Microglia and macrophage activation

focusing on myelin phagocytosis

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Microglia and macrophages are distinct populations (i)

- *Microglia* and *Macrophages* share a common mesodermal origin – the yolk sac
- *Microglia* and *Macrophages* are members of the Mononuclear Phagocytic System (MPS)
- *Microglia* and *Macrophages* share many receptors and functions
- Nonetheless, *Microglia* and *Macrophages* also differ in some of their functions

Microglia and macrophages are distinct populations (ii)

- *Microglia* originate from MPS-precursors that migrate during embryogenesis to the developing CNS parenchyma, giving rise to a self-sustained population of proliferating microglia
- *Monocytes* originate from MPS-precursors that migrate during embryogenesis to the bone-marrow
- *Tissue Macrophages* originate from circulating bone-marrow derived *monocytes* after their recruitment to tissues

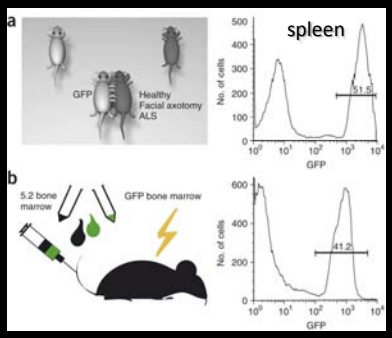
Microglia and macrophages are distinct populations (iii)

- *Microglia* and *Monocytes/Macrophages* populations become separated one from the other by the Blood-Brain-Barrier (BBB) at/or around birth
- Blood-borne *monocytes* enter the CNS when the BBB is functionally and/or anatomically disrupted (e.g. trauma, radiation and multiple sclerosis)

Microglia and macrophages are distinct populations (iv)

parabiosis

radiation



Ajami, Bennett, Krieger, Tetzlaff & Rossi, *Nature Neuroscience, 2007*

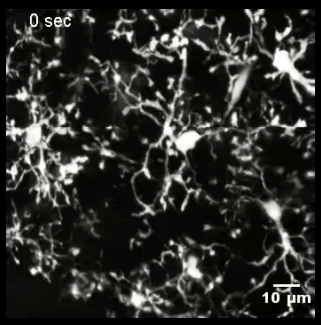
Microglia – the third element in the CNS

Santiago Ramon Y Cajal

Pio del Rio-Hortega

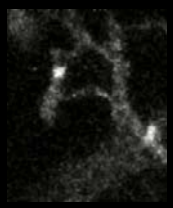
Wilder G. Penfield

In-vivo imaging "resting" microglia *GFP+* by time lapse two-photon microscopy



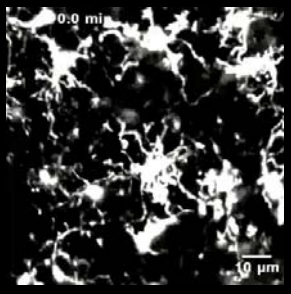
Davalos, Grutzendler, Yang, Kim, Zuo, Jung, Littman, Dustin & Gan, *Nature Neuroscience*, 2005

In-vivo imaging "resting" microglia *GFP+* by time lapse two-photon microscopy



Nimmerjahn, Kirchhoff & Fritjof, *Science*, 2005

In-vivo imaging of injury activated microglia *GFP+* by time lapse two-photon microscopy



Davalos, Grutzendler, Yang, Kim, Zuo, Jung, Littman, Dustin & Gan, *Nature Neuroscience*, 2005

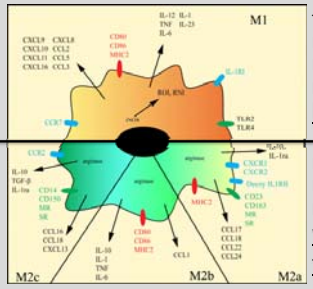
Microglia activation – what does it mean?

