Myelin-laden Macrophage: The True Villain Behind Spinal Cord Injury

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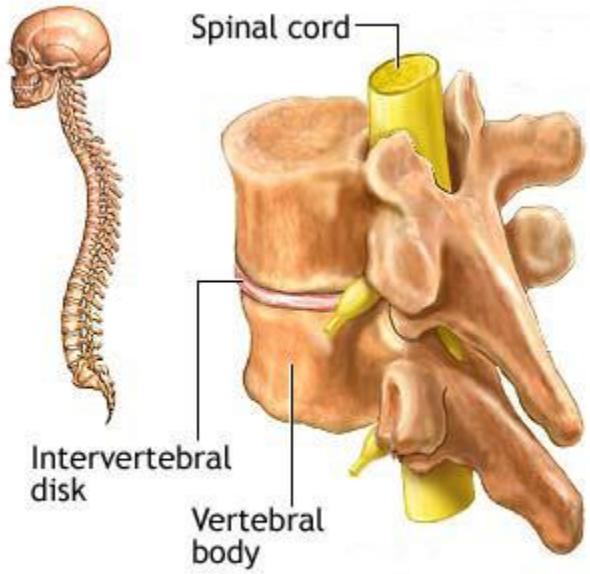
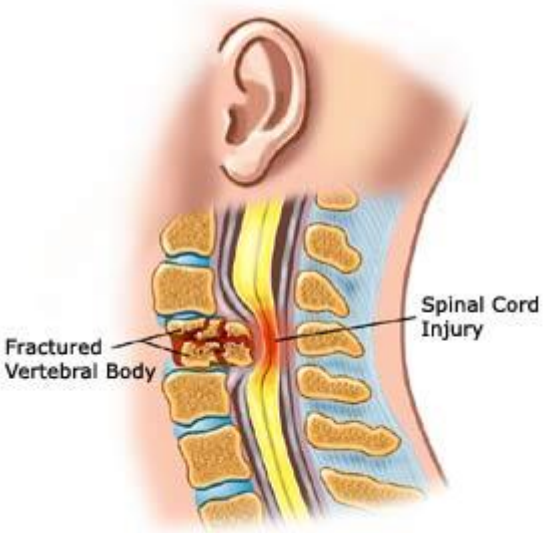
What is a Spinal Cord Injury?





Spinal Cord Injury

- **24% car accidents; >25% accidents working; gunshots, sporting accidents, *etc.***
- **0.4% of the US population or 1,275,000 people paralyzed due to SCIs**
- **Lifetime cost of SCI: $0.7-3 million for 25 year old patient**
- **Cost of enrollment in a clinical trial: $50,000 - $100,000/person**
- **Projected cost of phase 2 clinical trial: $5-10 million/candidate drug**
- **By developing therapies for patients and preventing potential new injuries, USA would save $400 billion on future direct and indirect lifetime costs**

Christopher & Dana Reeve Foundation

Currently no effective treatments for SCI

Current Research: Key Principles of Spinal Cord Repair

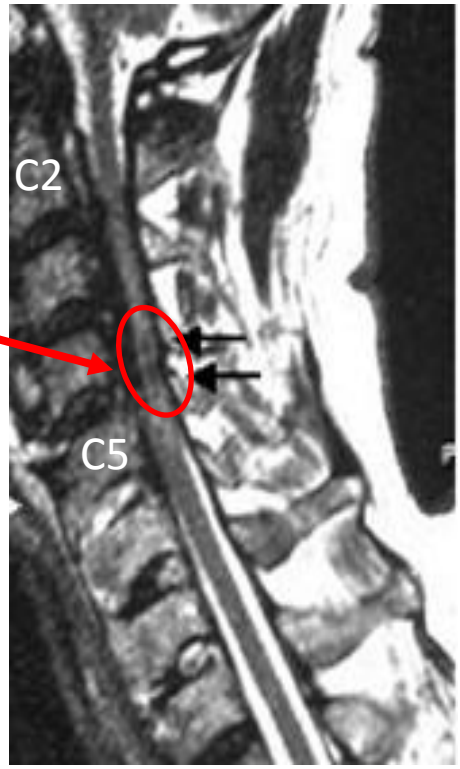
- **Neuroprotection—protecting surviving nerve cells from further damage**
- **Regeneration—stimulating the regrowth of axons and targeting their connections appropriately**
- **Cell replacement—replacing damaged nerve or glial cells**
- **Retraining CNS circuits and plasticity to restore body functions**
- **Improving microenvironment for regeneration**

http://www.ninds.nih.gov/disorders/sci/sci.htm

MRI of Cervical Spinal Cord on the T2-weighted Image

Intramedullary hemorrhage

Primary injury



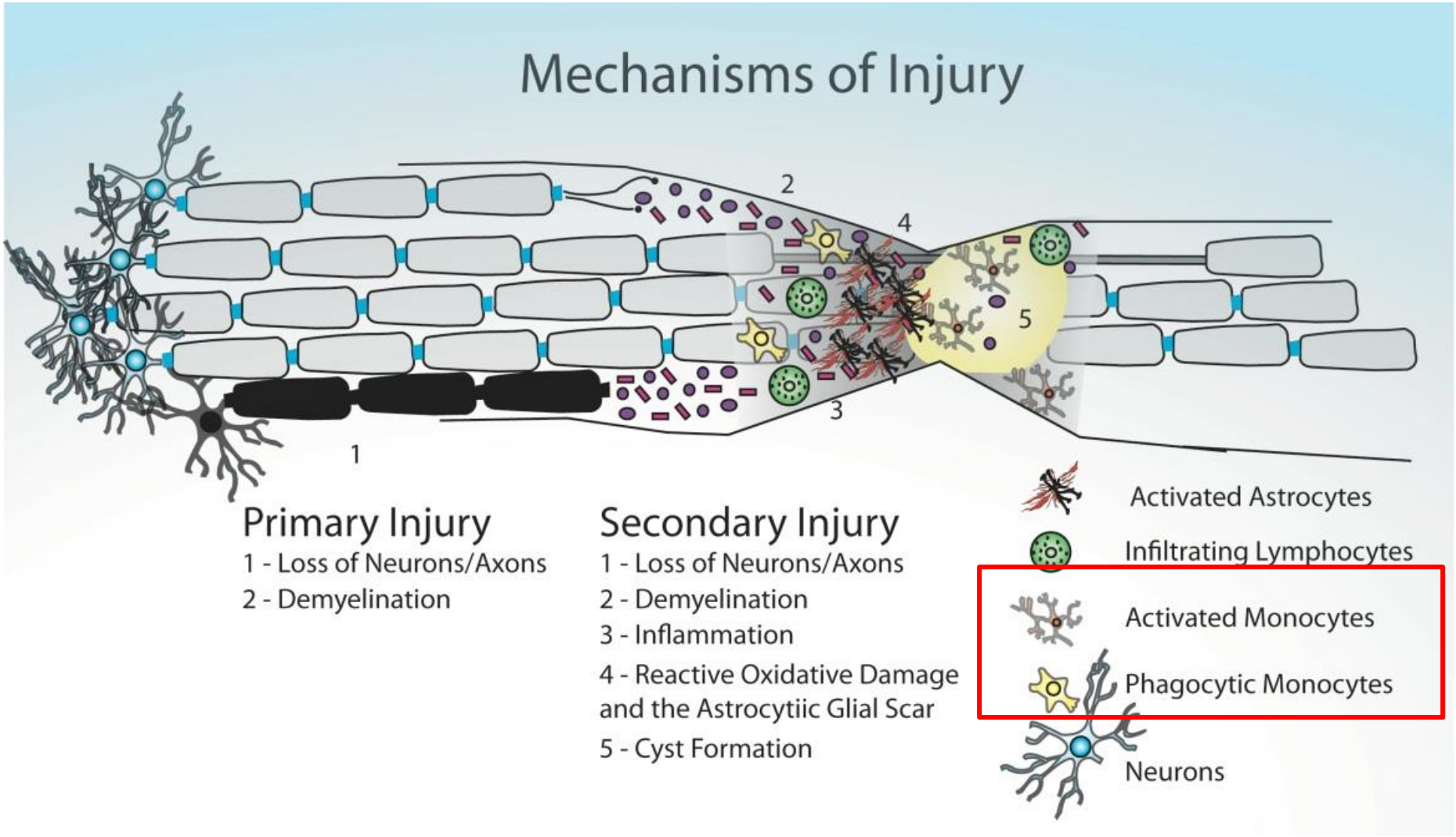
7h after injury

extensive gliosis

Secondary injury

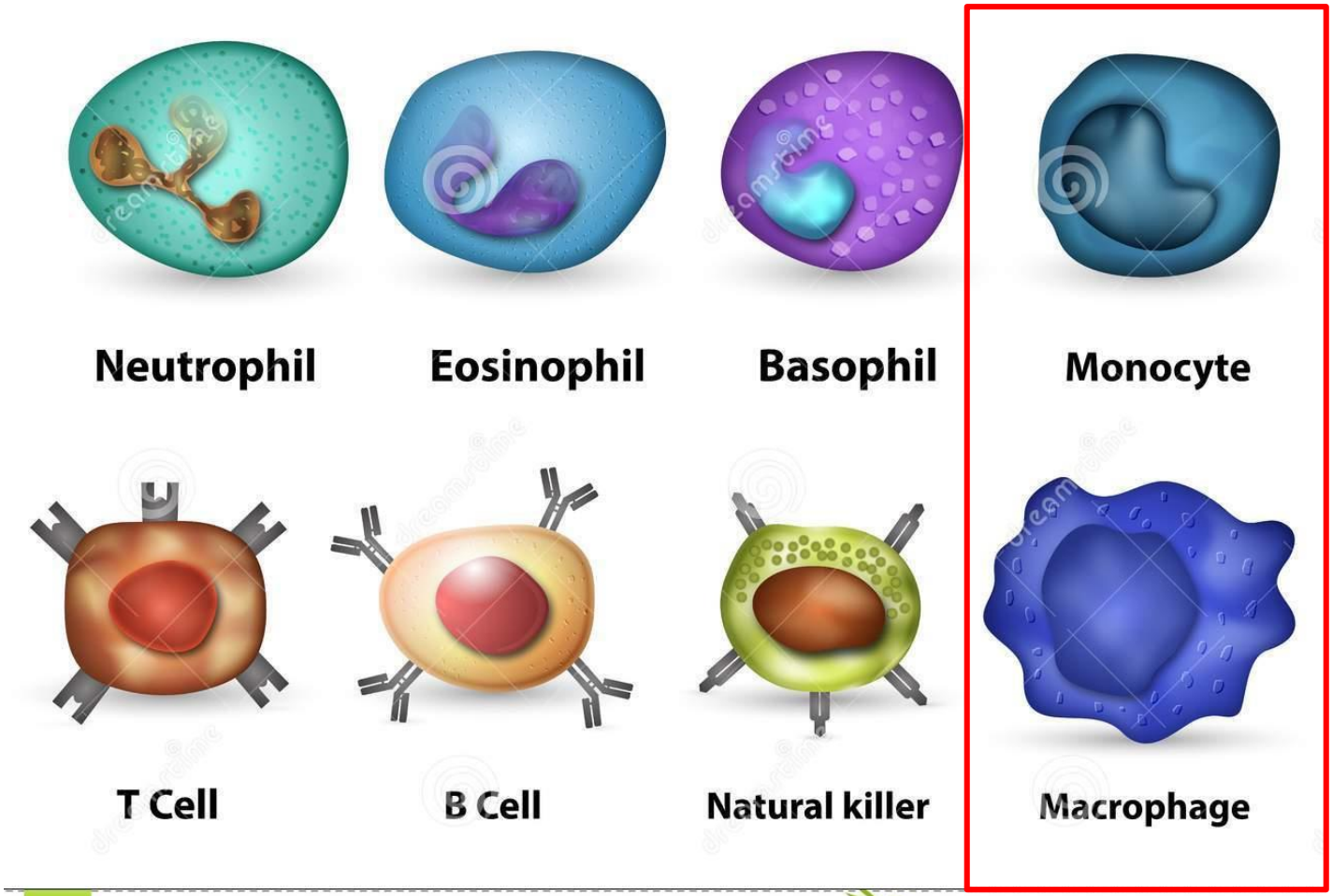
3 months after injury

Sudo *et al*. 2006

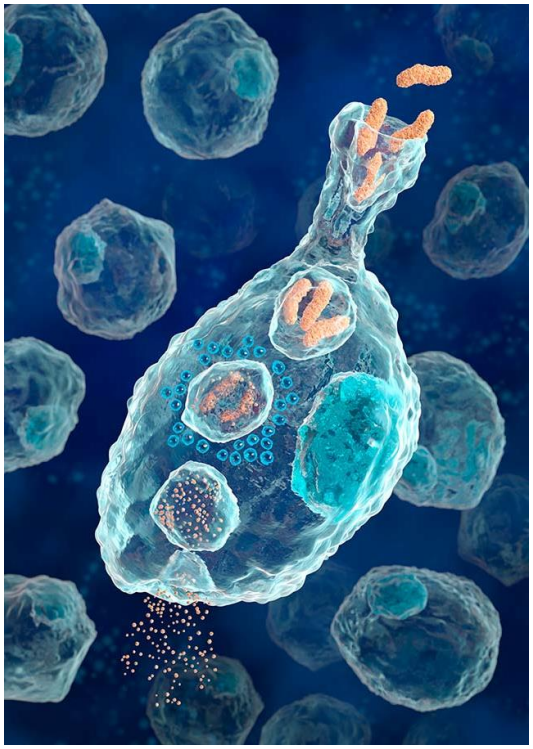


Salewski et al. 2013.

Main Leukocytes



Elie Metchnikoff, Russian Pathologist (1845–1916)



Nature Reviews | Molecular Cell Biology

Nature Reviews | Molecular Cell Biology

http://animatedhealthcare.com

His description of mobile cells battling invading pathogens was visually immediate and dramatic.

Selected Achievements of Metchnikoff

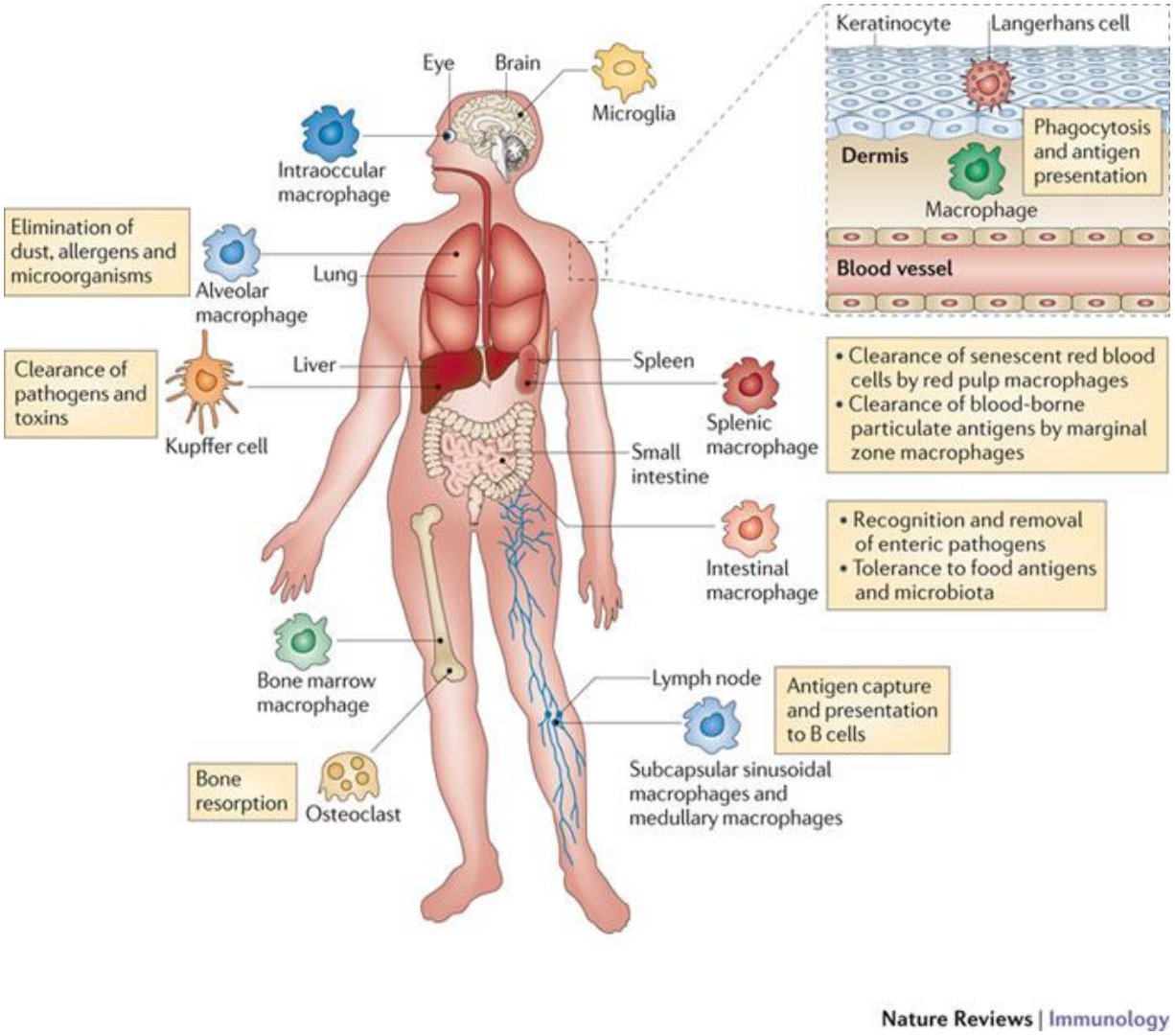
- **Description of phagocytosis as an active process and its role in host defense, across a wide range of organisms**
- **Description of natural immunity to infection (host-pathogen interaction with phagocytes playing a central role)**
- **Significance of inflammation as a beneficial process**
- **Description of cell migration and leukocyte recruitment**
- **Going from observations to hypothesis, for experimental testing**
- **Public outreach– popular writings, health promotion**

Gordon. *Eur. J. Immunol.* 2008. 38: 3257

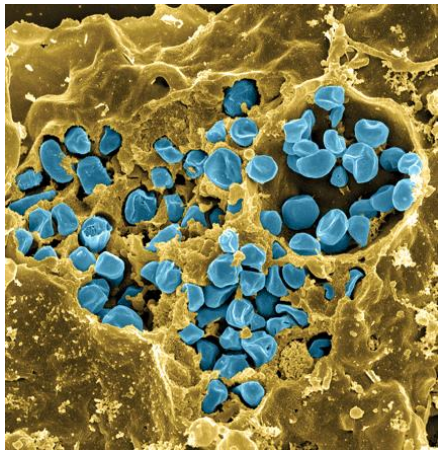
Macrophage

Functions of Macrophage:

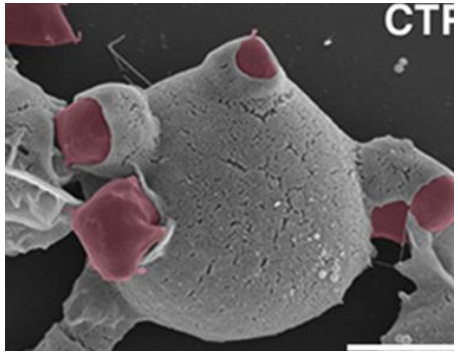
- Migration
- Phagocytosis
- Presentation of Ag
- Secretion

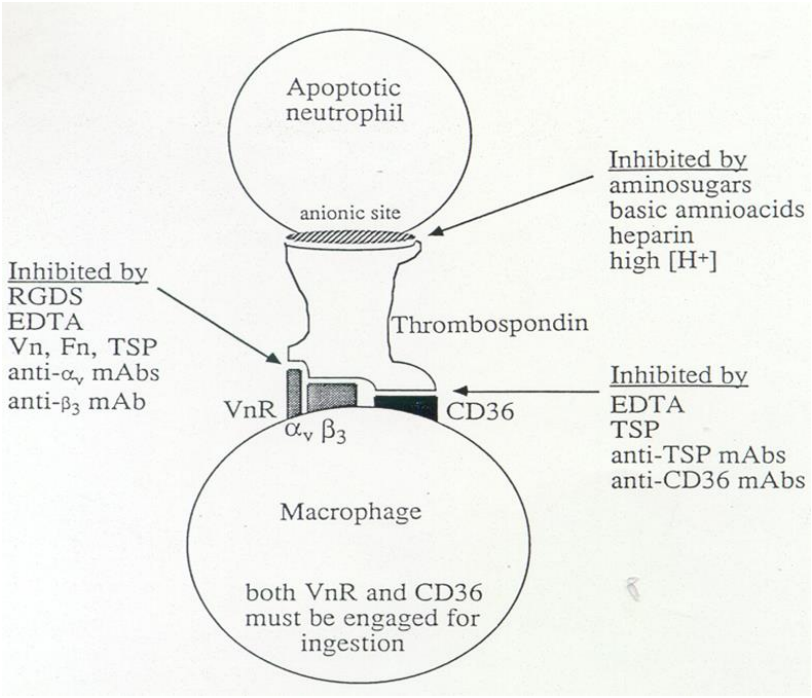


Macrophage: the Professional Phagocyte



Checroun *et al.* 2006

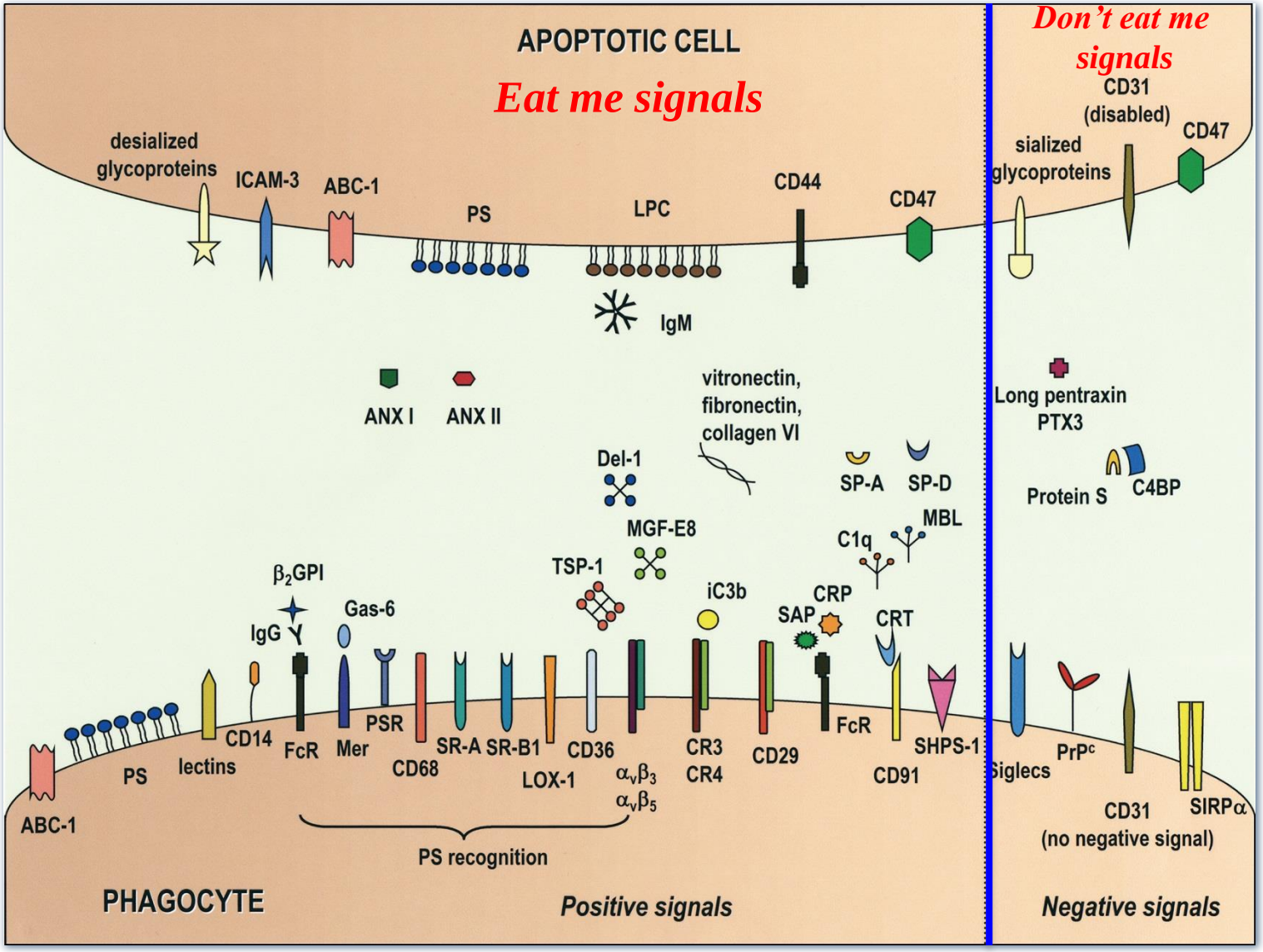




Sir John Savill

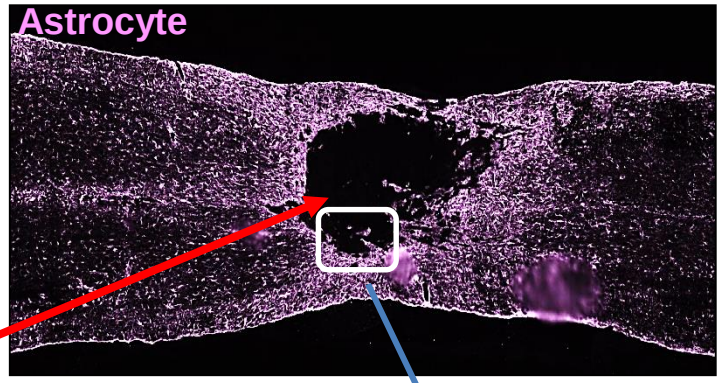
Dead men may tell no tales,
but dead cells certainly do,
the macrophage having the last word. ------Sir John Savill

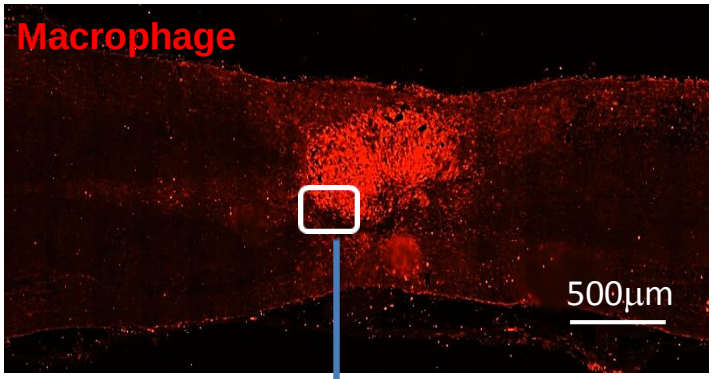
Signals that Regulate the Engulfment of Apoptotic Cells



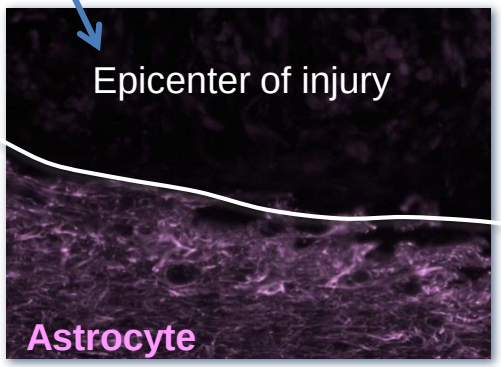
De Almeida &Linden. Cell Mol. Life. Sci. 2005. 62.

