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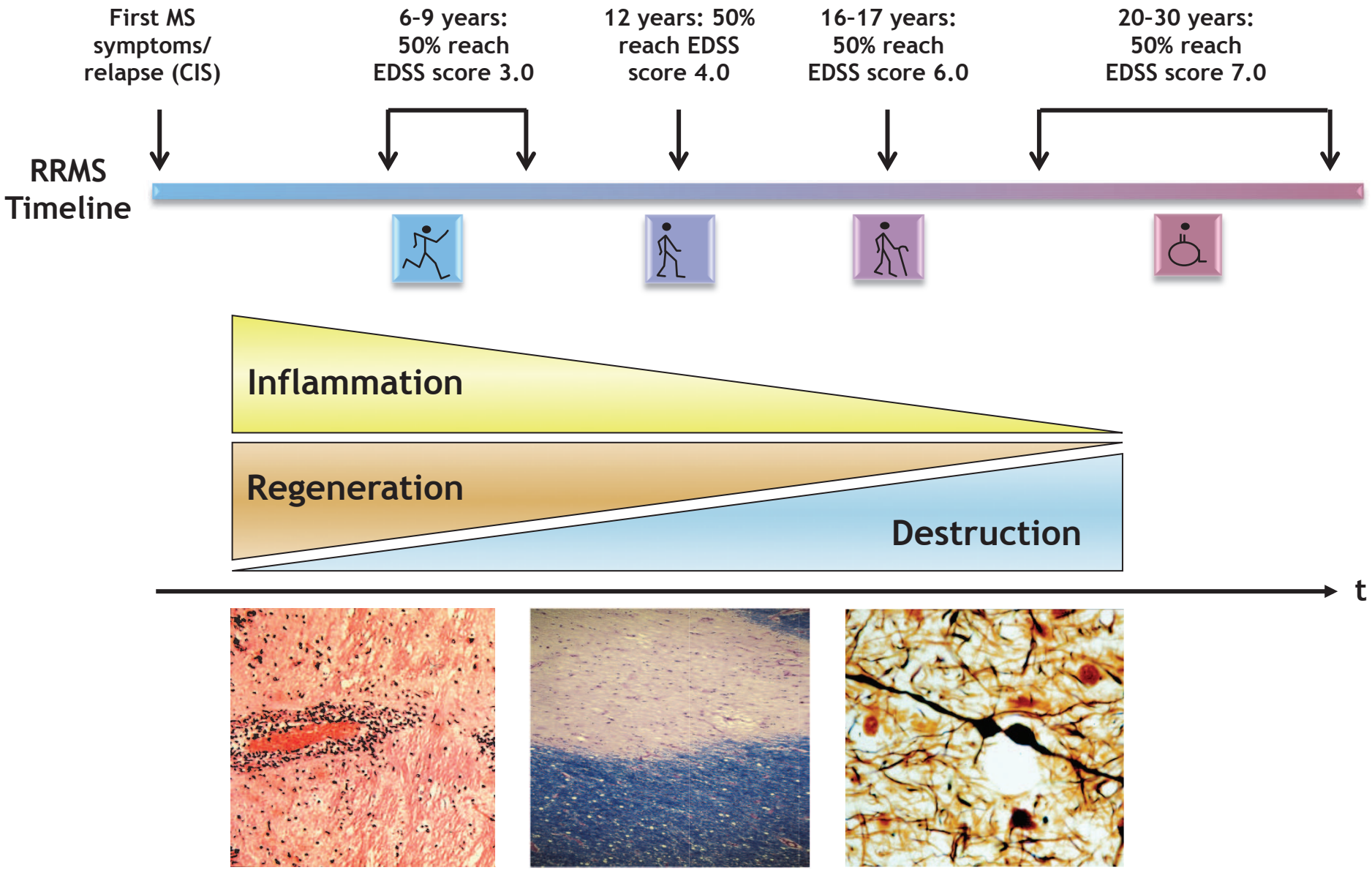
Infiammazione e neurodegenerazione nella sclerosi multipla

Orientarsi tra possibilità e rischi

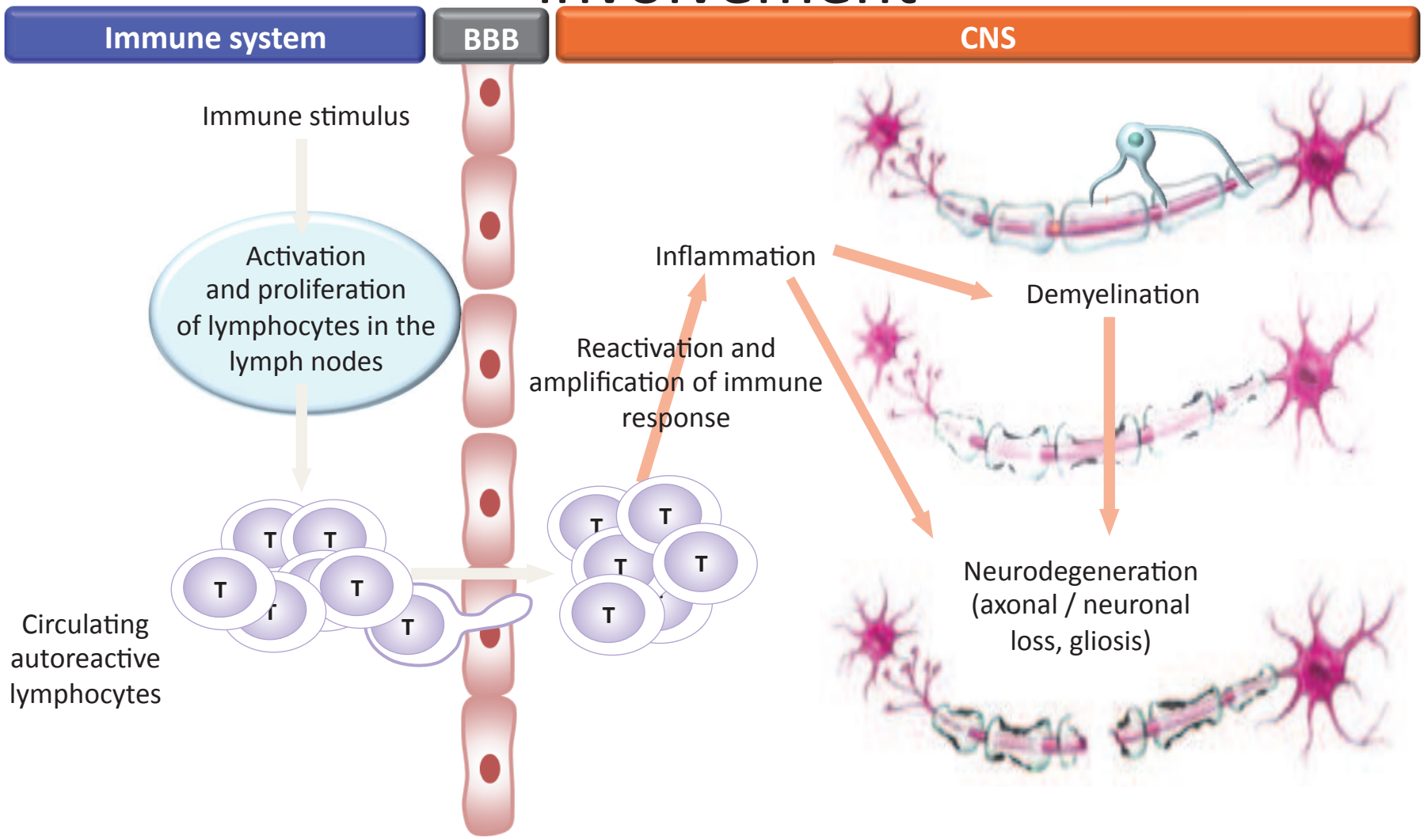
Monza Auditorium – 10 Giugno 2015

Center of Excellence for Biomedical Research
- University of Genoa -

The clinical course mirrors different stages of pathology



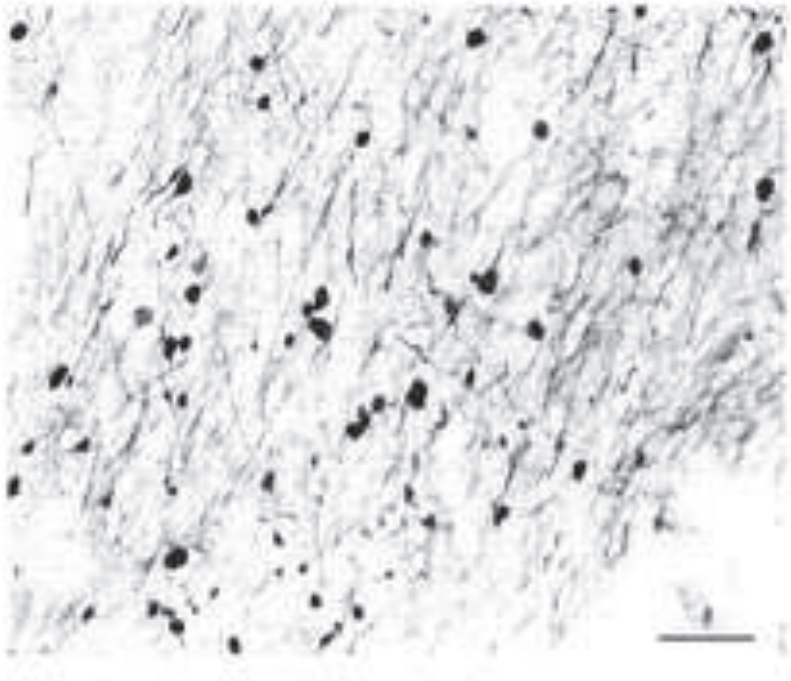
MS pathology: immune system and CNS involvement

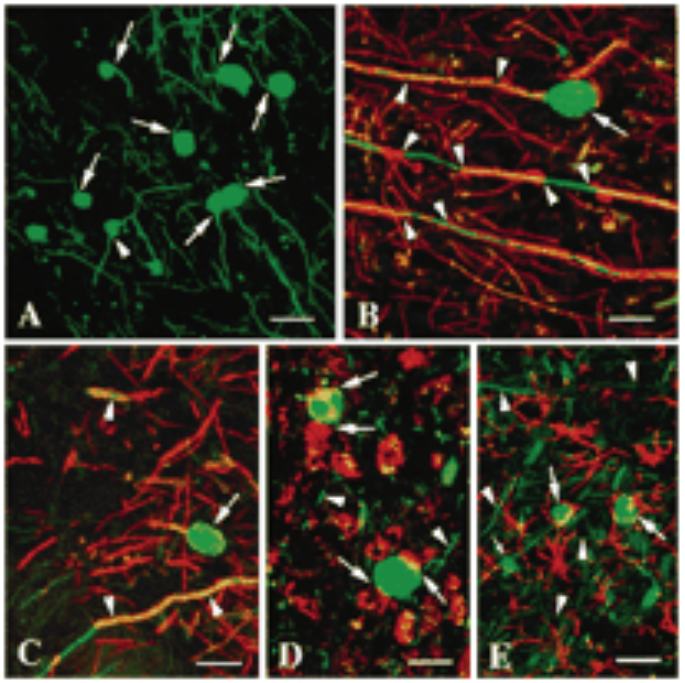


BBB, blood / brain barrier; CNS, central nervous system

Chun J, Hartung HP. *Clin Neuropharmacol* 2010; Mehling M *et al. Neurology* 2010

Il danno assonale è precoce, associato all'infiammazione ma nelle fasi iniziali della malattia è transitorio. Nelle fasi successive, la sofferenza cronica dell'assone porta alla degenerazione del neurone e alla perdita completa della funzione neurologica (FASE PROGRESSIVA)





Trapp, 1998

Pathological Differences between RRMS and Progressive MS (SPMS, PPMS)

- New waves of inflammation entering the CNS from circulation
- Focal demyelinating lesions with variable axonal injury and blood brain barrier injury mainly in the white matter

- Compartmentalized inflammation in the CNS
- Slow expansion of pre-existing white matter lesions
- Diffuse inflammation and axonal injury in NAWM
- Extensive cortical demyelination

Kutzelnigg et al 2005, Hochmeister et al 2006, Frischer et al 2009

Data suggesting a potential pathogenic role of myelin-reactive CD4 Th1 cells in humans

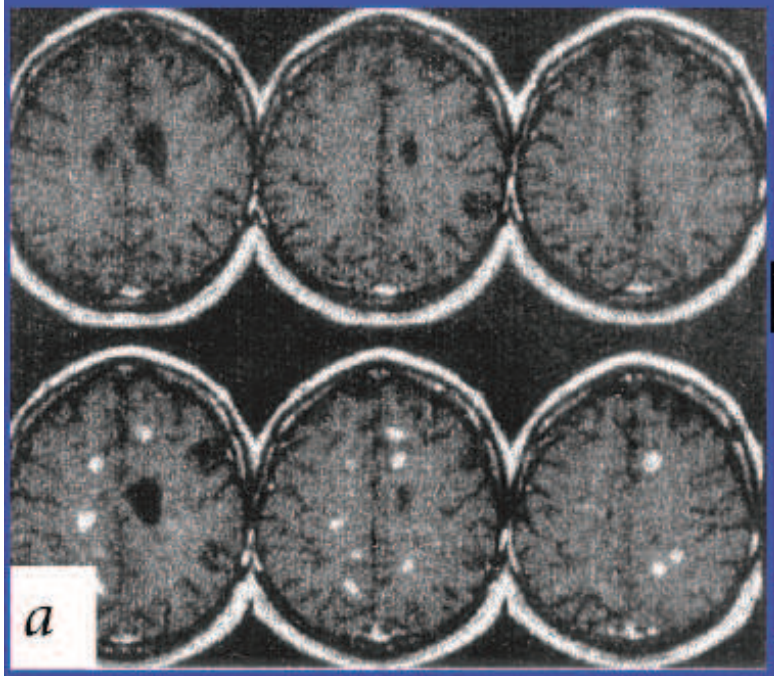


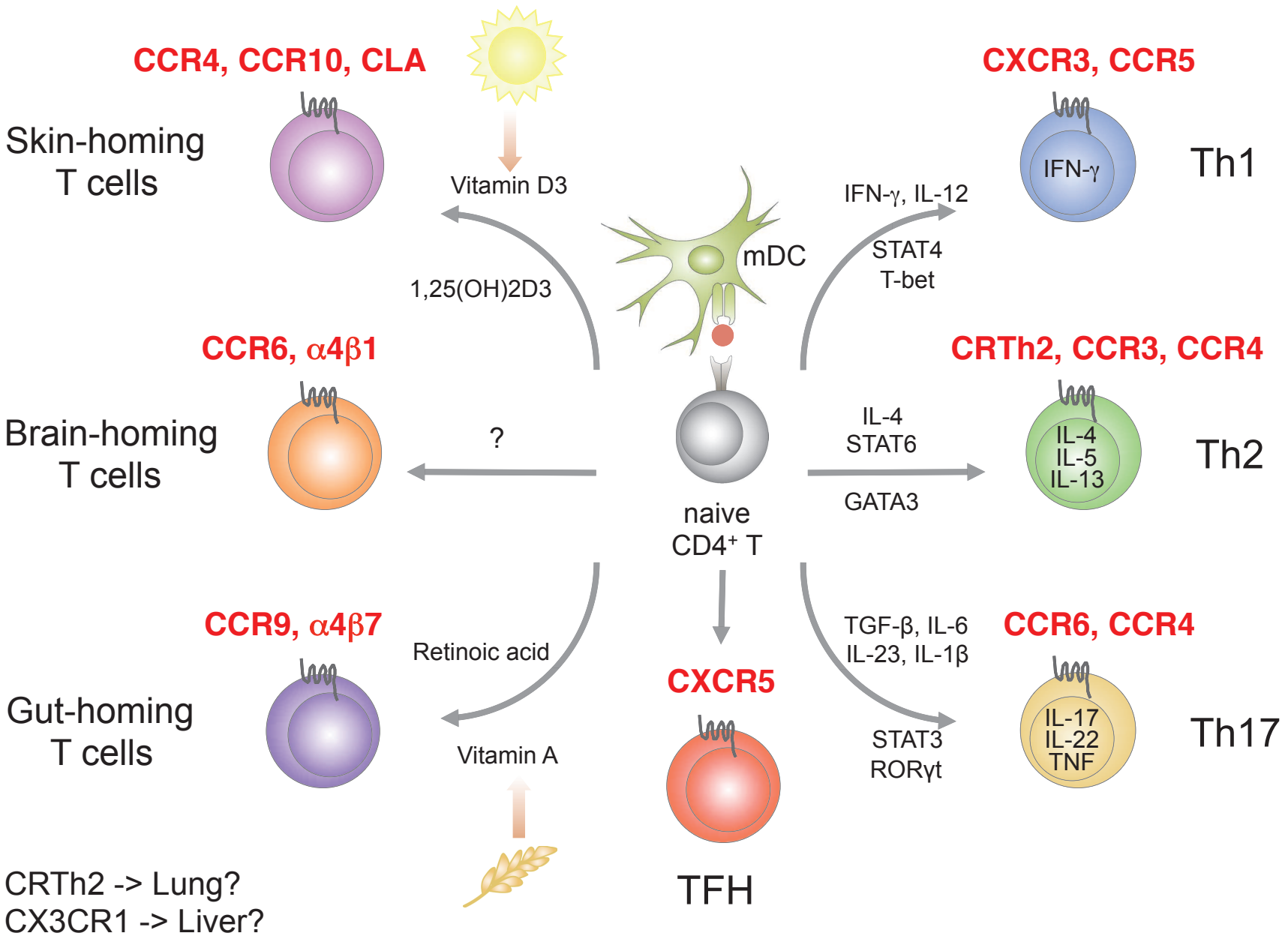
Table 2 Precursor frequencies of T cells specific for $MBP_{[83-99]}$ and APL in patients MS502 and MS601 during APL trial

MS patient	Ex vivo selecting antigen	Specificity and cross-reactivity	Pre-trial baseline	MS exacerbation during APL therapy	
			PBMC	PBMC	CSF
MS502	$MBP_{[83-99]}$	Specific for $MBP_{[83-99]}$ only	1 of 3.3×10^6	1 of 840	1 of 571
		Cross-reactive with APL	0	1 of 1400	1 of 800
	APL CGP771	Specific for APL only	1 of 1.2×10^6	1 of 620	1 of 1,200
		Cross-reactive with $MBP_{[83-99]}$	0	1 of 1320	1 of 2,400
MS601	$MBP_{[83-99]}$	Specific for $MBP_{[83-99]}$ only	1 of 3.0×10^6	1 of 0.4×10^6	1 of 667
		Cross-reactive with APL	0	1 of 1.2×10^6	NT
	APL CGP771	Specific for APL only	1 of 0.79×10^6	1 of 1.5×10^6	1 of 2,000
		Cross-reactive with $MBP_{[83-99]}$	1 of 2.8×10^6	1 of 3×10^6	NT

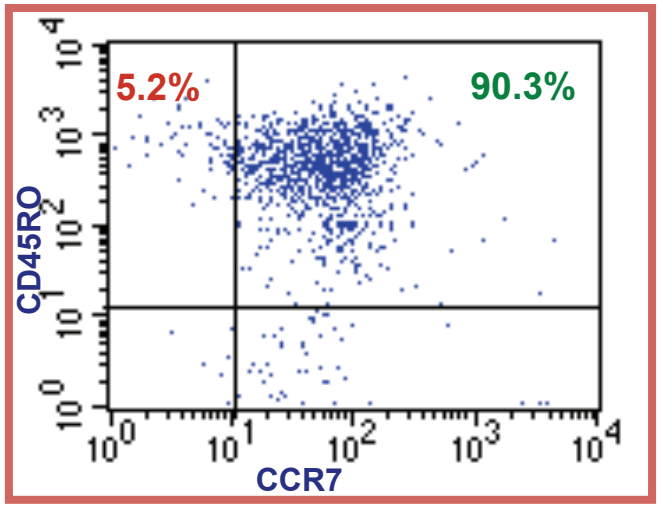
PBMC, peripheral blood mononuclear cell; NT, not tested.

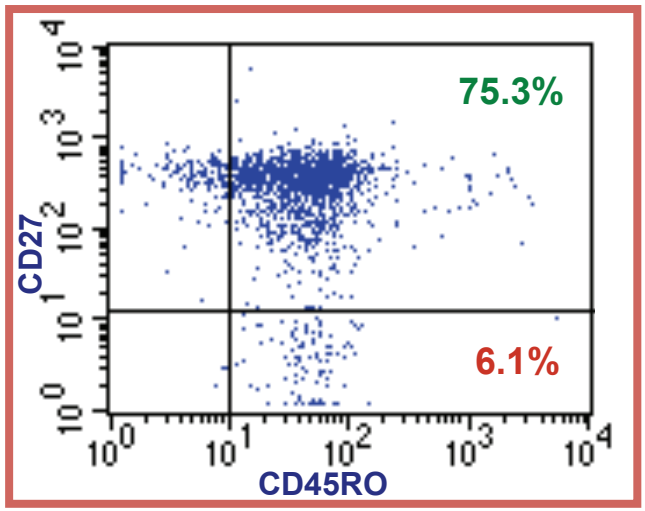
Bielekova et al. *Nat Med* 2000; 6: 1167

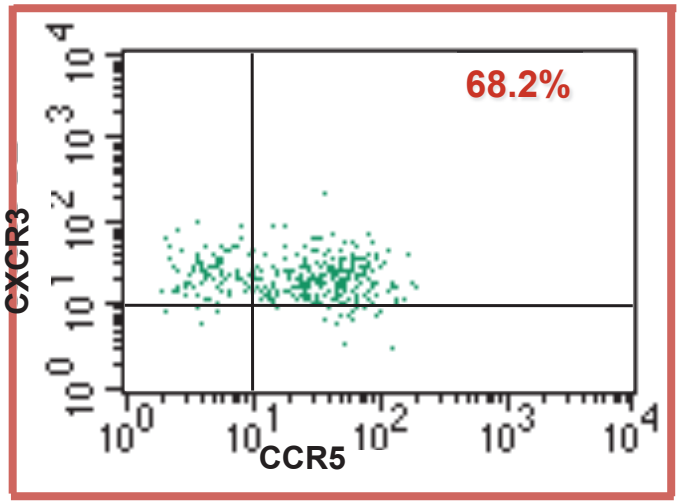
T helper cell differentiation: function and migration

