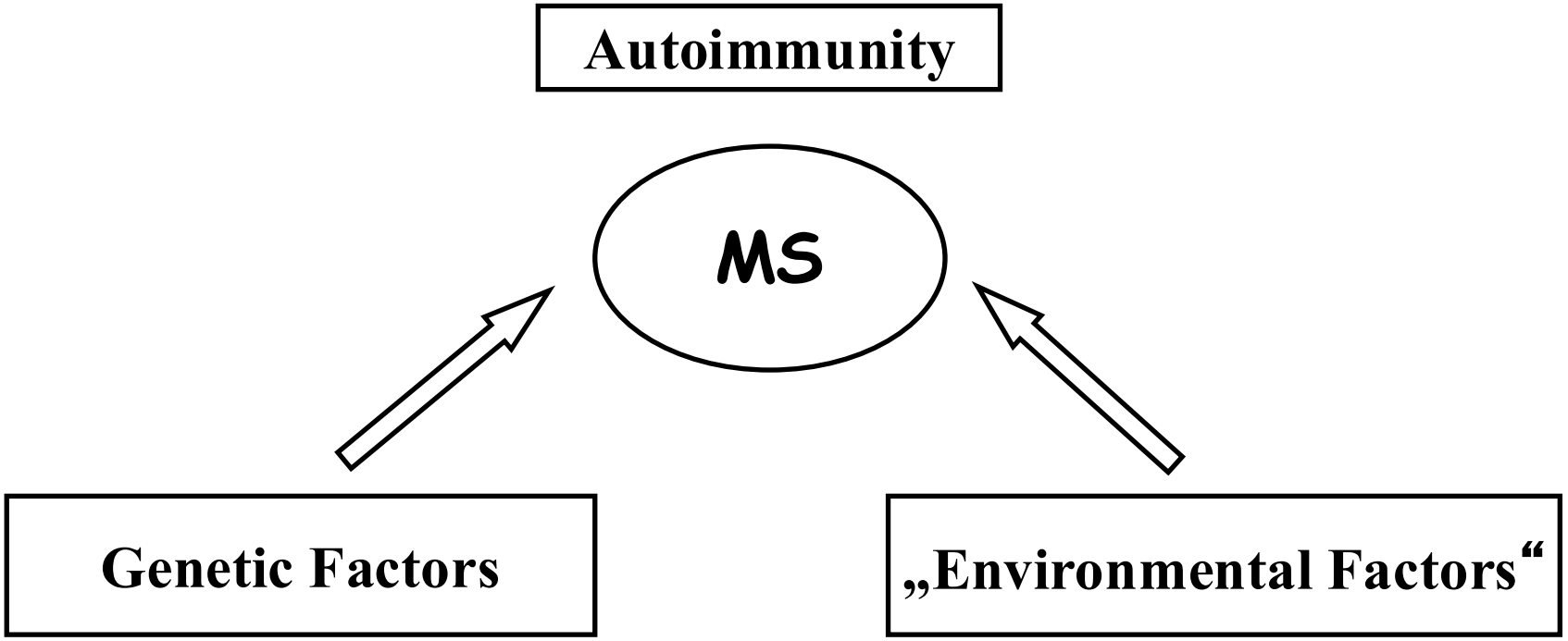
- Active/passive EAE (Mouse, Rat, Marmoset)
- Transgenic Models (MOG B/T Zell transgenic mice)
- Toxic models of demyelination: Cuprizone (oral), Lysolecithin/EtBr (Injection)
- Viral Models of demyelination: Theiler Virus Infection (Murine, CD8 cells)