- the term "activation" by itself is not informative
- microglia can be induced to upregulate the expression of various molecules and thereby be activated to perform different functions
- "activation" must be defined by the type of
  - molecules that are upregulated
    and / or
  - functions that are activated

M1-killer and M2-wound healing phenotypes

M1

Ly6C/Gr1⁺



TNFα & IL-1
ROS
IL-6
phagocytosis
killer

M2

Ly6C/Gr1⁻

IL-10
IL-6
phagocytosis
wound-healing
tissue remodeling

Benoit, Desnues & Mege, *J. Immunol.* 2008
Auffray, Sieweke, & Geissmann, *Ann. Rev. Immunol.* 2009

Are microglia and macrophages friend or foe?

M2 type – Wound healing

Anti-Inflammatory cytokines
- IL-10

Phagocytosis
- apoptotic cells
- pathogens
- neurotoxic products of neurodegeneration
- tissue debris

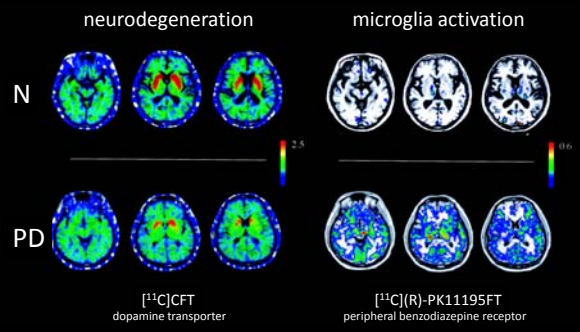
M1 type – Killer

Inflammatory cytokines
- TNFα, IL-1β

Neurotoxicity
- ROS
- PGE

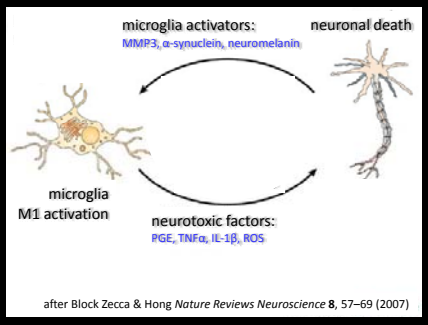
Phagocytosis

Microglia activation in Parkinson's diseases



Ouchi, Yoshikawa, Sekine, Futatsubashi, Kanno, Ogusu & Torizuka, *Ann Neurol* 2005

Neuroinflammation/microglia and neurodegeneration form a vicious circle



after Block Zecca & Hong *Nature Reviews Neuroscience* **8**, 57–69 (2007)

Are microglia and macrophages friend or foe?

M2 type – Wound healing

Anti-Inflammatory cytokines
- IL-10

Phagocytosis
- apoptotic cells
- pathogens
- neurotoxic products of neurodegeneration
- tissue debris