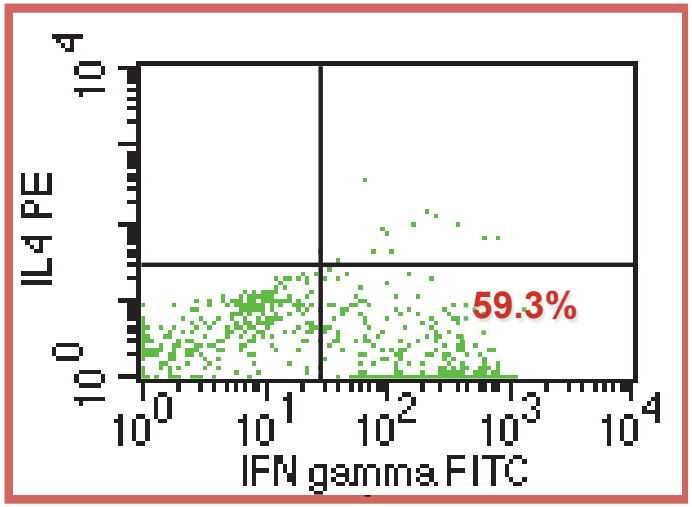


Memory Th1 cells represent the majority of cells into the CSF

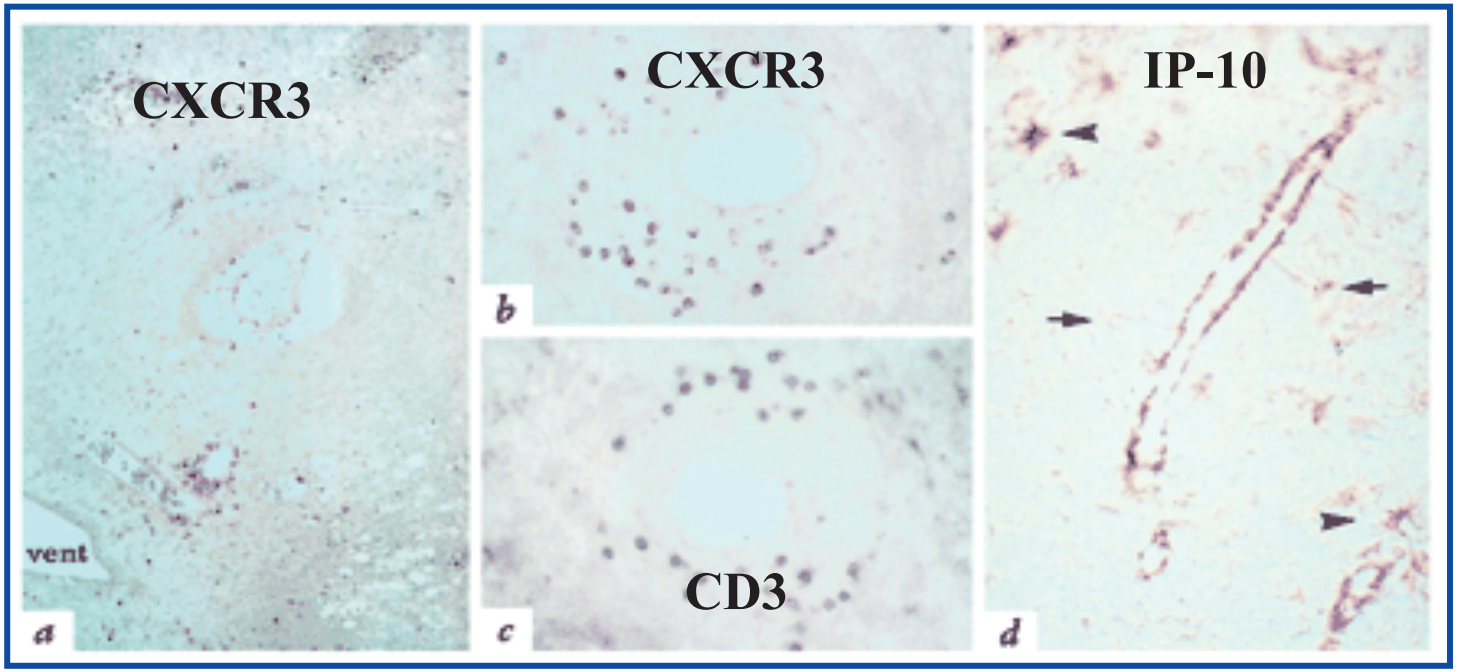


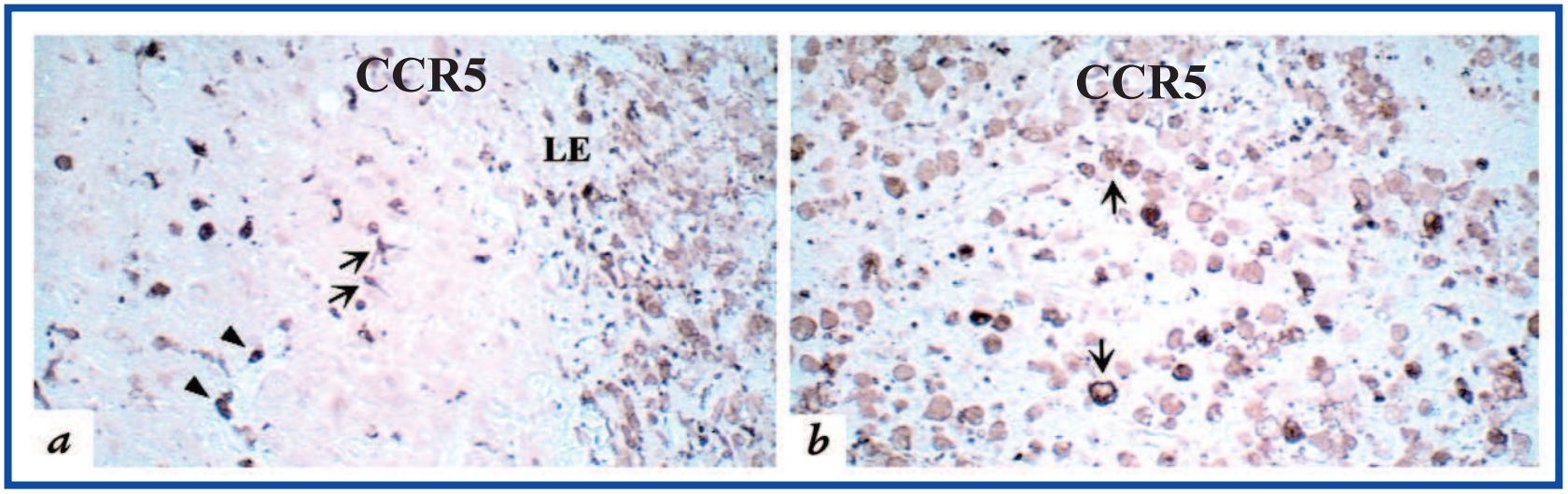






Giunti et al, J Leuk Biol 2003

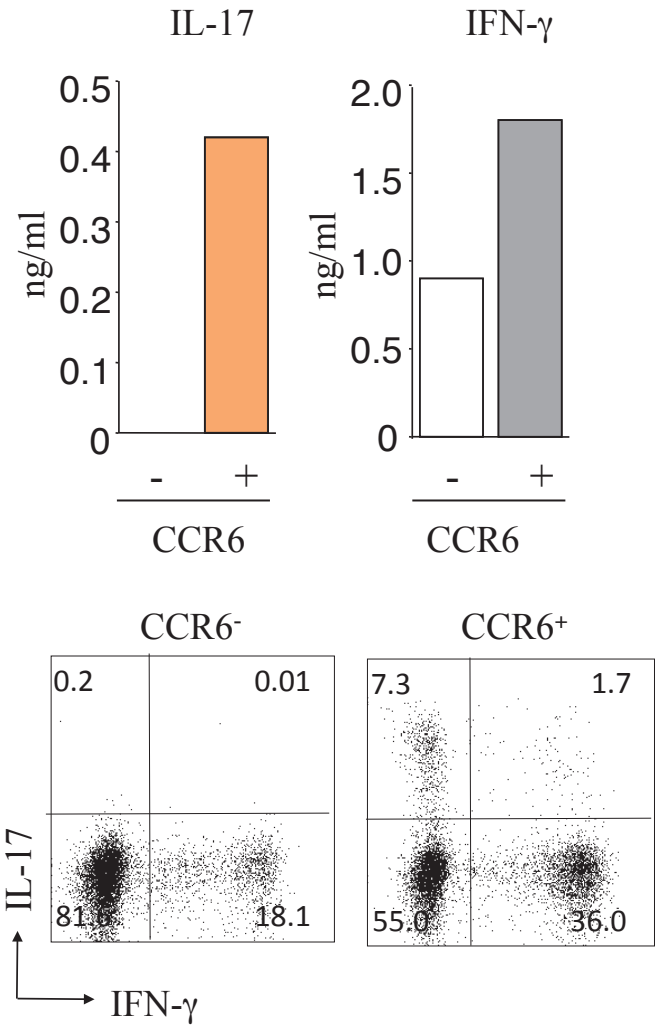




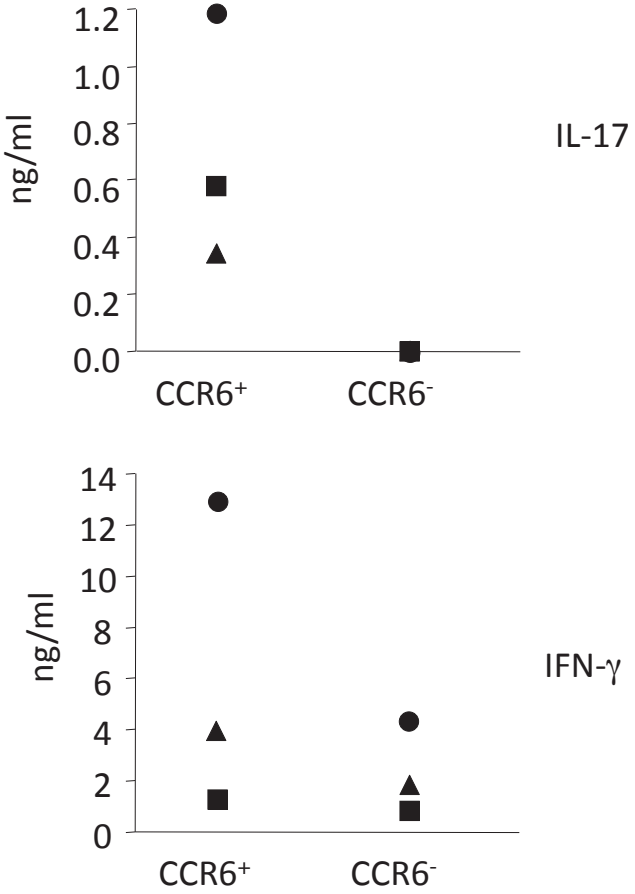
Sorensen et al, 1999

In humans, IL-17 production is restricted to CCR6$^+$ memory CD4$^+$ T cells

CD45RA- CD25- CD4+ memory T cells in blood



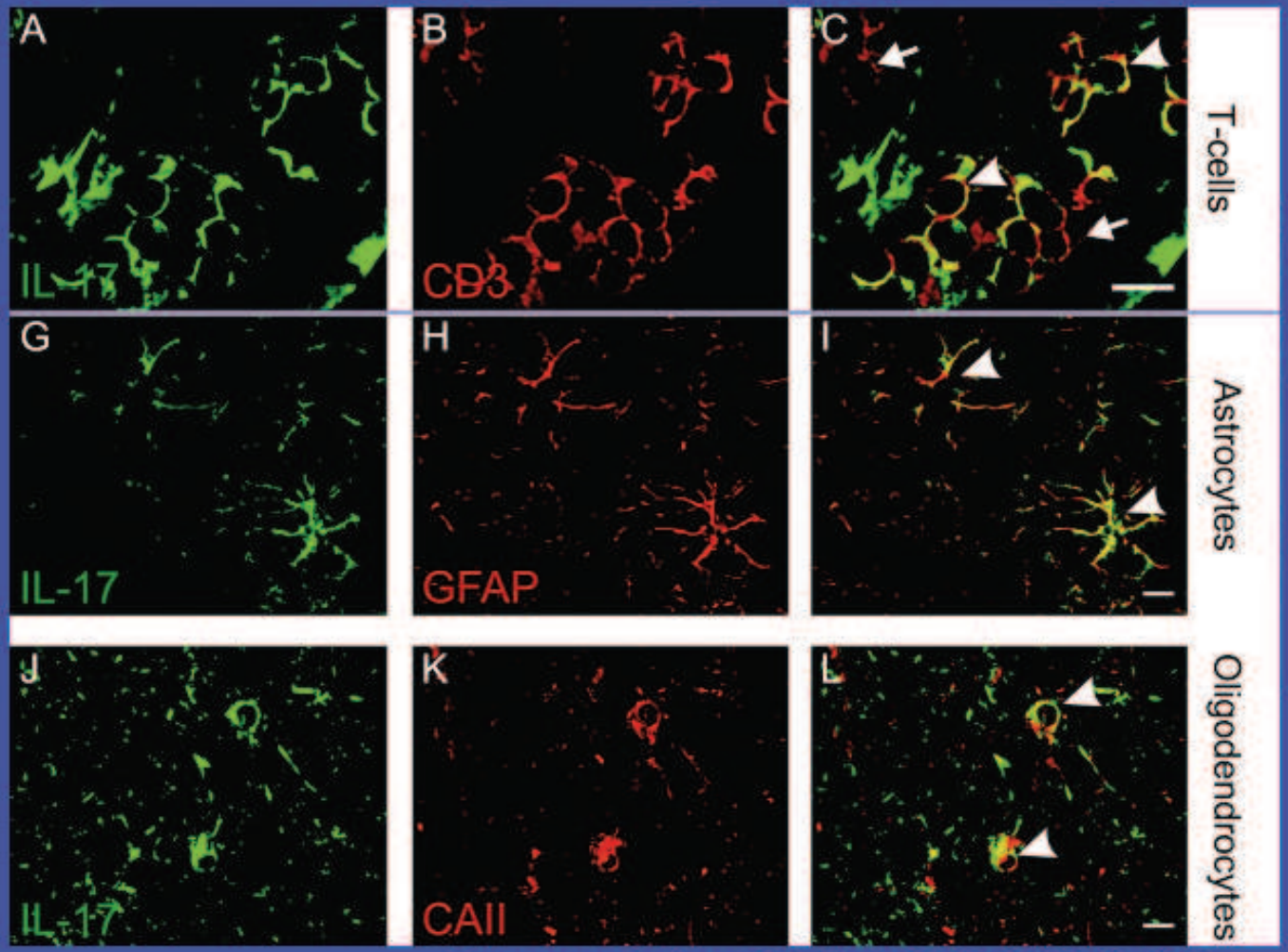
CD45RA- CD4+ effector T cells in inflamed tissue
(Collaboration with M. Gattorno, Gaslini, Genova)



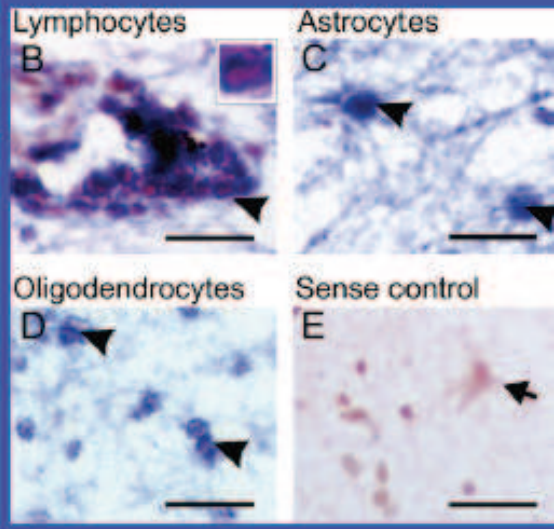
Acosta-Rodriguez et al, Nat Immunol, 2007

IL-17 production in T cells and glial cells in active MS lesions

Immunofluorescence by confocal microscopy

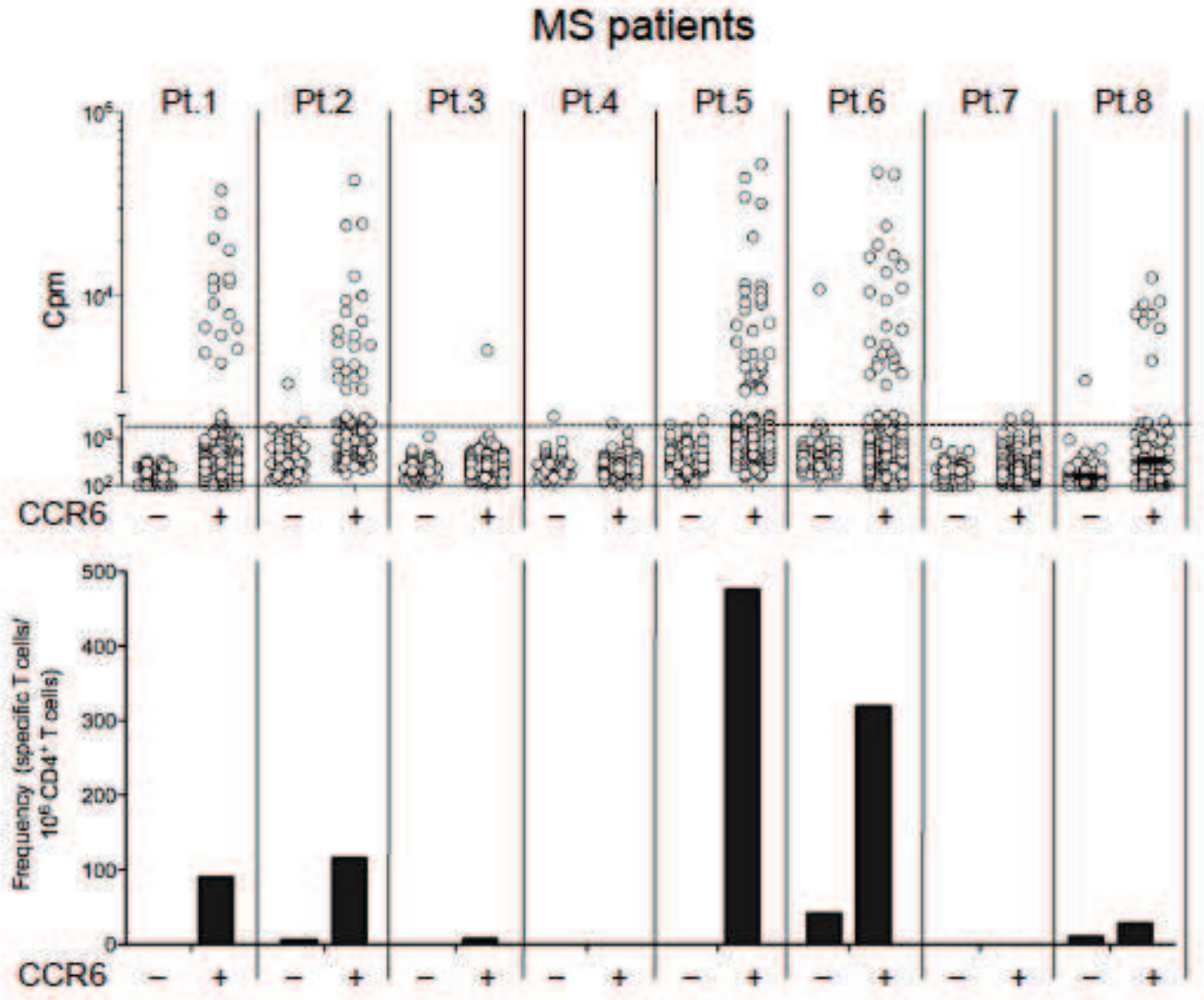


ISH for IL-17



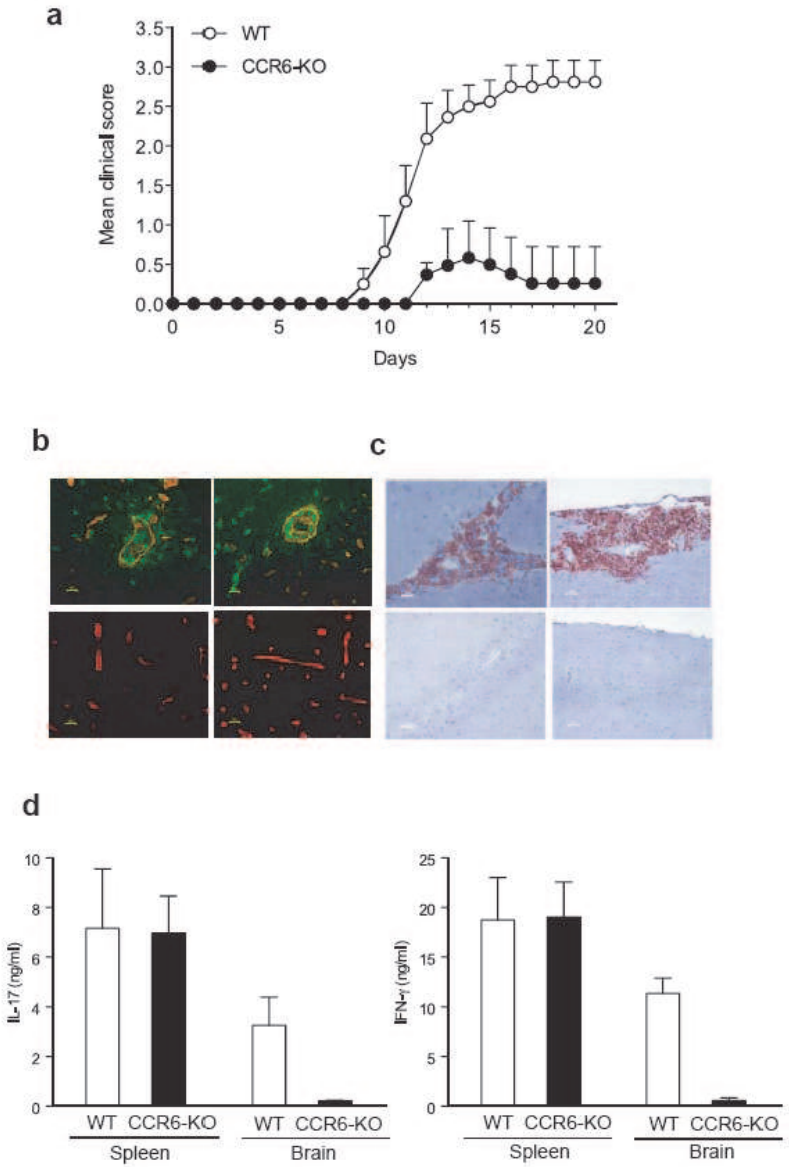
Tsartos et al. Am. J. Pathol. 2008;172:146-155

MOG-reactive T cells in MS patients are enriched in the CCR6 subset



Sallusto et al, Immunol Rev 2012

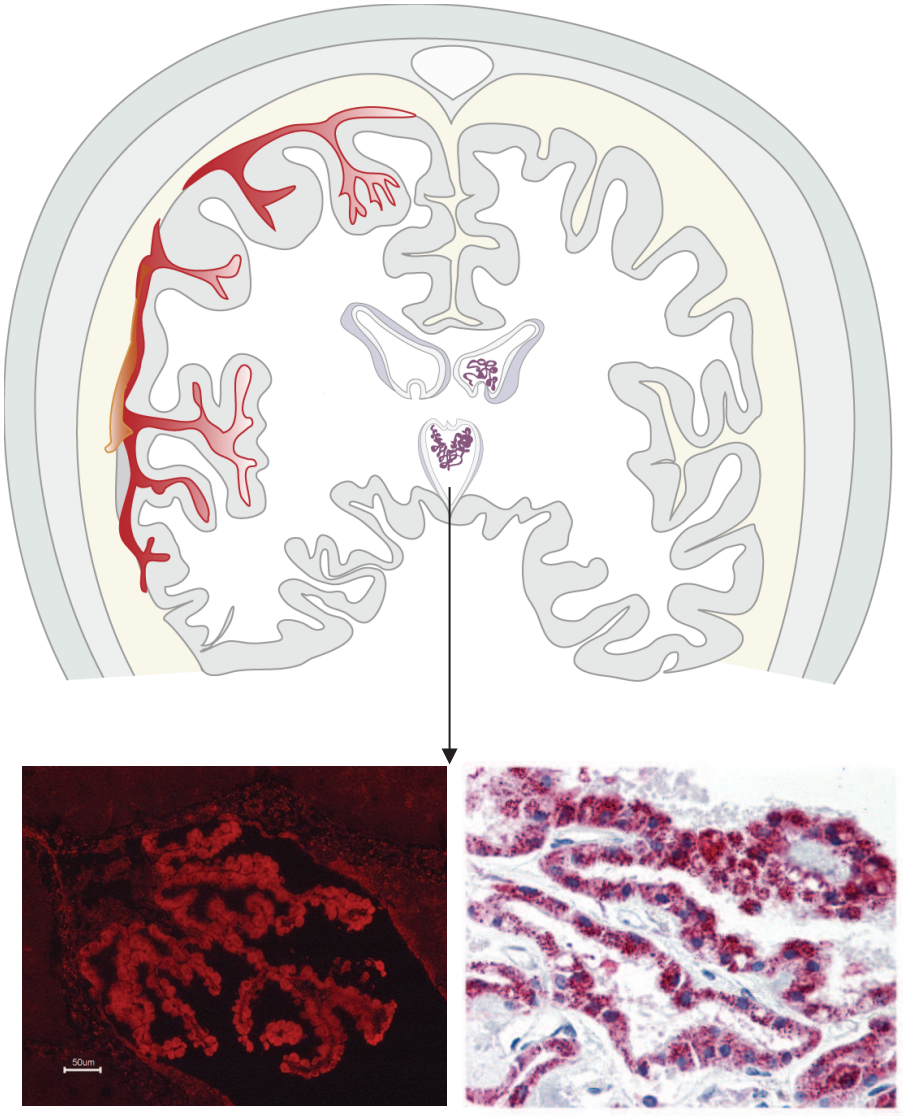
CCR6-deficient mice are resistent to EAE induction



Reboldi et al, Nat Immunol 2009

CCR6, Th17 and EAE pathogenesis

- CCR6 is expressed on human and mouse Th17 cells
- The CCR6 ligand CCL20 is expressed in the choroid plexus of mice and men
- CCR6 KO mice do not develop EAE
- CCR6+ Th17 cells enter the CNS at early time points through the choroid plexus and facilitate entry of CCR6 KO cells through the BBB
- CCR6 controls the constitutive traffic of T cells that may mediate immune surveillance in the CNS

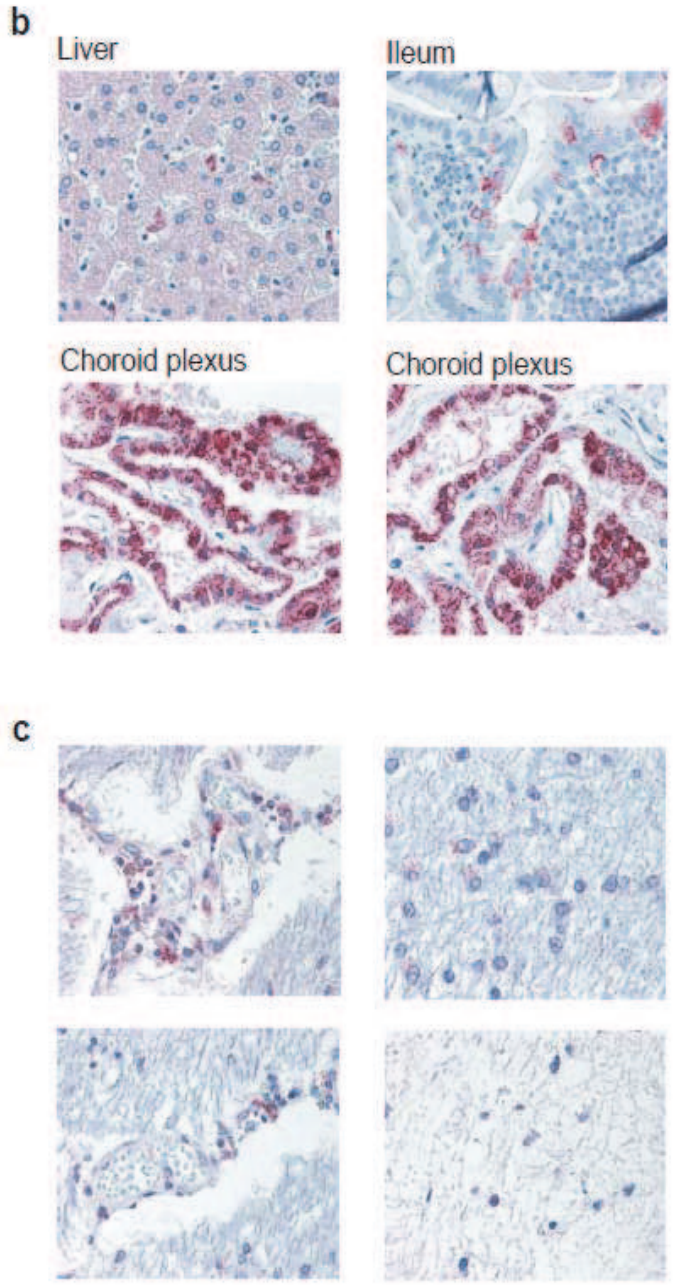


Mouse
choroid plexus

Human
choroid plexus

Reboldi et al Nature Immunol, 2009

CCL20 expression in human tissues: preferential distribution in the epithelial cells of the choroid plexus

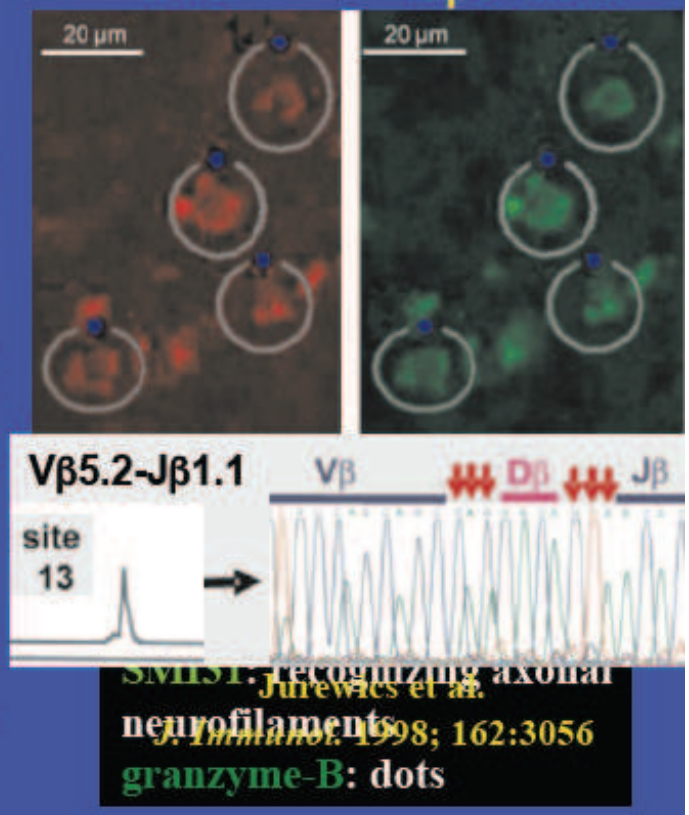


Reboldi et al Nature Immunol, 2009

A role for CD8 T cells in MS ?

HLA-class II: Association with the 'HLA-DR2 haplotype'
HLA-class I alleles: Independent influence on disease suceptibility

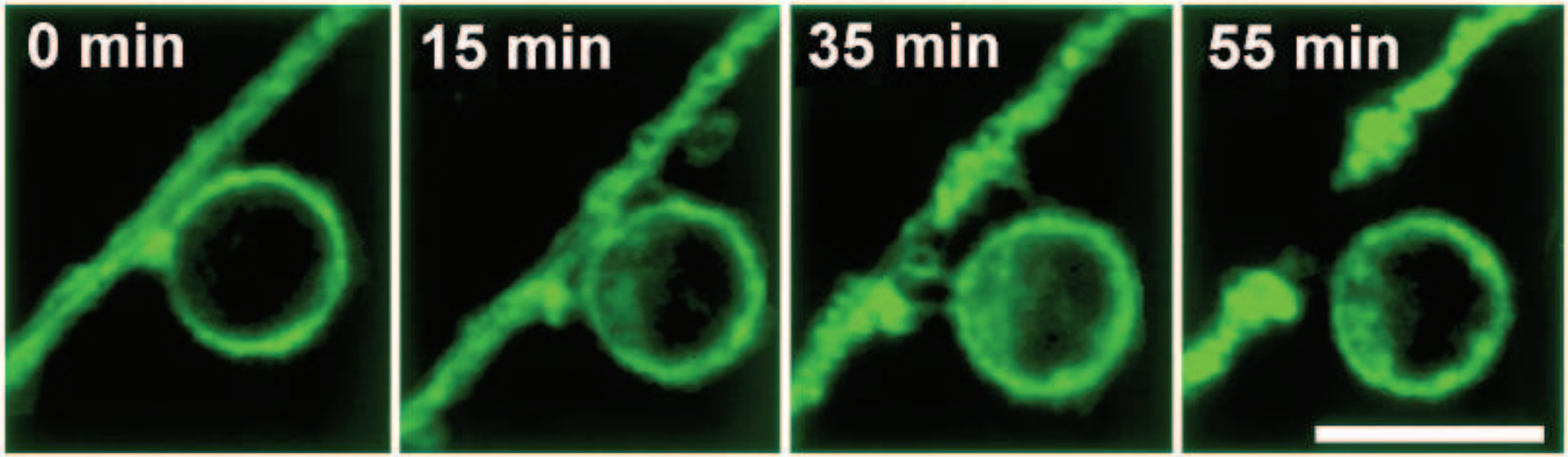
- **CD8$^+$ T cells dominate** in MS lesions.
- **Oligoclonal expansion** of CD8$^+$ T cells in the CSF and lesions of MS patients.
- Human **myelin-specific CD8 T cell clones** have been generated and, in mice, myelin-specific CD8 T cells **transfer EAE**.
- Correlation between axonal transection and CD8 T cell numbers ; contact between **granzyme+ T cell** and demyelinated **axons**.