MS – Worldwide distribution



Moving < 15: Risk of target country
Moving > 15: Risk of birth country

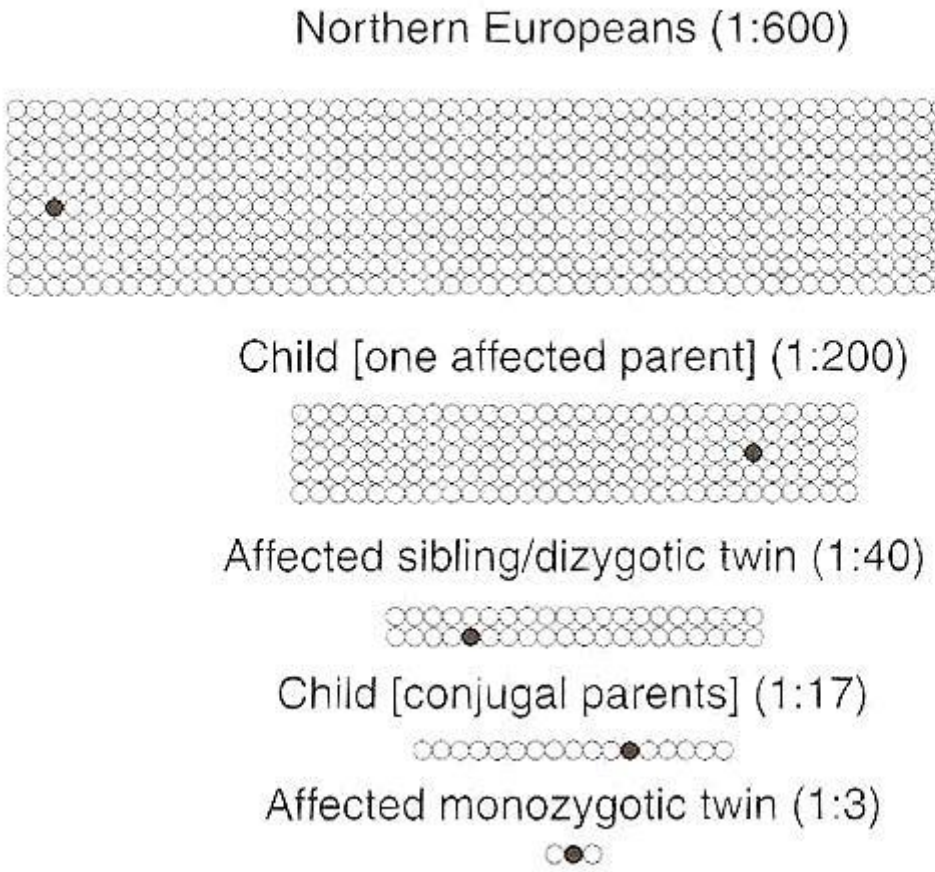
Multiple Sklerose - Etiology



1/3

2/3

Genetic factors– MS running in families



*HLA-DRB1*15:01* – OR 3,08

Genome wide association studys identified >200 susceptibility variants

Possible environmental factors increasing the risk of MS

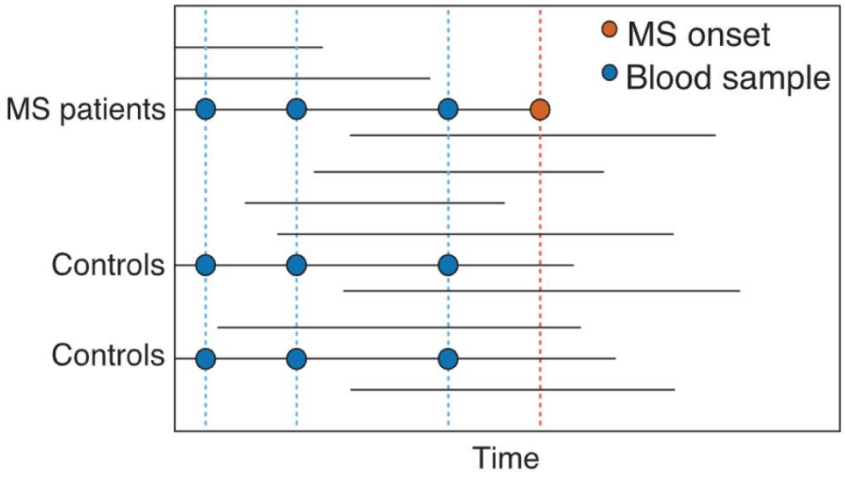
- Viruses: Epstein Barr Virus (EBV), Human endogenous retrovirus (HERV-W)
- Vitamin D deficiency/UV Light
- Dietary Factors: Fat, Salt
- Lifestyle: Smoking