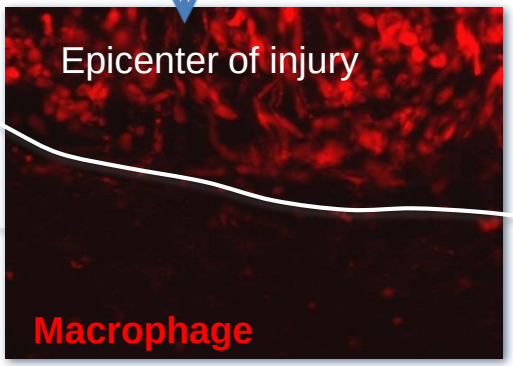
Injured Spinal Cord



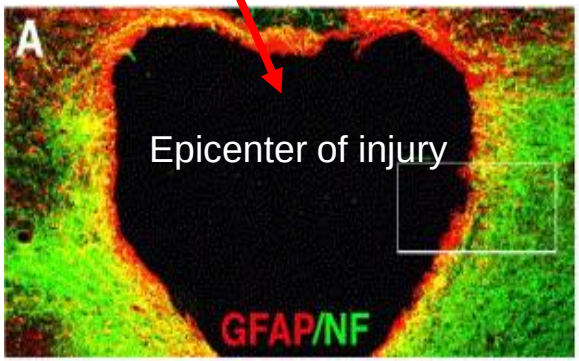


Desert?
Black hole?
Volcano?





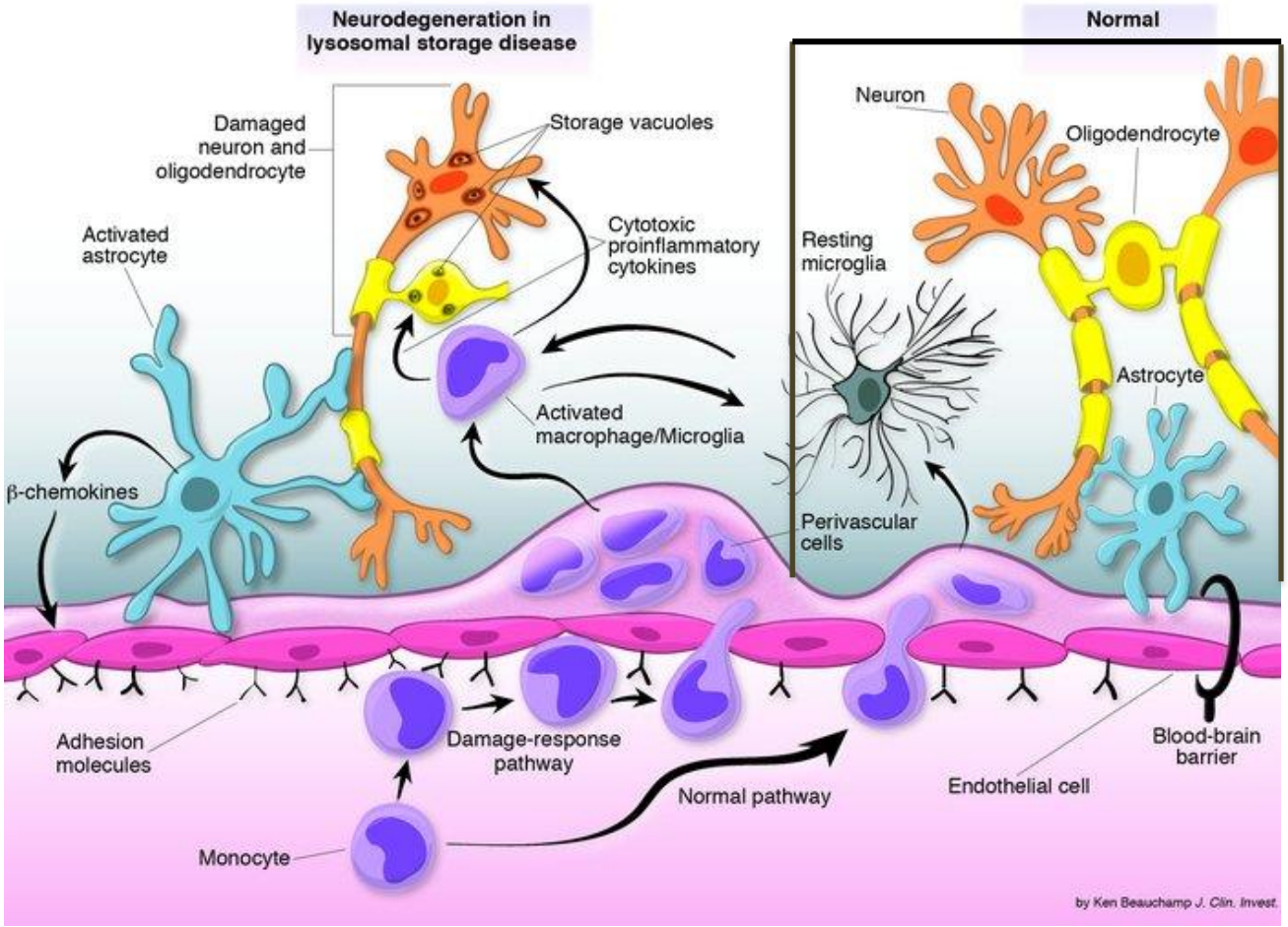
12w after SCI



6w after SCI

Lu *et al.* 2007. Exp. Neurol

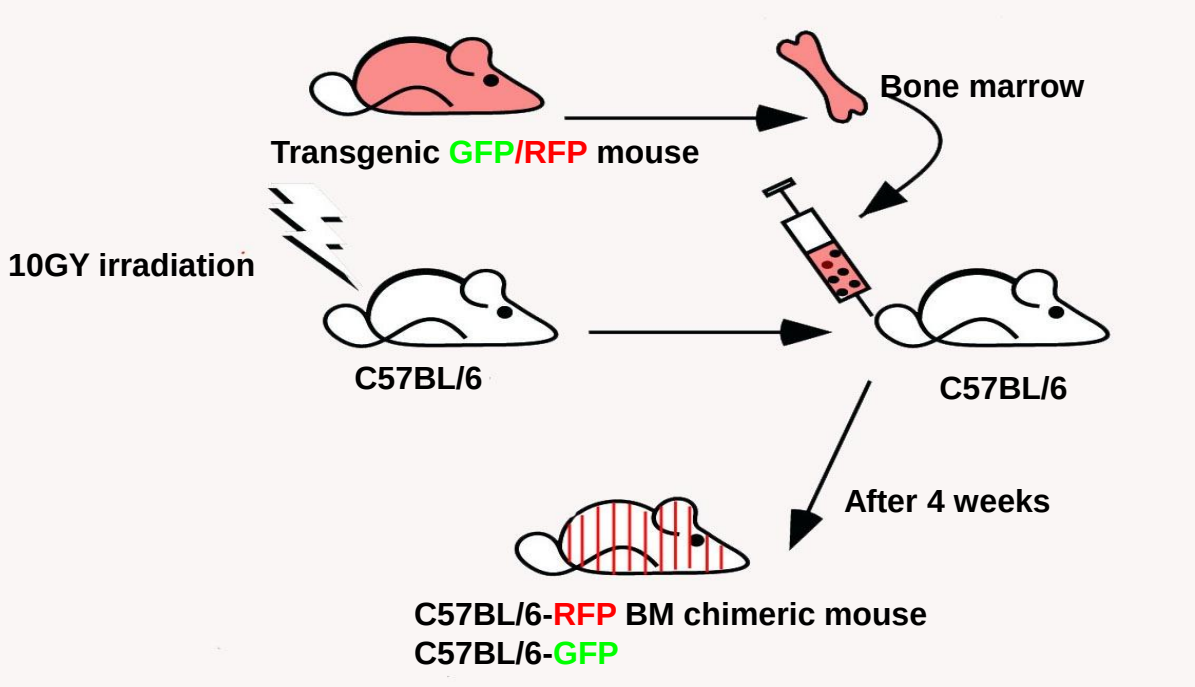
Macrophages and Microglia in CNS



Proia & Wu. JCI. 2004. 113.

Mouse Models

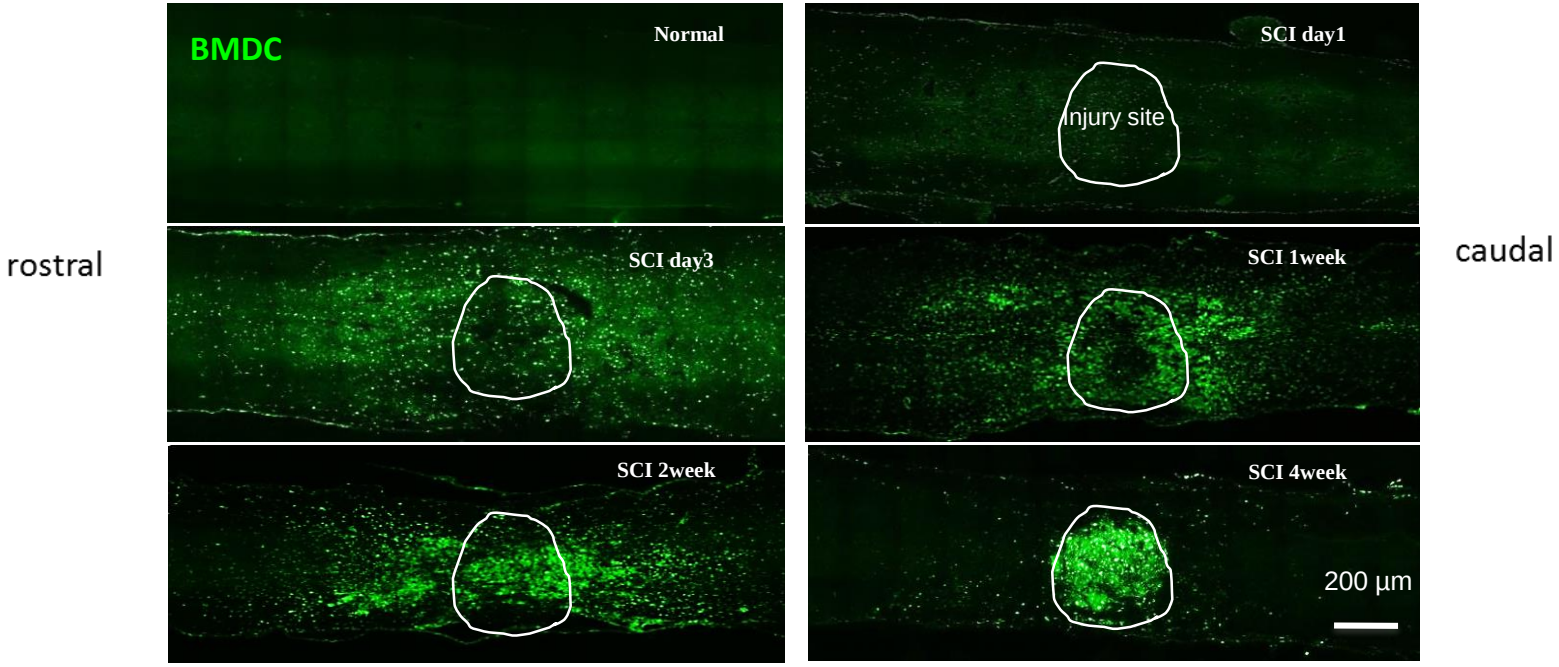
1. C57BL/6-RFP/GFP Bone Marrow (BM) Chimeric mice

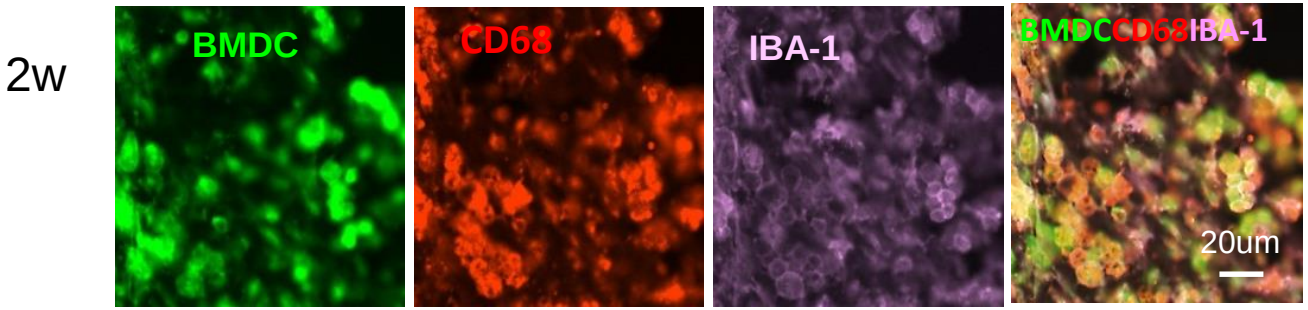


2. CX3CR1$^{GFP/+}$ (heterozygous) mice:

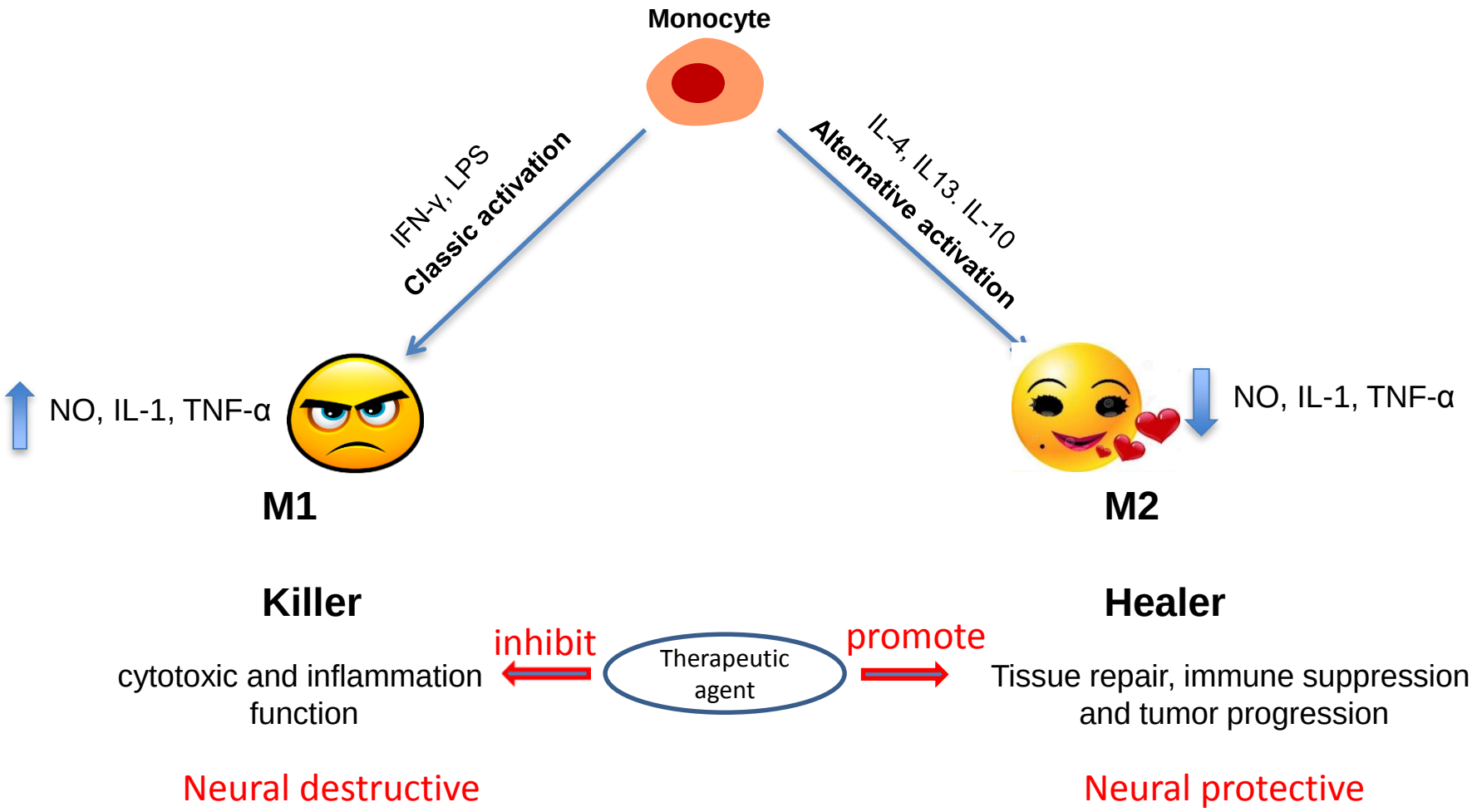
- GFP inserted in the CX3CR1 locus in one allele
- A normal allele enables the continued expression of CX3CR1 (maintains functional CX3CR1)