Jurewicz et al. *J. Immunol.* 1998; 162:3056

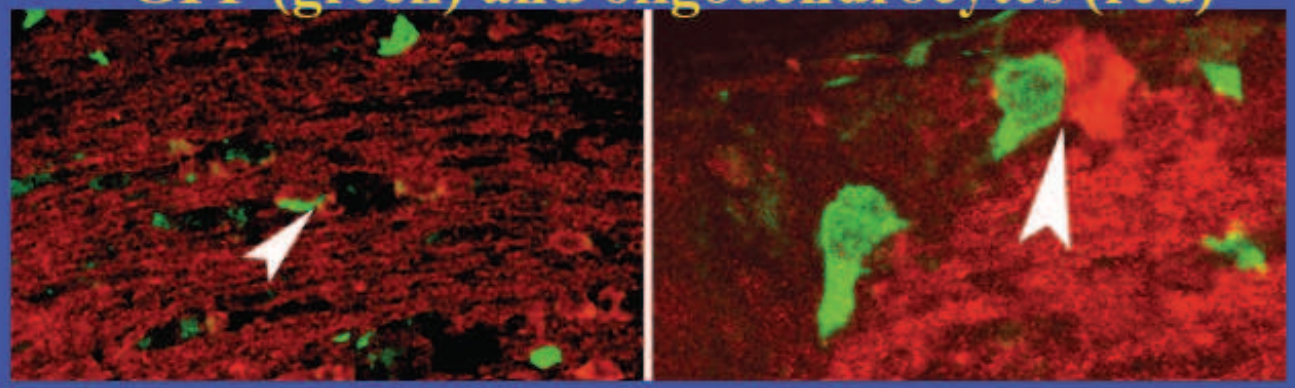
Reviewed in: Liblau et al. *Immunity* 2002, 17:1 ; Neumann et al. *Trends Neurosci* 2002, 25:313; Junker et al. *Brain* 2007, 130:2798.

Direct neurite membrane damage by MHC class I/peptide-restricted CD8+ T cells

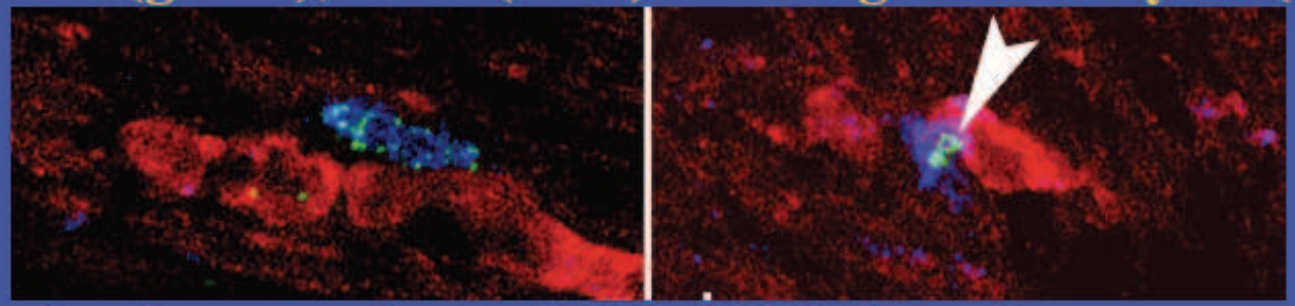


CNS damage by transfer of 'autoreactive' CD8 T cells targeting oligodendrocytes

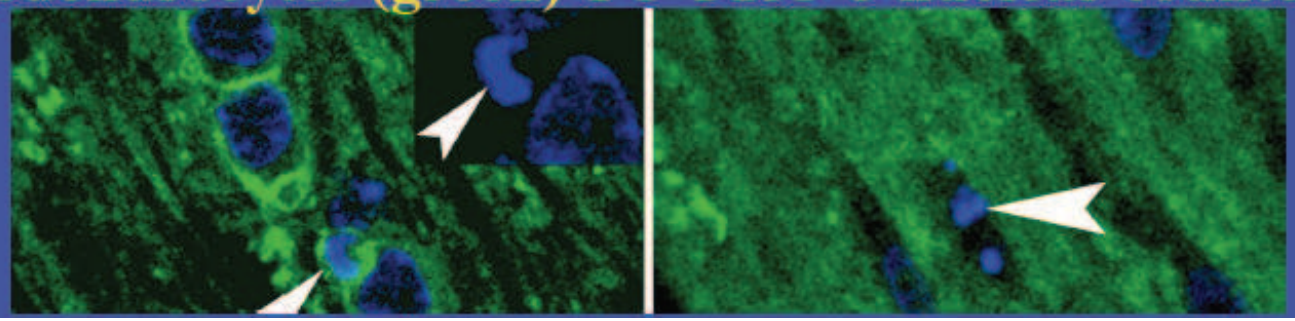
GFP (green) and oligodendrocytes (red)



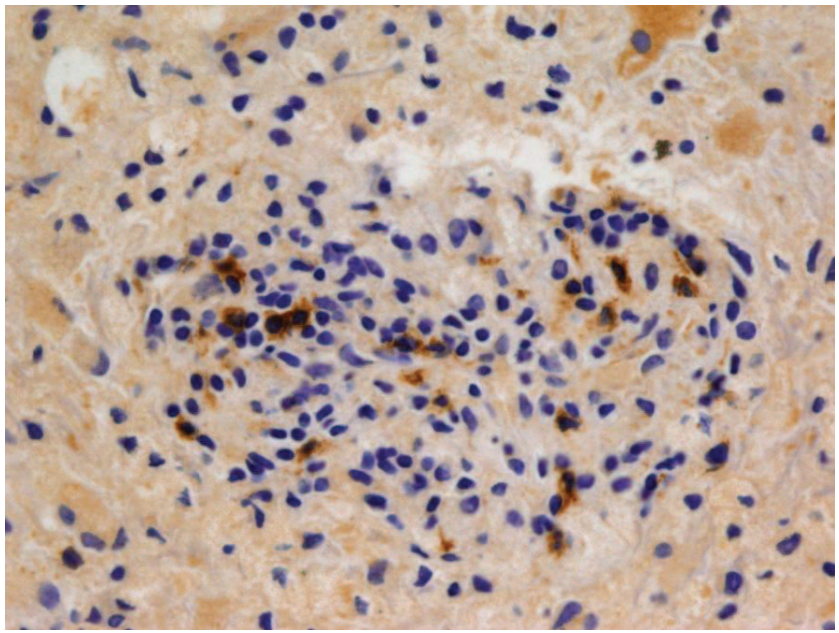
GrB (green), GFP (blue) and oligodendrocytes (red)

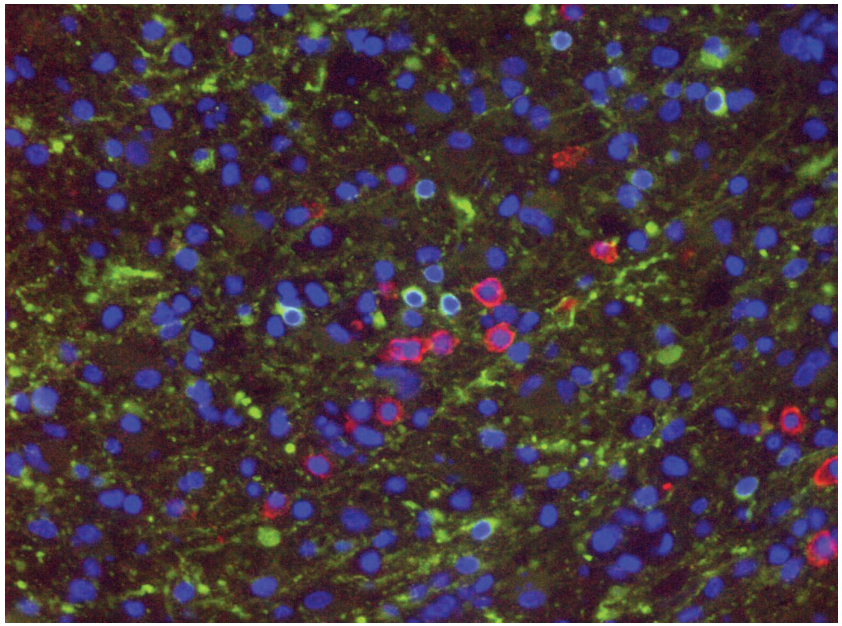


Oligodendrocytes (green) TO-PRO-3 nuclear counterstain.



$CD20^{+}$ B Cells (left) and CD138+ Plasma Cells (right) Are Present in MS Lesions





Blue=hematoxylin; brown=anti-CD20

Courtesy of Tonja Kuhlmann, 2008.

Identification of autoantibodies associated with myelin damage in multiple sclerosis

CLAUDE P. GENAIN[1], BARBARA CANNELLA[2], STEPHEN L. HAUSER[1] & CEDRIC S. RAINE[2]

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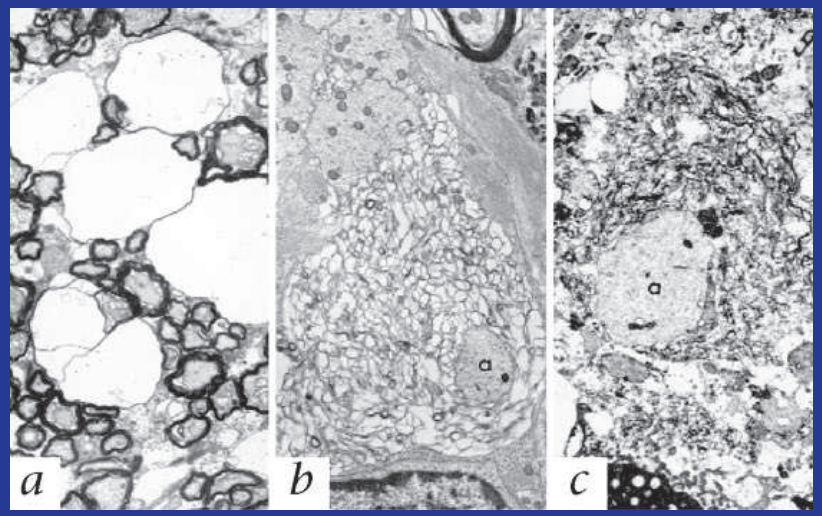
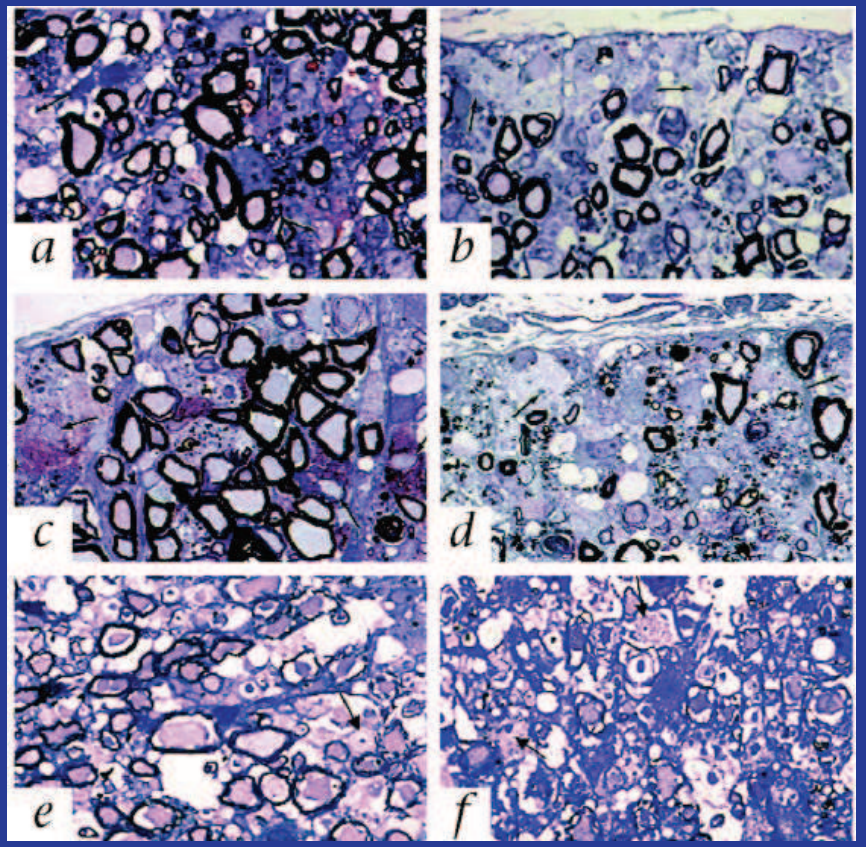
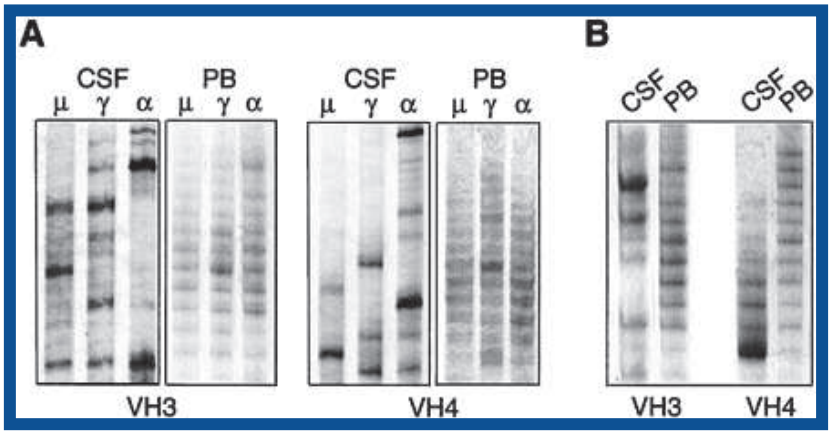
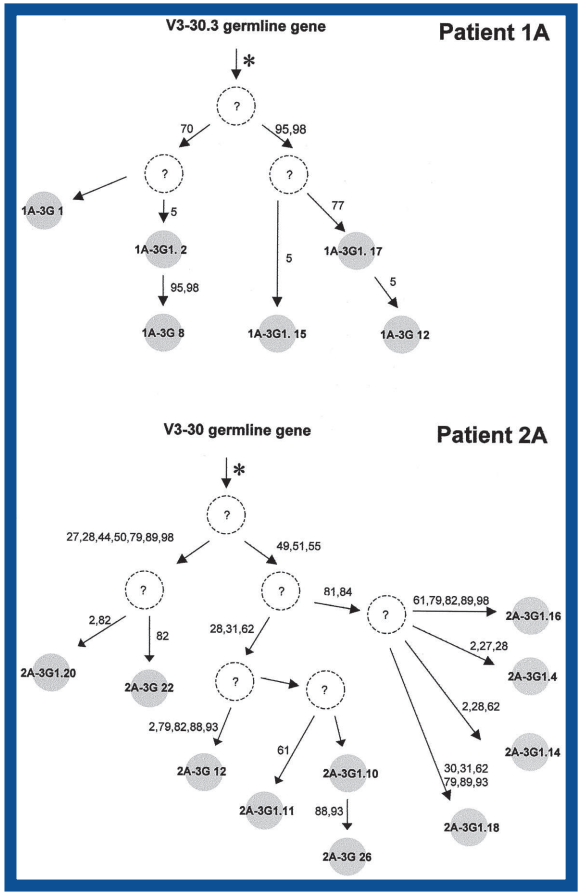
[2]Department of Pathology (Neuropathology), Albert Einstein College of Medicine, Bronx, New York 10461-1602, USA
Correspondence should be addressed to C.P. Genain; email: claudeg@itsa.ucsf.edu



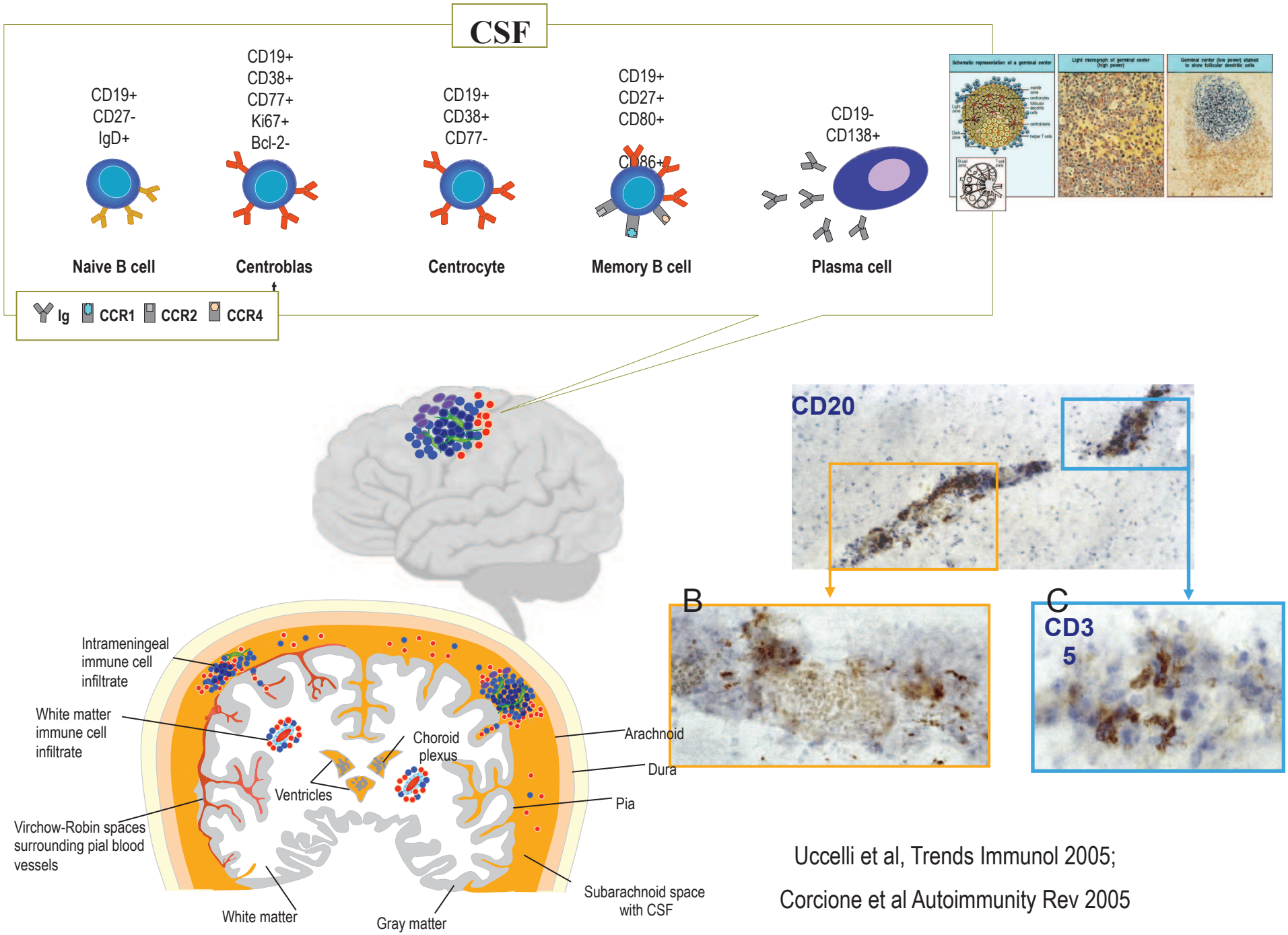


Clonally related B lymphocytes accumulates in the CSF of MS patients





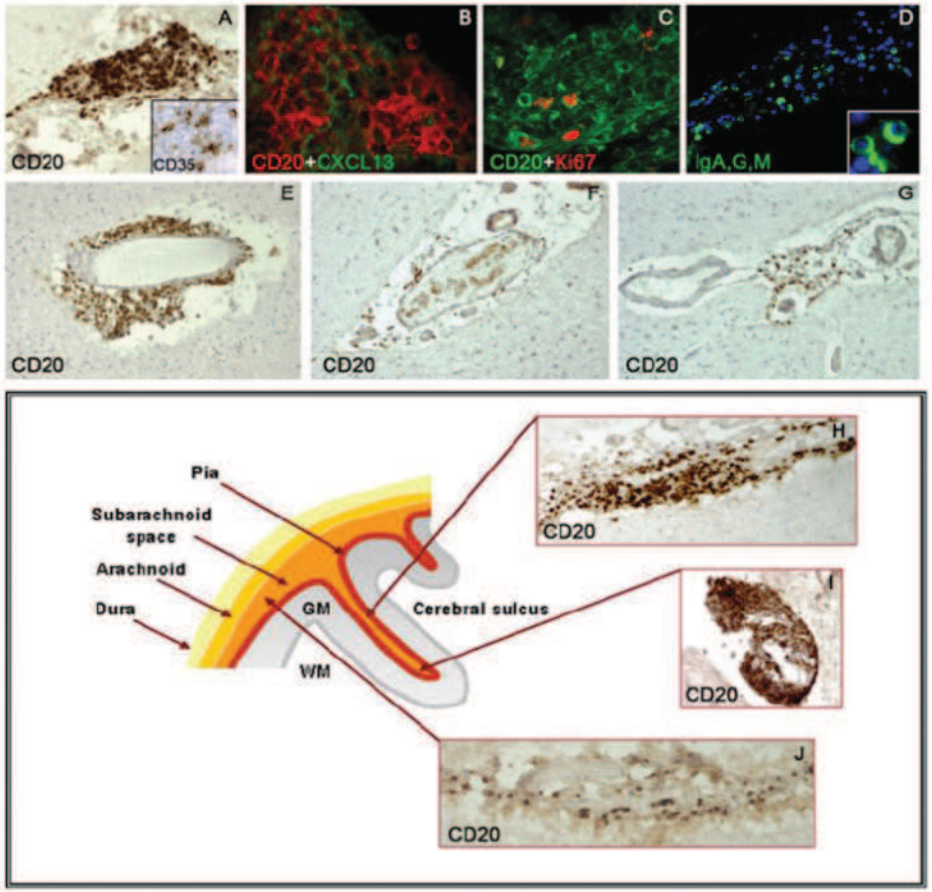
Colombo et al, J Immunol 2000

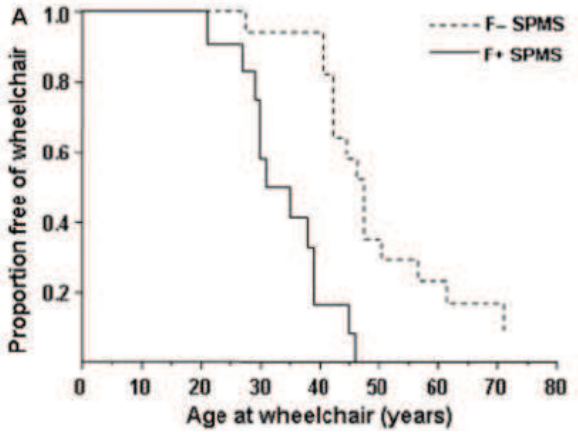


Uccelli et al, Trends Immunol 2005;

Corcione et al Autoimmunity Rev 2005

B cell follicles contain CD20^{+} B cells



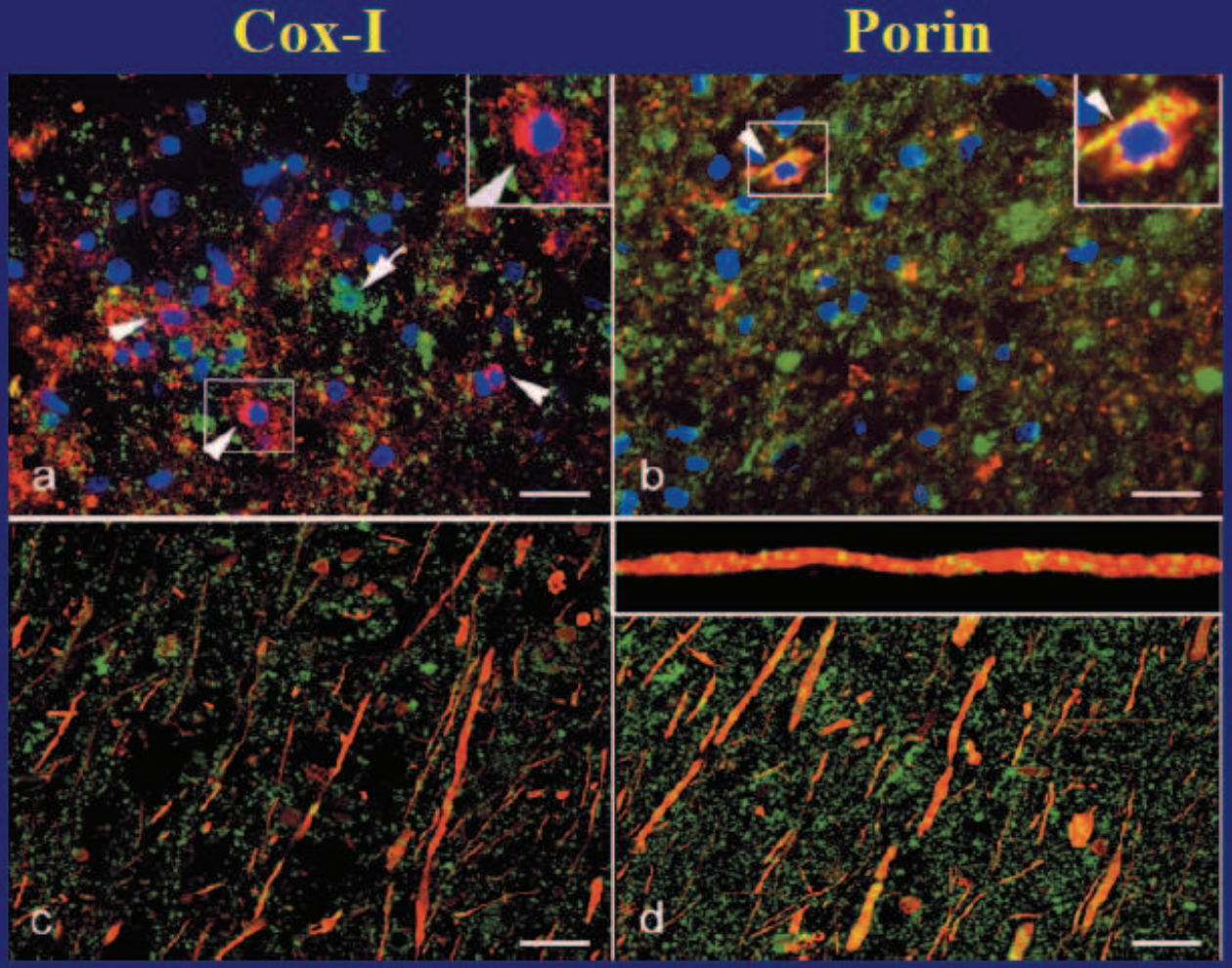


Serafini 2004, Magliozzi 2007

Mitochondrial Injury in MS Lesions
(Mahad et al Brain 2008, 2009)