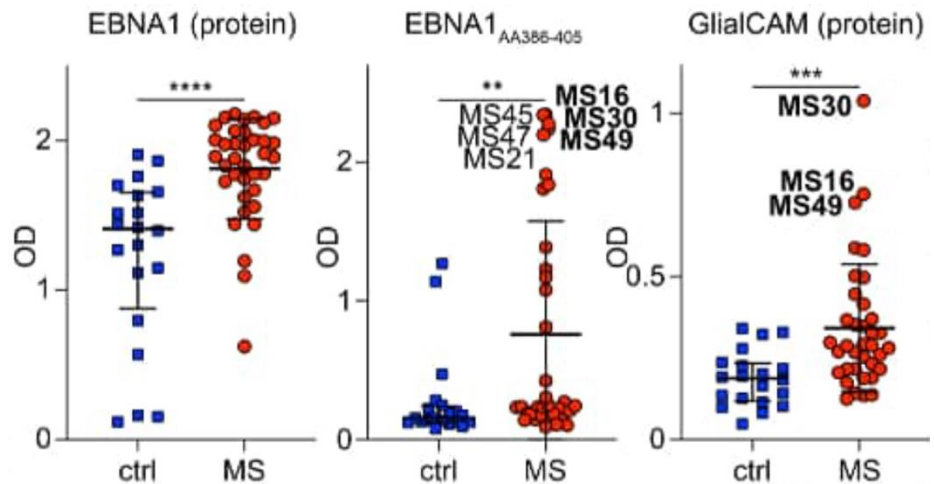
Science

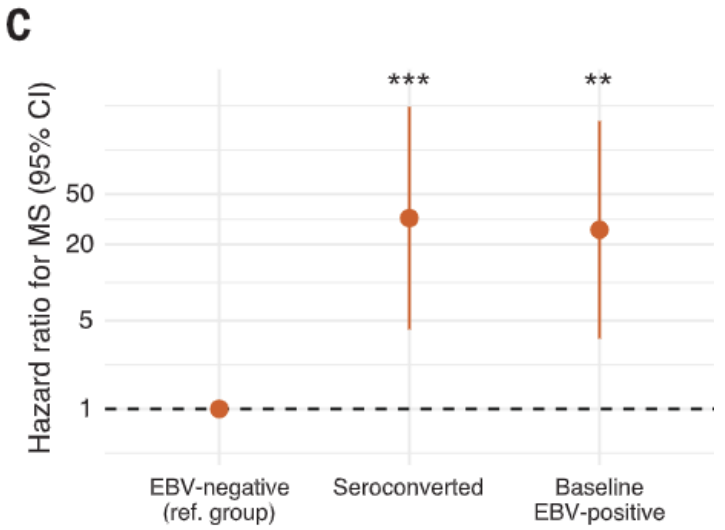
Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis

Kjetil Bjornevik et al.

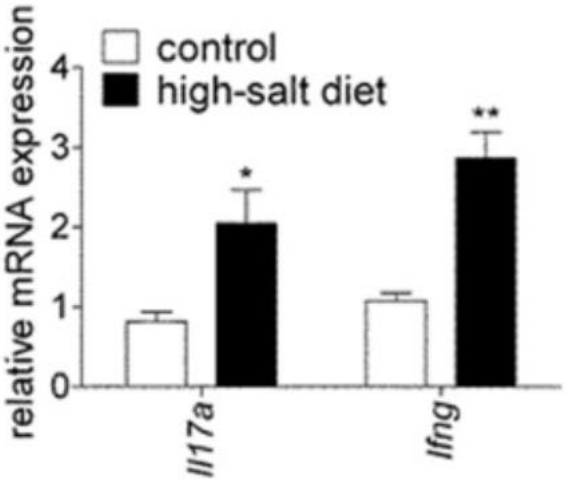
- 62x10^6 samples
- 1 /801 soldiers was EBV neg. before MS diagnosis
- HR for MS in EBV pos. vs. neg: 32
- Possible cross-reactivity?