Bone Marrow-derived Macrophages (BMDM) in Injured Spinal Cord

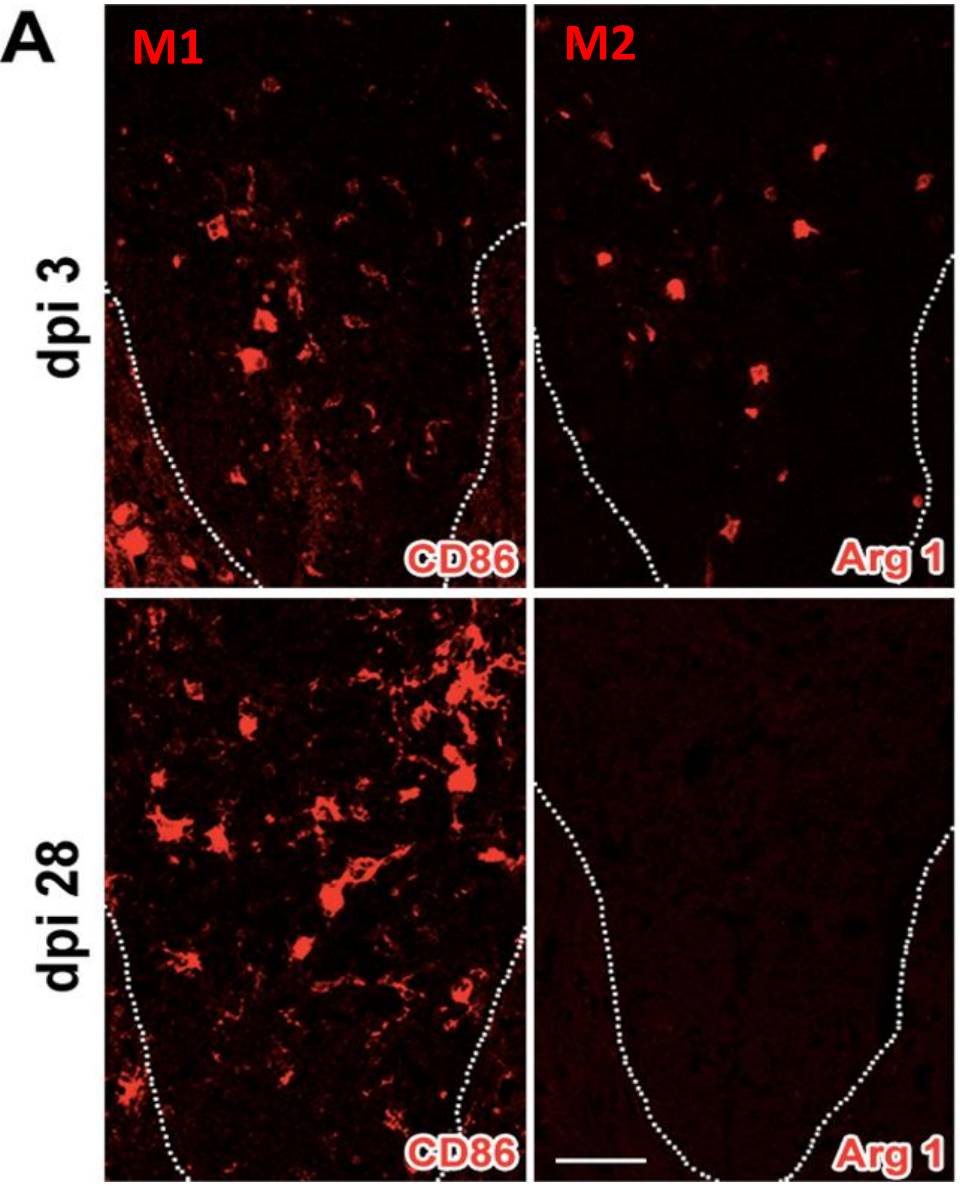


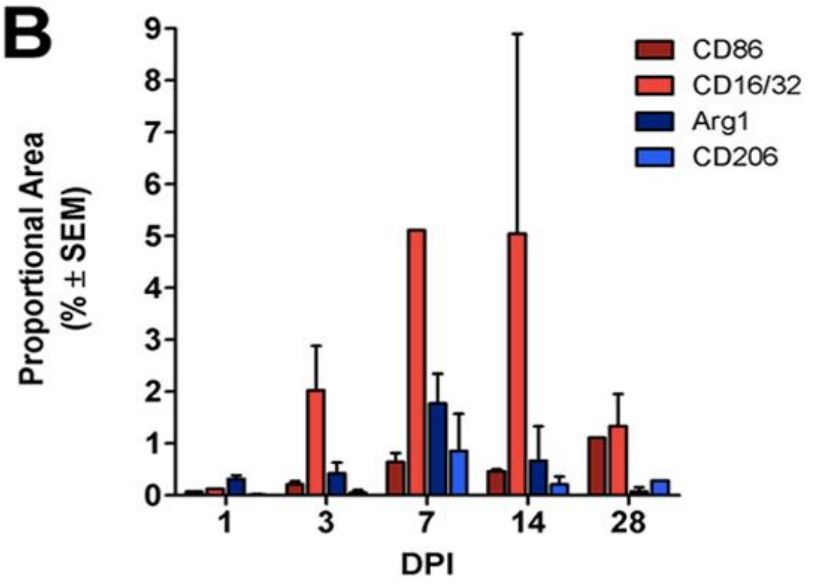


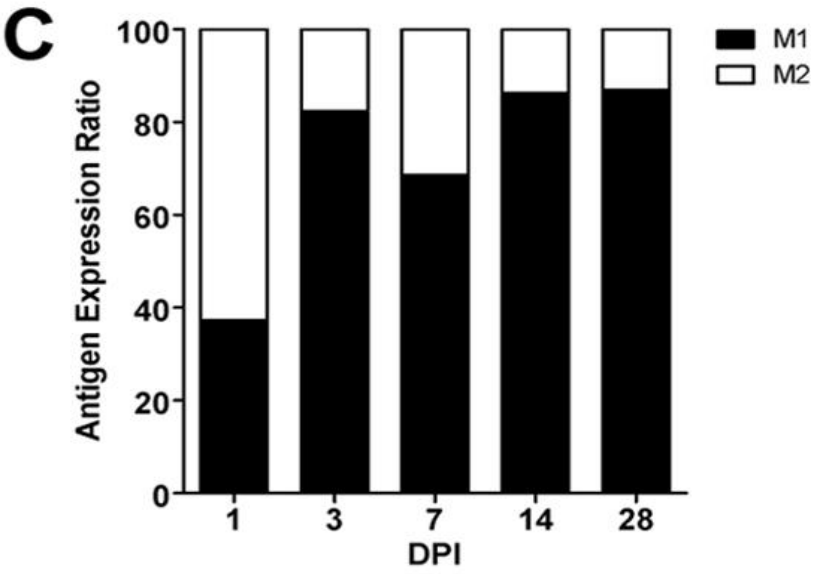
Macrophage Activation



Macrophages in Injured Spinal Cord

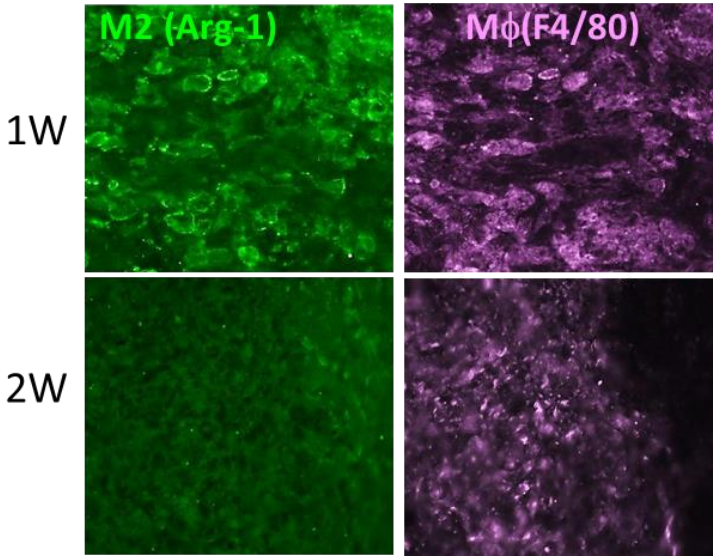




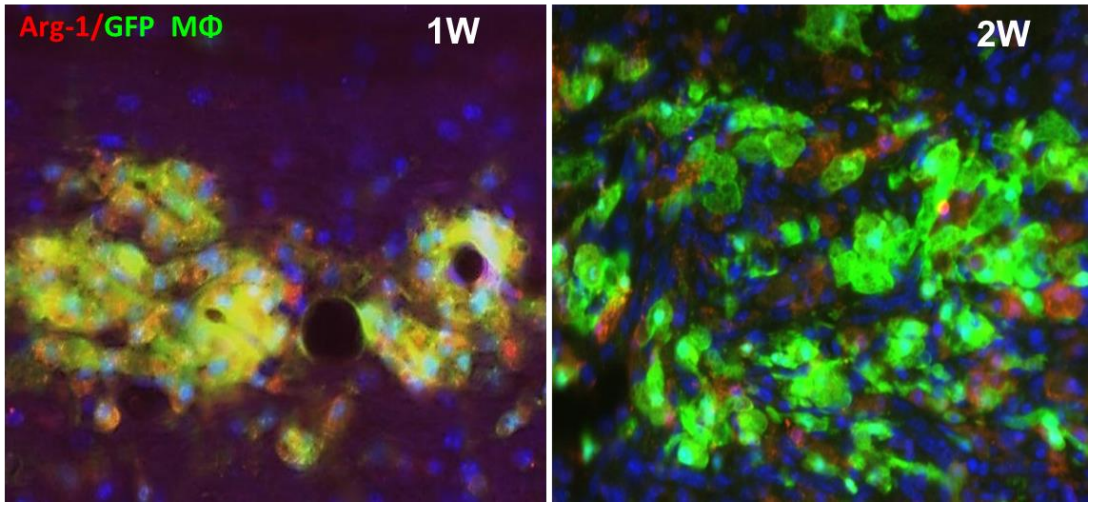


Kigerl et al. J Neuronsci. 2009

Macrophages in Injured Spinal Cord

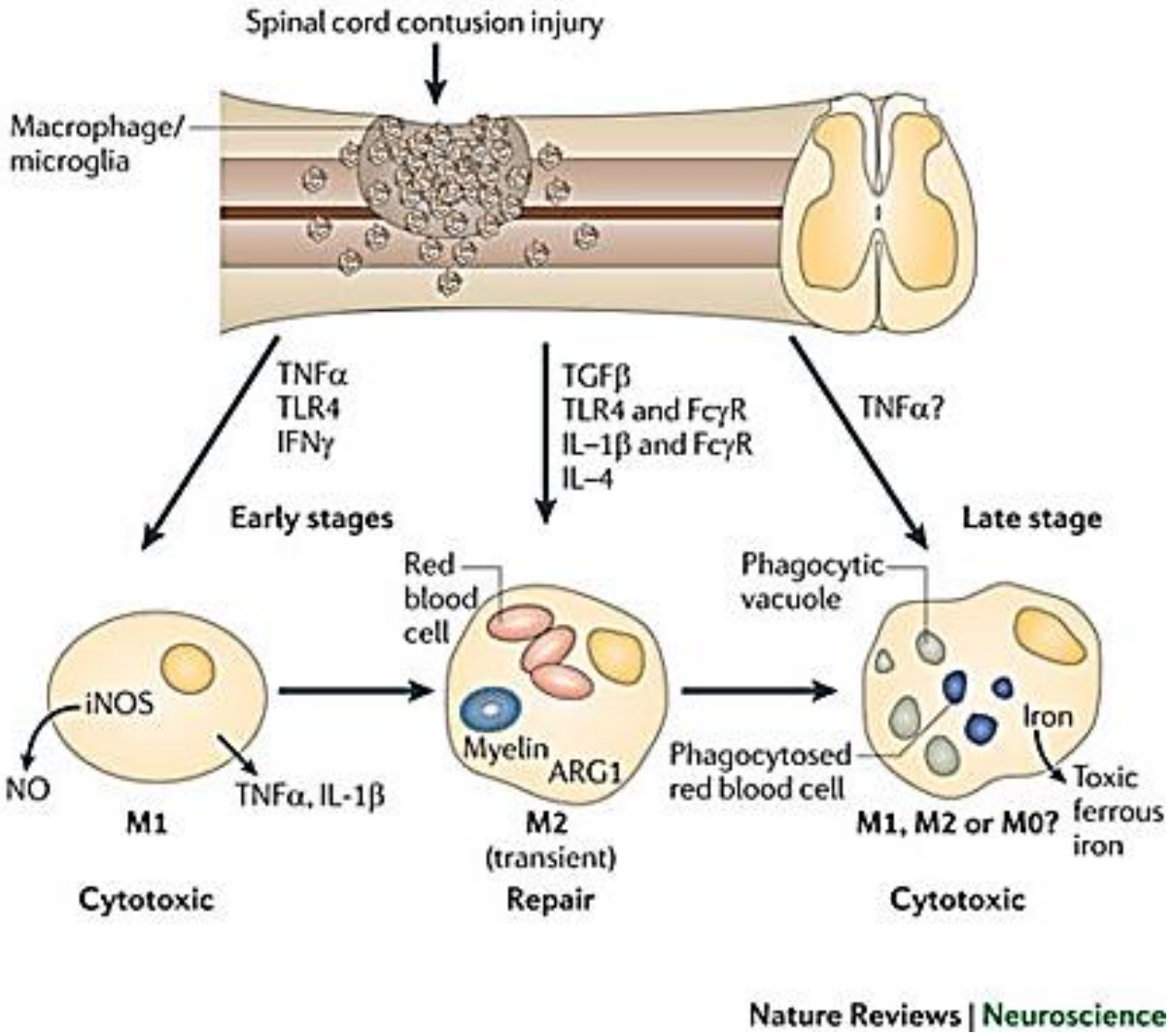


GFP-M2 injection after SCI



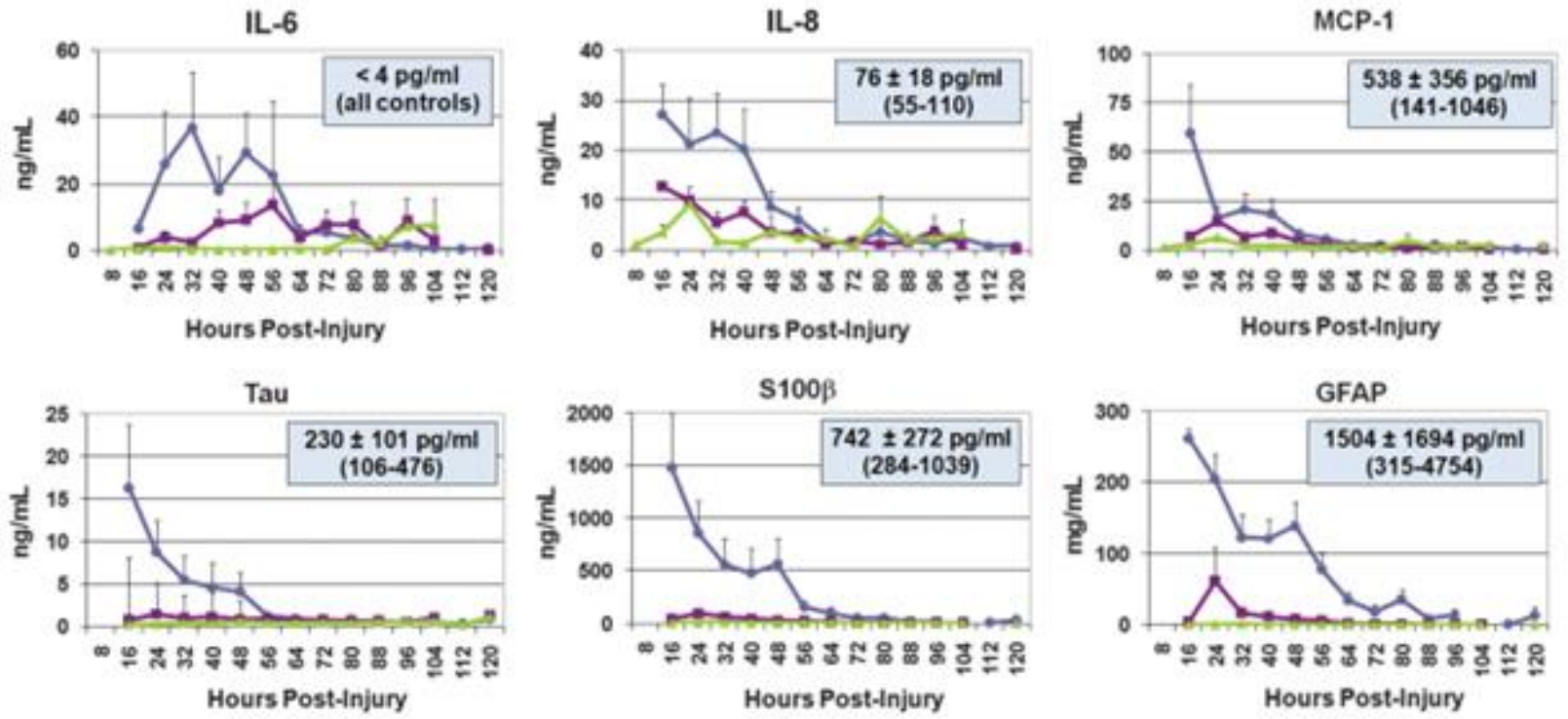
Wang et al. Glia. 2015

Macrophage Polarization in the Injured Spinal Cord



David & Kroner. 2011. 12: 382

A Severity-Dependent Expression of Inflammatory Mediator in Cerebrospinal Fluid (CSF) Post-SCI



American Spinal Injury Association (ASIA) to classify SCI patients

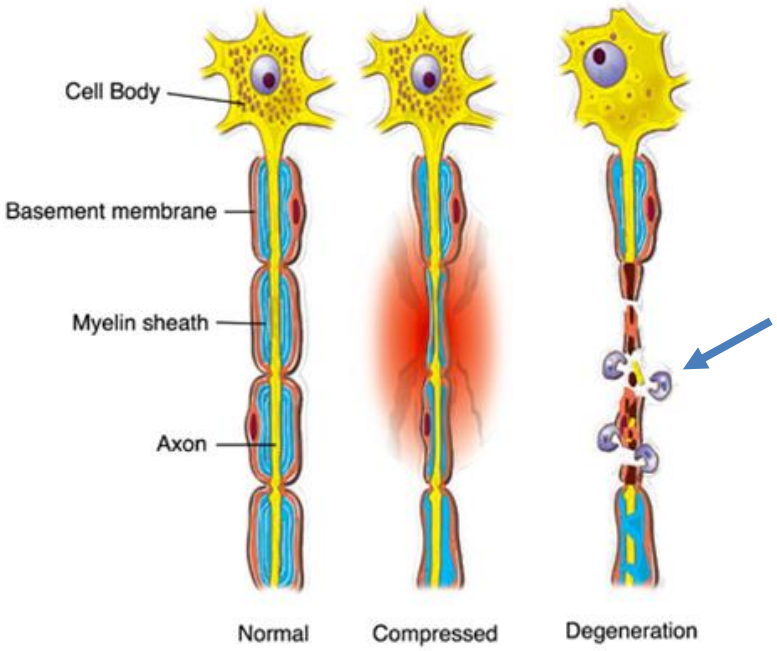
Grade A: motor and sensory complete paralysis

Grade B: motor complete, sensory incomplete paralysis

Grade C: incomplete motor and sensory paralysis

Kwon et al. J. Neurotrauma. 2009. 27:669

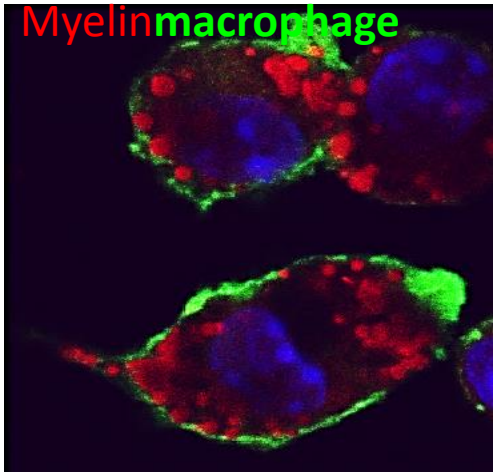
Myelin

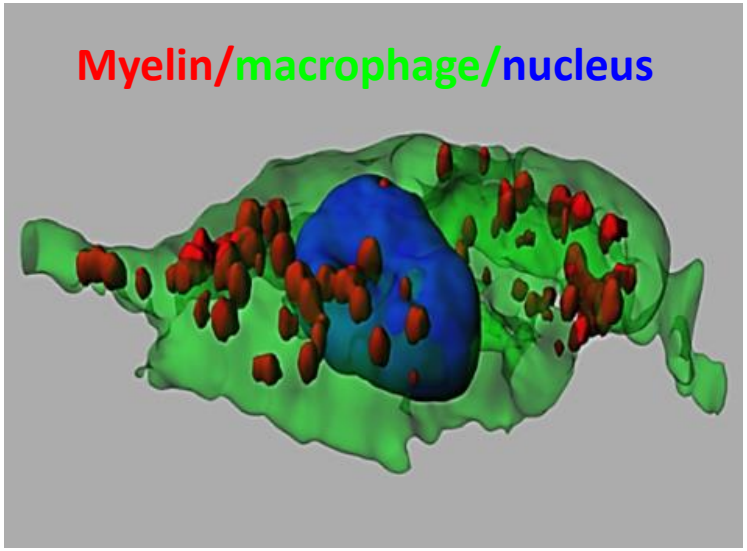


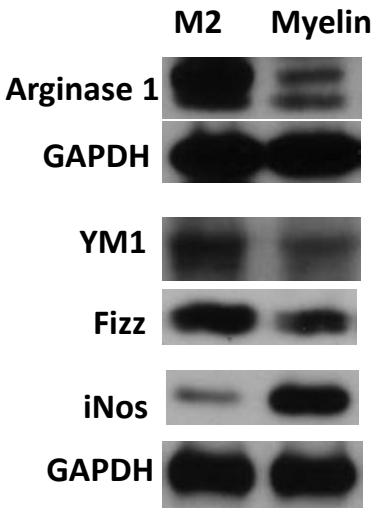
Myelin debris can be generated immediately after injury

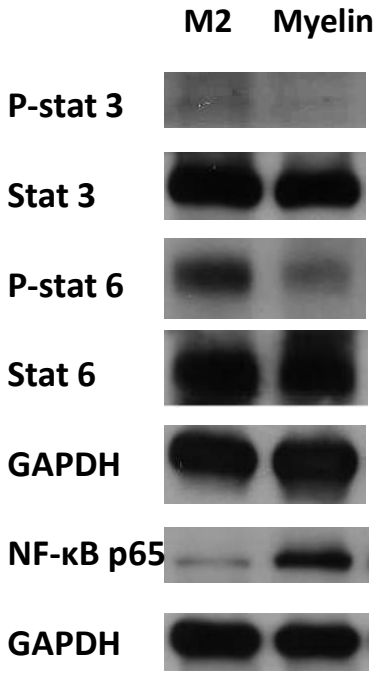
- **Composed of *lipids* and *proteins* (myelin basic protein, MBP; proteolipid protein, PLP; myelin-associated glycoprotein, MAG; myelin-oligodendrocyte glycoprotein, MOG)**
- **Myelin debris is an inhibitory signal for regeneration**
- **Myelin debris can stimulate inflammation**
- **Axons are directly exposed to inflammatory environment**