- Active MS lesions (Pattern III, Pattern II and chronic active) versus stroke lesions
- Immuncytochemistry for mitochondrial proteins (Porin, MTND6, NDUFS3, SDHA, COX-I, COX-IV)
- Enzyme histochemistry for Complex II and IV
- Quantitative assessment of mitochondrial protein expression and function

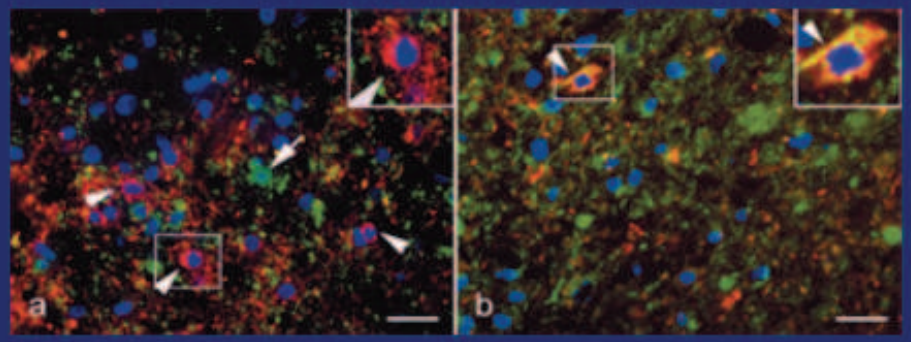
COX-I Deficiency in Active Multiple Sclerosis Lesions



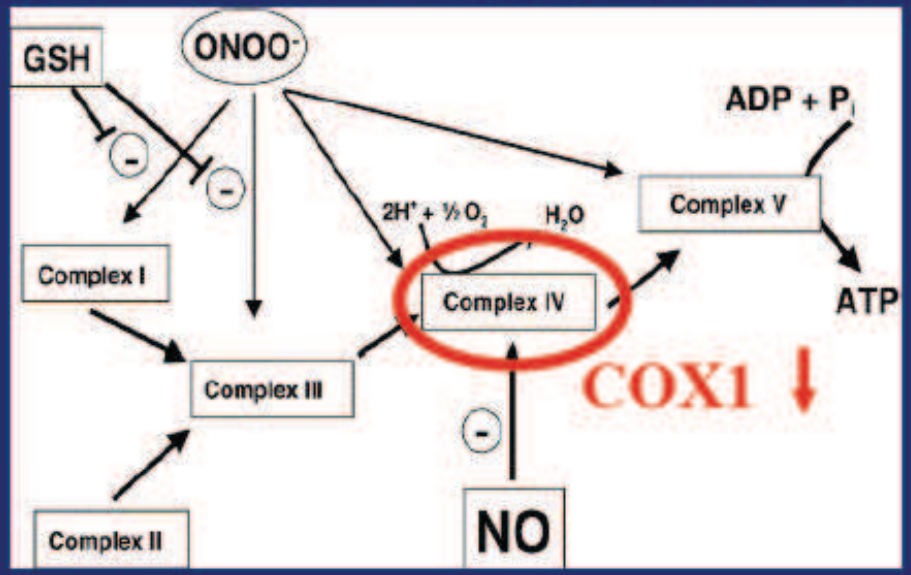
Mahad et al, Brain 2008

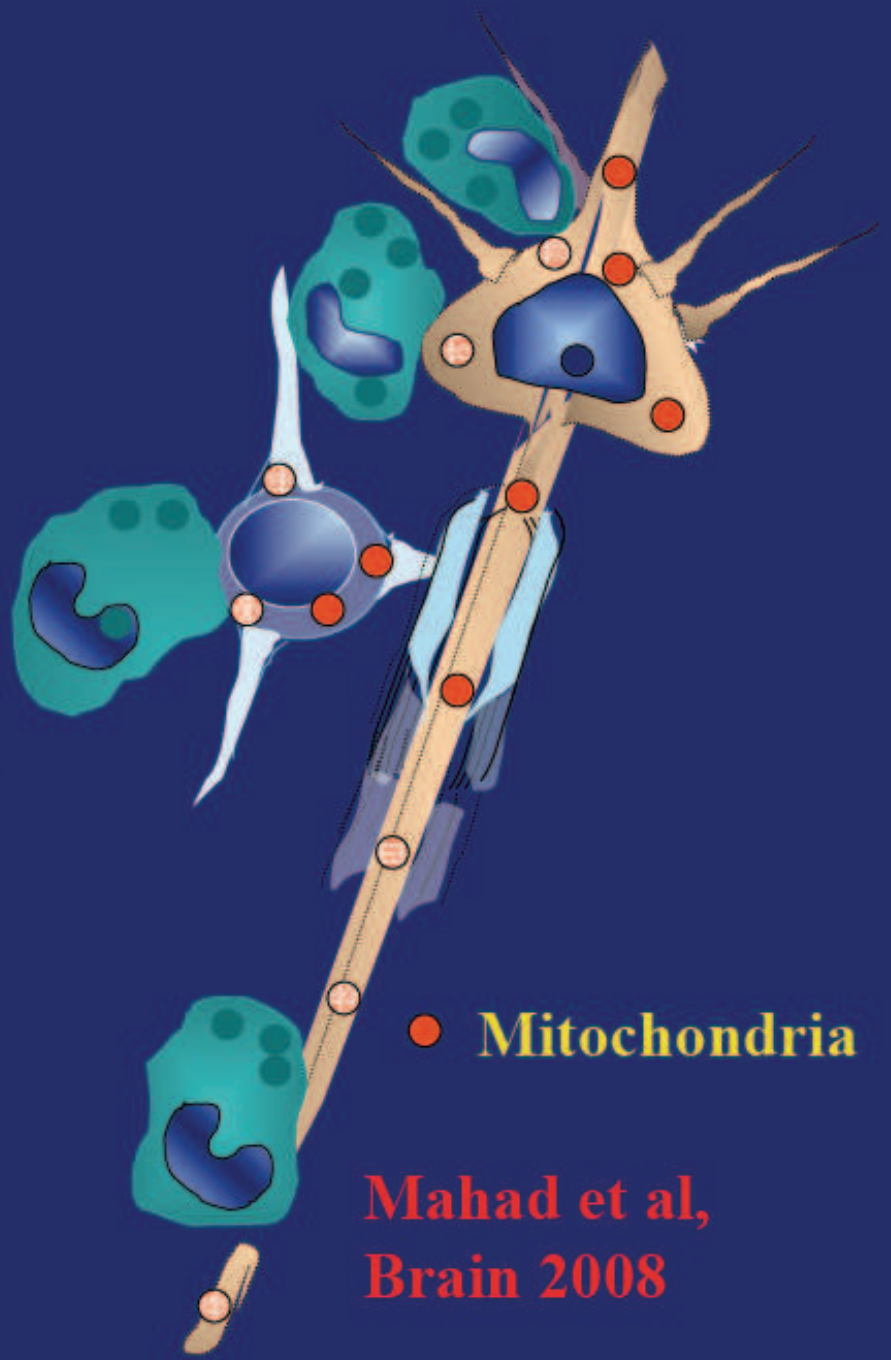
60% loss of cytochrome C oxidase 1 (COX-1) from mitochondria in oligodendrocytes and axons in pattern III MS lesions

HISTOTOXIC HYPOXIA IN MS



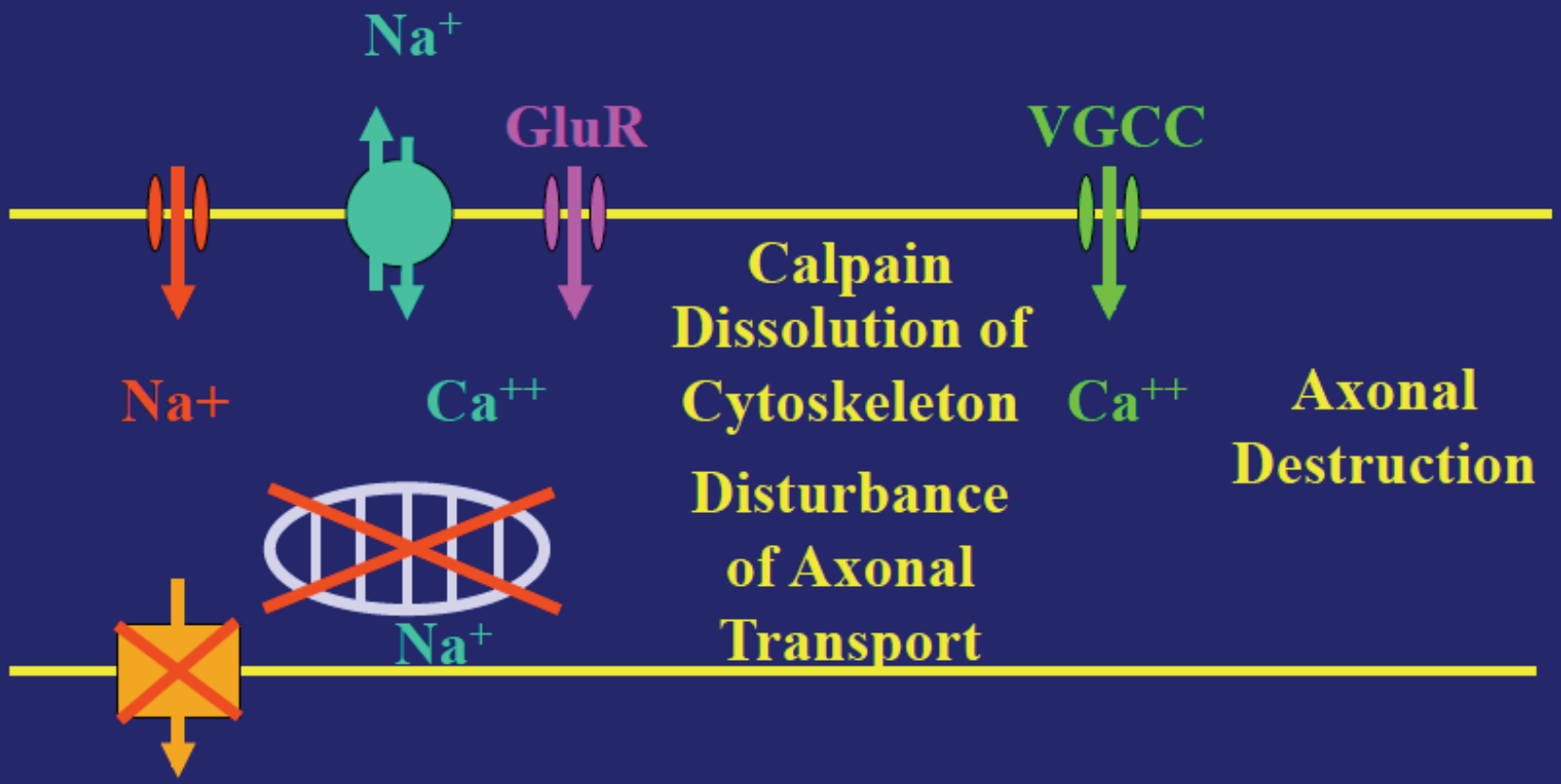
COX1 < COX4 < Complex I or II < Porin





Mahad et al, Brain 2008

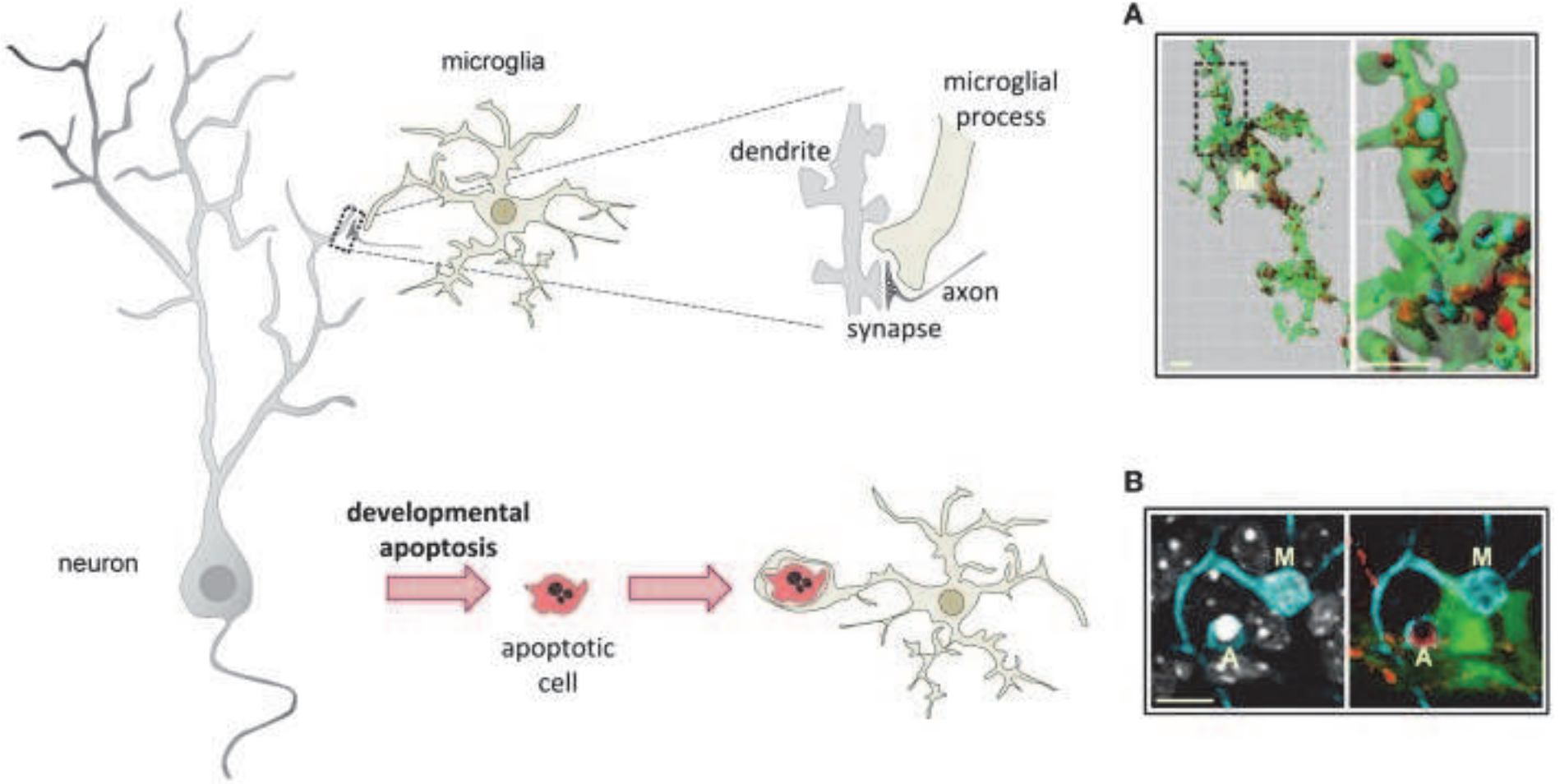
Axonal Injury in Multiple Sclerosis Lesions



What induces Mitochondrial Injury in Inflammatory Lesions ?

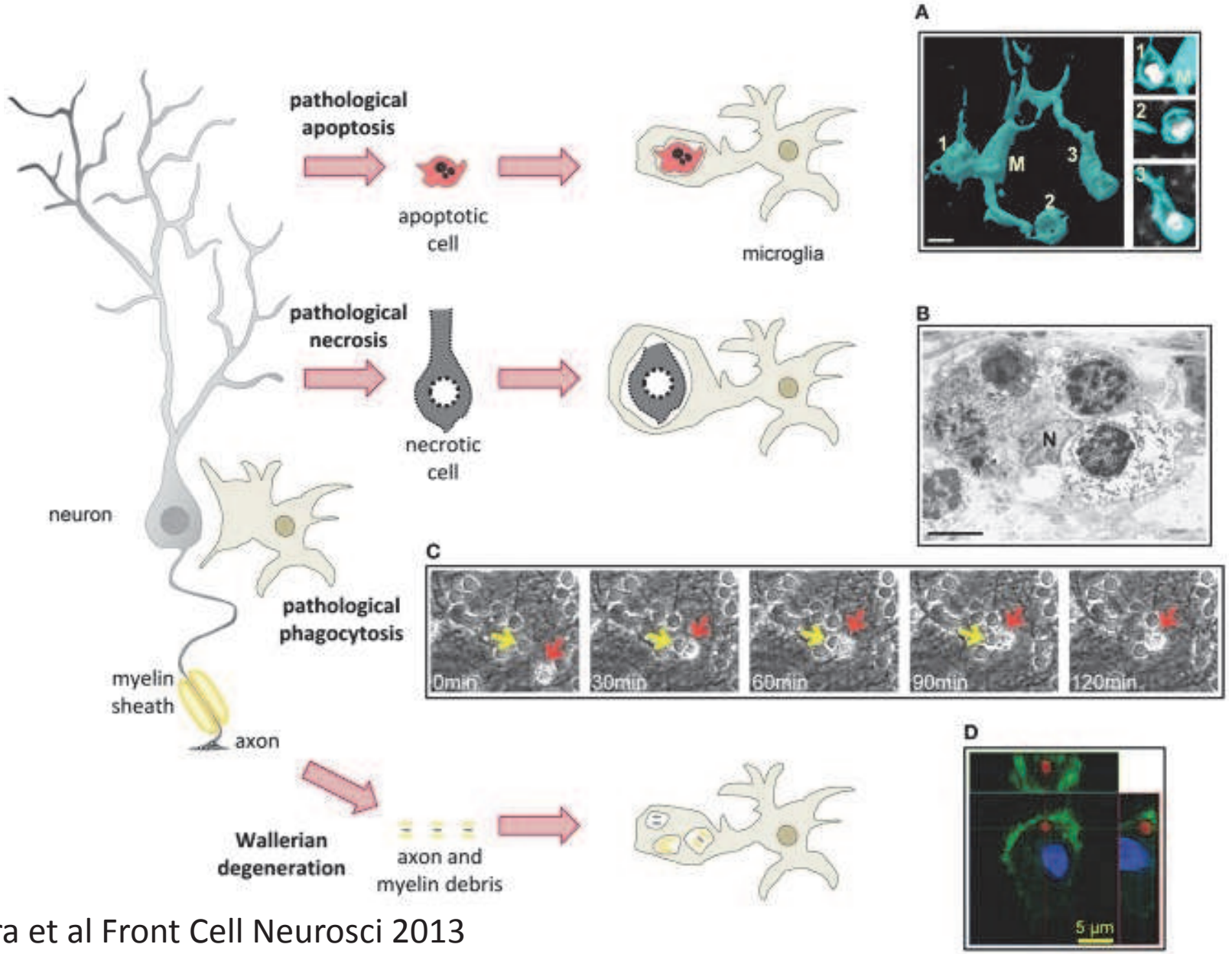
- Neurodegeneration in MS is associated with activated macrophages / microglia
  - Proinflammatory cytokines (indirect ?)
    - Th1,Th2 or MM cytokines (Microarray; Lisak et al 2009)
  - Nitric oxide radicals
  - Reactive oxygen species
  - Others ??

Microglial phagocytosis in health



Sierra et al Front Cell Neurosci 2013

Microglial phagocytosis in disease



Sierra et al Front Cell Neurosci 2013

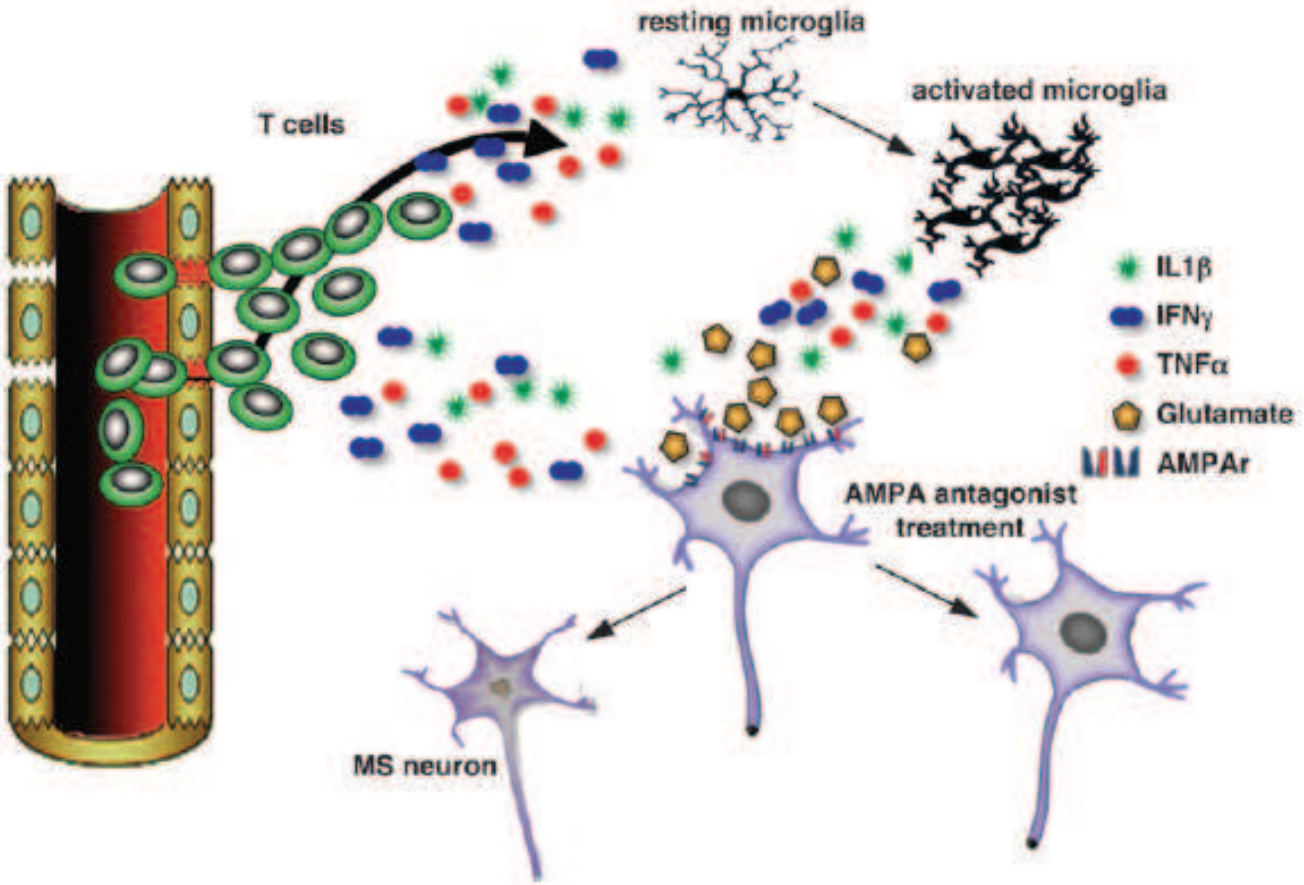


Figure 1 Glutamate is released not only by excitatory synaptic terminals but also by infiltrating lymphocytes and by activated microglia in MS brains. Glutamate binds to and activates abnormally sensitive AMPA receptors, leading to synaptic and neuronal degeneration. Blockade of AMPA receptors preserves neuronal integrity and ameliorates the clinical course of mice with EAE

Microglia nodules in MS are associated with degenerating axons

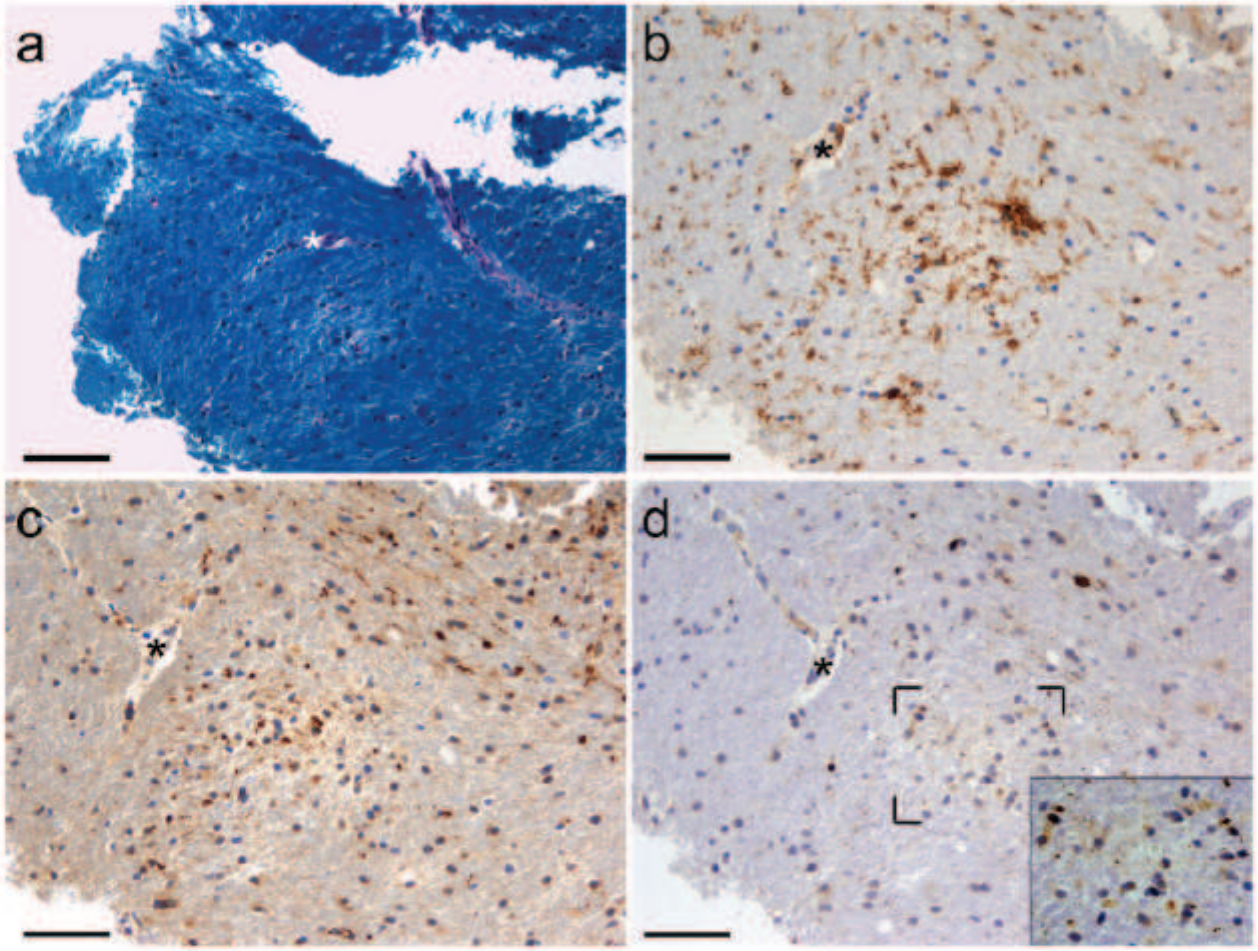


Fig. 2 Microglial nodules are associated with underlying axonal pathology in early multiple sclerosis. LFB/PAS stain and HLA-DR immunohistochemistry were used to identify microglial nodules in PPWM (**a**, **b**). Sequential tissue sections (**a**, **b**, **c**, **d**) were matched using a blood vessel (*asterisk*) (MS no. 5). The region of interest exhibits intact myelin (**a**). Microglial nodule representing cluster of activated HLA-DR expressing microglia/macrophages localized in normally myelinated PPWM tissue (**b**). Sequential section identified injured/damaged axons associated with microglial nodules (**c**, **d**). APP^+, acutely damaged axons are detected in close association with the microglial nodule (**c**). $SMI32^+$, axonal ovoids occur in the same region (**d** and *inset*). *Inset* in (**d**) shows higher magnification of the marked region. *Scale bars* = (**a**) 100 μm; (**b**–**d**) 50 μm