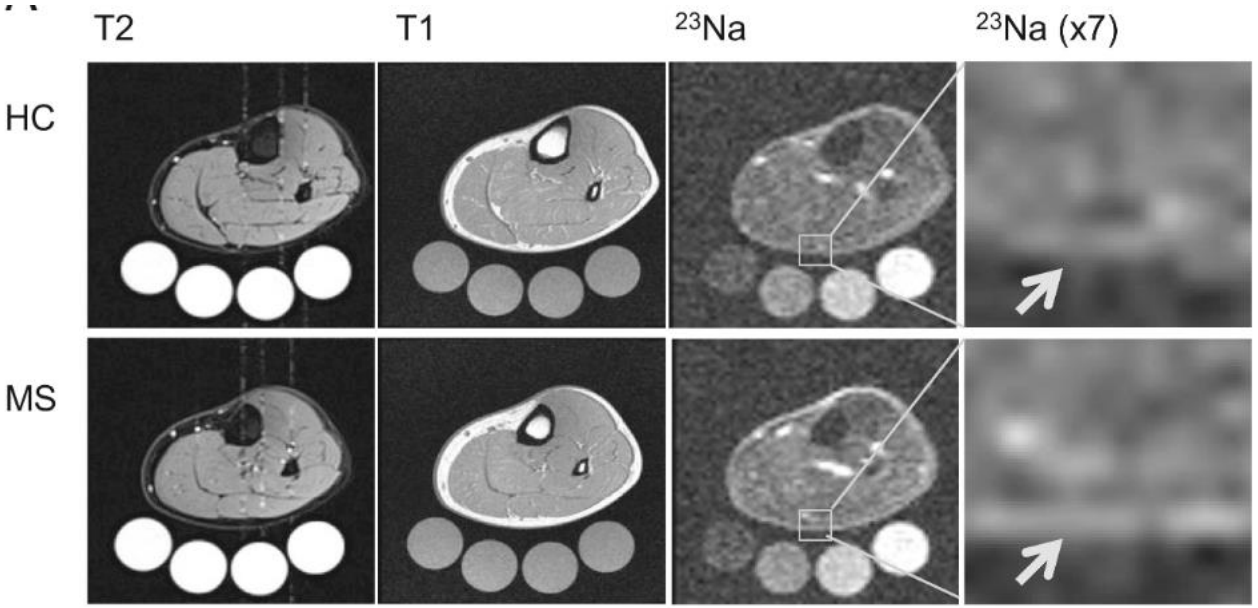
High salt diet in experimental neuroinflammation

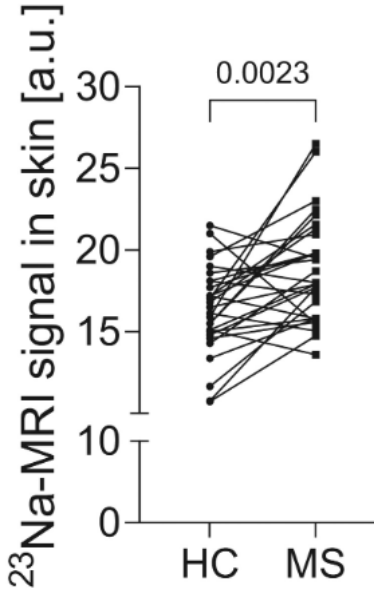


- Mice, which are fed a high-salt diet develop a more severe EAE
- this correlates witch an increased expression of IL-17 and IFNy in CNS-infiltrating lymphocytes

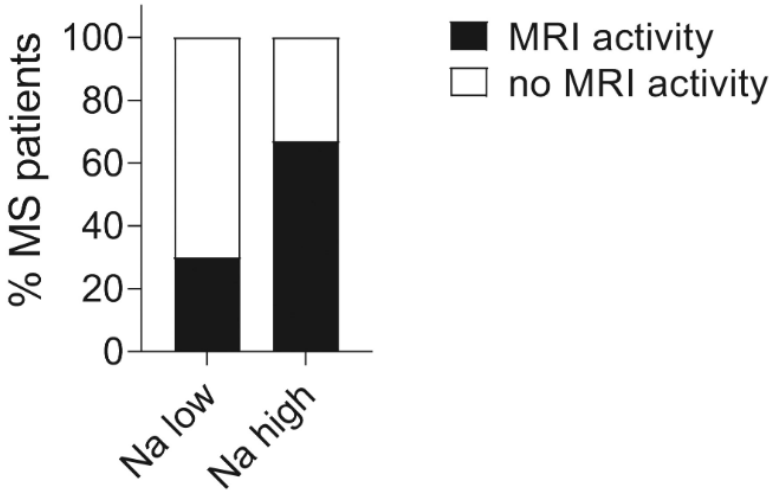
- Is high salt also relevant in MS?