Macrophages Uptake Myelin Debris



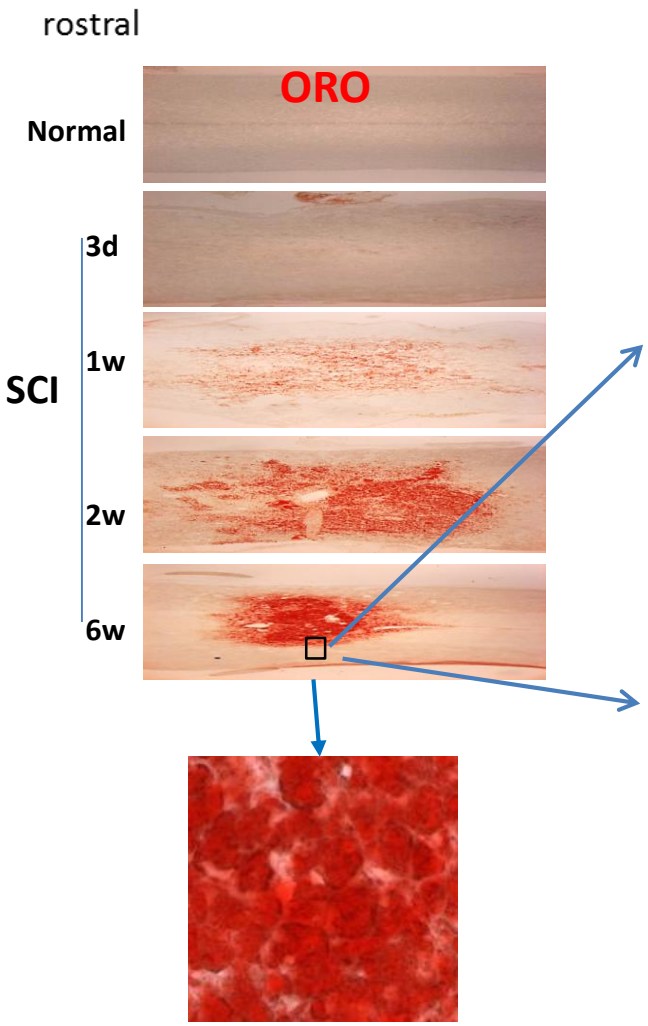


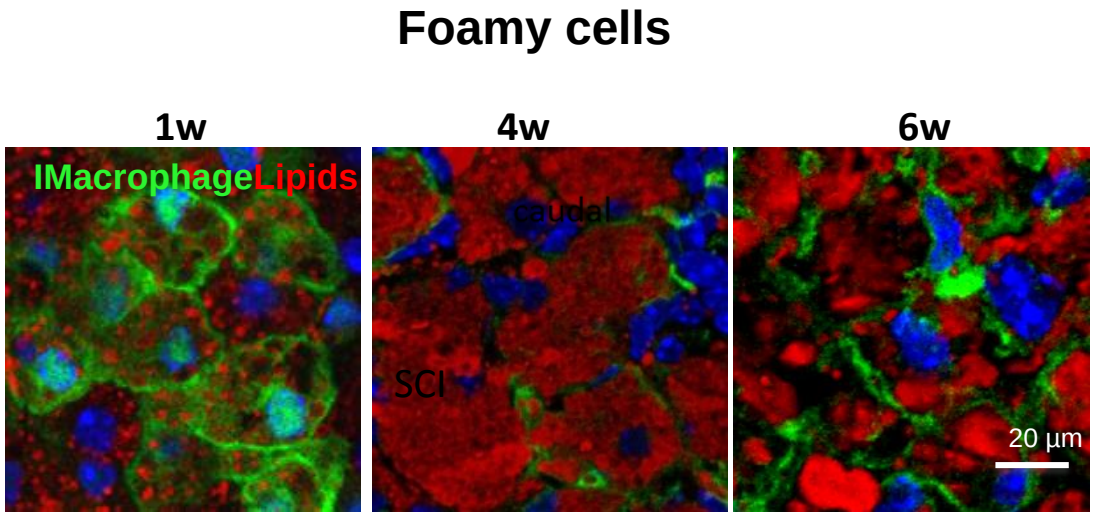


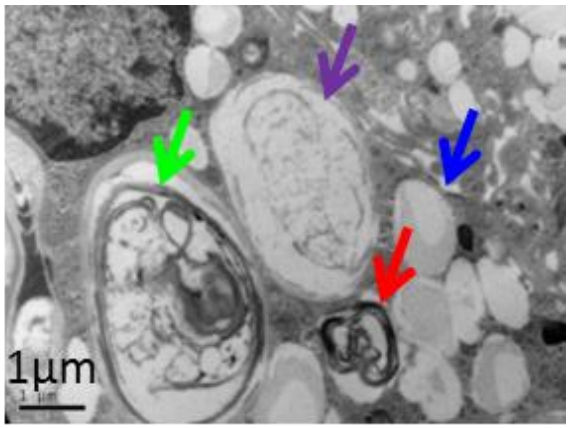


Wang *et al*. Glia. 2015

Lipid Accumulation in Macrophages at Injury Site





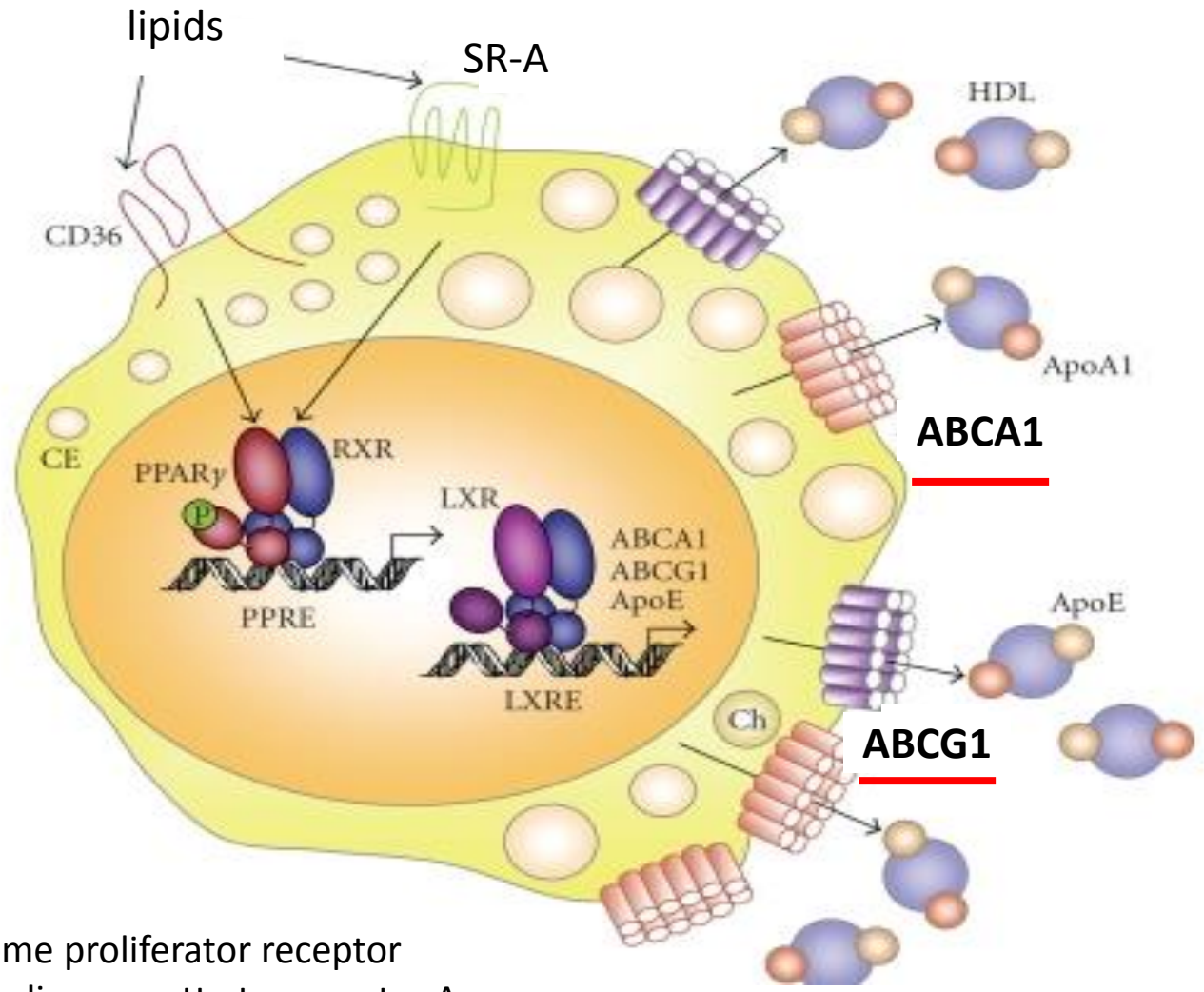


Wang *et al*. Glia. 2015

Why do these lipids remain in the Mϕ?

Is the path for lipids to leave blocked?

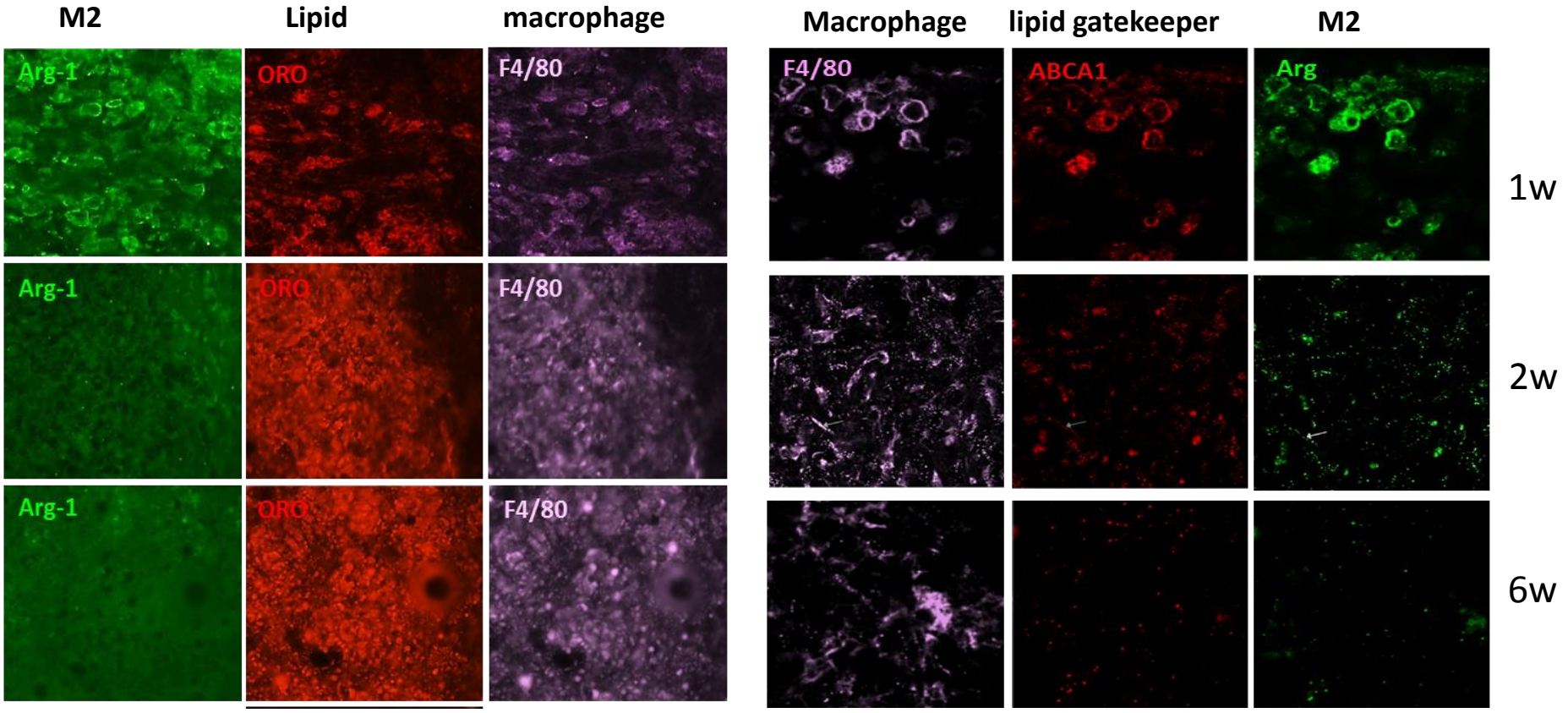
Lipid transporters: ABCA1 and ABCG1



PPAR: peroxisome proliferator receptor
ABCA1: ATP-binding cassette transporter A
LXR: liver X receptor
RXR: retinoid X receptor

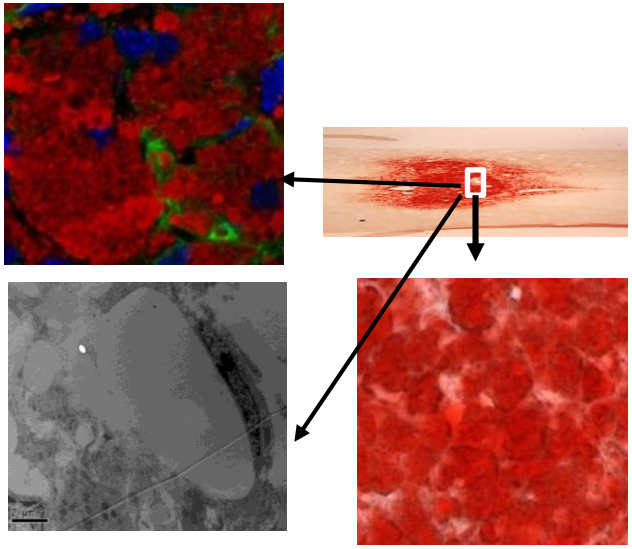
Demers *et al.* **PPAR Research.** 2008.

Macrophages at Lesion Site



Wang *et al*. Glia. 2015

Do Myelin-laden Macrophages Have Normal Function?

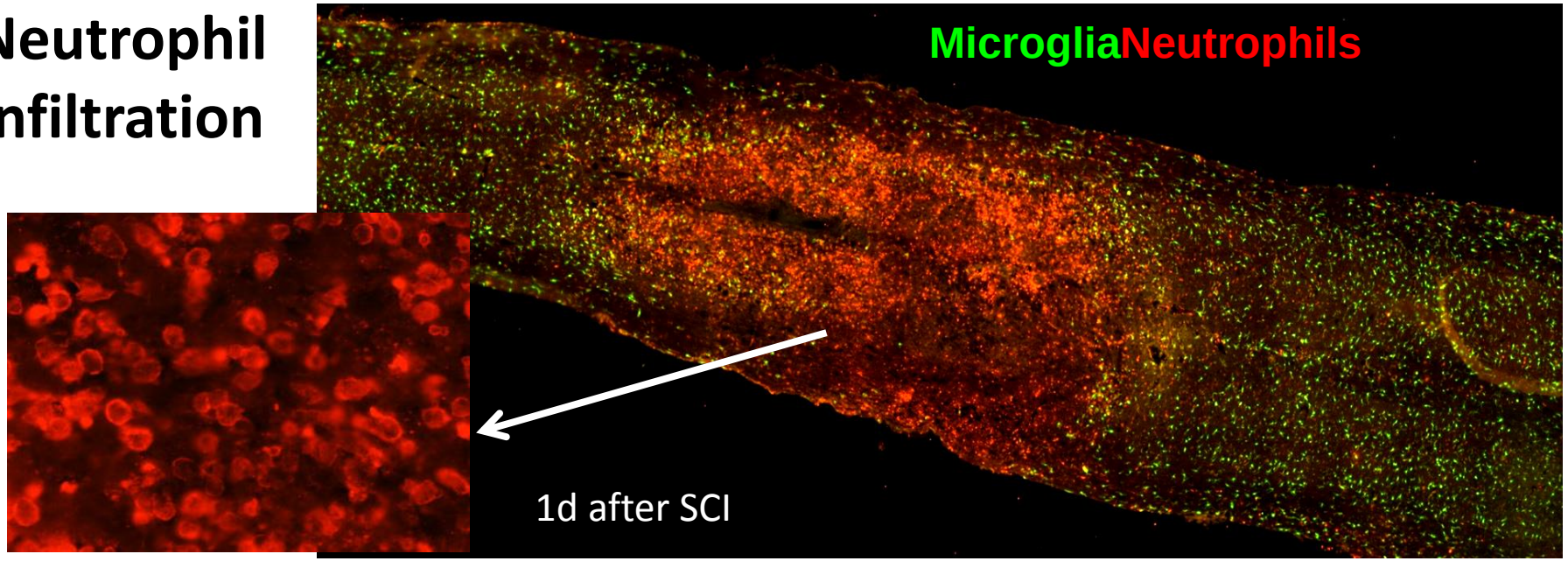


Phagocytic capacity

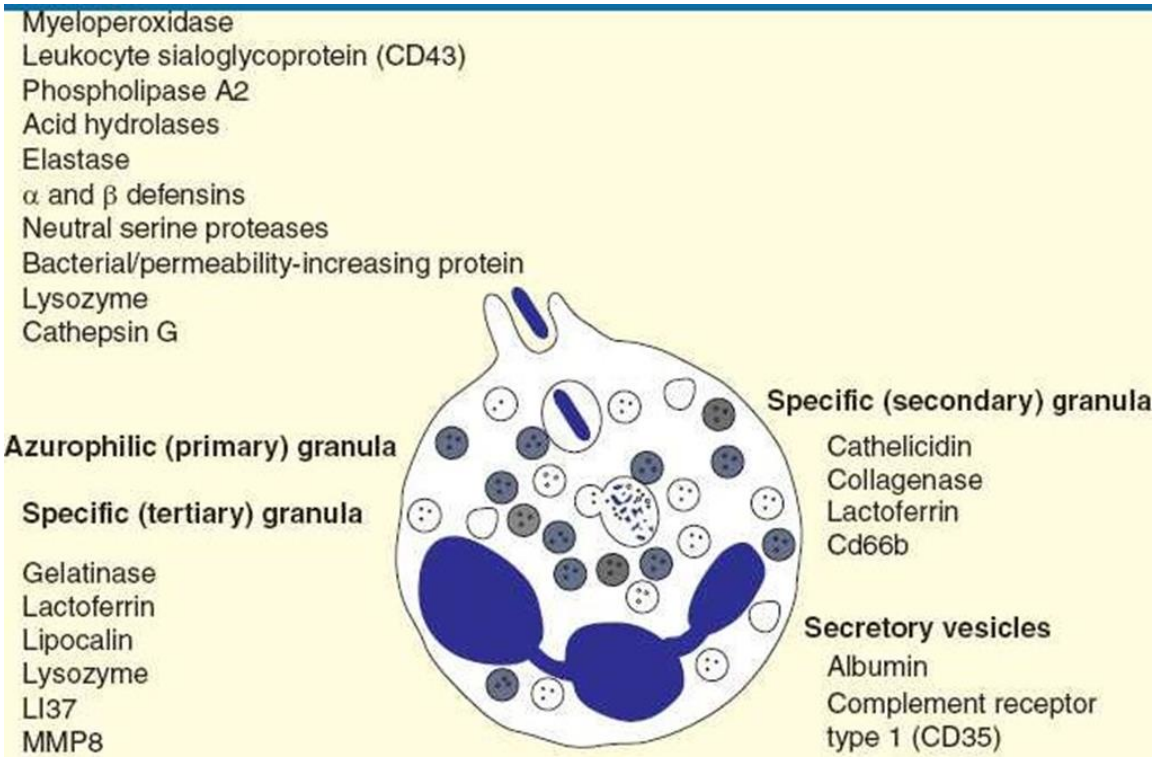
Can mye-Mϕ eat more?

For example: apoptotic neutrophils

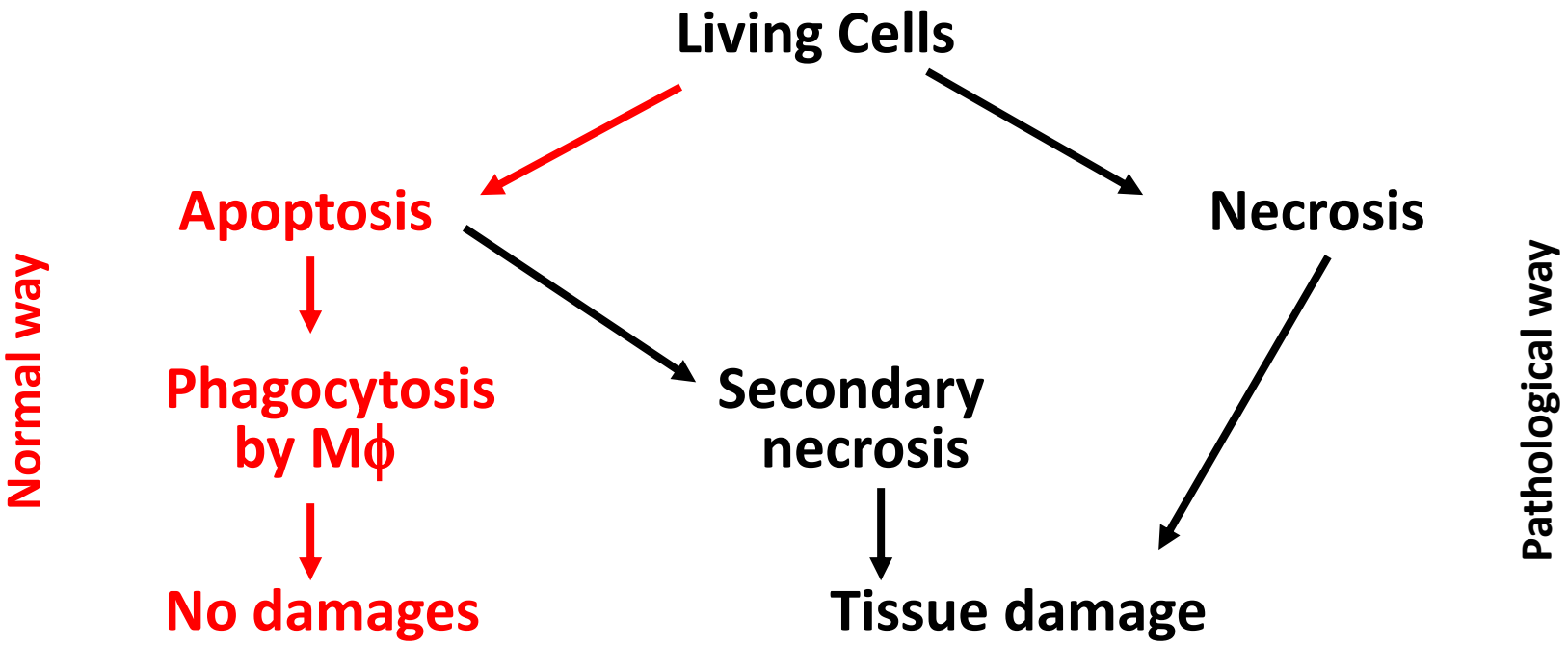
Neutrophil Infiltration



- **<u>Short lifespan - 12 hours</u>**
- **Present in blood (60-70% of WBC)** (Not found in healthy tissues)
- **Granules in the cytoplasm are responsible for killing microbes (primary and secondary granules)**

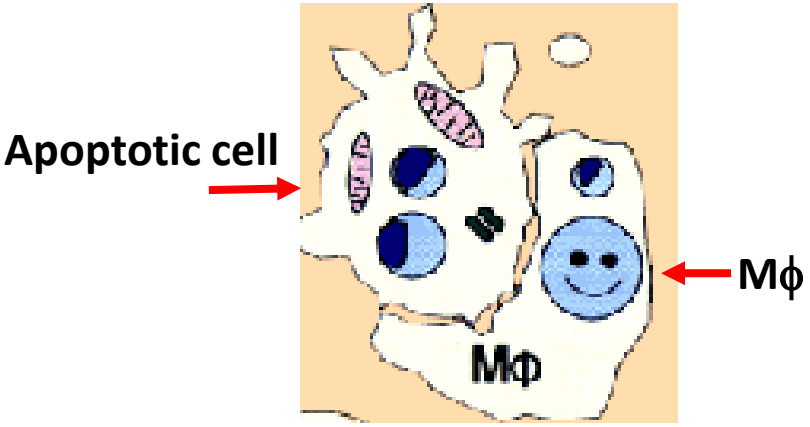


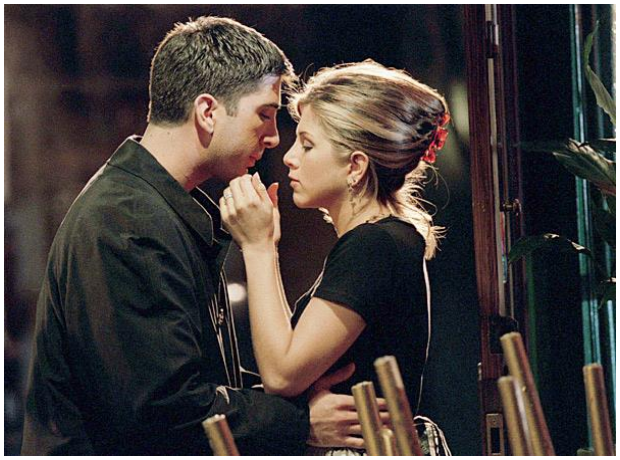
Apoptosis and Phagocytosis



Our bodies produce 5-10 × 10^{10} neutrophils every day. Effective removal of apoptotic neutrophils is important for "making space" for living cells and for maintaining the function of tissue.

Macrophages and Apoptotic Cells: A Love Story



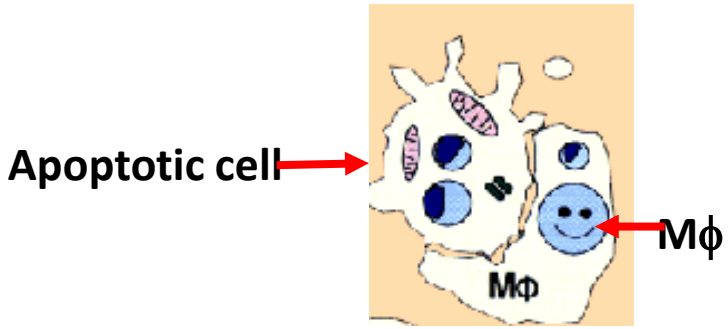


Signals for Apoptotic Cell Clearance

- **Come here signals:** chemoattactants (MCP-1)
- **Find me signals:** fractalkine, ATP and UTP (apoptotic cells attract macrophages are the beginnings of fatal attraction)
- **Eat me signals** (Phosphatidylserine):
- **Don't go away signals** (MIF)

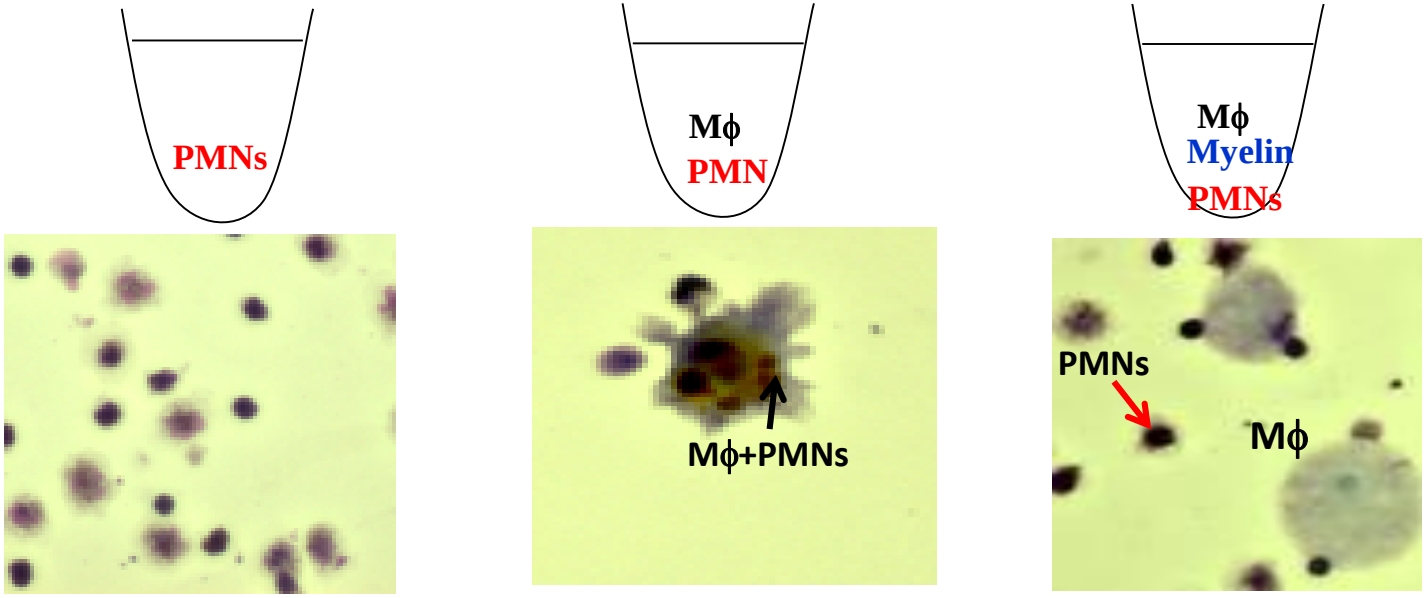
Apoptotic cells make an active effort to attract phagocytes.