Singh et al, Acta Neuropath 2013

Microglia nodules in MS are associated with degenerating axons

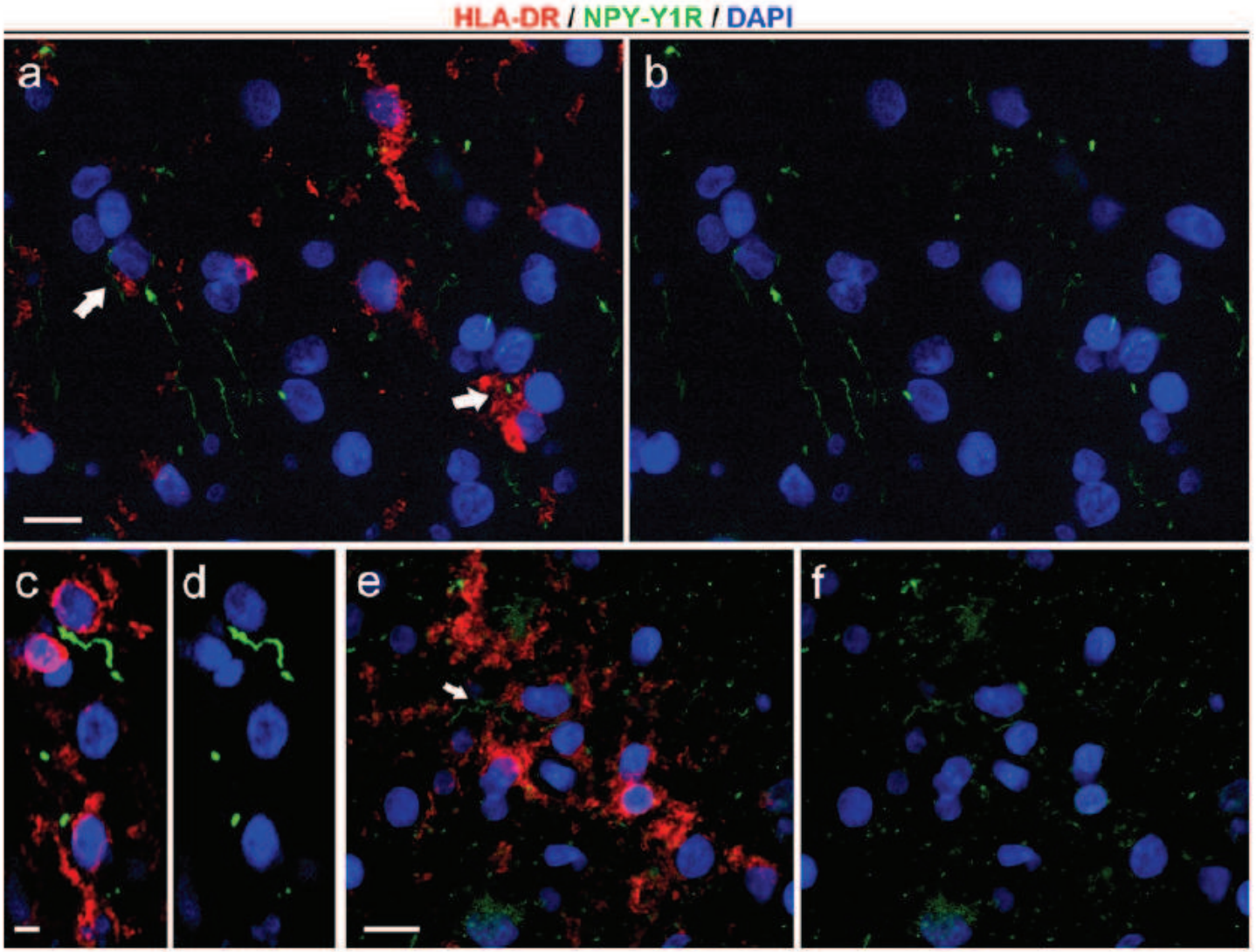


Fig. 3 Axons undergoing Wallerian degeneration in close spatial association with activated microglia/macrophages in MS PPWM. NPY-Y1R$^+$ axons (*green*) undergoing Wallerian degeneration apposed to HLA-DR$^+$ microglia/macrophages (*red*) with an activated morphology (*arrows*, **a–b**) (MS no. 14). NPY-Y1R$^+$ axons were frequently surrounded by activated microglia/macrophages throughout the PPWM (**c–d**) (MS no. 1). Activated microglia/macrophage cells were visualized clustering along the length of the NPY-Y1R$^+$ axonal segment (*arrow*, **e–f**) (MS no. 4). Stainings are merged with DAPI, which stains the nuclei (**a–f**, *blue*). *Scale bars* = (**a–b**) 25 μm; (**c–d**) 10 μm; (**e–f**) 20 μm

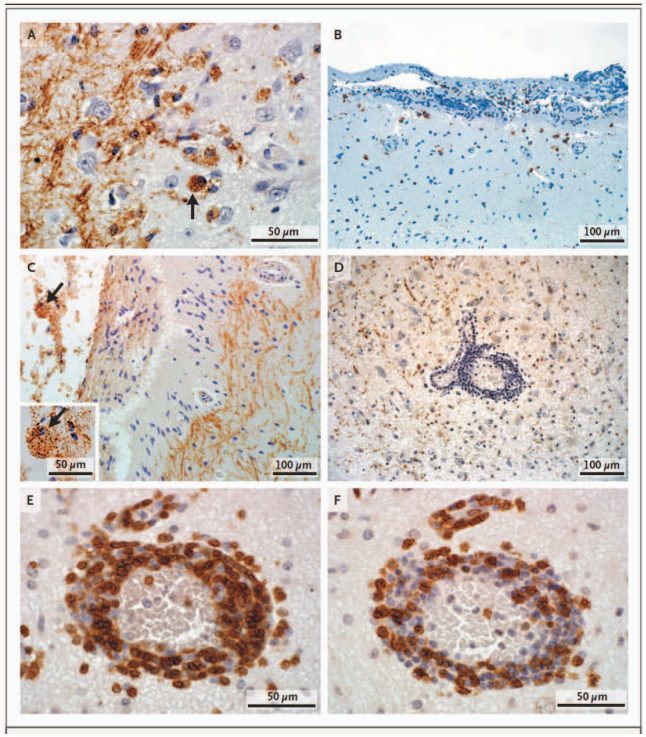
Singh et al 2013

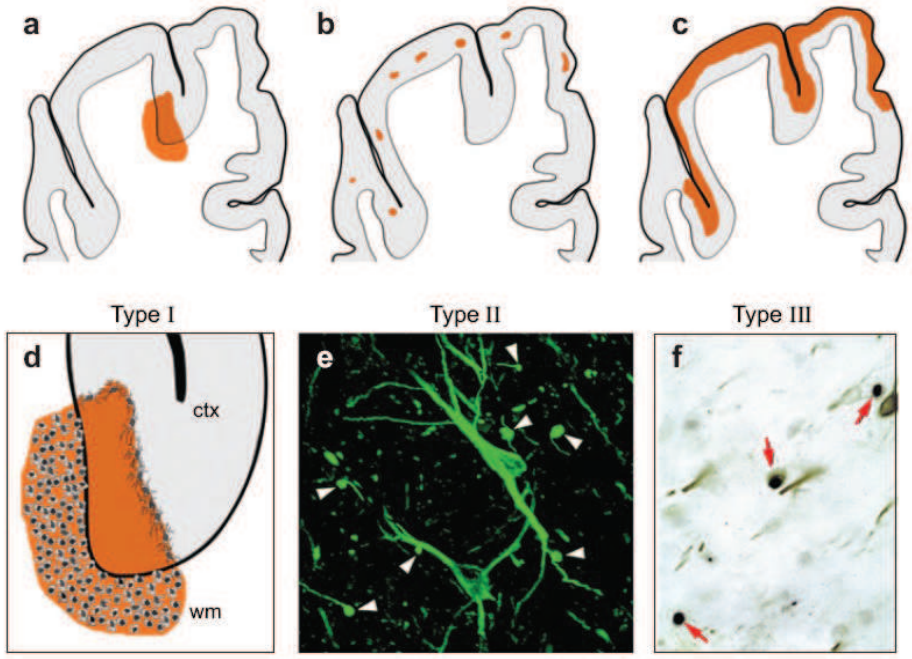
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Inflammatory Cortical Demyelination in Early Multiple Sclerosis

Claudia F. Lucchinetti, M.D., Bogdan F.G. Popescu, M.D., Ph.D., Reem F. Bunyan, M.D., Natalia M. Moll, M.D., Ph.D., Shanu F. Roemer, M.D., Hans Lassmann, M.D., Wolfgang Brück, M.D., Joseph E. Parisi, M.D., Bernd W. Scheithauer, M.D., Caterina Giannini, M.D., Stephen D. Weigand, M.S., Jay Mandrekar, Ph.D., and Richard M. Ransohoff, M.D.





Trapp and Nave 2008

Iron and neurodegeneration in the MS brain

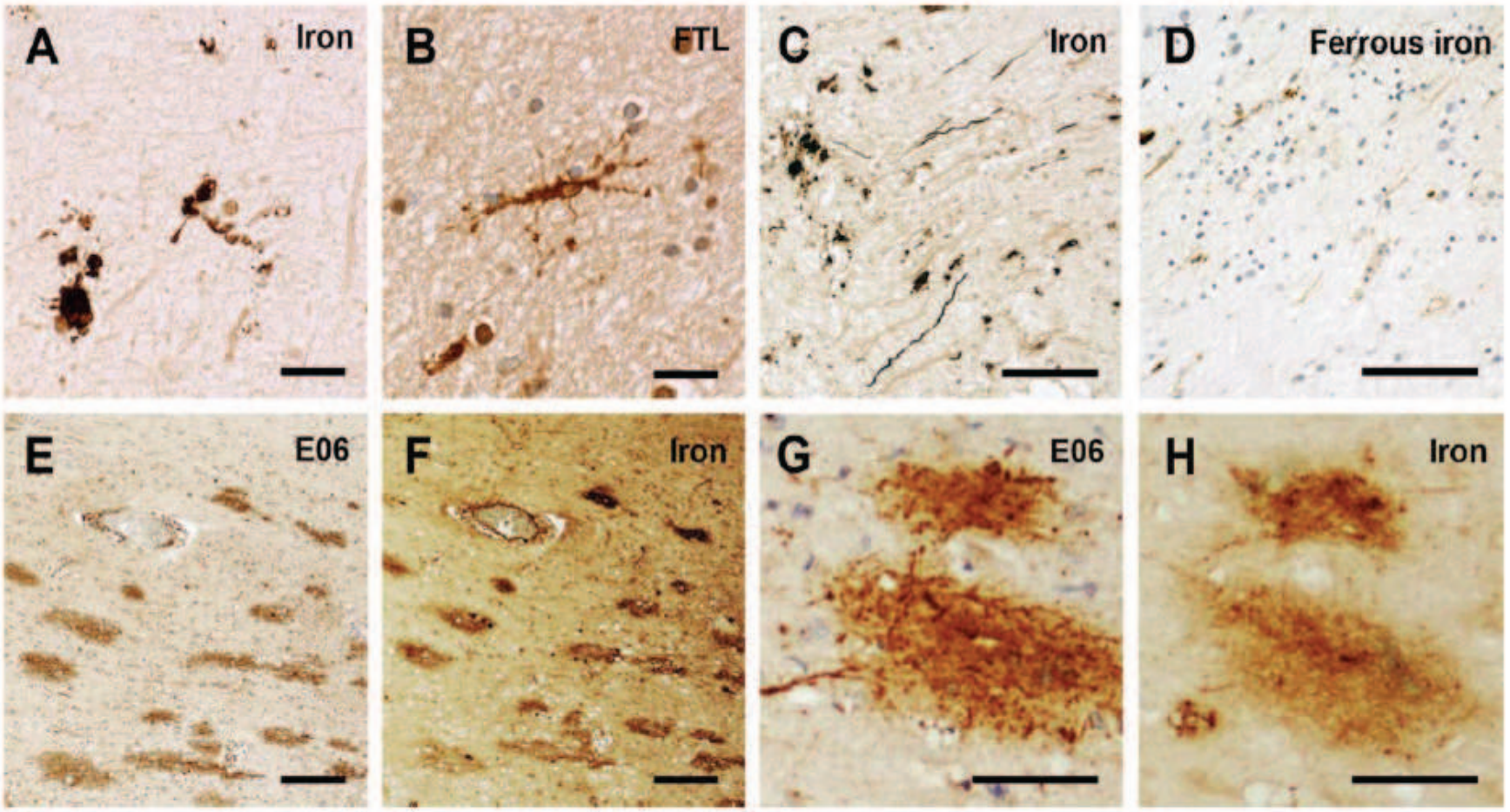
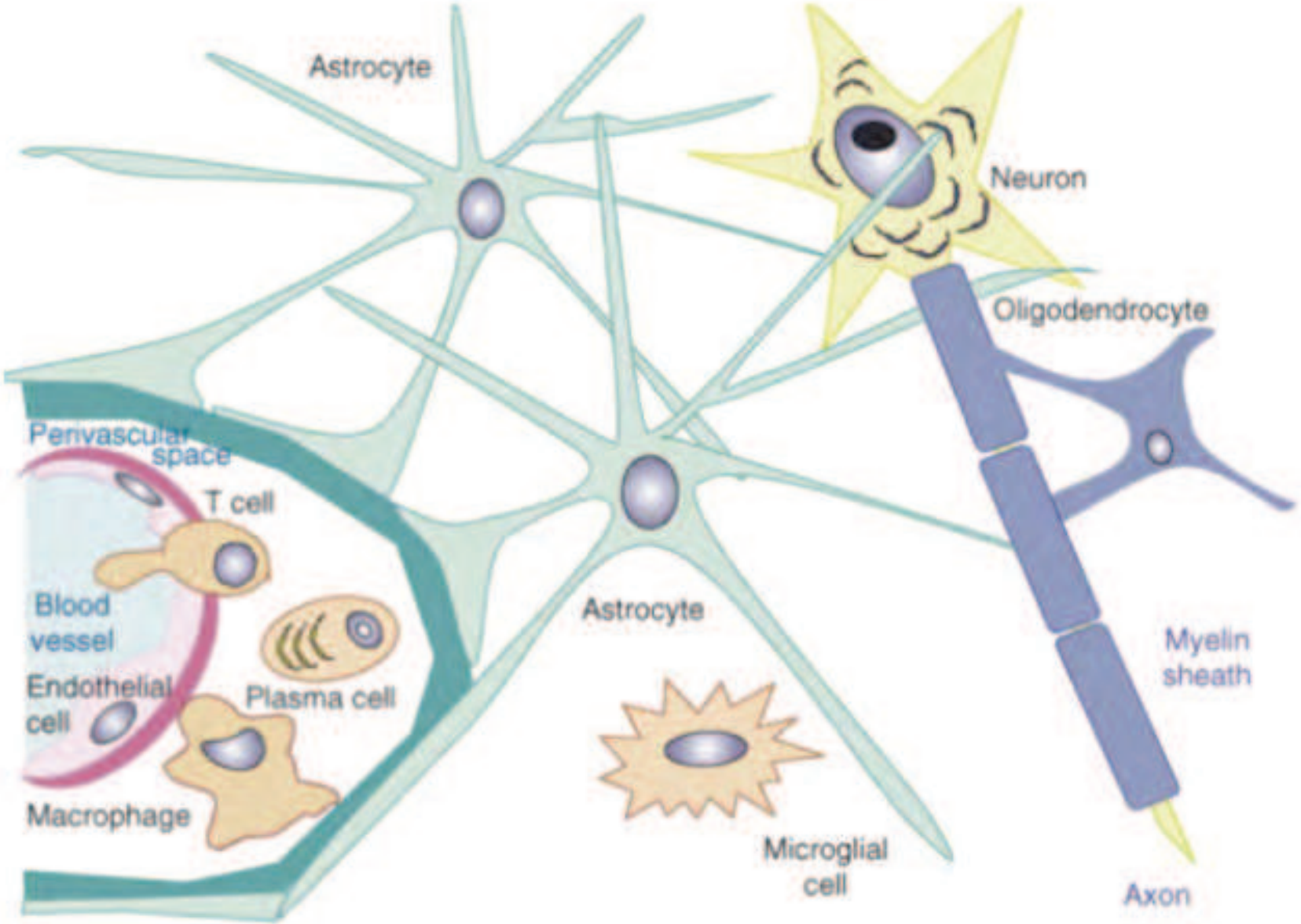


FIGURE 4: Dystrophic microglia, axonal iron, and oxidized phospholipids in multiple sclerosis (MS) lesions. Iron-loaded microglia and macrophages in active MS lesions show signs of degeneration (dystrophy) with process beading, retraction, and fragmentation (A), which is also visible in microglia stained for ferritin light polypeptide (FTL; B, brown). At active lesion edges, total (C) and rarely also ferrous nonheme iron (D, brown) accumulates in axons. (E–H) Oxidized phospholipids (E06 reactivity; E and G; brown) are detected in lesions with high iron content (total nonheme iron staining in F and H). Scale bars = 20μm (A, B); 100μm (C, D); 200μm (E, F); 75μm (G, H).

Hametner et al, Ann Neurol 2013

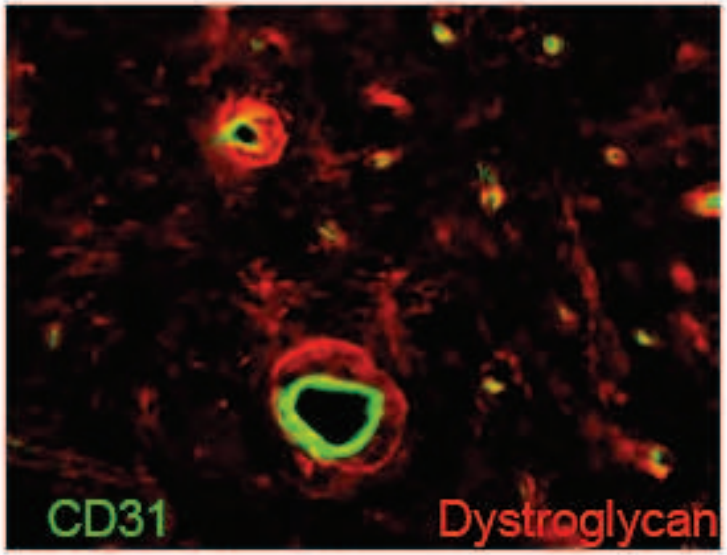


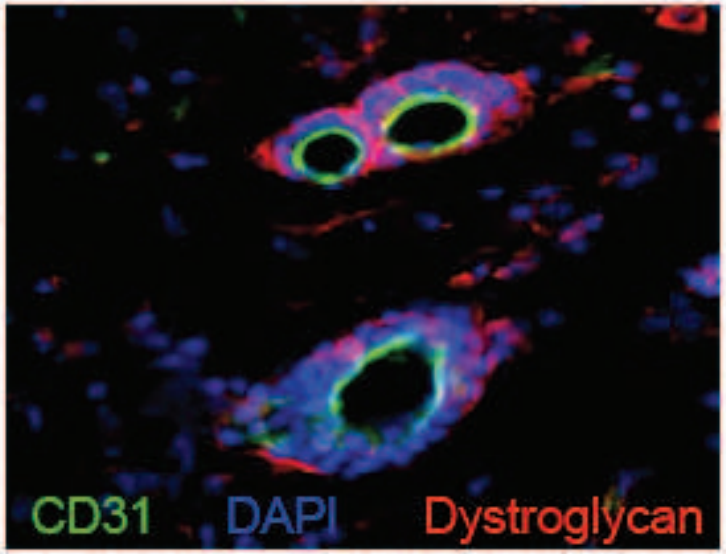
TRENDS in Immunology

Farina Aloisi Meinl 2007

Dystroglycan is expressed by astrocyte end-feet at glia limitans

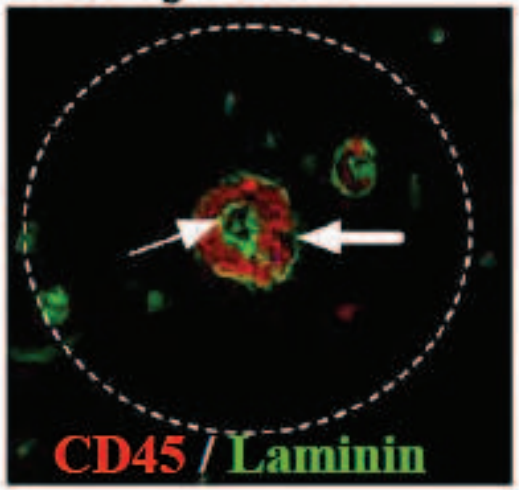
Dystroglycan is degraded by MMP2, 9 in CNS inflammatory diseases (Agarwal/Sorokin, 2006)



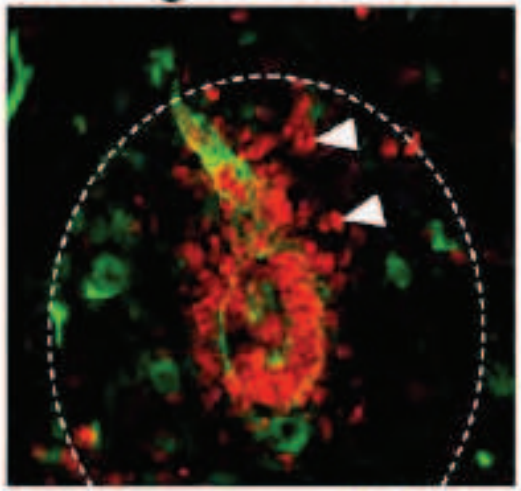


Füchtbauer

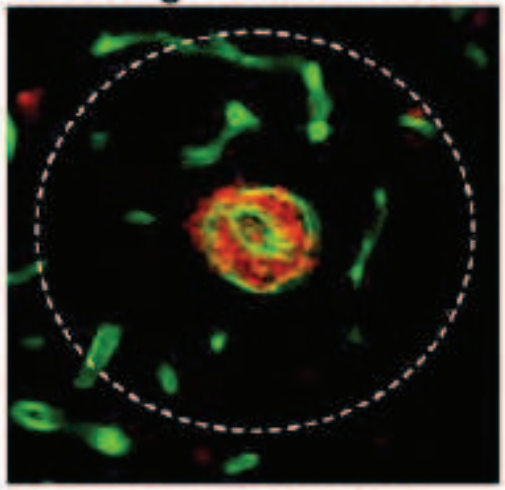
CCL2 Tg Control



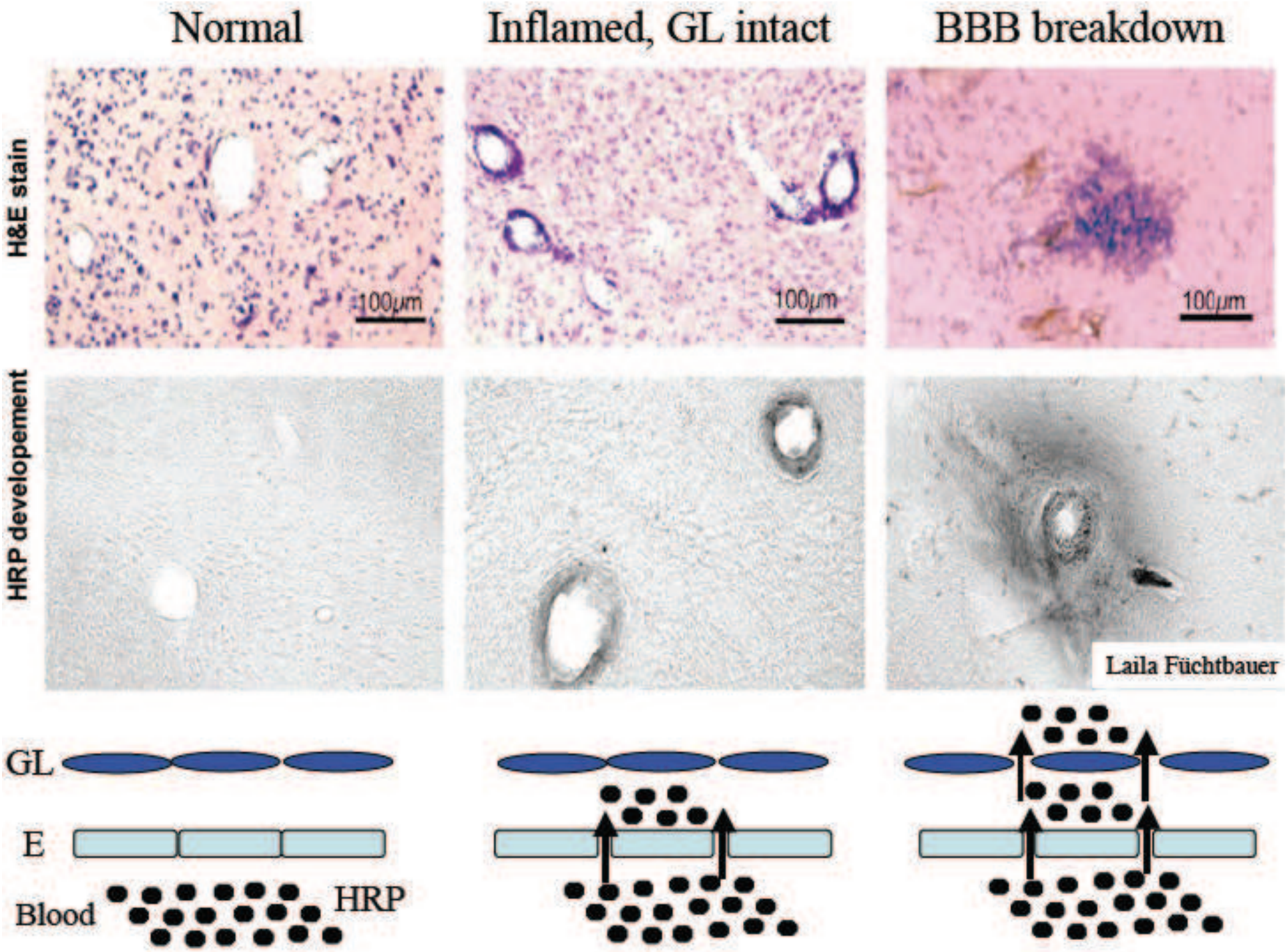
CCL2 Tg + PTx + PBS



CCL2 Tg + PTx + BB-94

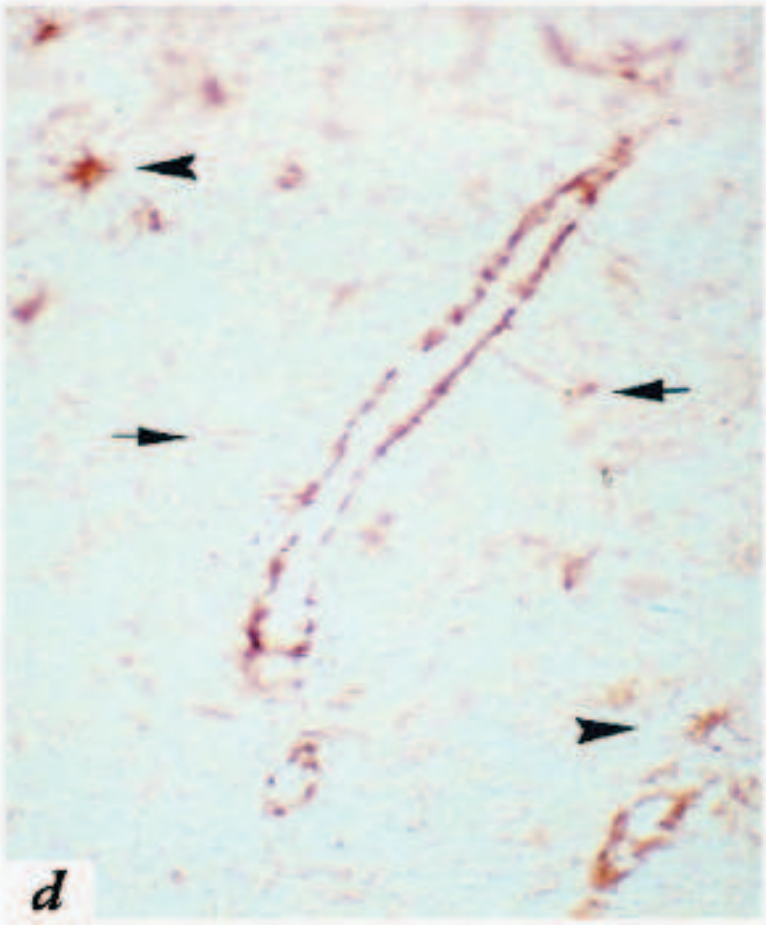


Toft-Hansen et al, J Immunol. 177:7242 (2006)