High salt diet in experimental neuroinflammation

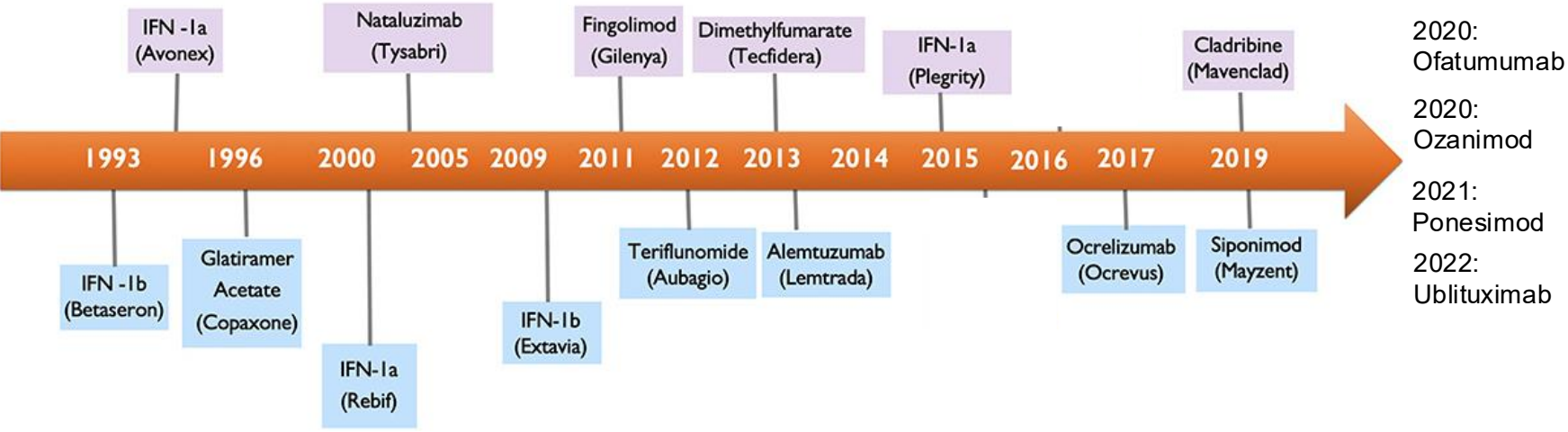




- Skin of MS patients contains more salt than HC
- High skin salt correlates with disease-activity