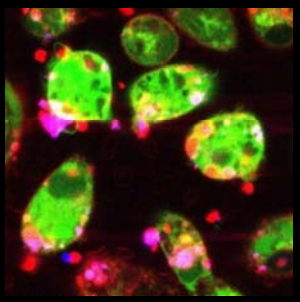
M1 type – Killer

Inflammatory cytokines
- TNFα, IL-1β

Neurotoxicity
- ROS
- PGE

Phagocytosis

Phagocytosis of degenerated-myelin time-lapse confocal microscopy

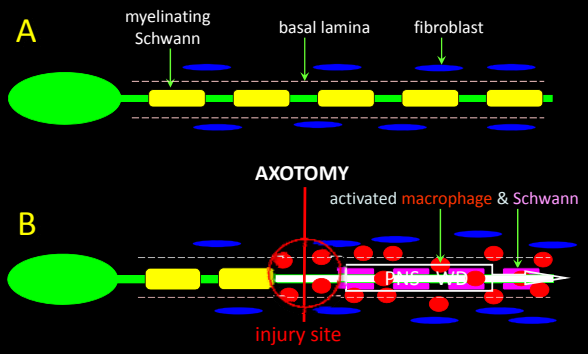


Hadas, Spira, Hanisch, Reichert , Rotshenker, *J. Neuroinflammation* 2012

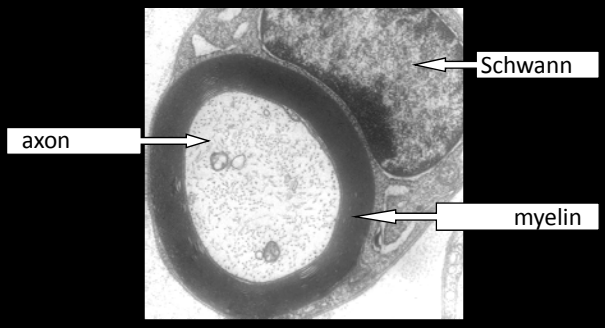
Mechanisms that regulate the phagocytosis of degenerated-myelin

- Galectin-3 / MAC-2
- Cytoskeleton; actin and myosin
- Immune inhibitory receptors
  - SIRPα and CD47 interactions

PNS: (A) intact and (B) Wallerian degeneration

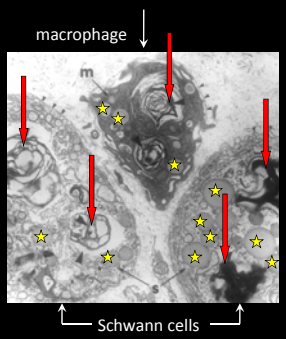


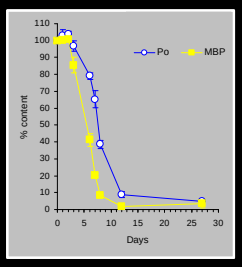
Intact PNS: myelinated axon



Reichert, Saada & Rotshenker, *J. Neurosci.* 1994

Myelin phagocytosis and degradation in normal PNS Wallerian degeneration (WD)

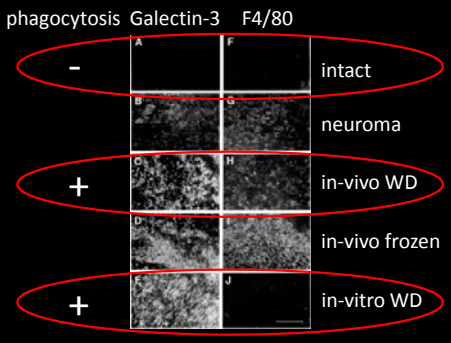




Reichert, Saada & Rotshenker, *J. Neurosci.* 1994

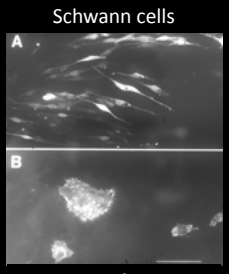
Be'eri, Reichert, Saada & Rotshenker, *Eur. J. Neurosci.* 1998

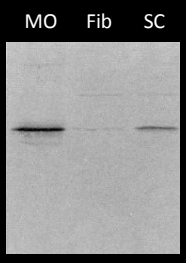
Galectin-3/MAC-2 expression is correlated with myelin phagocytosis in injured PNS



Reichert, Saada & Rotshenker, *J. Neurosci.* 1994

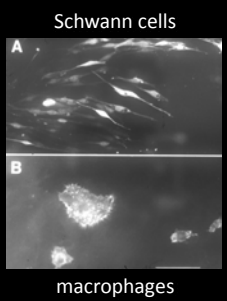
Macrophages and Schwann cells express Galectin-3 / MAC-2 as they clear degenerated-myelin

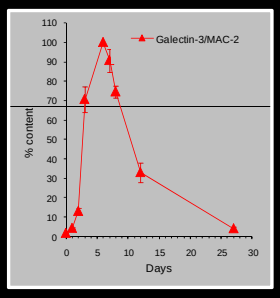




Reichert, Saada & Rotshenker, *J. Neurosci.* 1994

Macrophage and Schwann cell activation in normal Wallerian degeneration – Galactin-3 / MAC-2