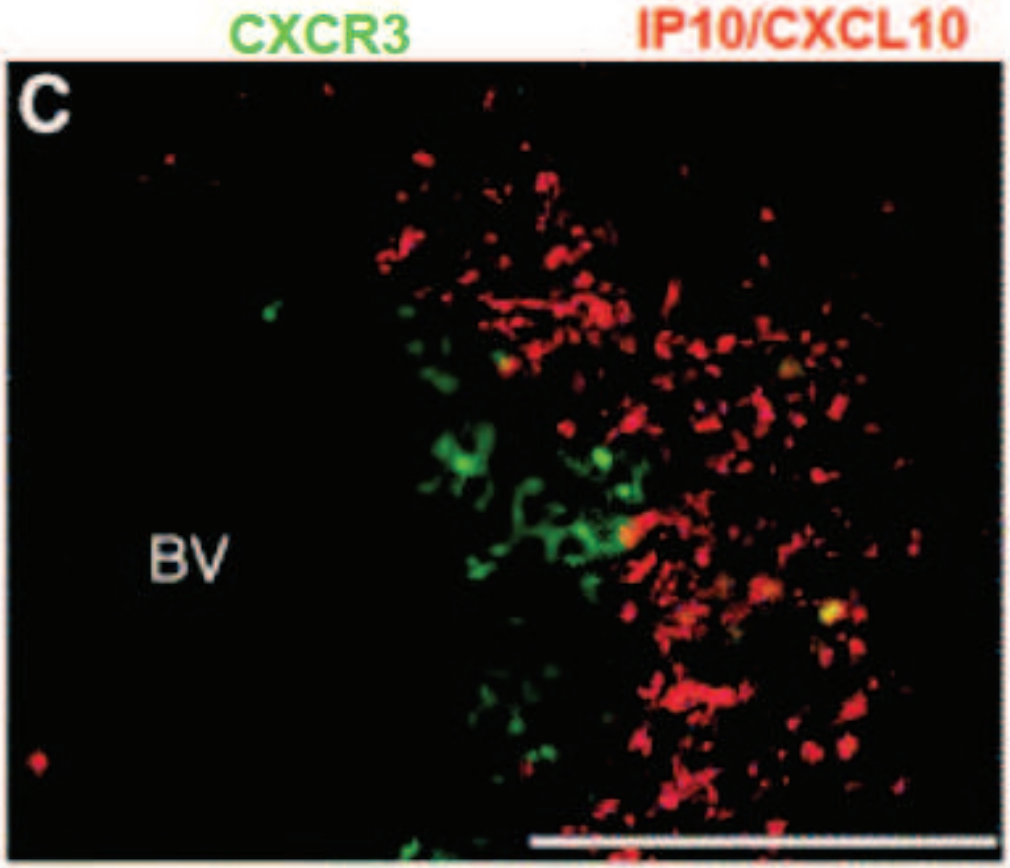

Normal
Inflamed, GL intact
BBB breakdown
H&E stain
HRP developement
100µm
100µm
100µm
Laila Füchtbauer
GL
E
Blood
HRP

Astrocytes - BBB-relevant source of chemokines

- Attract T-cells, monocytes and PMN to infiltrate the CNS
- Exert chemotactic effects on microglia in CNS

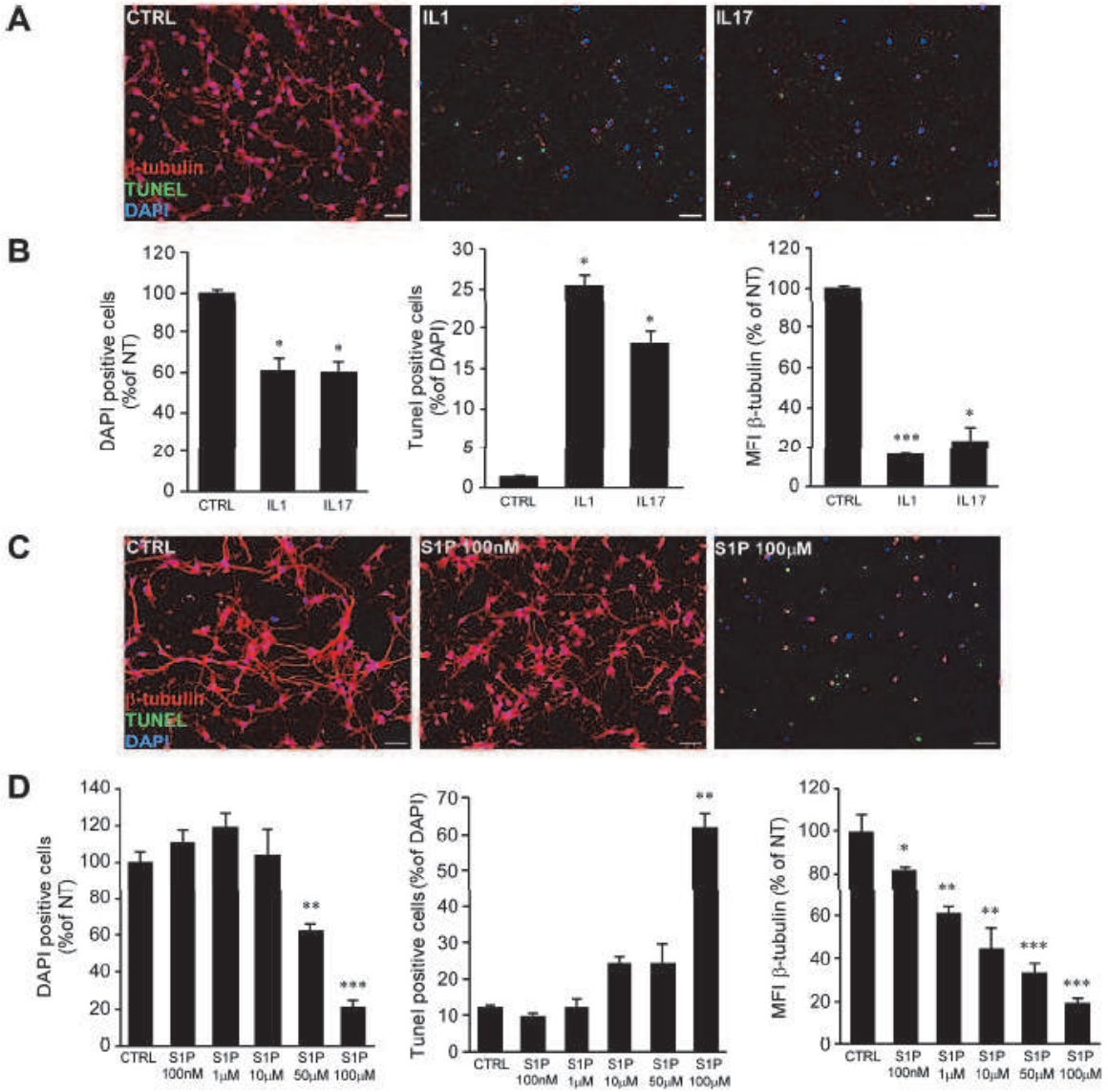


Sørensen et al. J. Clin. Invest. 1999

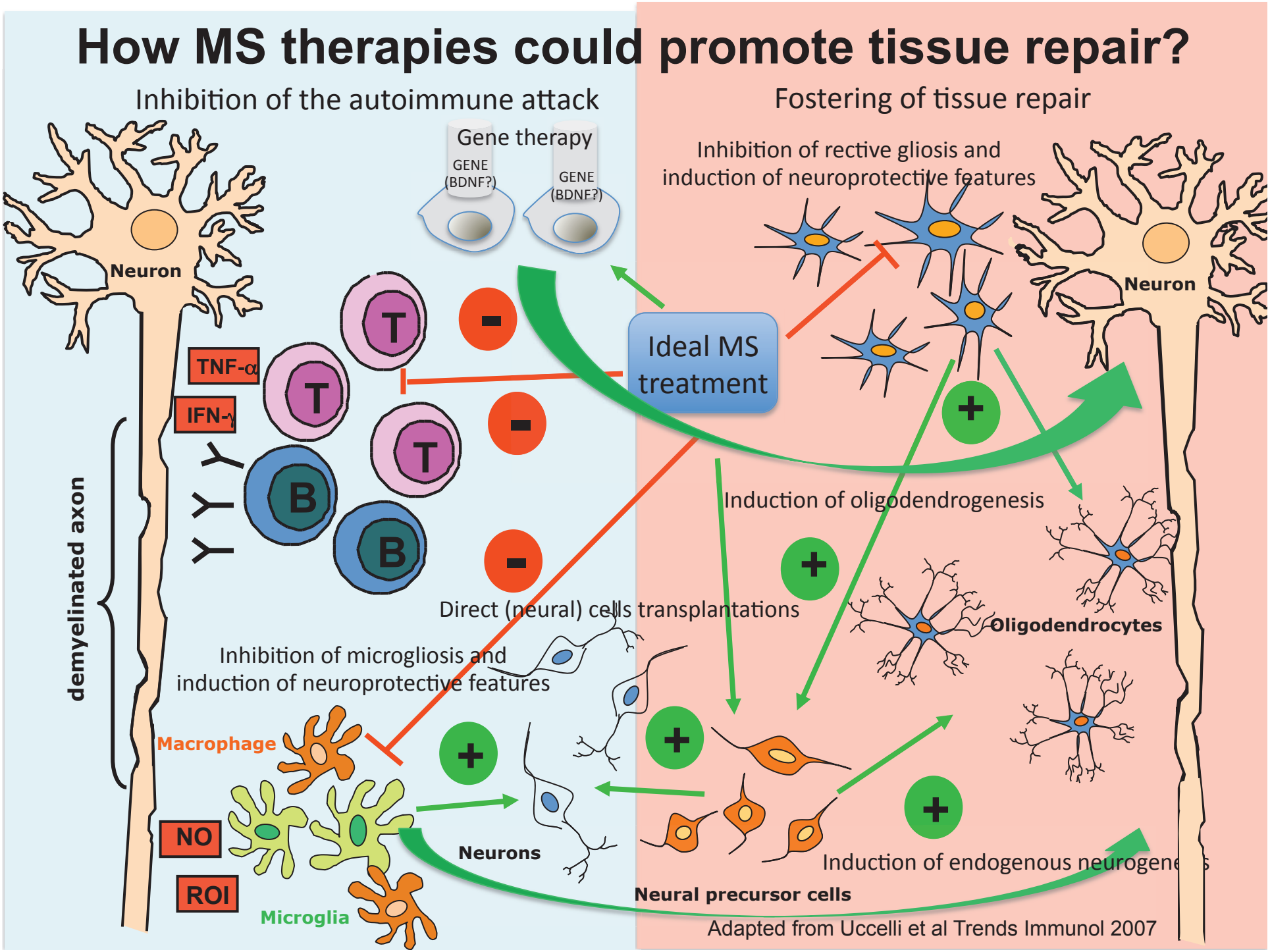


Sørensen et al. J. Neuroimmunol. 2002

Astrocyte responses to inflammatory cytokines or S1P induce neurite fragmentation and neuronal death



Colombo et al Ann Neurol 2014


How MS therapies could promote tissue repair?
Inhibition of the autoimmune attack
Fostering of tissue repair
Gene therapy
GENE (BDNF?)
GENE (BDNF?)
Inhibition of rective gliosis and induction of neuroprotective features
Neuron
Neuron
TNF-α
IFN-γ
T
T
T
B
B
Ideal MS treatment
demyelinated axon
Induction of oligodendrogenesis
Direct (neural) cells transplantations
Oligodendrocytes
Inhibition of microgliosis and induction of neuroprotective features
Macrophage
NO
ROI
Microglia
Neurons
Neural precursor cells
Induction of endogenous neurogenesis
Adapted from Uccelli et al Trends Immunol 2007

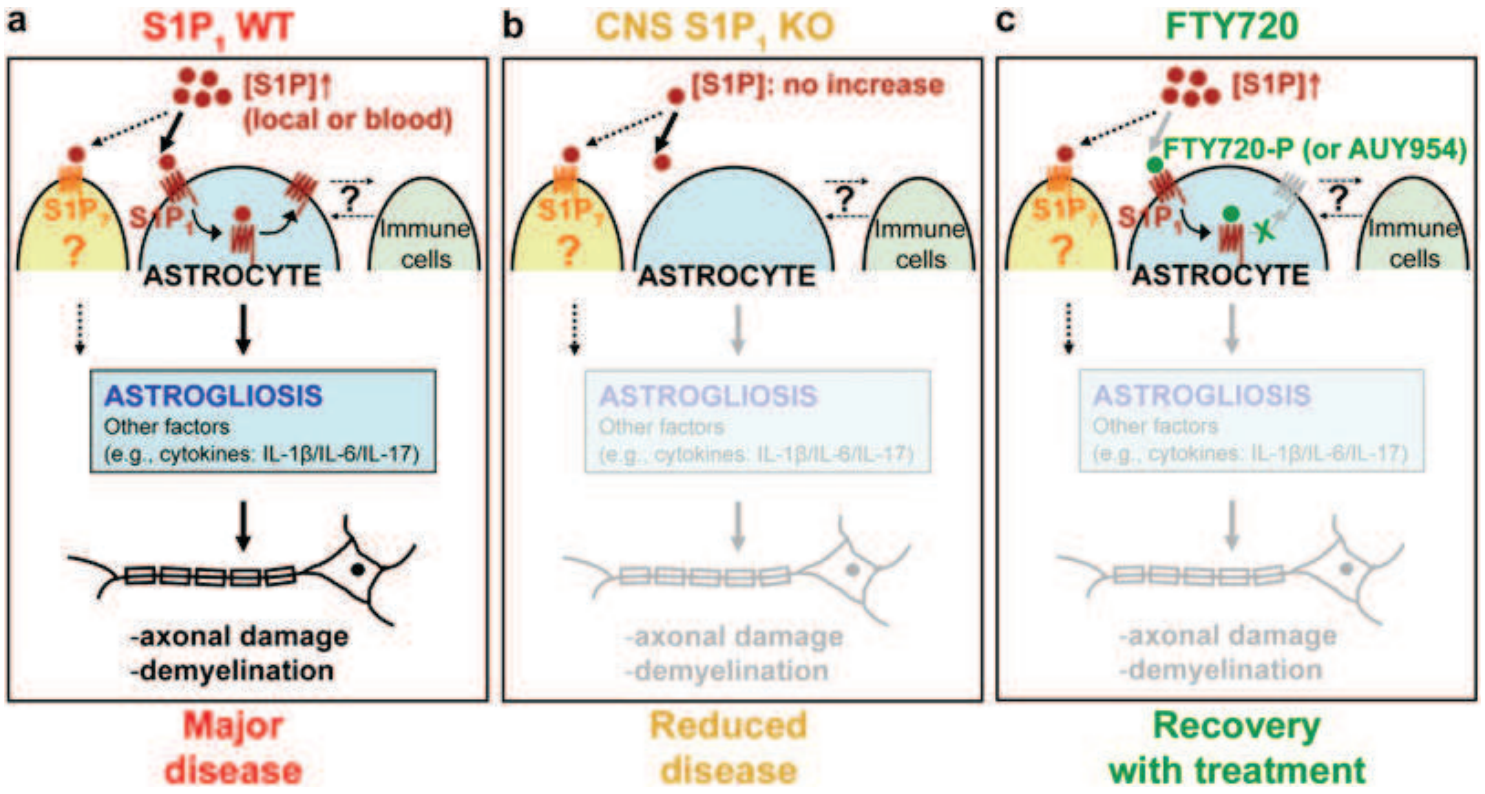
PNAS

FTY720 (fingolimod) efficacy in an animal model of multiple sclerosis requires astrocyte sphingosine 1-phosphate receptor 1 ($S1P_1$) modulation

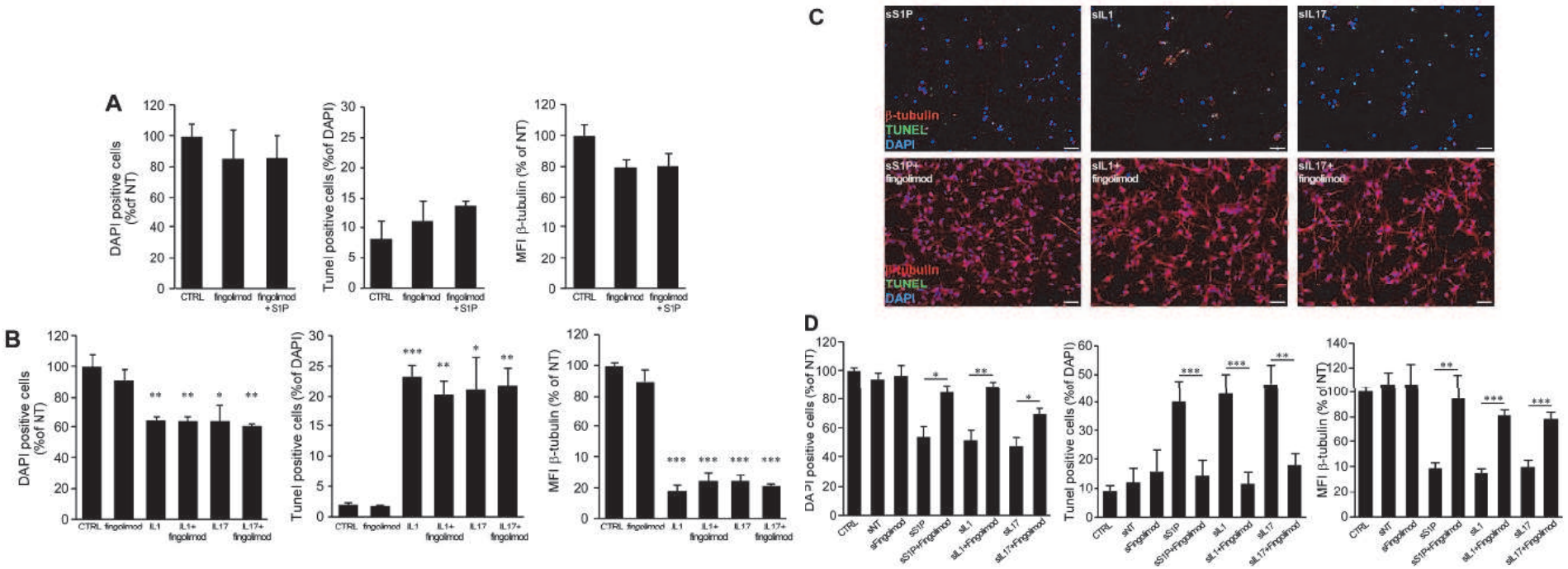
Ji Woong Choi[a,1,2], Shannon E. Gardell[a,1], Deron R. Herr[a,1], Richard Rivera[a], Chang-Wook Lee[a], Kyoko Noguchi[a], Siew Teng Teo[a,b], Yun C. Yung[a,b], Melissa Lu[a], Grace Kennedy[a], and Jerold Chun[a,3]

[a]Department of Molecular Biology, Dorris Neuroscience Center, The Scripps Research Institute, La Jolla, CA 92037; and [b]Biomedical Sciences Program, University of California at San Diego, La Jolla, CA 92093

Edited by Michael Sela, Weizmann Institute of Science, Rehovot, Israel, and approved November 10, 2010 (received for review September 21, 2010)



Fingolimod blocks neurodegeneration induced by astrocyte responses to cytokines and S1P



Colombo et al Ann Neurol 2014

Therapeutic administration of fingolimod to EAE mice reduces cytokines and S1P receptor expression and NO production in vivo

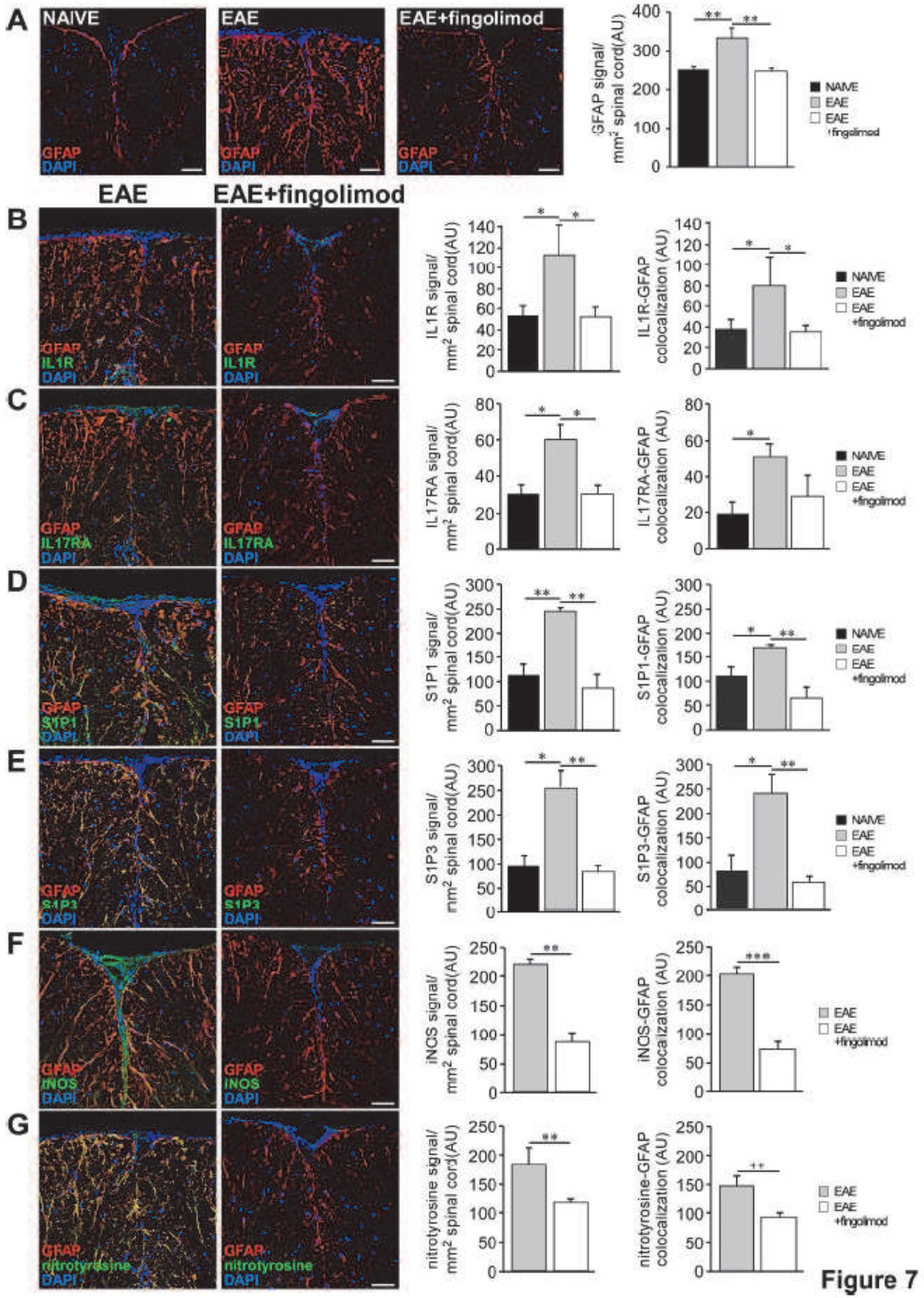
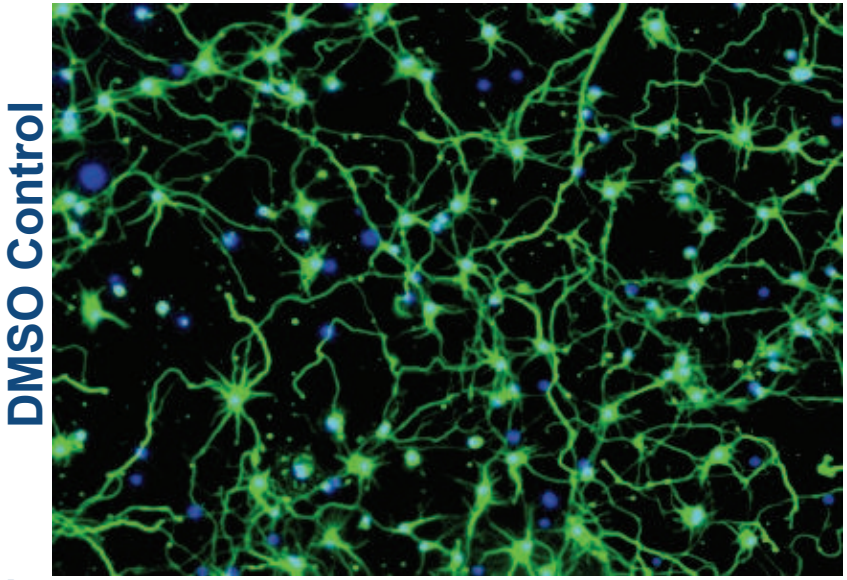
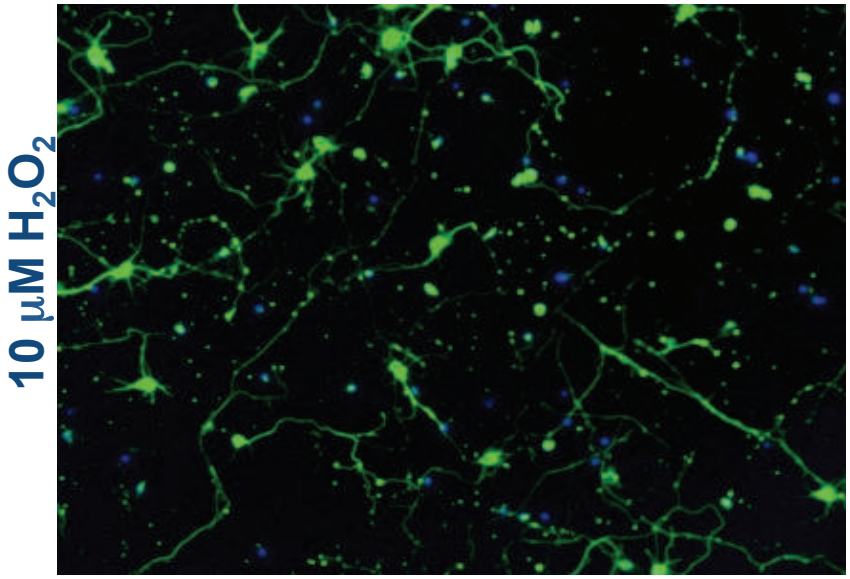