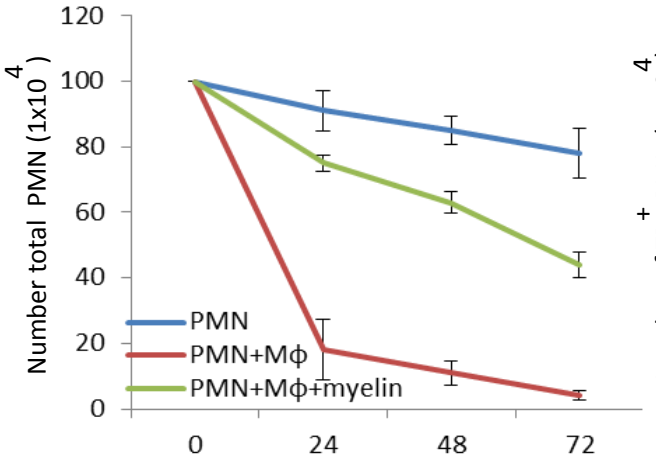
After-the-meal : clearance of apoptotic cells is "immunological silent"

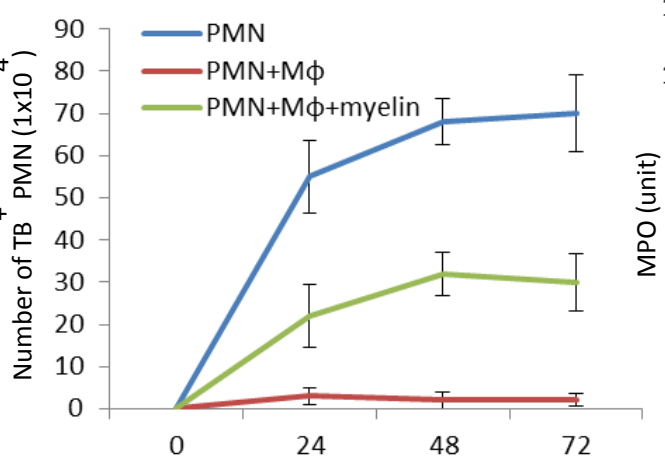


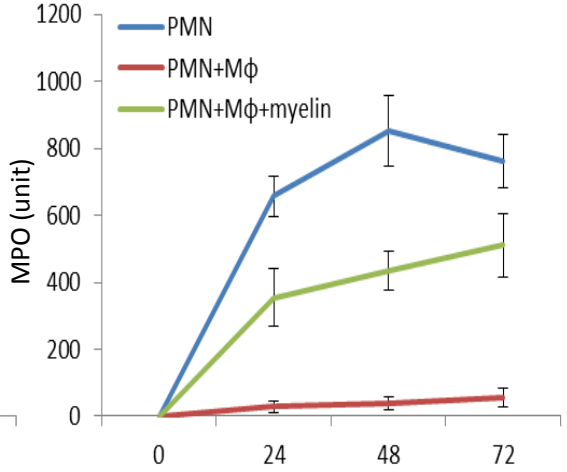
However, their bond can be easily broken…

Interaction of Mϕ, Neutrophils (PMN) and Myelin Debris









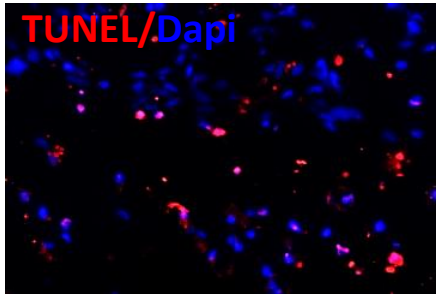
Wang et al. Glia. 2015

The Tragedy of the Neutrophil

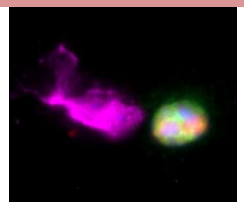
Neutrophil

No one wants me…
I feel like dying.

Injury site (3d post SCI)



PMNTUNELMϕ



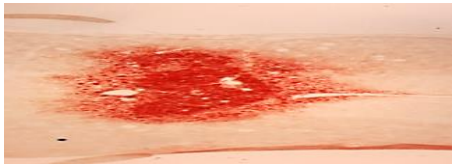
1w after SCI

The horrible consequence of the broken relationship

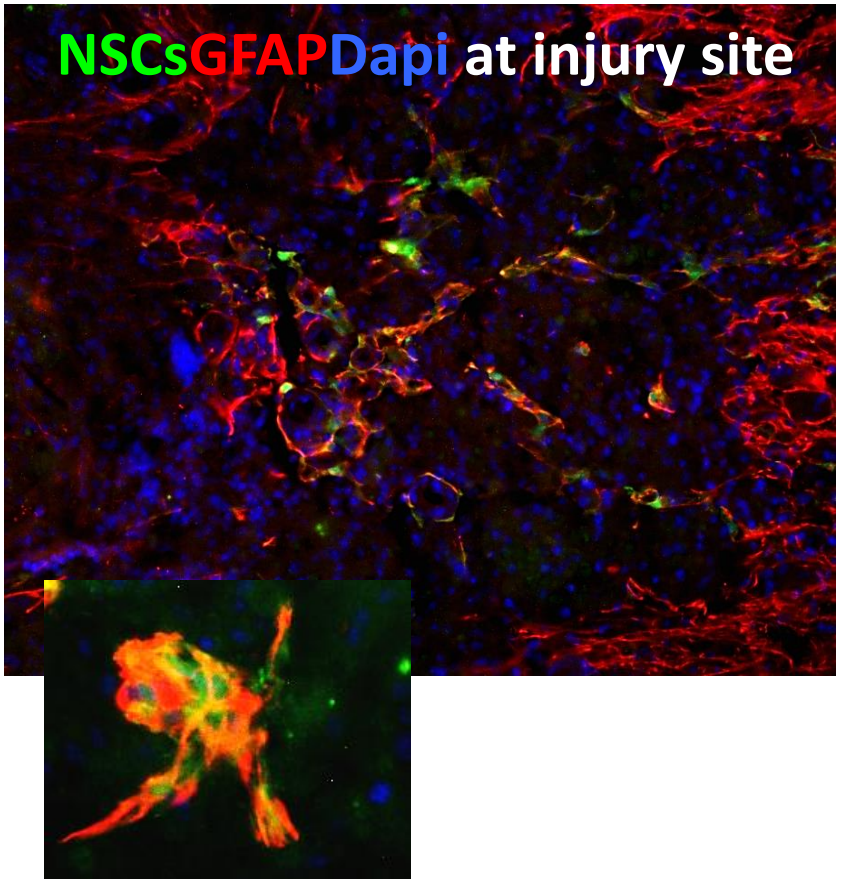
Tissue damage

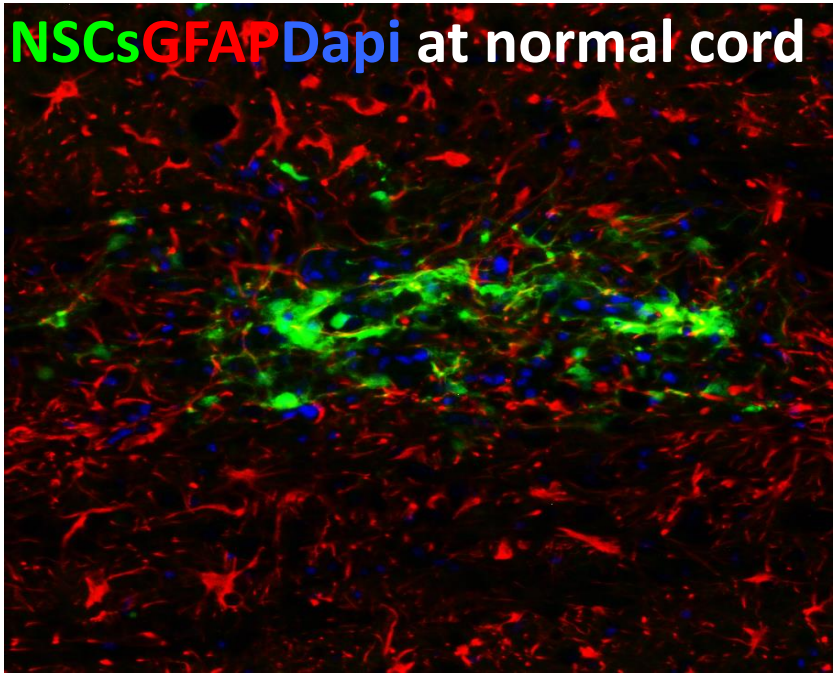
NSCs Injection into Injured vs. Normal Spinal cord

Injured cord

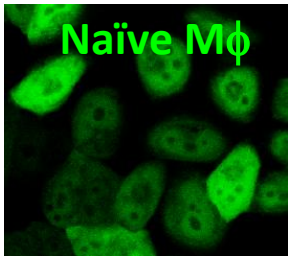


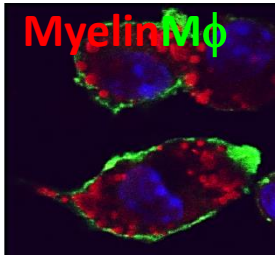
normal cord

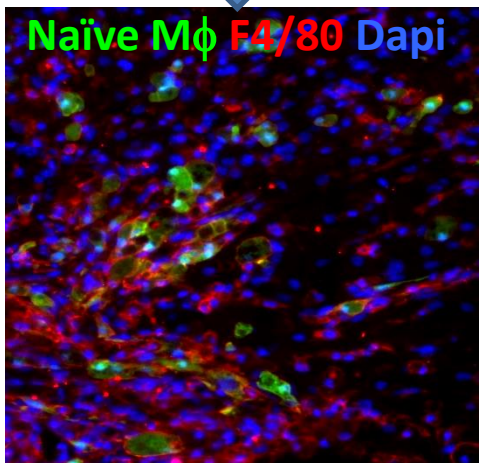


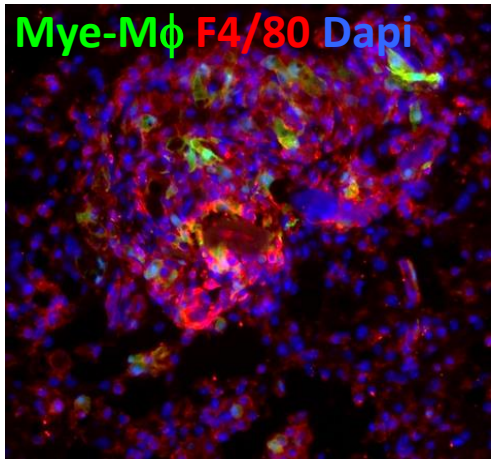


Distribution of Injected Naïve Mϕ and Mye-Mϕ in the Normal Cord

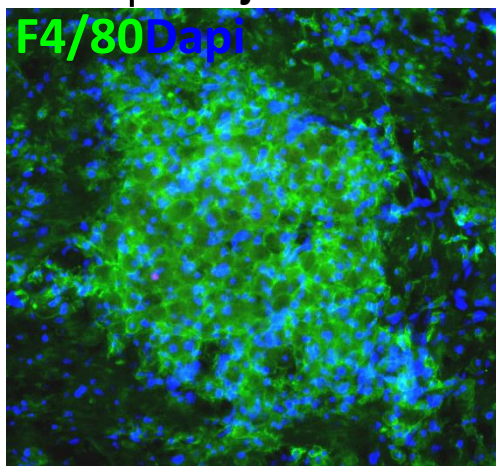








Mϕ in injured cord



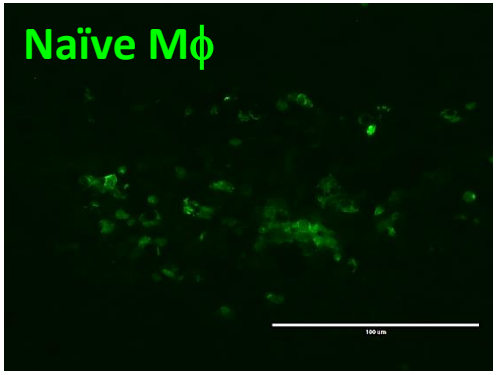
Normal spinal cord

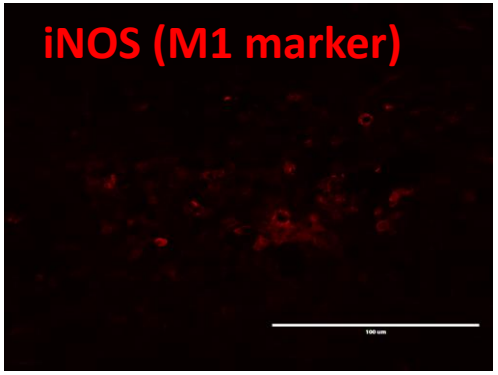
Normal spinal cord

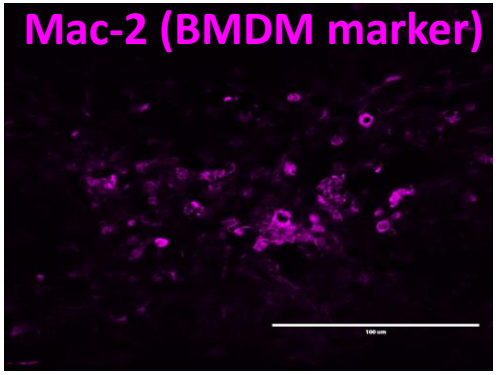
Spinal cord injury

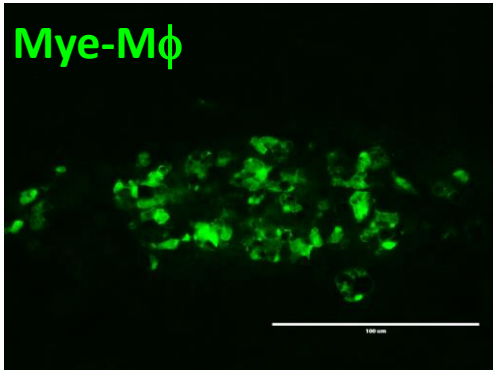
5 days after injection

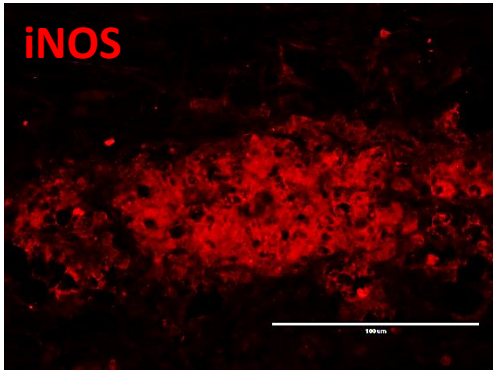
Inflammation in the Normal Cord Injected With Naïve- vs. Myelin-macrophages

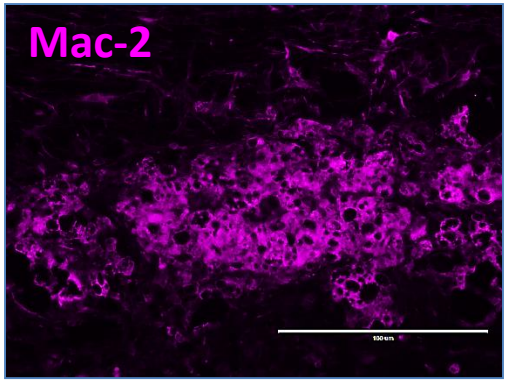










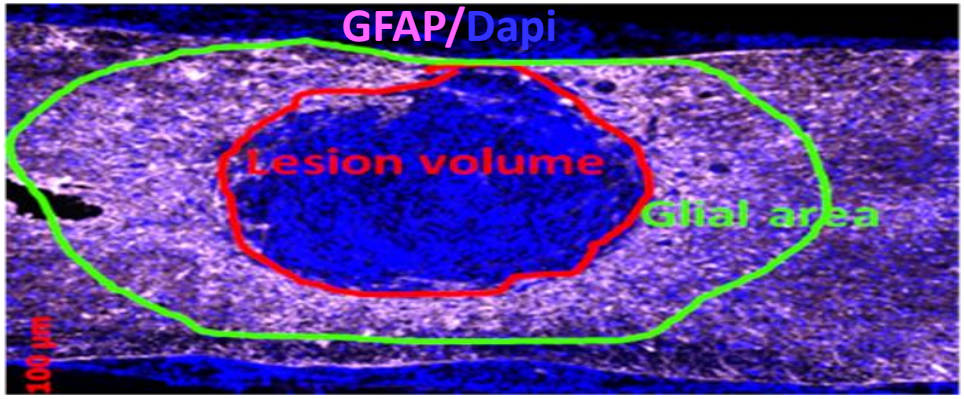


5 days after injection

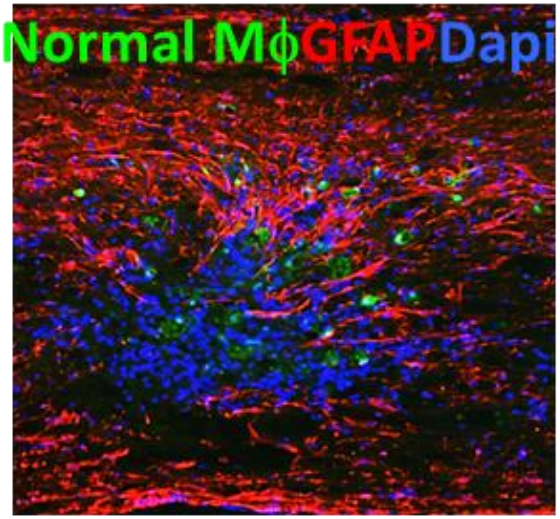
Spinal Cords Staining for GFAP to Quantify Lesion Volume

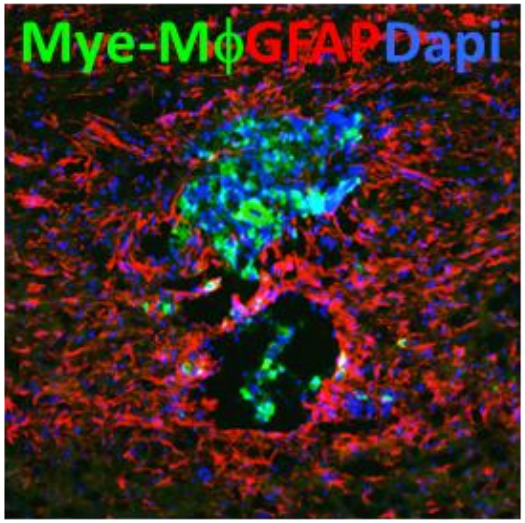
Lesion area: GFAP-

Gliosis: GFAP+++



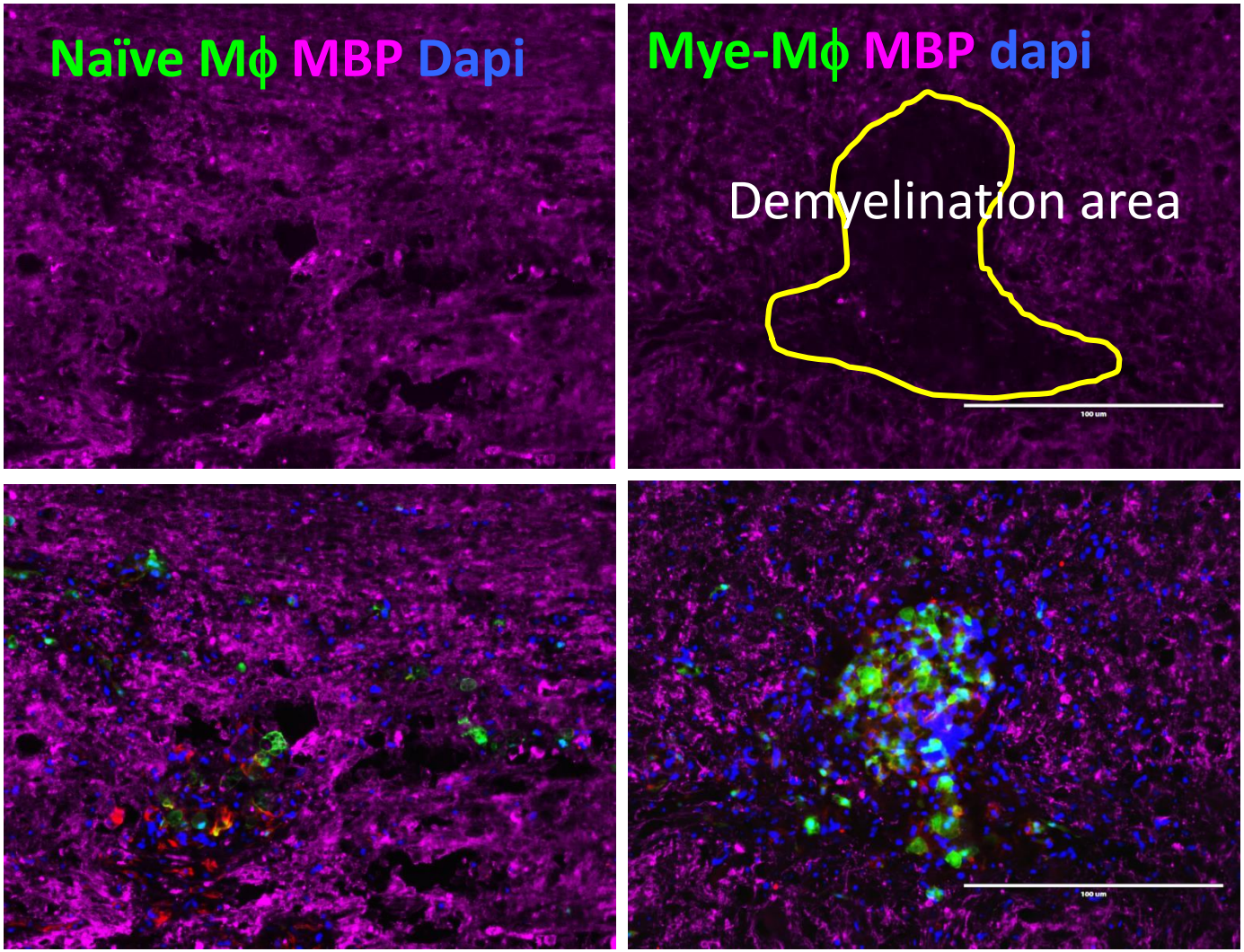
SCI





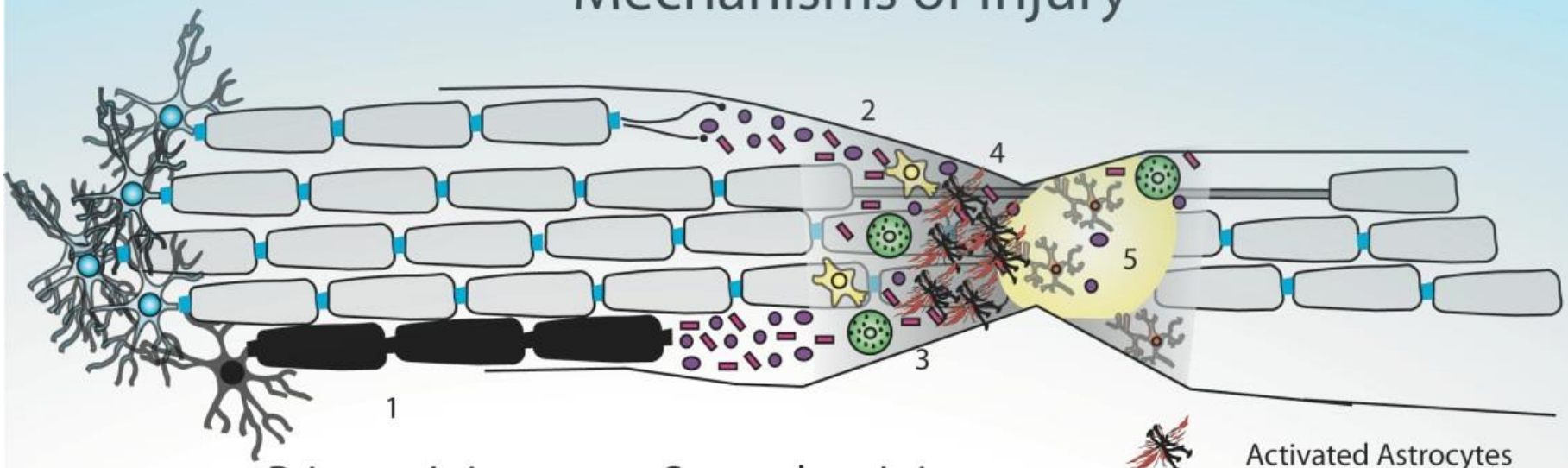
5 days after cell injection

Spinal Cords Staining for Myelin Basic Protein (MBP) to Quantify Demyelination Area