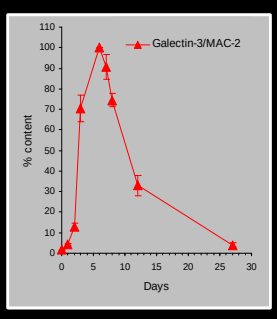


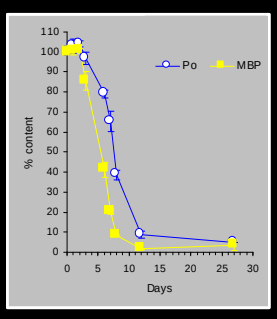


Reichert, Saada & Rotshenker, *J. Neurosci.* 1994

Be'eri, Reichert, Saada & Rotshenker, *Eur. J. Neurosci.* 1998

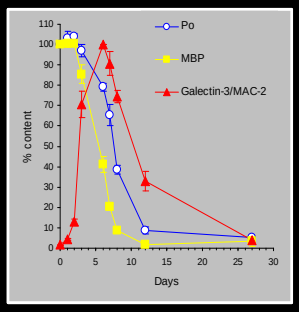
Schwann cell & macrophage activation myelin phagocytosis & degradation





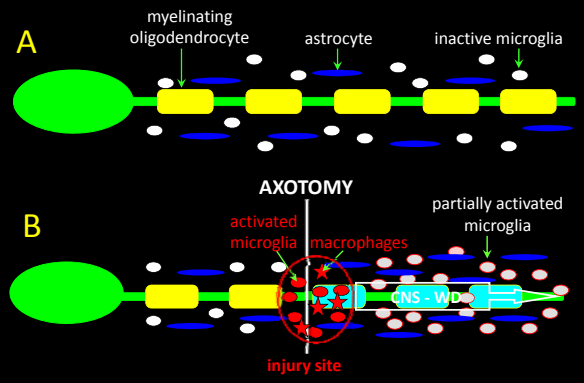
Be'eri, Reichert, Saada & Rotshenker, *Eur. J. Neurosci.* 1998

Schwann cell & macrophage activation myelin phagocytosis & degradation are orchestrated in time

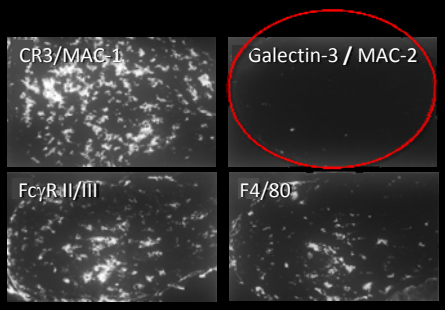


Be'eri, Reichert, Saada & Rotshenker, *Eur. J. Neurosci.* 1998

CNS: (A) intact and (B) Wallerian degeneration

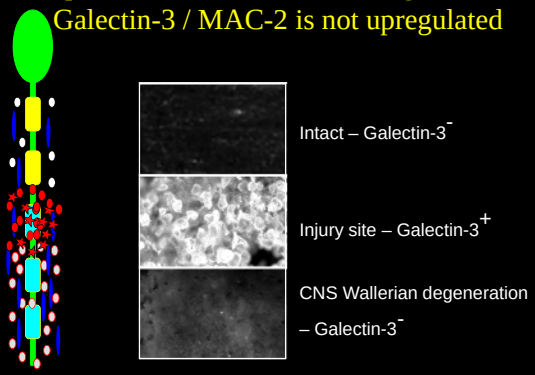


Deficient activation of microglia in optic nerve CNS Wallerian degeneration; Galectin-3 / MAC-2 is not upregulated