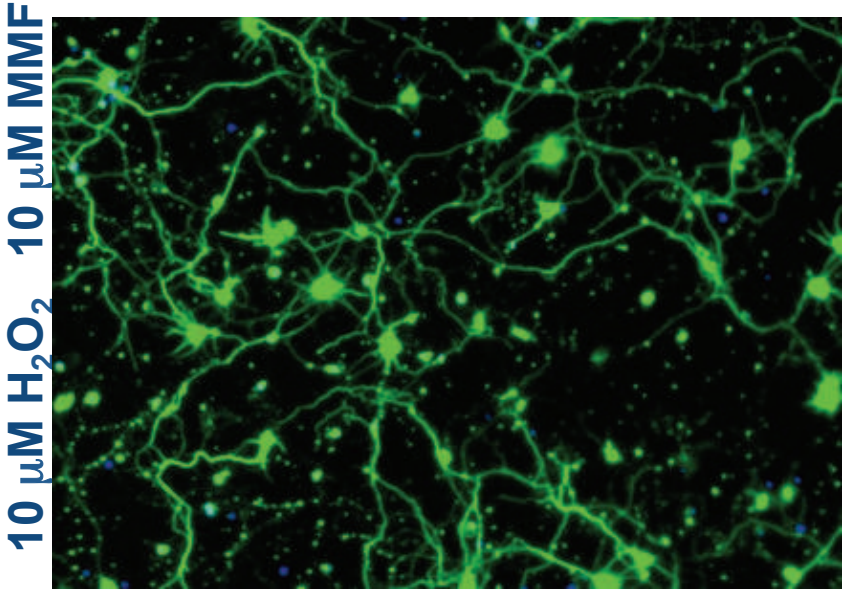
Figure 7

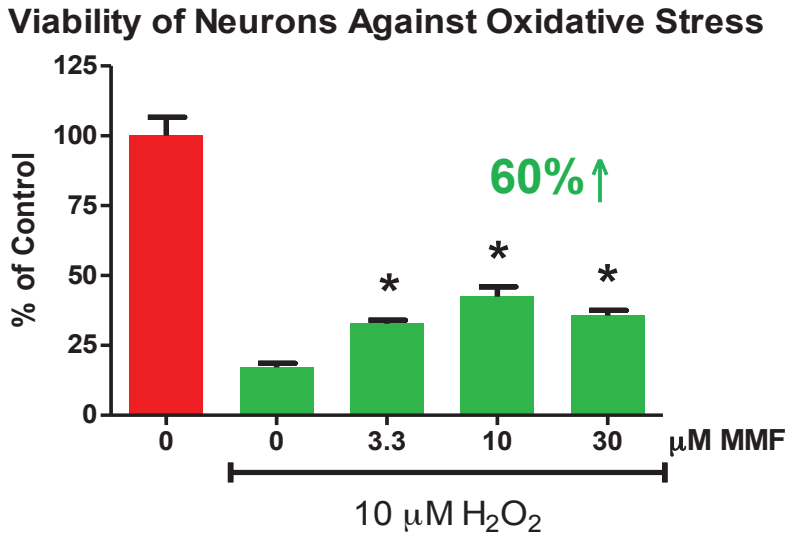
Colombo et al Ann Neurol 2014

Dimethyl Fumarate Improved Neuron Survival After Oxidative Challenge



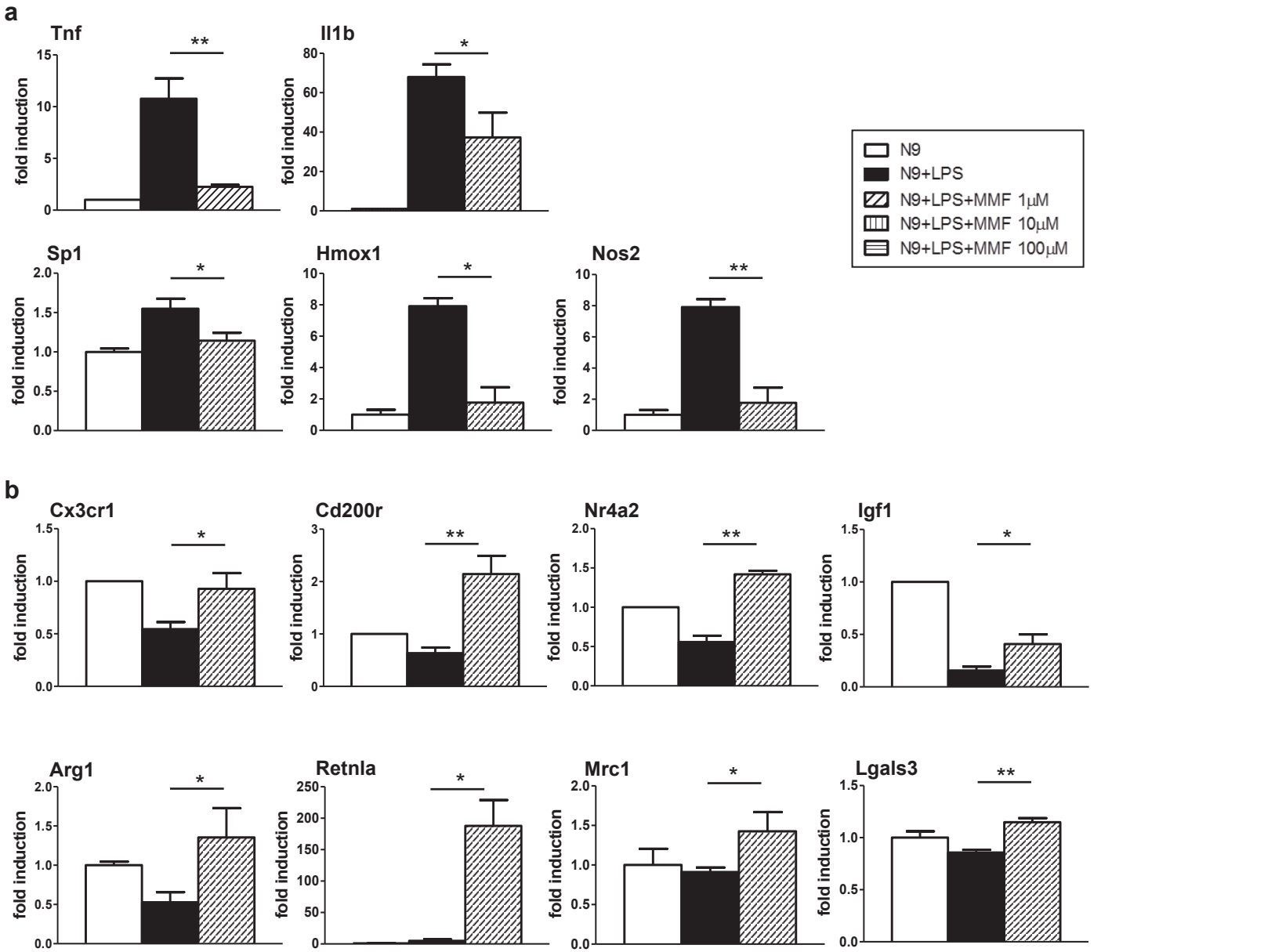






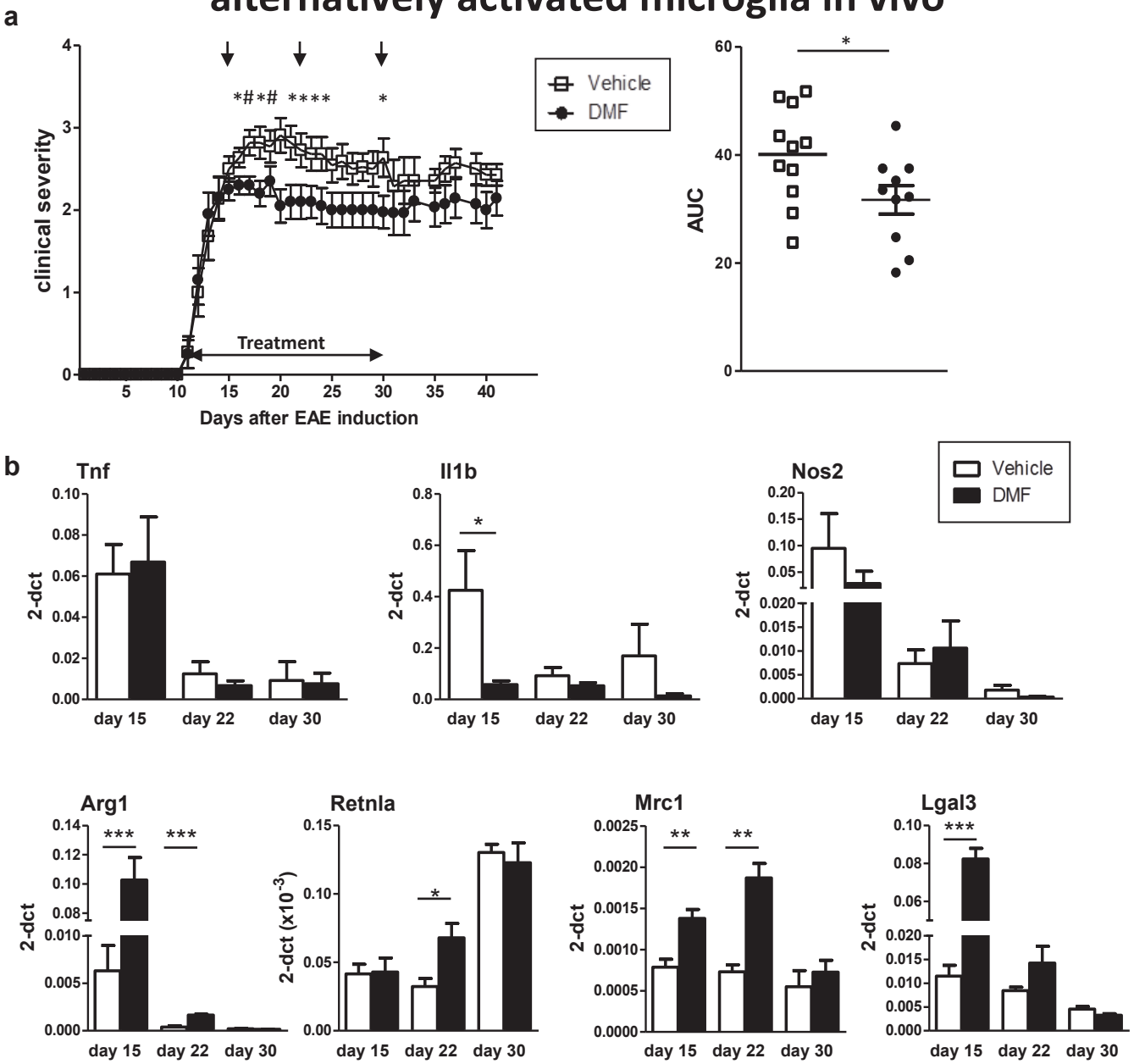
Scannevin et al. (2012) JPET

MMF induces a molecular switch in activated microglia from a pro-inflammatory to an alternatively activated phenotype



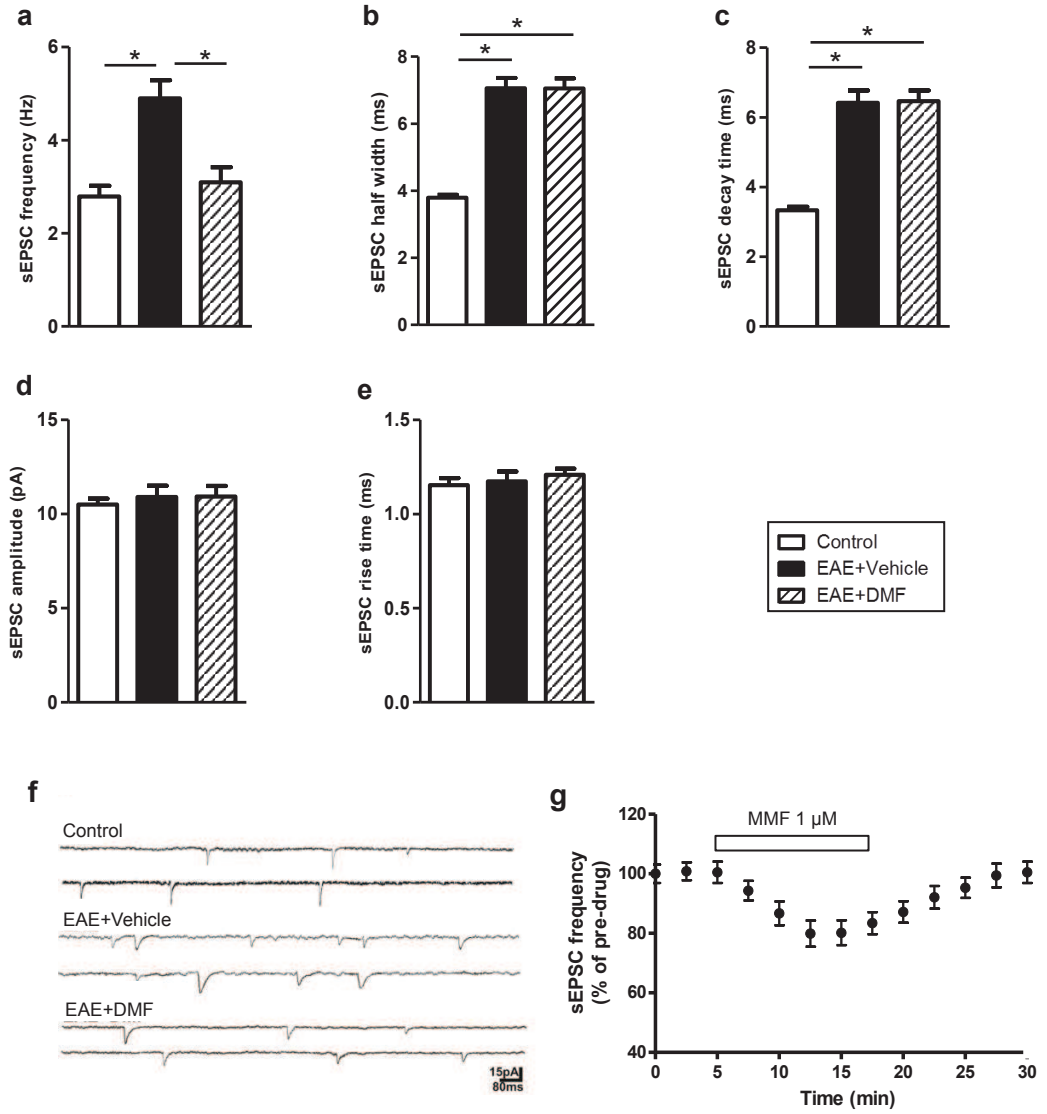
Parodi et al, Acta Neuropathol 2015

Treatment with DMF ameliorates EAE and induces an increase in markers of alternatively activated microglia in vivo



Parodi et al, Acta Neuropathol 2015

DMF treatment normalizes pre-synaptic abnormalities of glutamatergic transmission in EAE mice

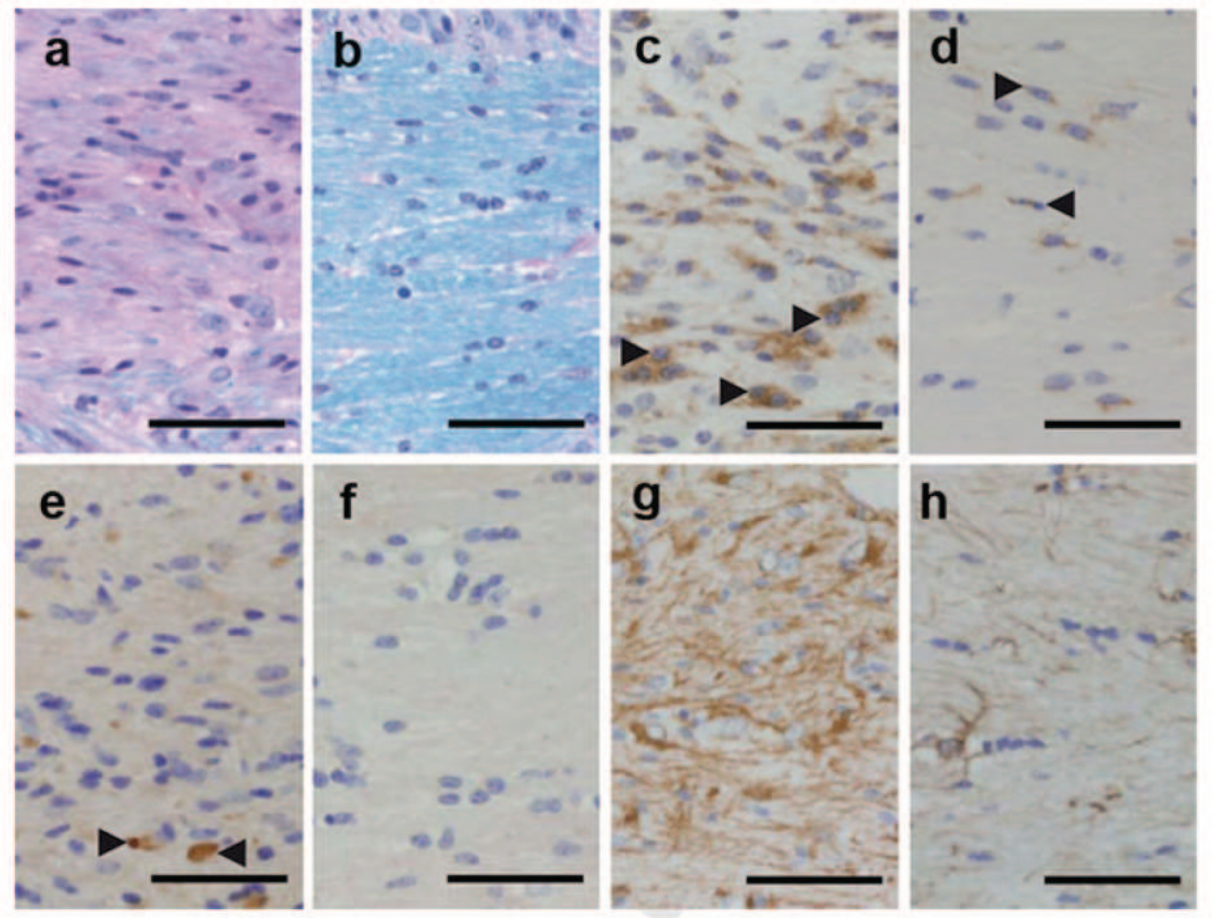


In collaboration with D. Centonze and S. Rossi

Parodi et al, Acta Neuropathol 2015

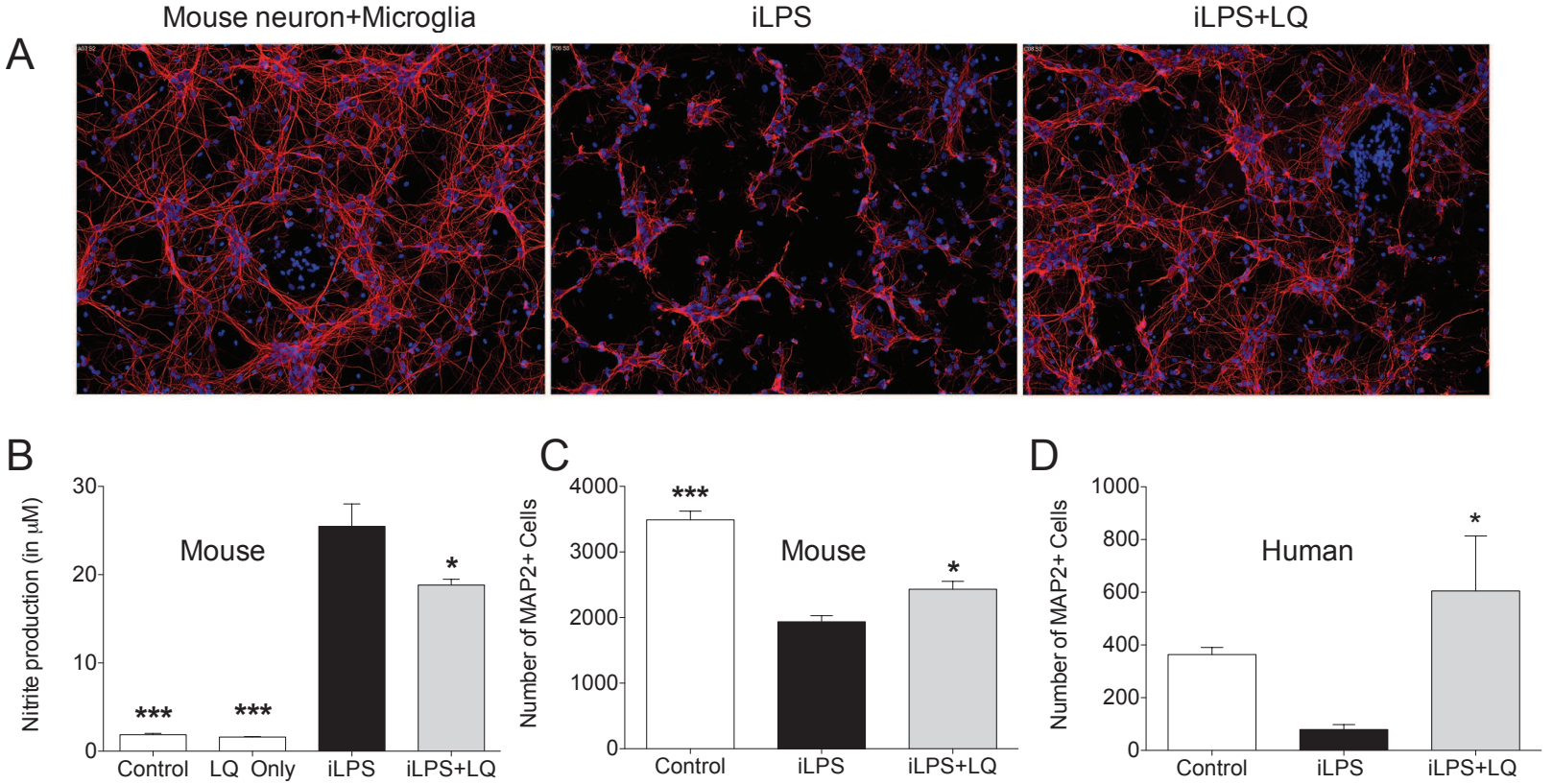
Laquinimod reduces demyelination, microglia infiltration, acute axonal damage and gliosis independent of T and B cells

In comparison to controls, LAQ-treated Rag1 -/- mice displayed markedly reduced demyelination in the corpus callosum (Fig. 4a,b), fewer callosal microglia (Fig. 4c, d), fewer APP-positive axonal spheroids (Fig. 4e, f) and less fiber gliosis (Fig. 4g, h).



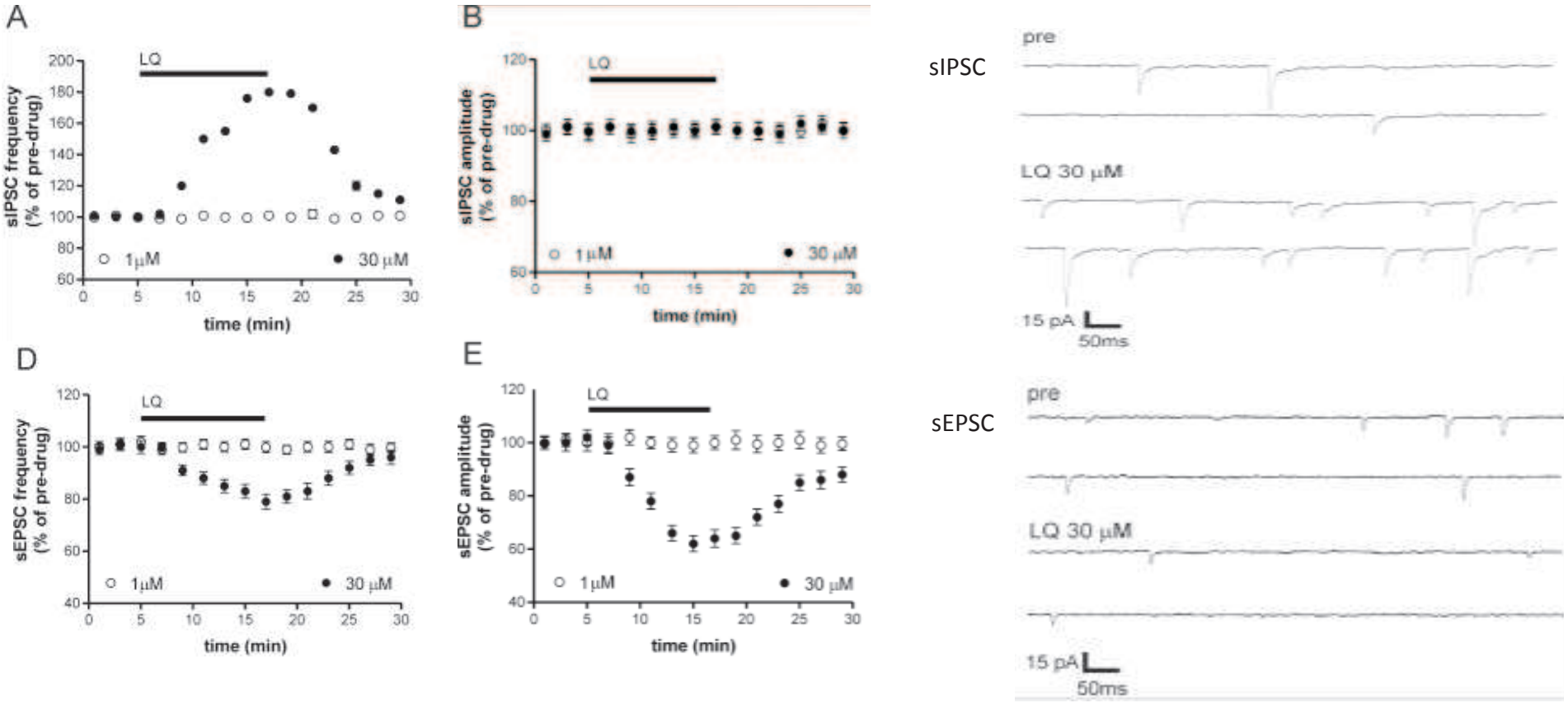
Brück et al., Acta Neuropathol. 2012 September; 124(3): 411–424.