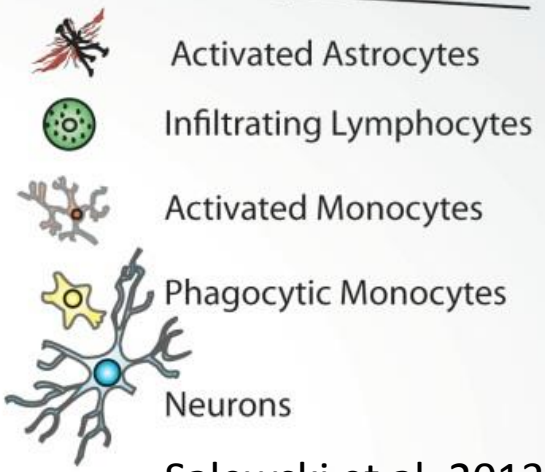
Normal cord

Mechanisms of Injury



Primary Injury
1 - Loss of Neurons/Axons
2 - Demyelination

Secondary Injury
1 - Loss of Neurons/Axons
2 - Demyelination
3 - Inflammation
4 - Reactive Oxidative Damage and the Astrocytiic Glial Scar
5 - Cyst Formation



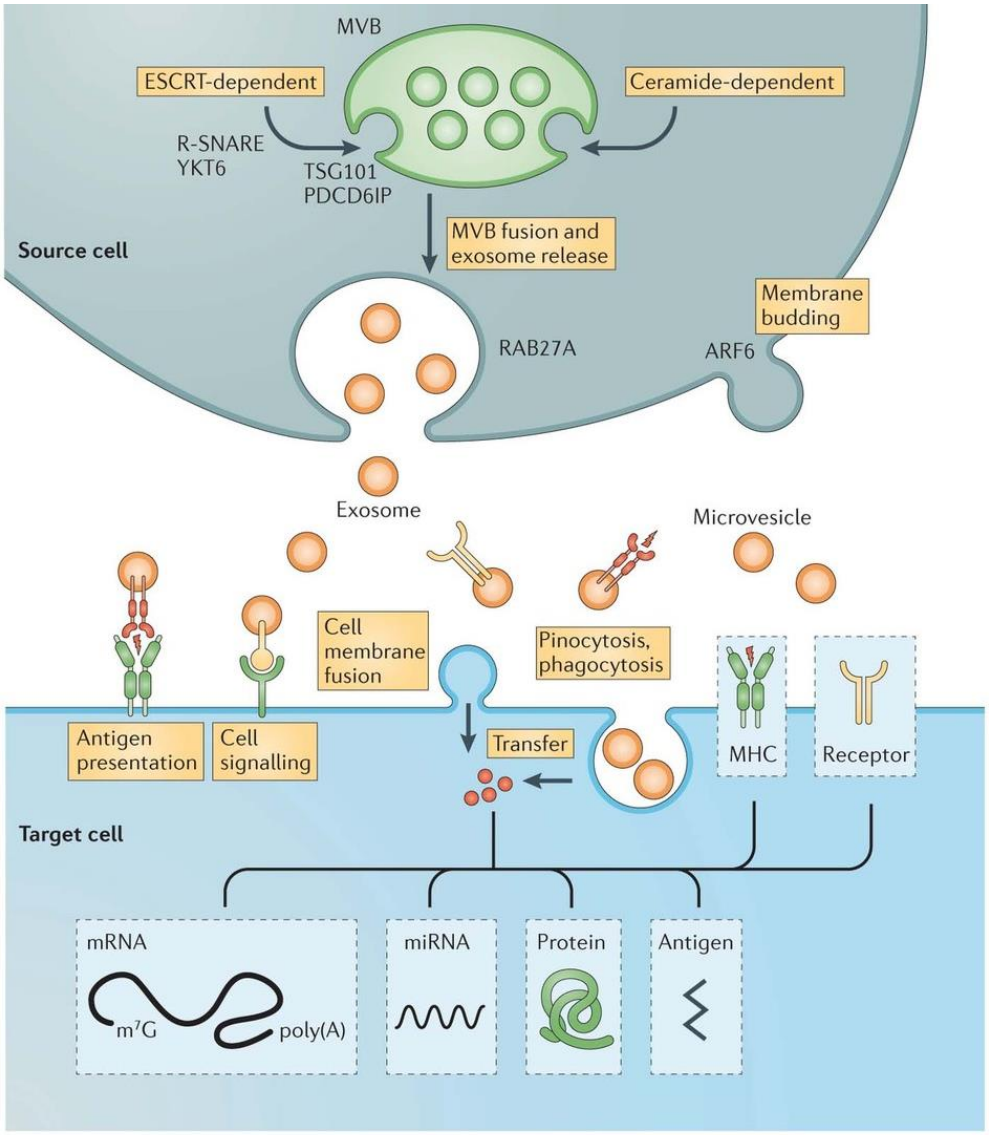
Salewski et al. 2013.

Mechanisms of Secondary Injury

- Vascular change
- Free radicals
- Excitotoxicity
- Calcium influx
- Cell death
- **Myelin-laden macrophages and impaired phagocytic capacity**

This is the first example of a single cell population that can cause pathogenic change

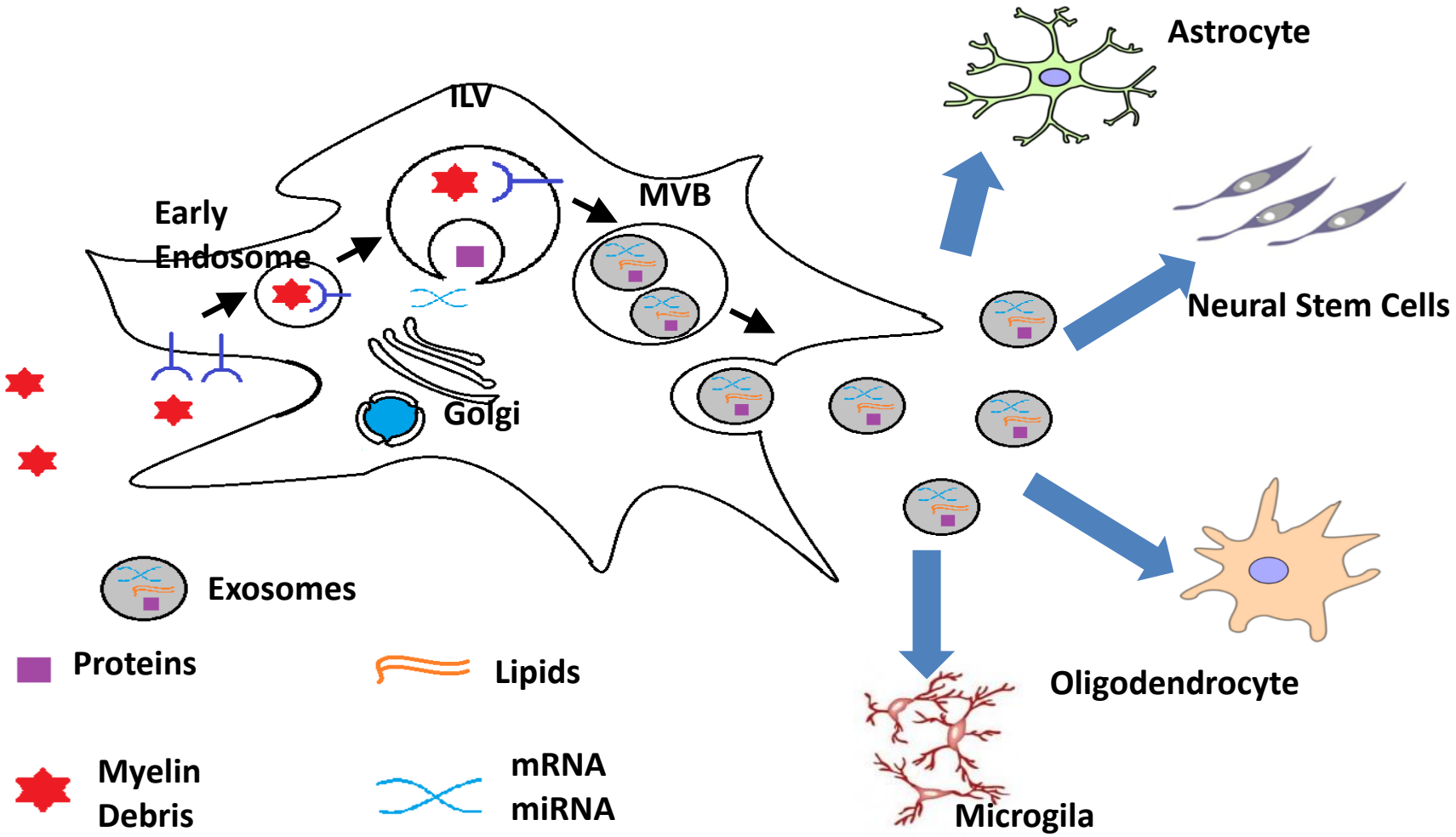
Exosomes and Their Interactions with Recipient Cells



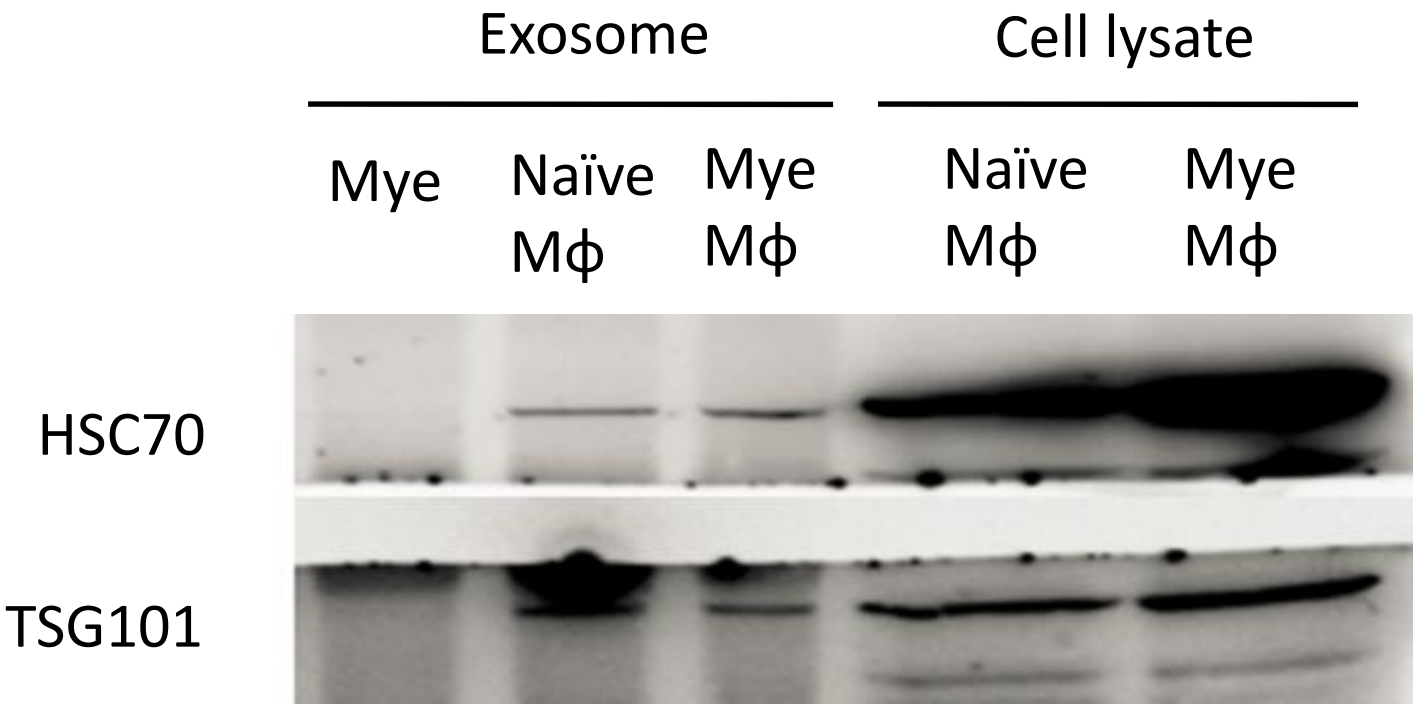
Nature Reviews | Drug Discovery

- Were considered as rubbish bags
- Exosome size: 30-150nm
- Can transport proteins, mRNA/miRNA, lipids
- Participate in pathophysiological processes

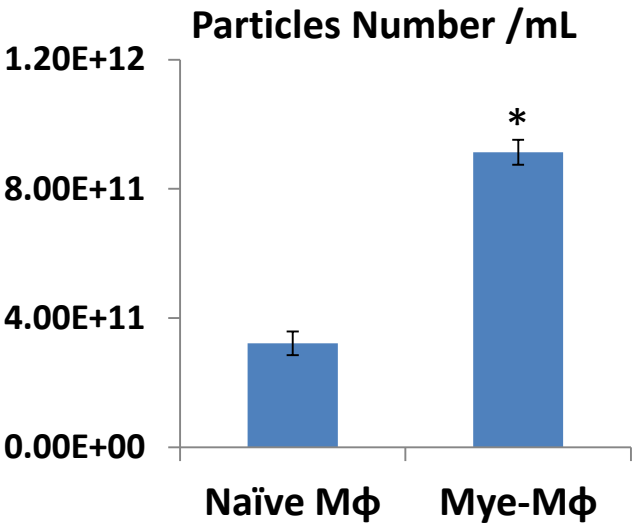
Mye-Mϕ Exosome Secretion and its Possible Roles in Communication With Recipient Cells in Spinal Cord