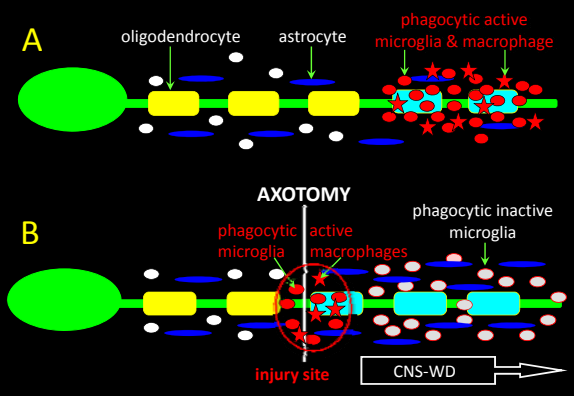
Reichert & Rotshenker, *J. Neuroimmunol.*, *1996*

Deficient activation of microglia in spinal cord CNS Wallerian degeneration: Galectin-3 / MAC-2 is not upregulated

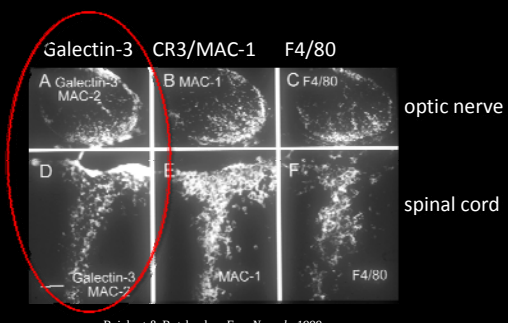


Liraz-Zaltsmann, Reichert & Rotshenker, *unpublished*

Microglia are activated to phagocytose myelin in EAE and at injury sites, but not at CNS-WD



EAE activated microglia & macrophages

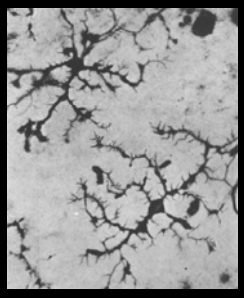


Reichert & Rotshenker, *Exp. Neurol.*, *1999*

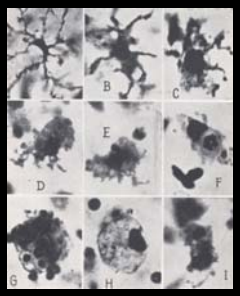
... microglia phagocytose myelin in EAE better than macrophages...
Rinner, Bauer, Schmidts, Lassmann & Hickey, *Glia*, 1995

Phagocytic active and phagocytic inactive microglia

Branched forms of the normal microglia of the cerebral cortex

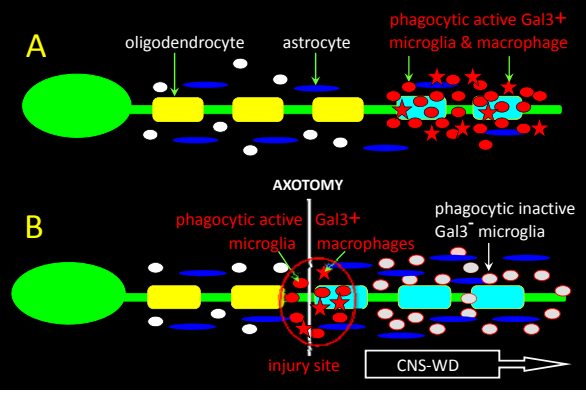


Evolution of microglia during its phagocytic activity; amoeboid



Pio del Rio-Hortega, *Microglia, in Cytology & Cellular Pathology of the Nervous System*, 1932

Galectin-3 / MAC-2 is expressed in (A) EAE and (B) injury sites, but not in CNS Wallerian degeneration



	F4/80	FcRγII/III	SRAI/II	CR3/MAC-1	Galectin-3/ MAC-2	myelin phagocytosis
in-vivo CNS-Wallerian degeneration	+++	+++	+++	+++	—	—
in-vivo CNS-EAE & in-vitro	+++	+++	+++	+++	+++	+++