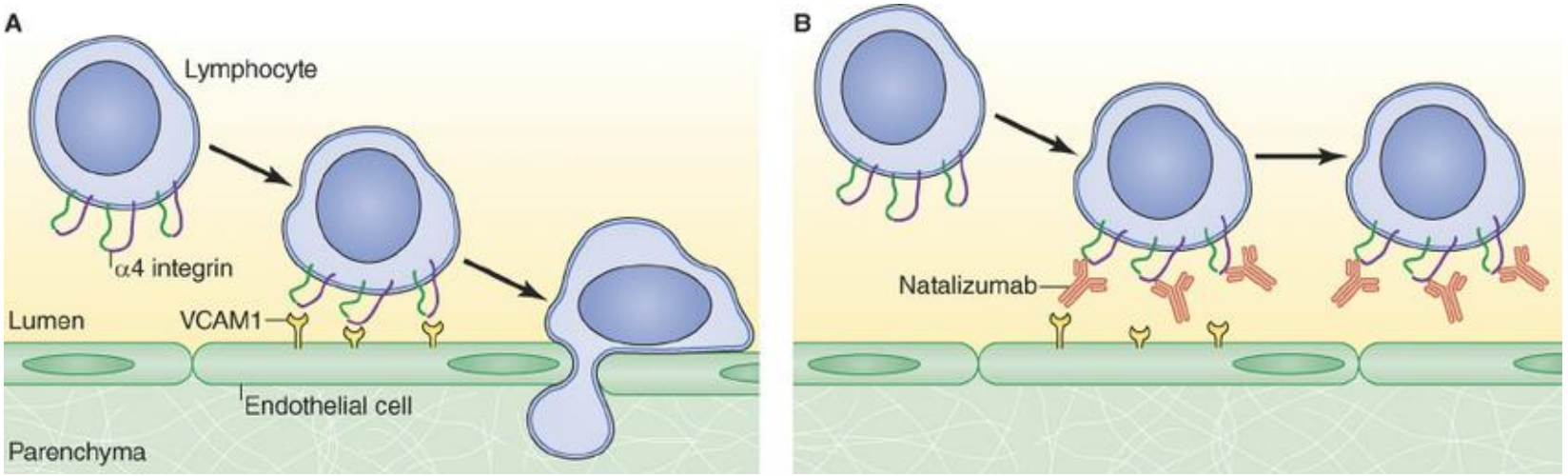


Disease modifying therapies in MS

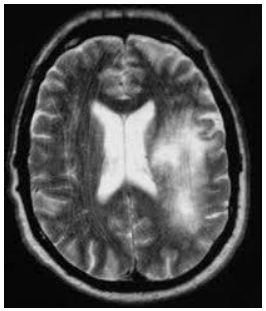


- DMT are used to prevent relapses, new lesions and increase in disability
- Efficacy, mode of action and mode of application differ

Natalizumab



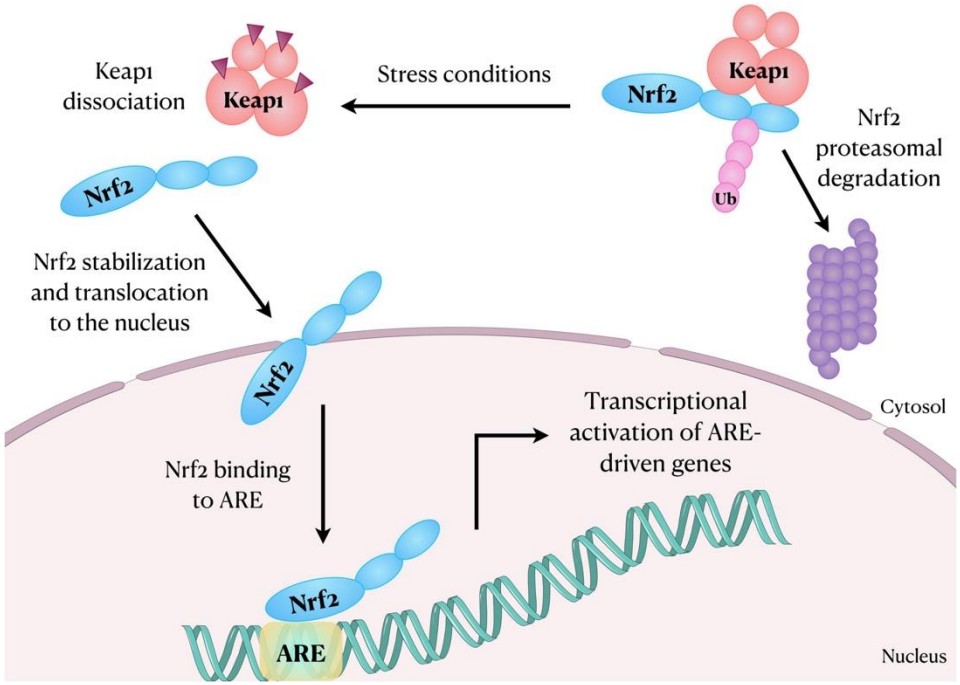
- mAB blocks α4-integrin on lymphocytes to prevent adhesion to endothel and therefore diapedesis
- Highly effective in prevention of MS, but risk of cerebral JC virus infection: PML



Dimethylfumarate - DMF

- DMF mimics oxidative stress
- Oxidation of cystein in Keap-1 → disinhibition of Nrf2 → upregulation of anti-oxidative pathways
- Oxidation of cystein in NFkB prevents phosphorylation and translocation to the nucleus

  → reduction of Th1 and Th17
- Lower efficiency compared to CD20 AB or Natalizumab



Stefanie today

- Therapy with Ofatumumab (s.c. CD20 ab), 1 injection/month, well tolerated
- No relapses, no disability
- Finishing studies

- Hopefully happy end..

.. no long term data on highly effective therapies

Summary: MS as network disease