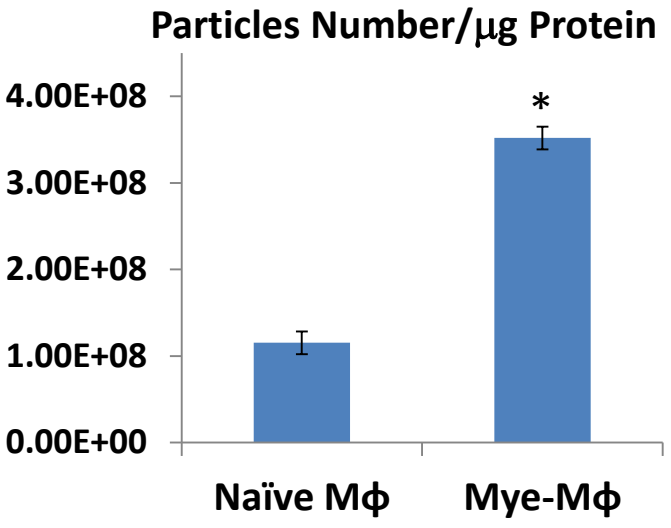


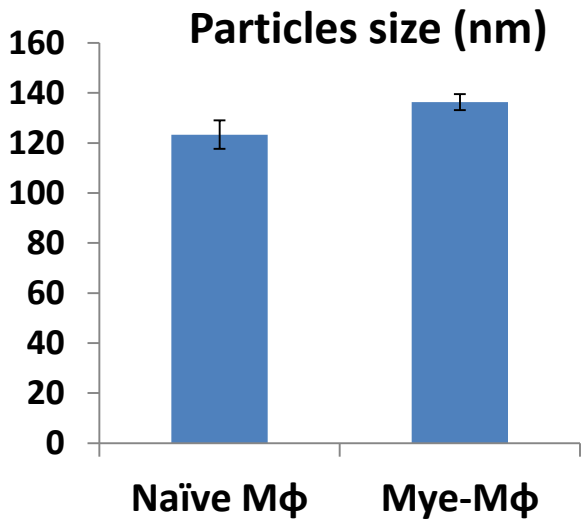
Exosome Secretion from Mye-Mϕ

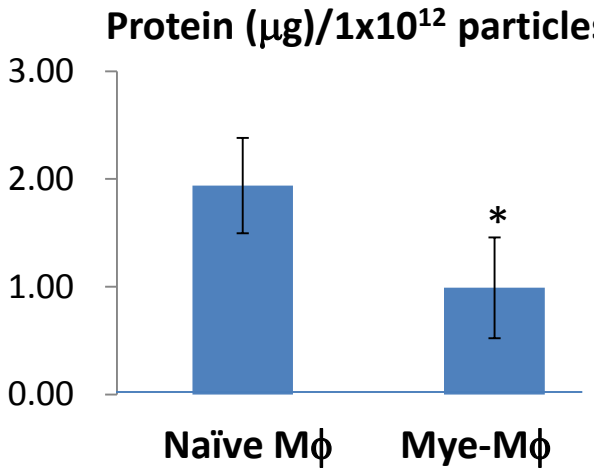


Characterization of Exosomes from Macrophages

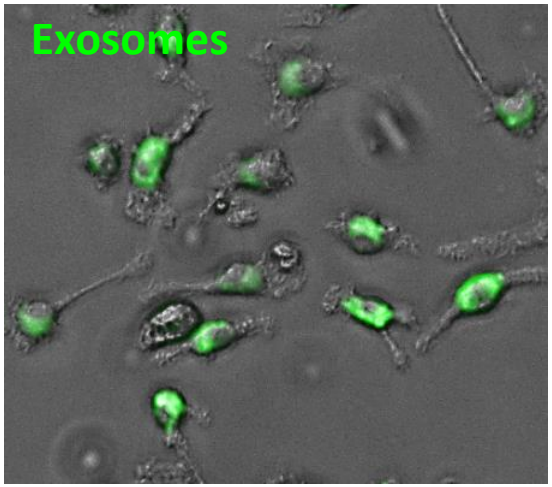


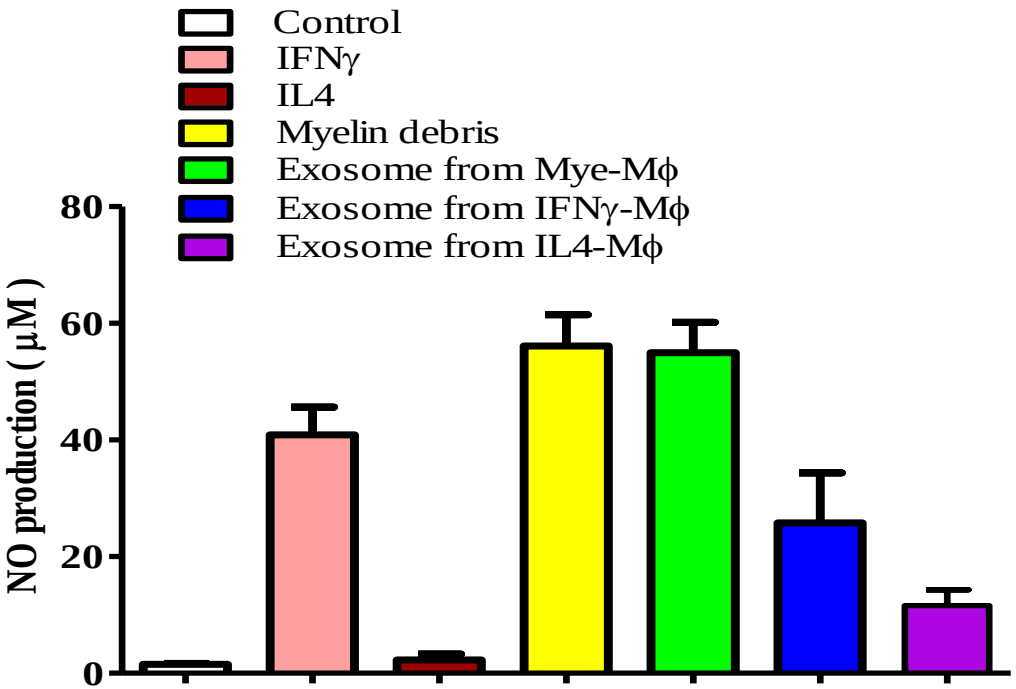




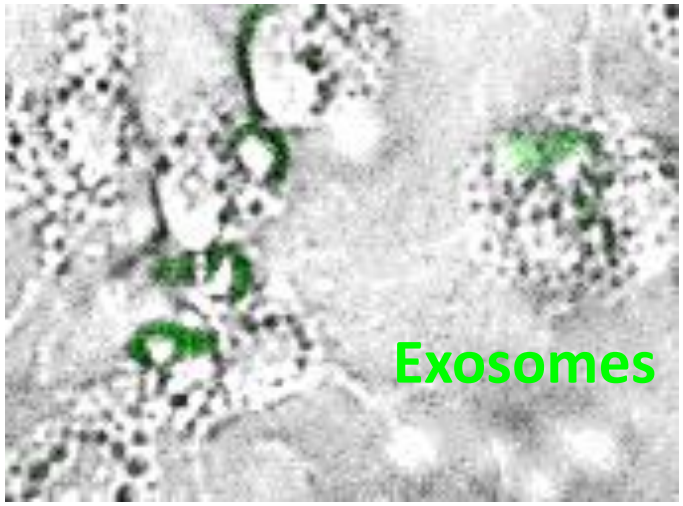


The Effect of Exosomes on Regulation of NO Production in Naïve Macrophages

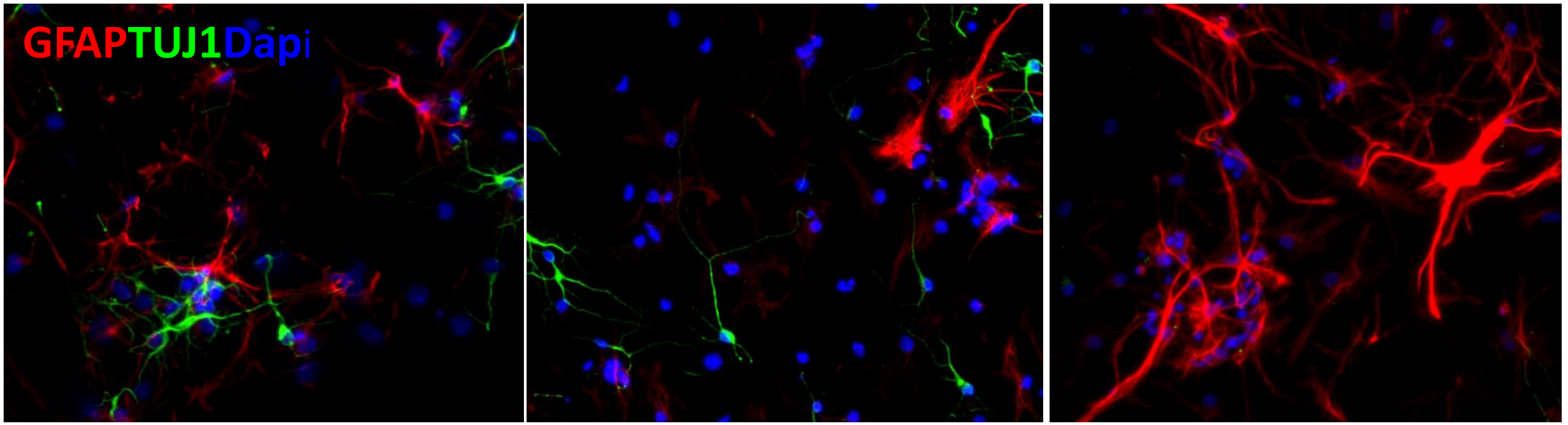




The Effect of Exosomes on Regulation of NSC Differentiation

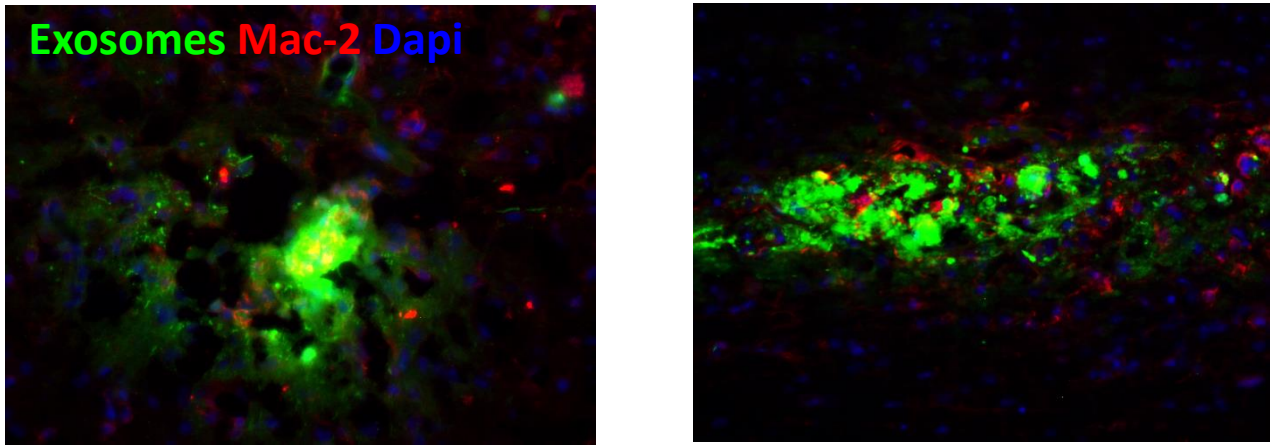


Control | exosomes from naïve Mϕ | exosomes from Mye-Mϕ

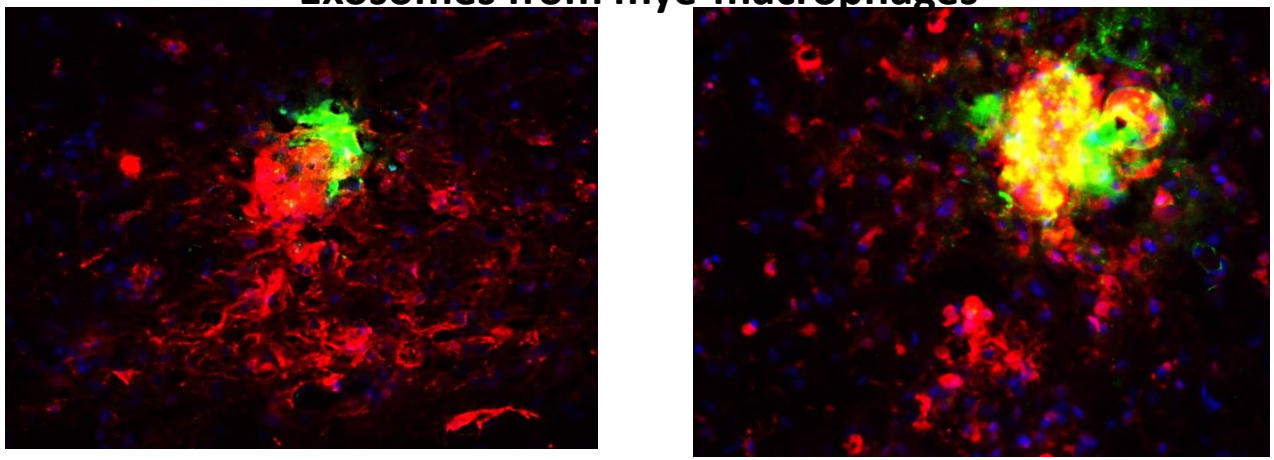


Exosomes Induce Mϕ Infiltration in Normal Cord

Exosomes from naïve macrophages



Exosomes from mye-macrophages

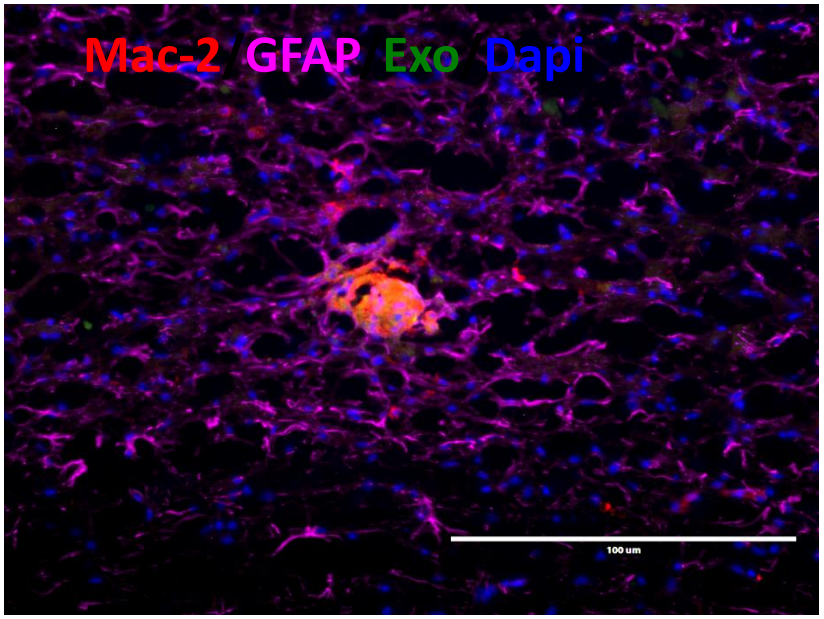


Mouse 1 Mouse 2

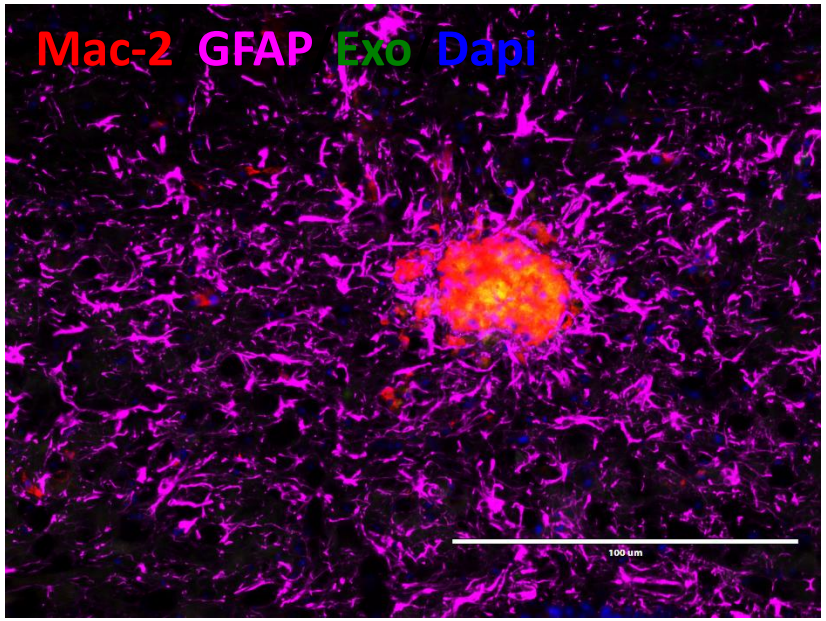
1w after SCI

Exosomes Induce Reactive Astrocyte (gliosis) in Normal Cord

Exosomes from naïve-Mϕ



Exosomes from Mye-Mϕ



2w after SCI

Does targeting mye-Mϕ…

inhibit secondary injury?

promote motor neuron function recovery?

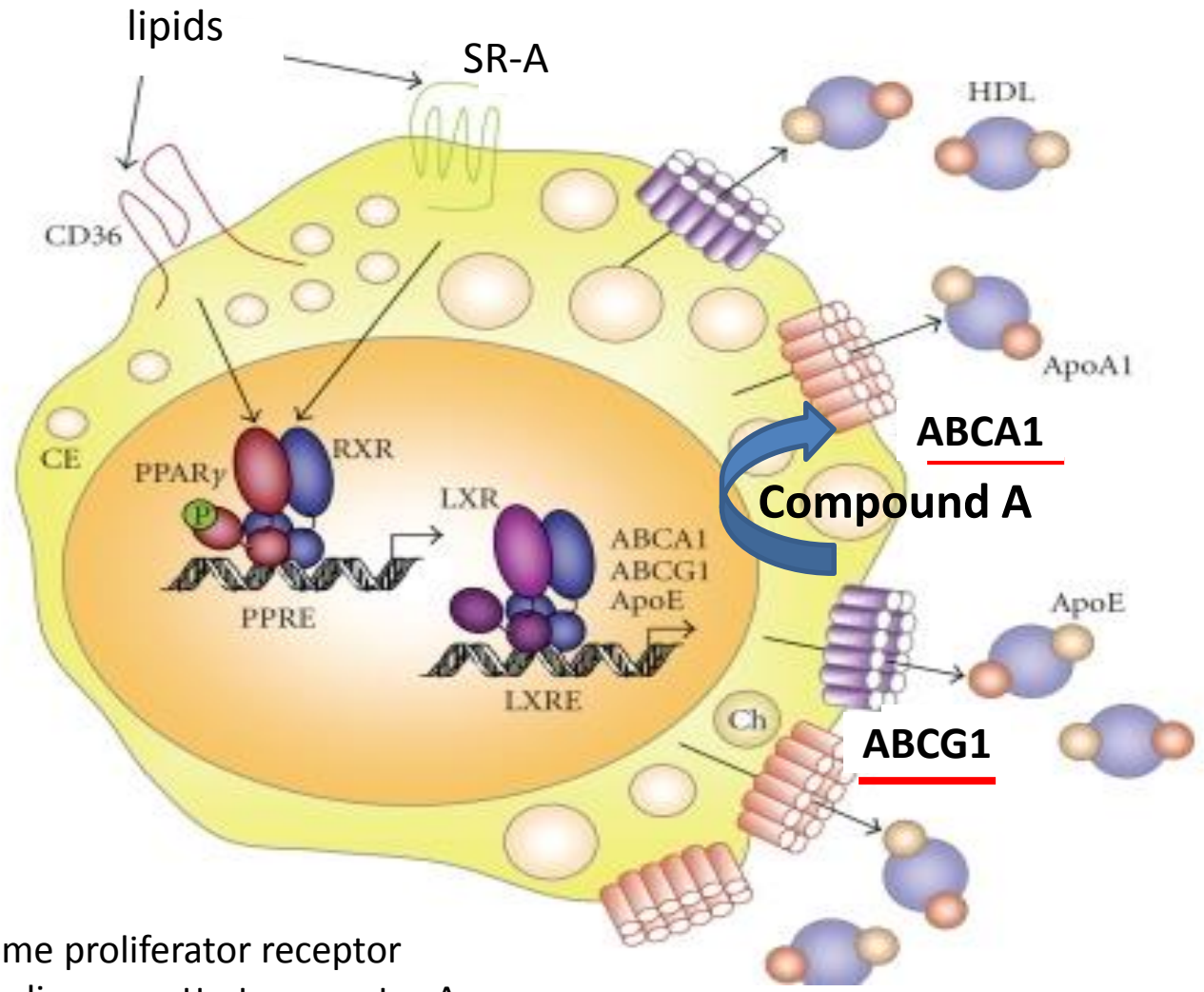
Exploring New Therapeutic Strategies Targeting Myelin-Laden Macrophages In SCI

- **Inhibition of circulating monocyte migration?**
- **Promotion of M2 Mϕ activation**
- **Pharmacologic manipulation of ABCA1 and Mϕ lipid efflux *in vivo***
- **Promotion of Mϕ emigration**
- **Transplantation of "appropriate or beneficial" Mϕ (anti-inflammatory macrophages with intact phagocytic capacity)**

Strategy I

Pharmacologic manipulation of ABCA1 and macrophage lipid efflux *in vivo*

Lipid Transporters: ABCA1 and ABCG1

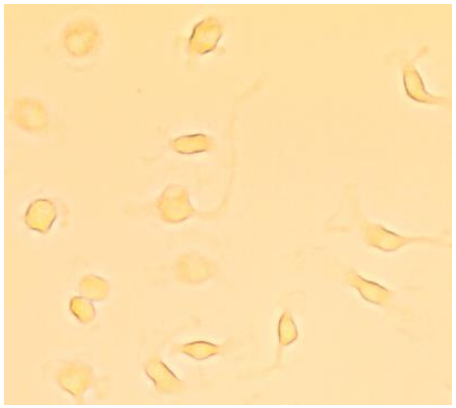


PPAR: peroxisome proliferator receptor
ABCA1: ATP-binding cassette transporter A
LXR: liver X receptor
RXR: retinoid X receptor

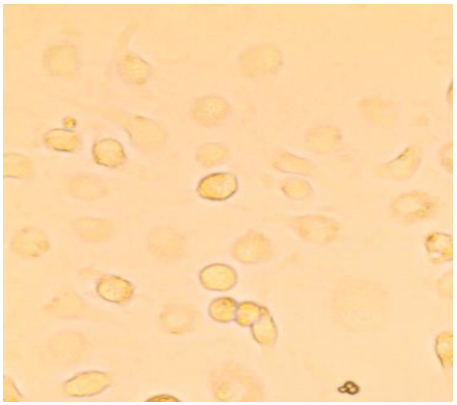
Demers *et al.* **PPAR Research.** 2008.

Compound A Rescues Myelin-Inhibited Arginase-1 Activity

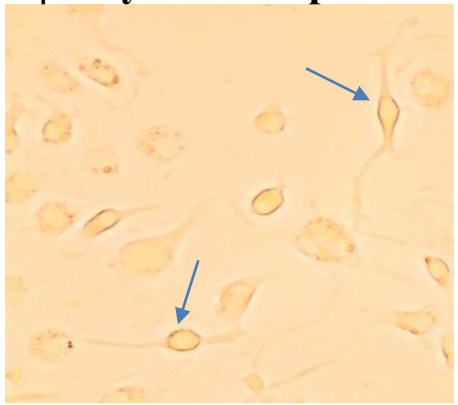
Mφ alone



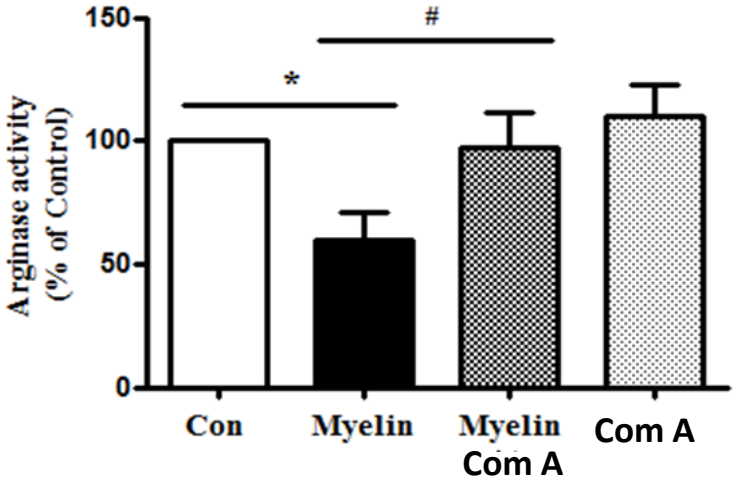
Mφ+Myelin

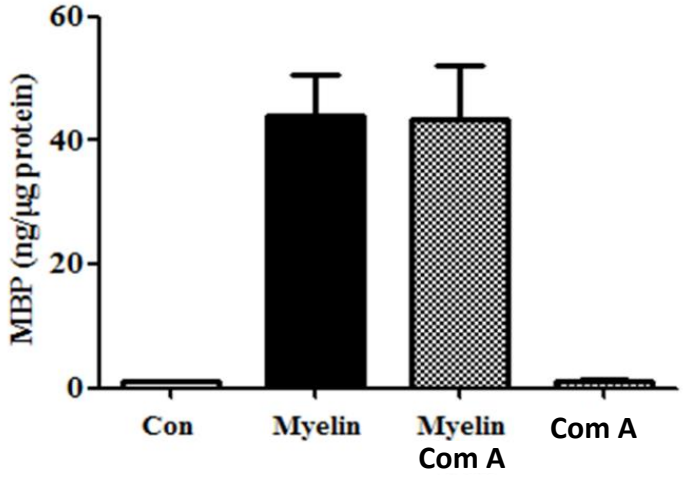


Mφ+Myelin+compound A

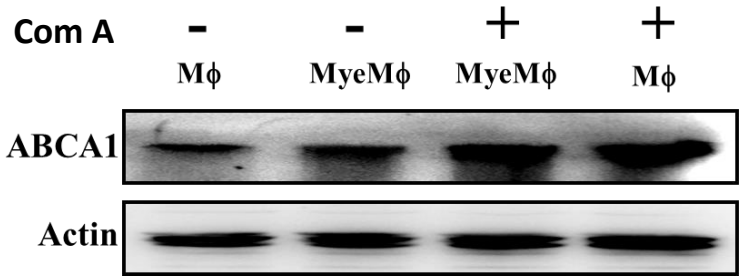


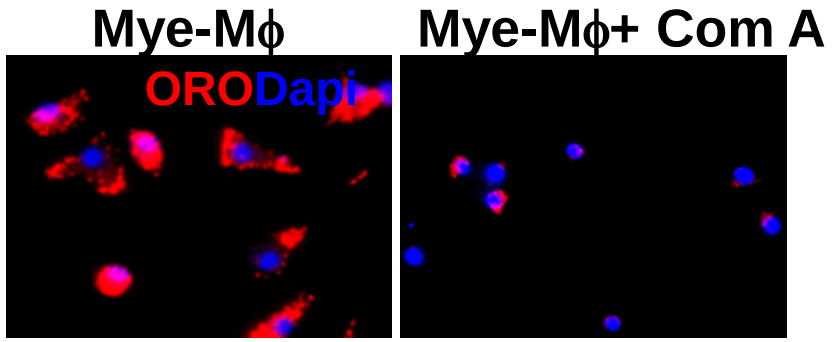
Mφ+Compound A

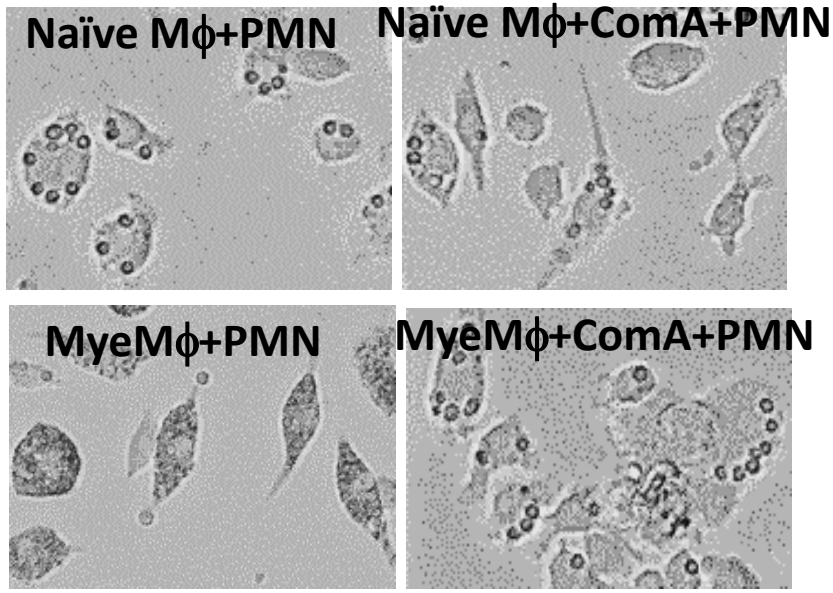


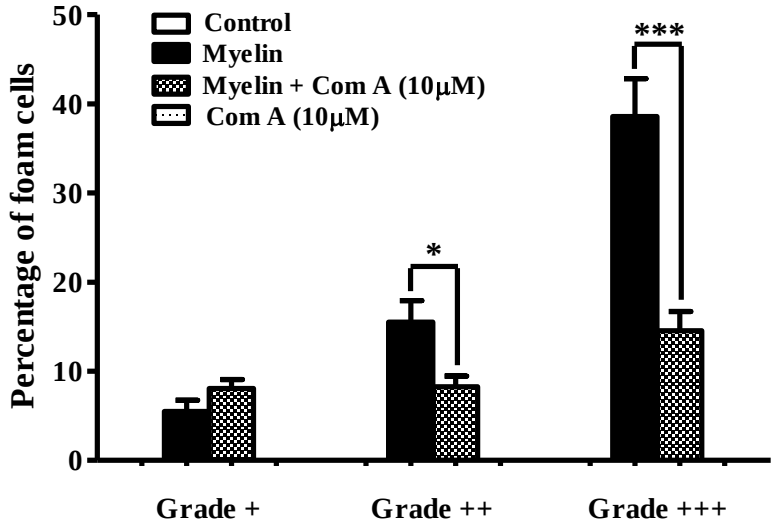


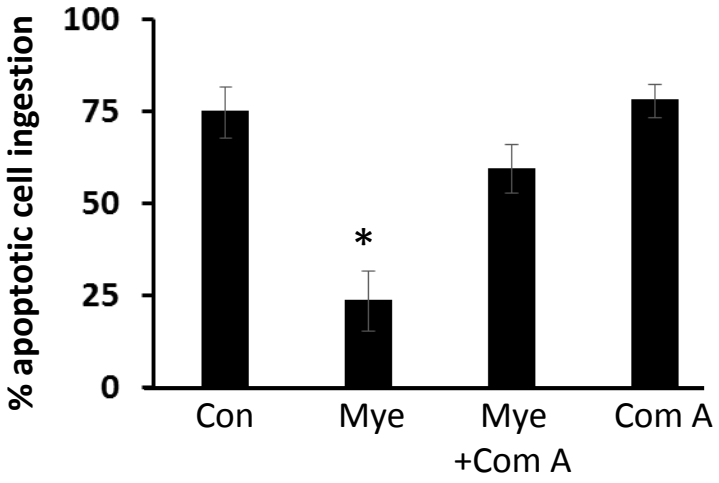
Compound A Enhances Lipid Efflux and Apoptotic Cell Uptake