In co-culture of Neurons and Microglia, Activated Microglia Kill Neurons and this Is Reduced by Laquinimod

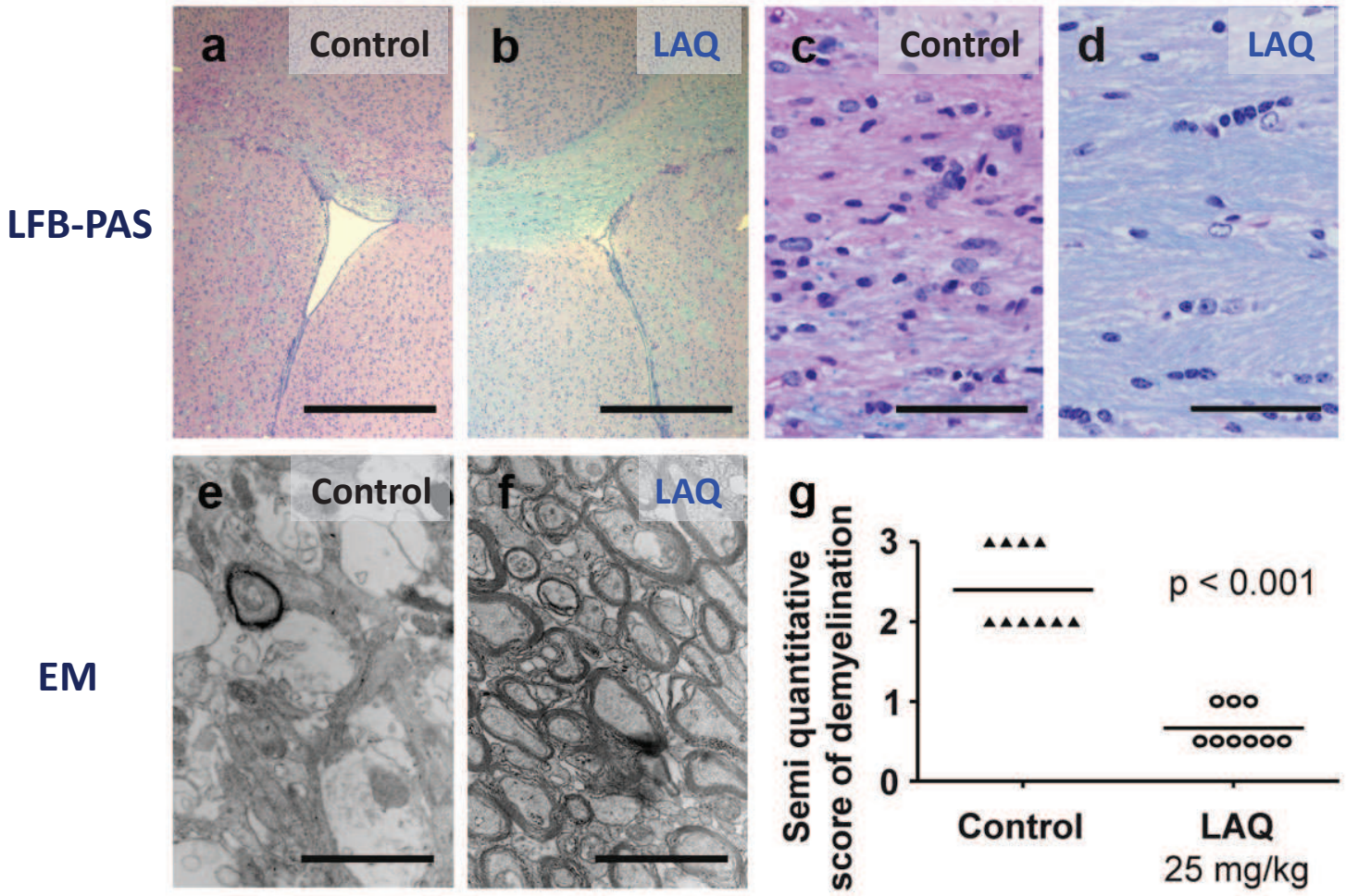


- Laquinimod pretreatment of microglia reduced the generation of NO and loss of neurons caused by iLPS.
- iLPS activation of mouse or human neuronal microglia co-culture leads to the reduction of neuronal counts, and this was attenuated by laquinimod treatment

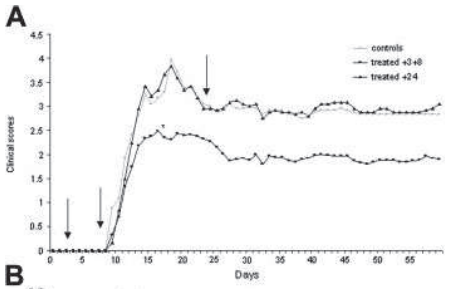
Laquinimod regulates synaptic transmission by reducing glutamate-mediated excitatory currents and increasing GABA-mediated inhibitory currents



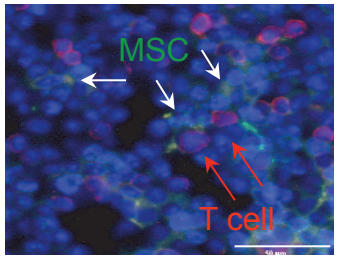
Effect of Laquinimod on Demyelination in the Cuprizone Model

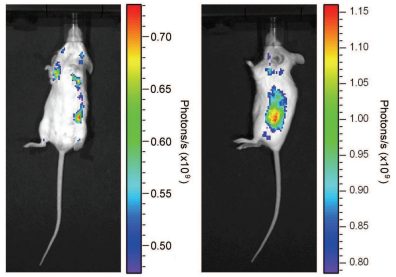


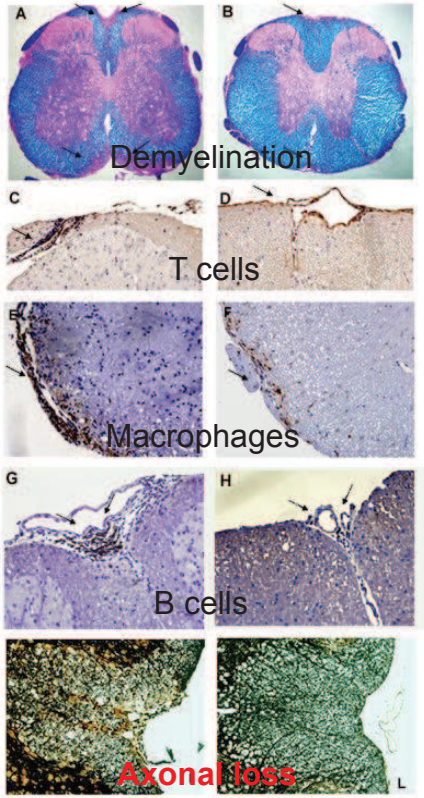
MSCs ameliorate EAE inducing in vivo tolerance to myelin antigens inside secondary lymphoid organs (Zappia et al 2005; Gerdoni et al 2007)



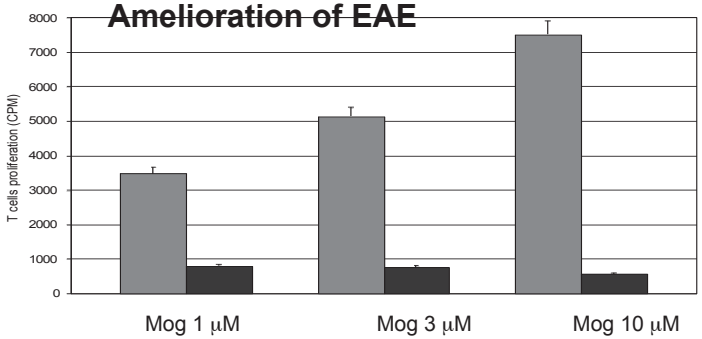
Lymph nodes engraftment

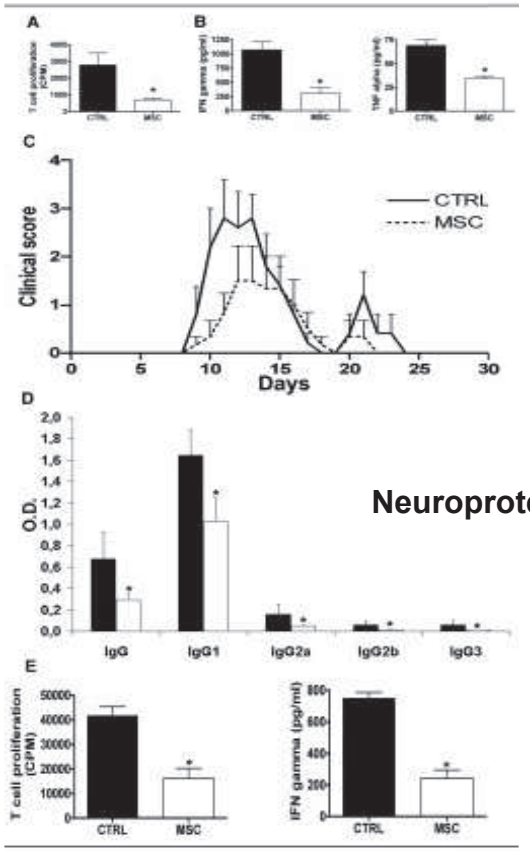




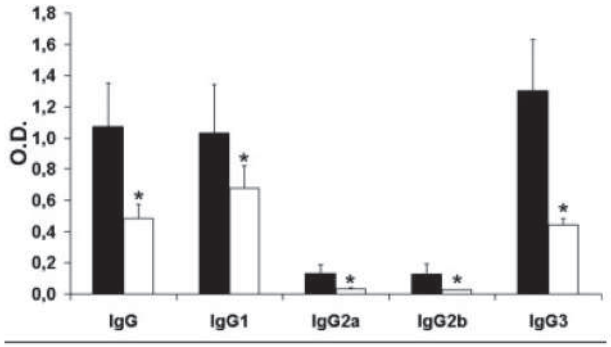


Amelioration of EAE

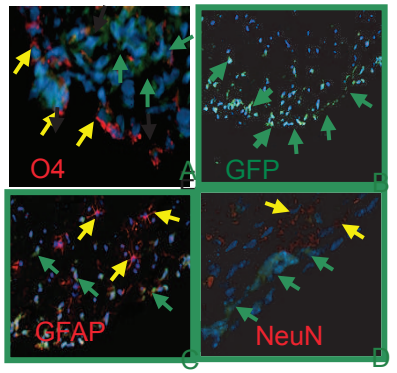




Inhibition of Ag-specific T and B cell response

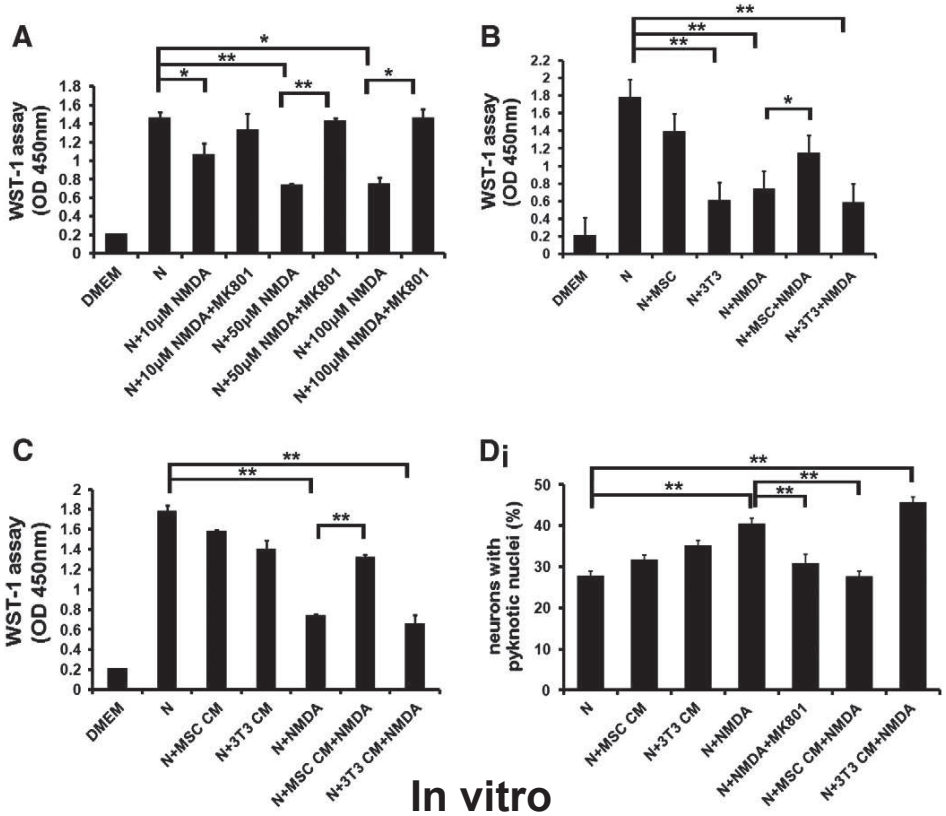


Neuroprotection without transdifferentiation

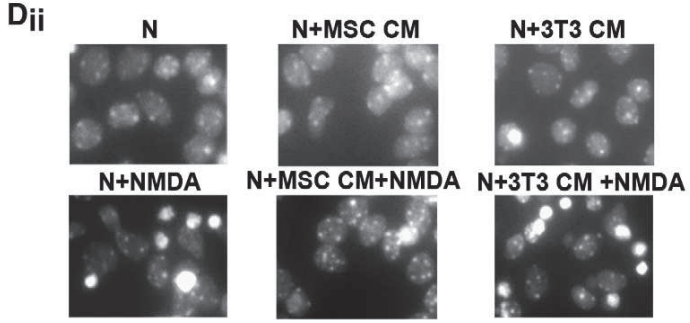


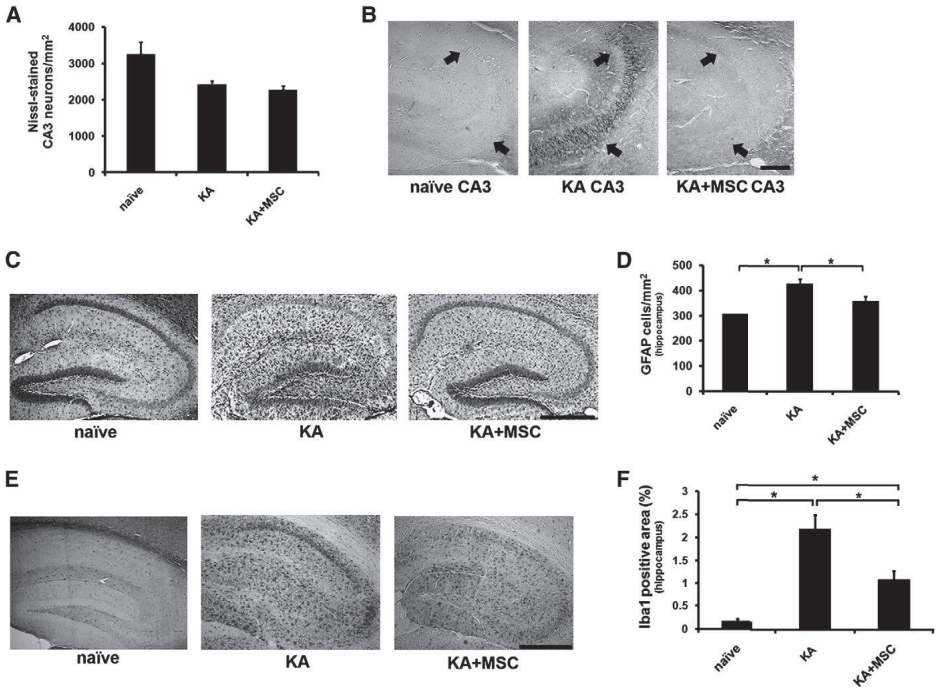
Inhibition of T cell encephalitogenic potential

Mesenchymal stem cells protect CNS neurons against glutamate excitotoxicity



In vitro

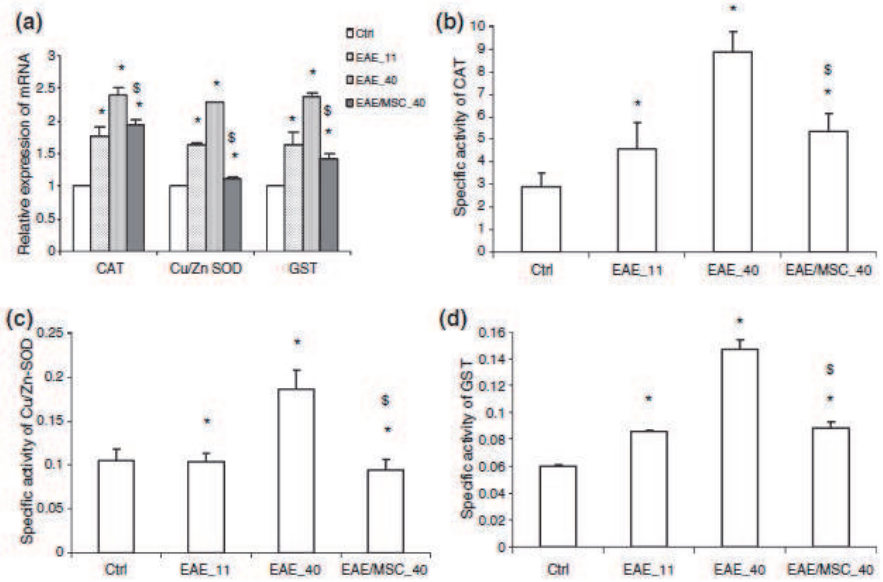


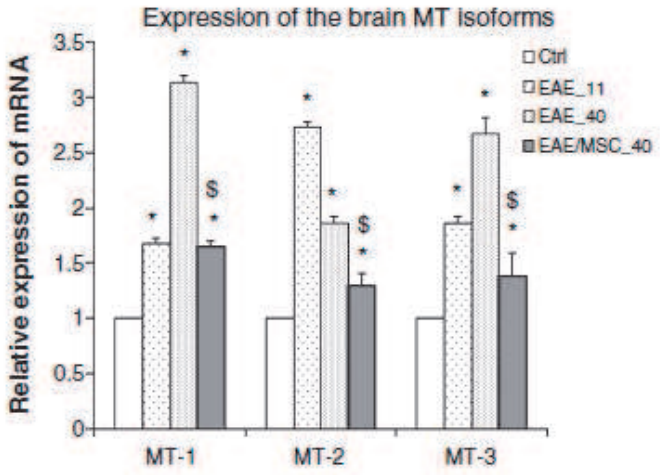


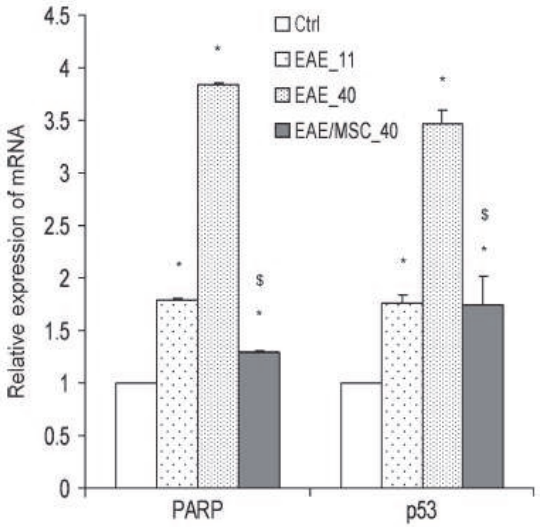
In vivo

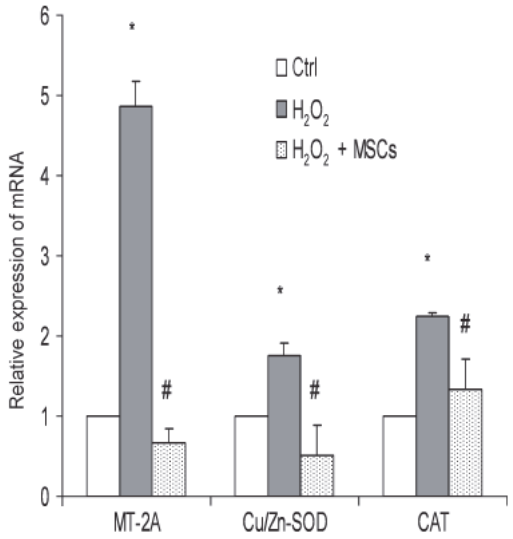
Voulgari-Kokota et al
Exp Neurol 2012

MSC reduce the EAE-dependent oxidative stress in the CNS



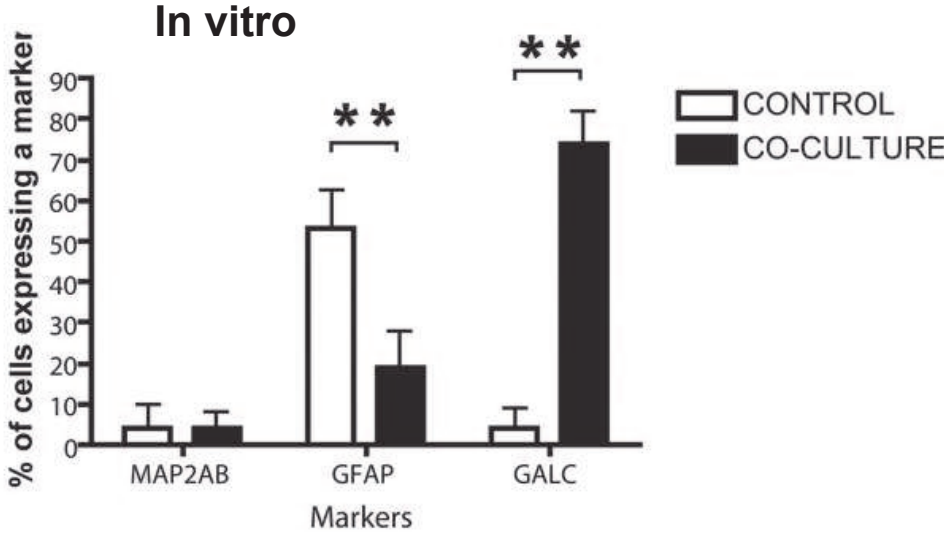


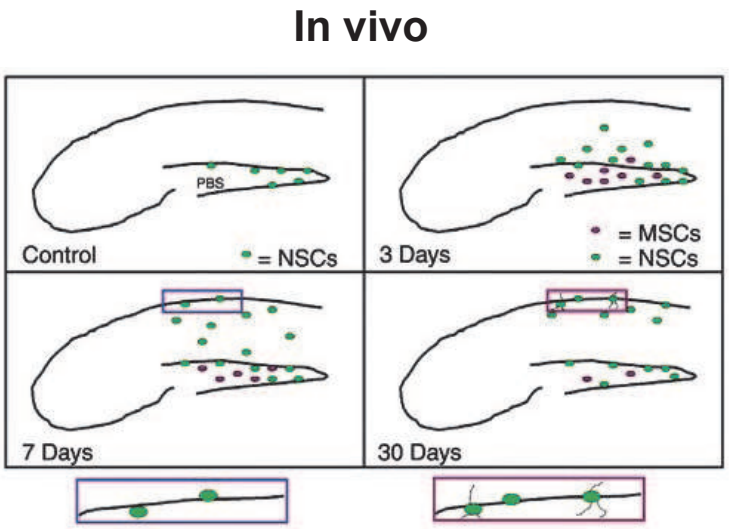


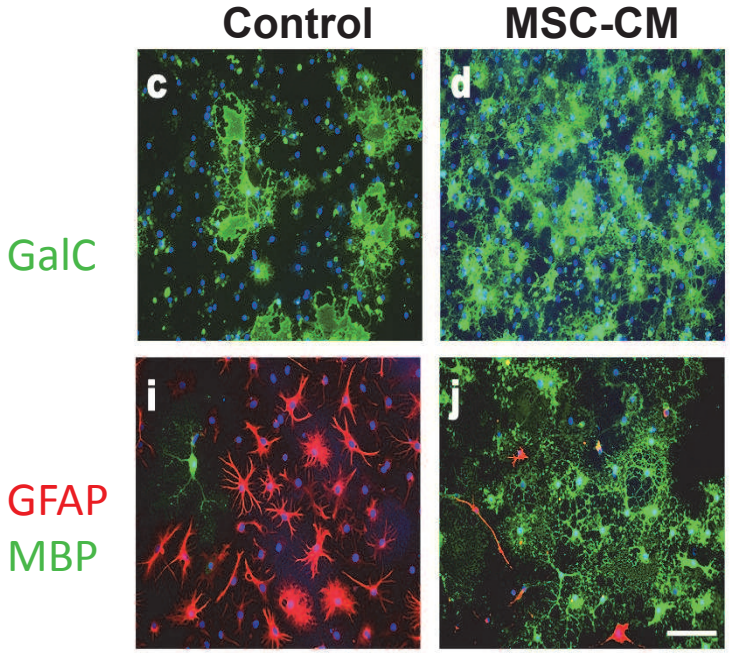


Lanza et al, JNC 2009

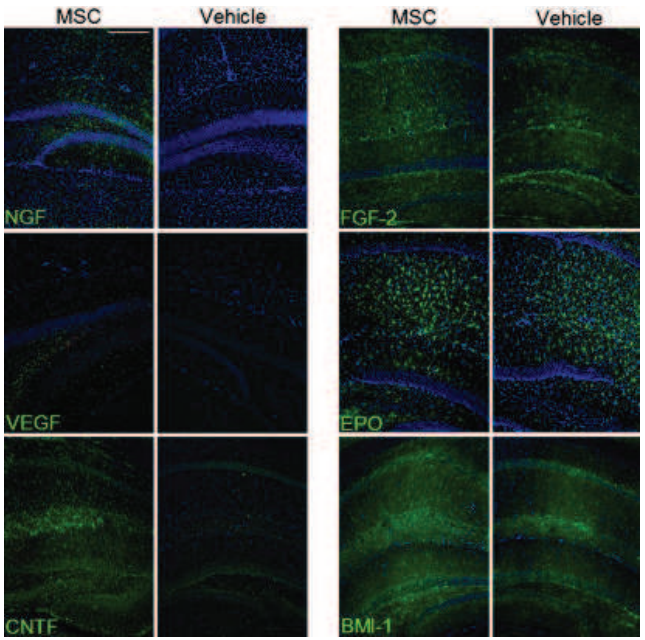
Mesenchymal stem cells promote neurogenesis





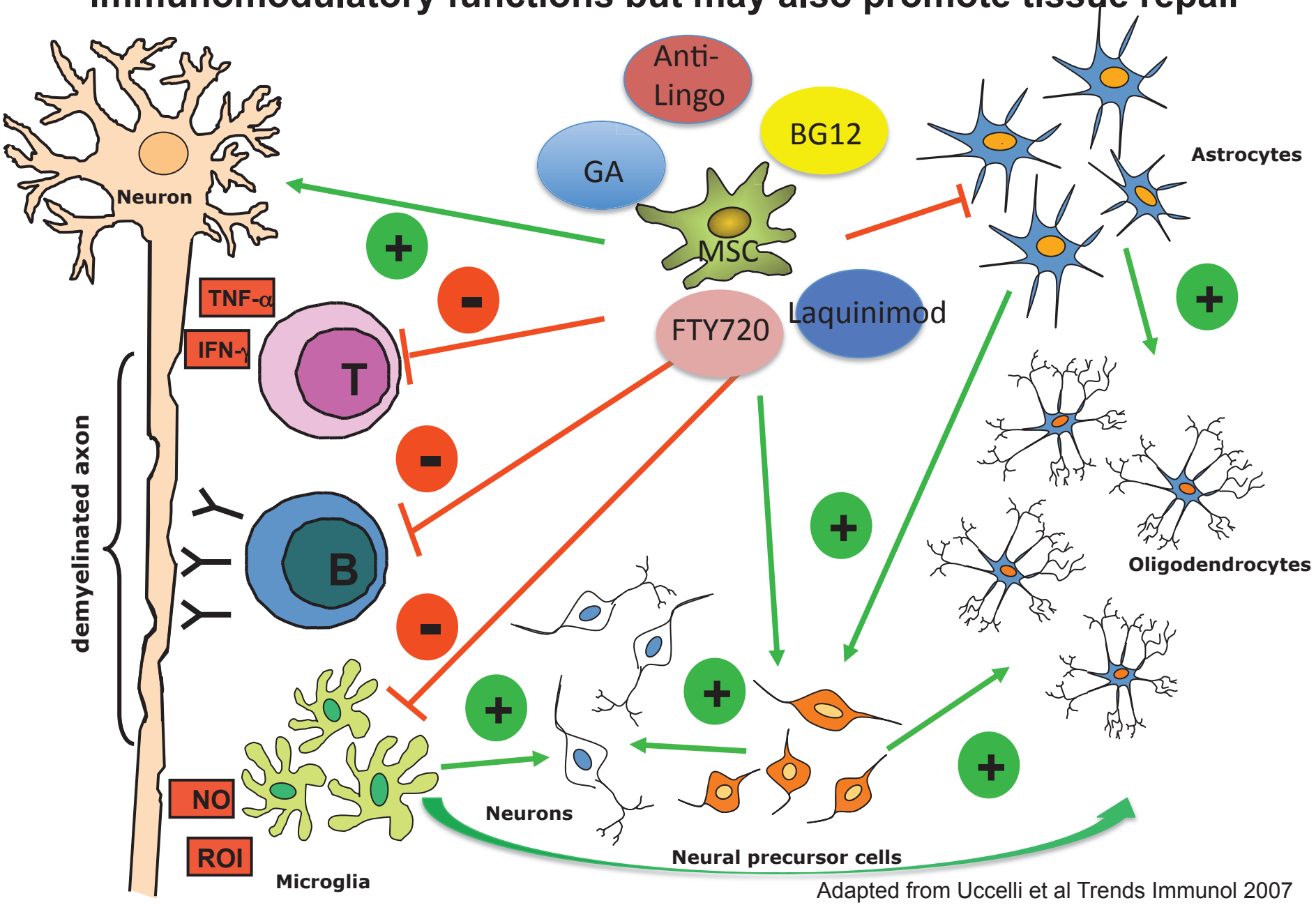


Rivera et al., Stem Cells, 2006



Munoz et al, PNAS 2006

Treatments for multiple sclerosis mainly display immunomodulatory functions but may also promote tissue repair



Adapted from Uccelli et al Trends Immunol 2007