1. Galectin-3 expression and myelin phagocytosis are correlated

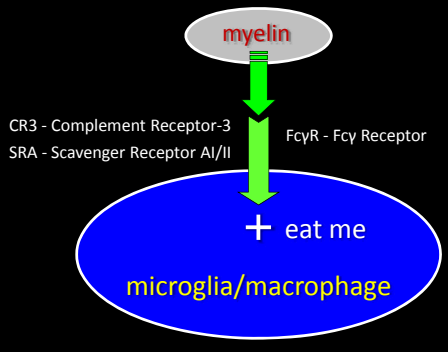
	F4/80	FcRγII/III	SRAI/II	CR3/MAC-1	Galectin-3/ MAC-2	myelin phagocytosis
in-vivo CNS-Wallerian degeneration	+++	+++	+++	+++	—	—
in-vivo CNS-EAE & in-vitro	+++	+++	+++	+++	+++	+++

1. Galectin-3 expression and myelin phagocytosis are correlated
2. CR3 and SRA can either be
   - "active" and mediate phagocytosis
     or
   - "inactive" and not mediate phagocytosis

	F4/80	FcRγII/III	SRAI/II	CR3/MAC-1	Galectin-3/ MAC-2	myelin phagocytosis
in-vivo CNS-Wallerian degeneration	+++	+++	+++	+++	—	—
in-vivo CNS-EAE & in-vitro	+++	+++	+++	+++	+++	+++

1. Galectin-3 expression and myelin phagocytosis are correlated
2. CR3 and SRA can either be active and mediate phagocytosis or inactive and not mediate phagocytosis
3. Galectin-3 marks a state of activation where CR3 and SRA mediate myelin phagocytosis
4. Working hypothesis: Galectin-3 activates CR3 and SRA mediated myelin phagocytosis

Phagocytosis is a receptor mediated event whereby phagocytes internalize particles > 0.5µM



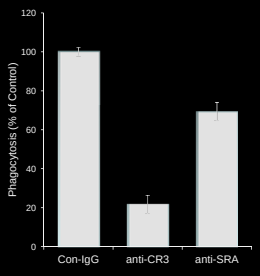
CR3; αM/β2 integrin

CR3 and SRA mediate phagocytosis of degenerated-myelin by Gal-3^{+} microglia

Galectin-3 (MAC-2)

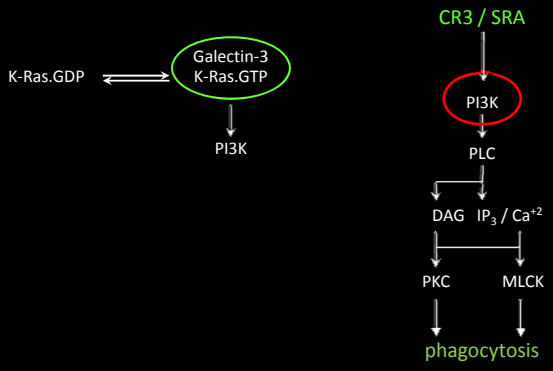
CR3 (complement receptor-3)

SRAI/II (scavenger receptor-A/II)



Rotshenker, *J. Mol. Neurosci., 2003*
Reichert & Rotshenker, *Neurobiol. Dis., 2003*
Reichert, Slovodov, Makranz & Rotshenker, *Neurobiol. Dis., 2001*

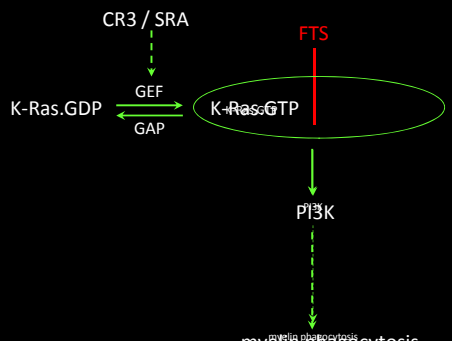
The signaling network of myelin phagocytosis