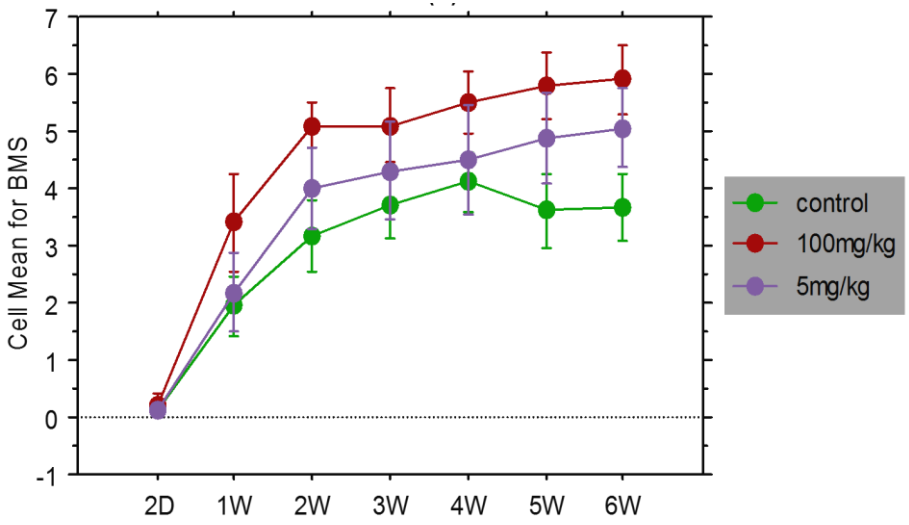


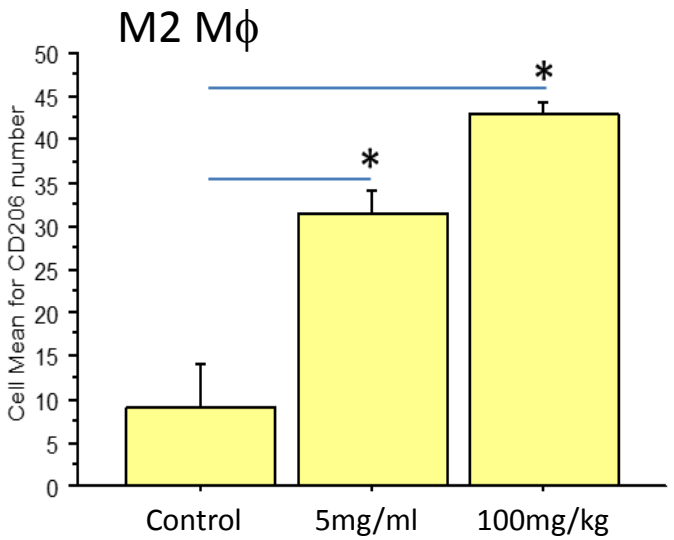


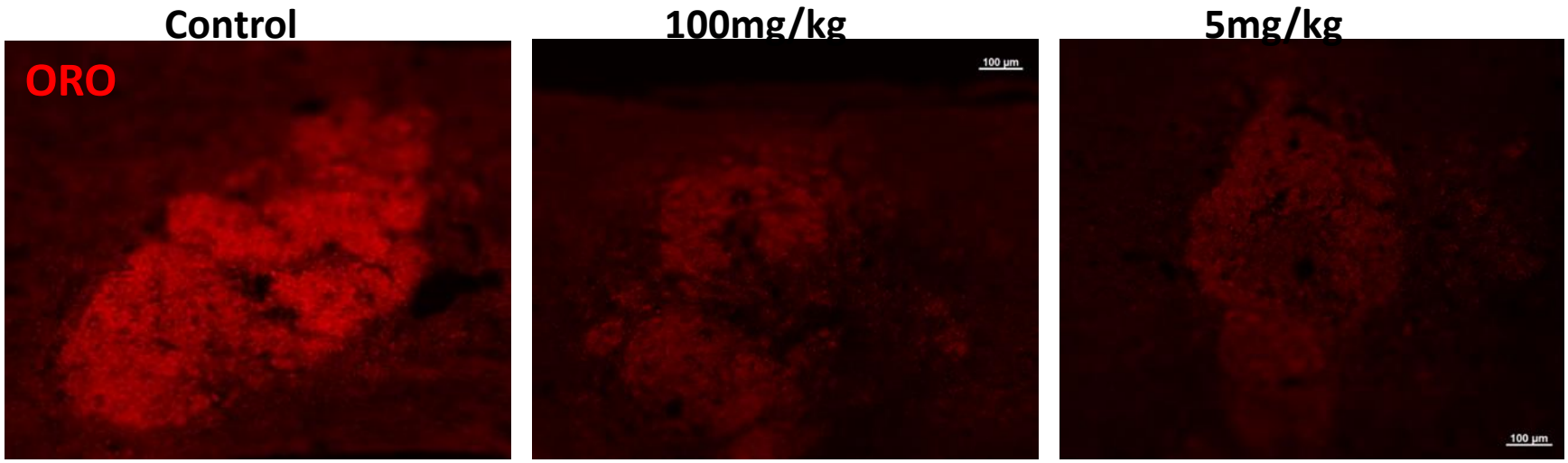




Compound A Significantly Increased BMS Score





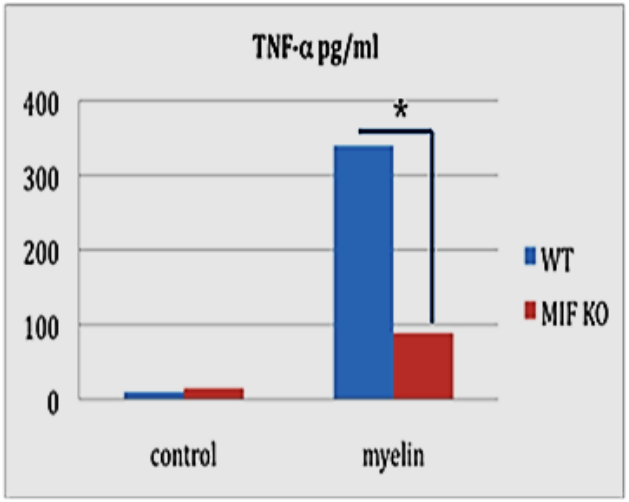


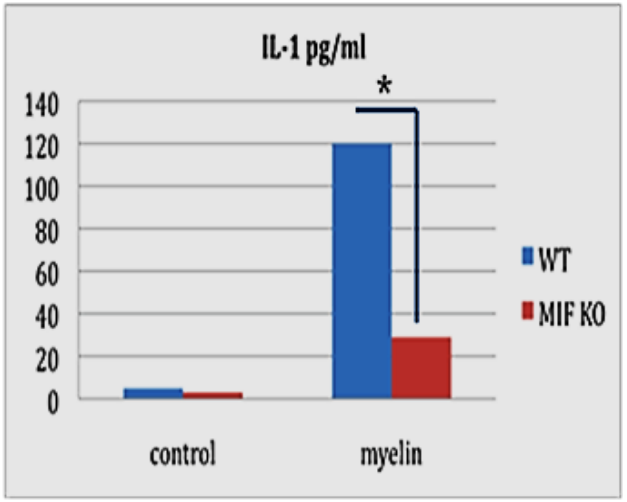
4 weeks after treatment

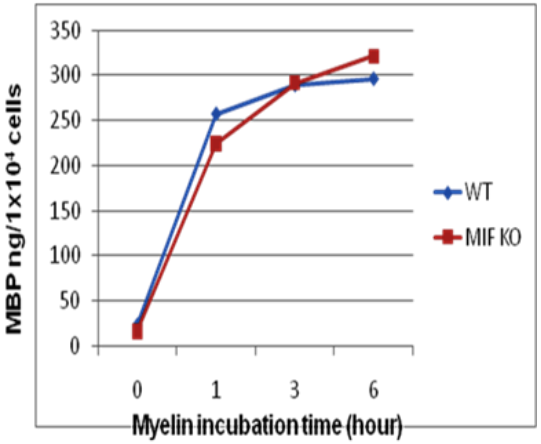
Strategy II

Transplantation of “appropriate or beneficial” Mϕ
(anti-inflammatory Mϕ with intact phagocytic capacity)

Macrophage Migration Inhibitory Factor (MIF) KO Macrophages

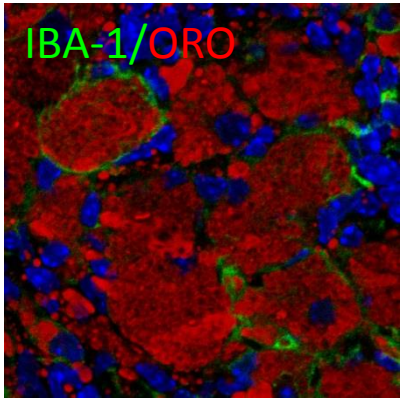


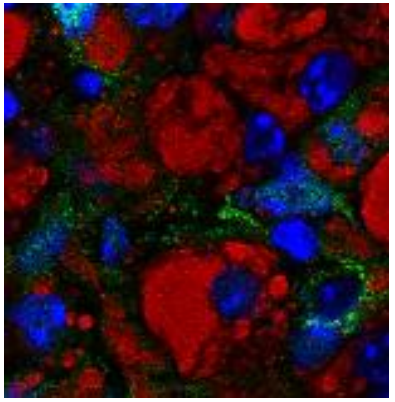




WT Mϕ

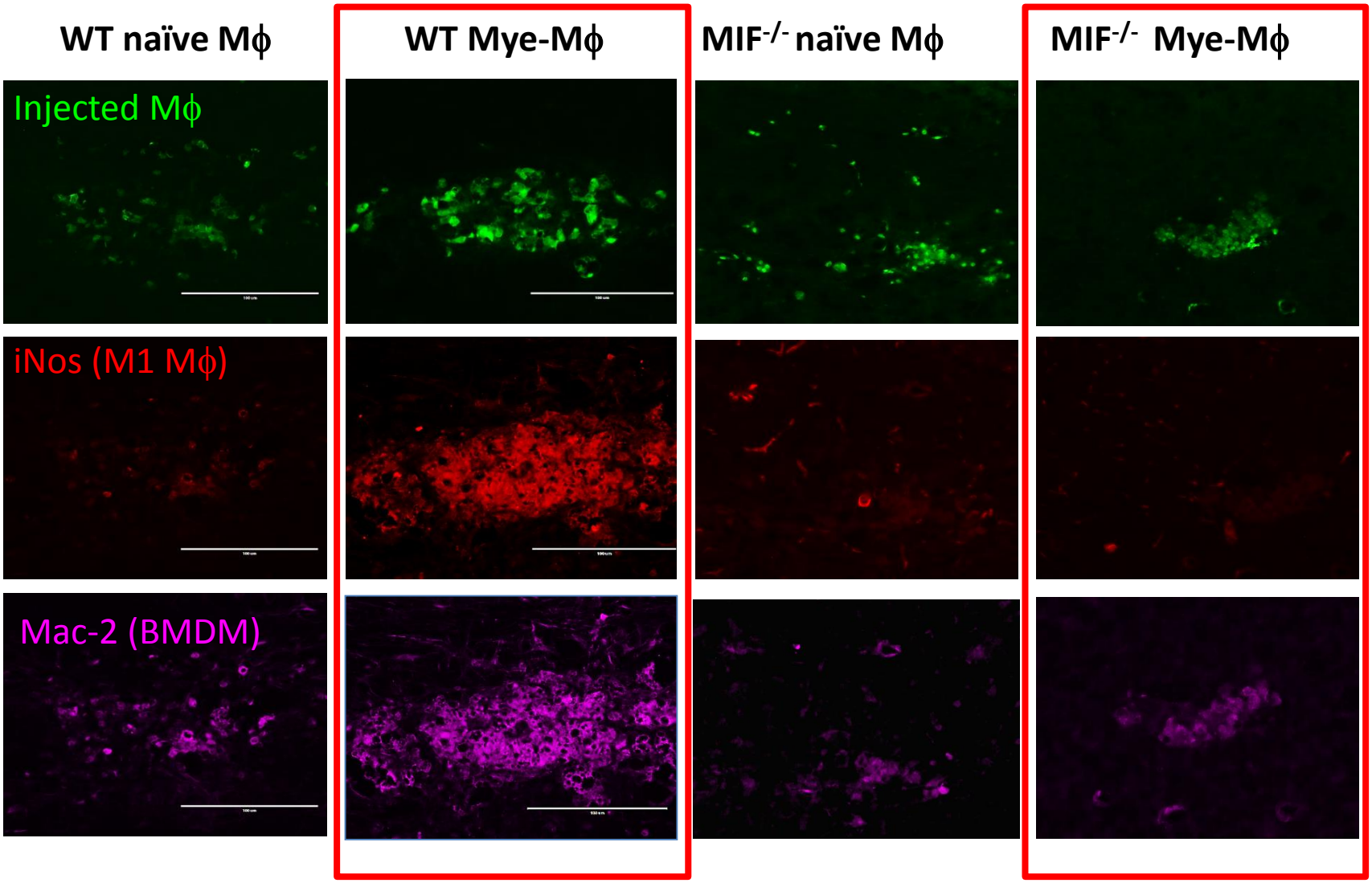
MIF KO Mϕ





4W after SCI

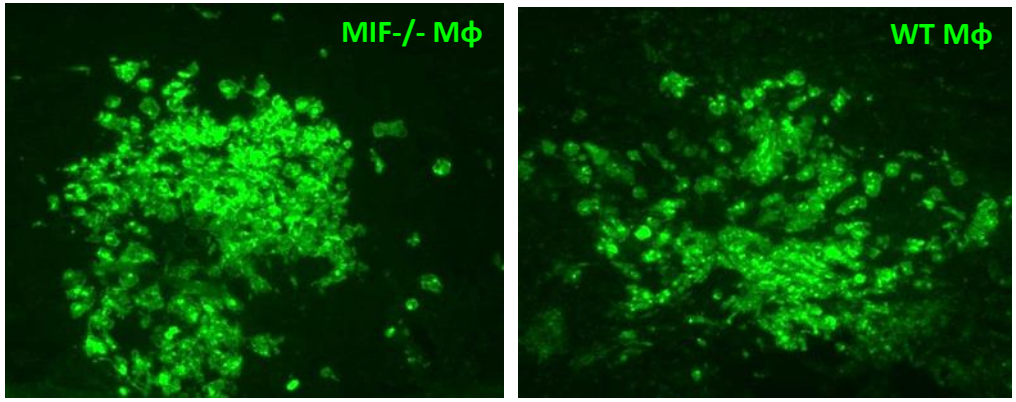
GFP-Mϕ Injection in the Injured Spinal Cord

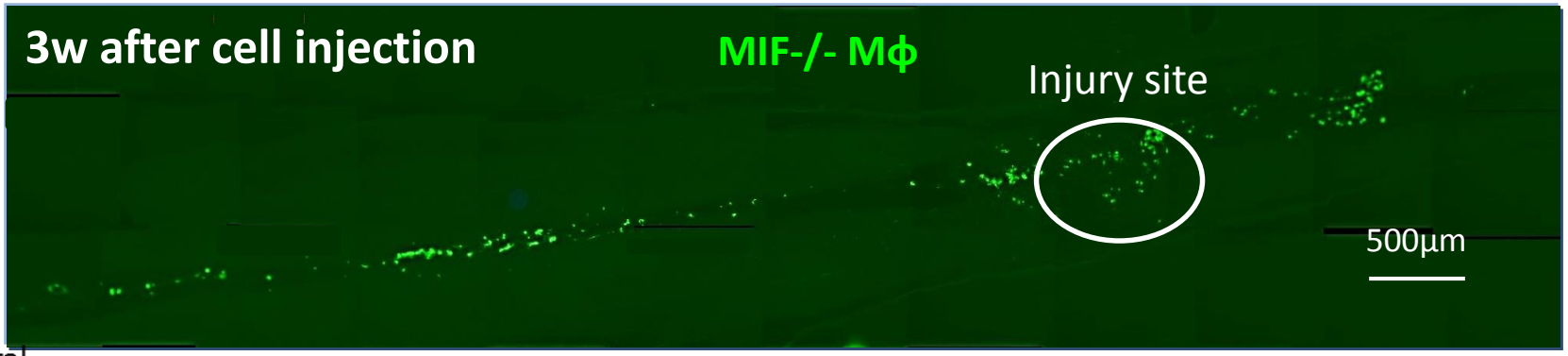


7 days after SCI

GFP-macrophage Injection in the Injured Spinal Cord

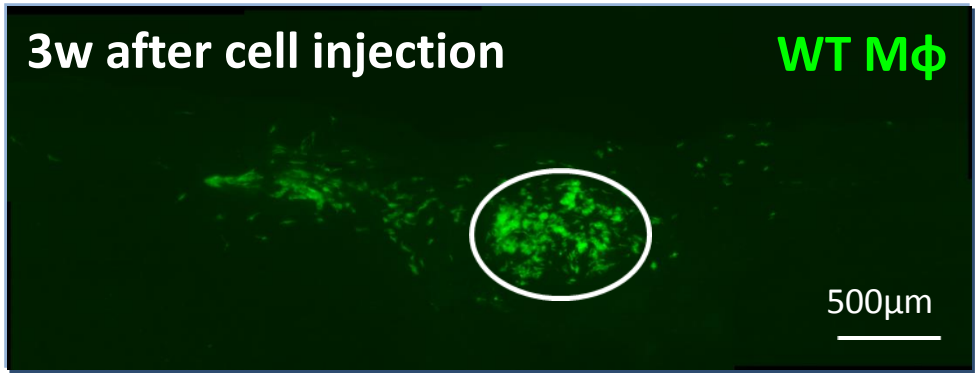
3d after injection



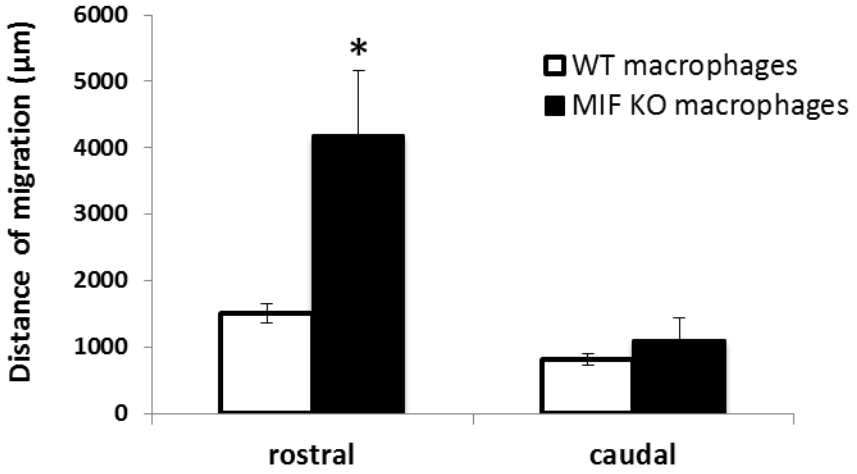


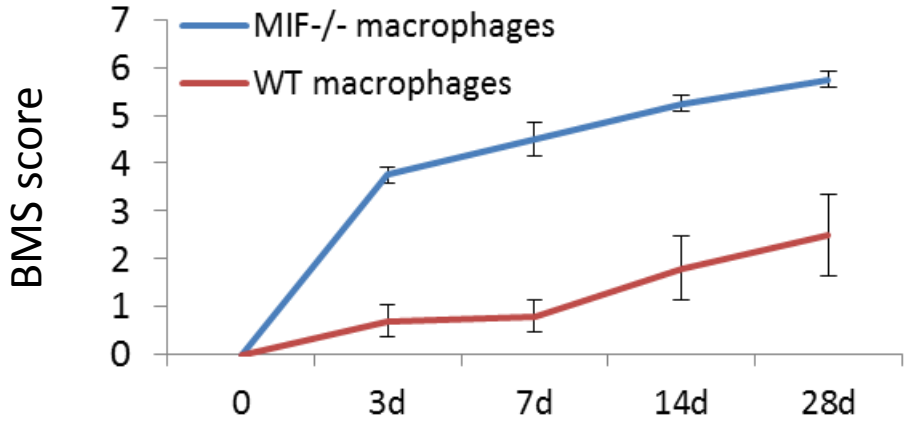
rostral

caudal

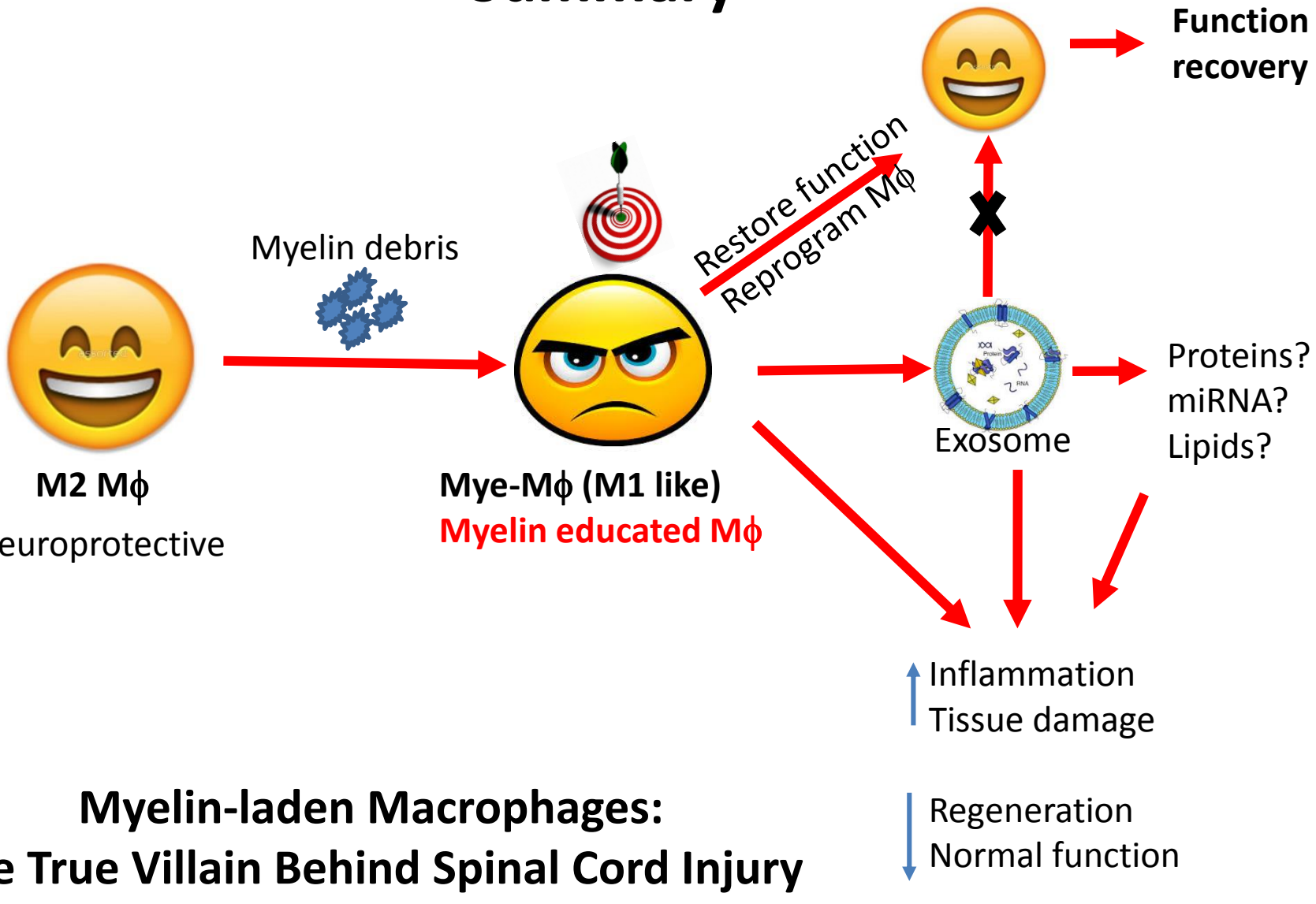


Effect of Macrophage on Locomotion after SCI



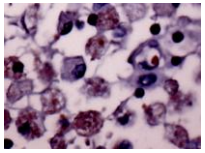


Summary

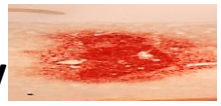


Myelin-laden Macrophages:
The True Villain Behind Spinal Cord Injury

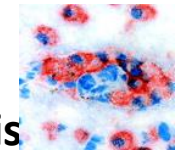
CNS Disorders that Generate Myelin Debris



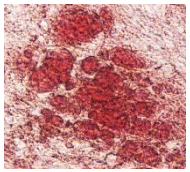
Stroke



Injury
(SCI,TBI, optic nerve injury)



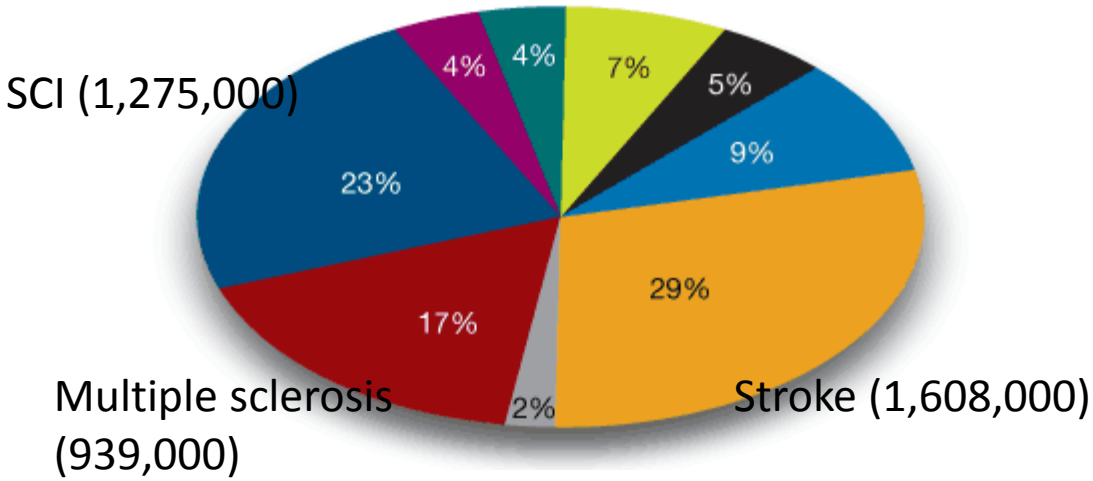
multiple sclerosis



Neurodegenerative diseases
(Alzheimer's,
Parkinson's and ALS)

Demyelination → **Mye-Mϕ**

Cause of Paralysis Among US Adults



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NIH R01 GM100474
NSF DMS-0714589
NJ Commission on SCR CSCR13IRG006
FSU College of Medicine