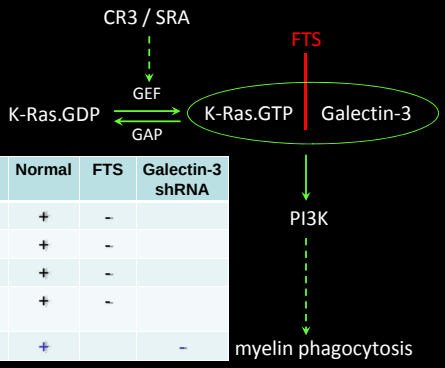
Kloog *et al.*

Makranz, Cohen, …Reichert & Rotshenker, *Neurobiol. Dis., 2004*
Cohen, Makranz,….Maya Spira, Reichert & Rotshenker, *Glia, 2006*

Galectin-3 / MAC-2 activates phagocytosis through K-Ras.GTP / PI3K



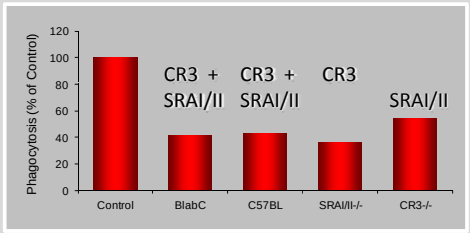
Galectin-3 / MAC-2 activates phagocytosis through K-Ras.GTP / PI3K



	Normal	FTS	Galectin-3 shRNA
phagocytosis	+	-	
K-Ras.GTP	+	-	
PI3K	+	-	
K-Ras.GTP Galectin-3	+	-	
phagocytosis	+		-

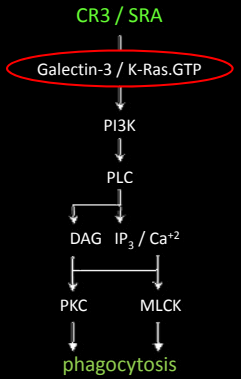
Rotshenker, Reichert, Gitik, Haklai, Elad-Sfadia & Kloog, *Glia, 2008*

FTS inhibits myelin phagocytosis that is mediated by CR3 and SRAI/II

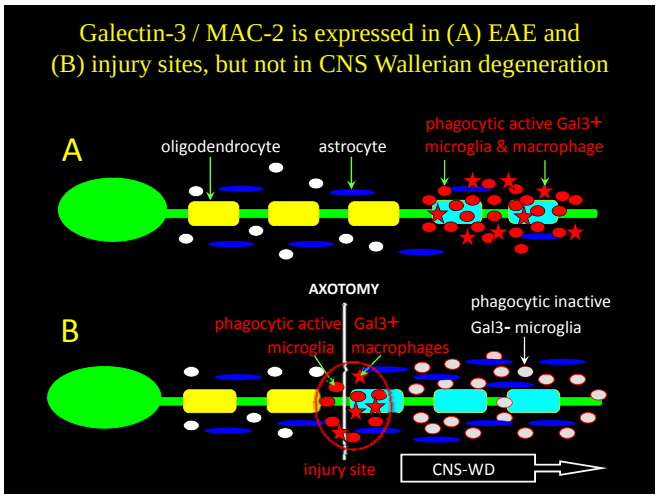


Rotshenker, Reichert, Gitik, Haklai, Elad-Sfadia & Kloog, *Glia, 2008*

The signaling network of myelin phagocytosis



Makranz, Cohen, …Reichert & Rotshenker, *Neurobiol. Dis., 2004*
Cohen, Makranz,….Maya Spira, Reichert & Rotshenker, *Glia, 2006*


Galectin-3 / MAC-2 is expressed in (A) EAE and
(B) injury sites, but not in CNS Wallerian degeneration
A
oligodendrocyte
astrocyte
phagocytic active Gal3+
microglia & macrophage
AXOTOMY
B
phagocytic active
microglia
Gal3+
macrophages
phagocytic inactive
Gal3- microglia
injury site
CNS-WD