Biologics/Regenerative Medicine:

The Science of Stem Cells and Regenerative Therapies

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Disclosure

- **No conflicts of interest to disclose; No off-label or investigational use of a therapeutic product**
- Member, National Academy of Medicine Action Collaborative on Countering the Opioid Epidemic
- Member, US Department of Health and Human Services (HHS) Pain Management Best Practice Inter-Agency Task Force (PMTF)
- Member, HHS Centers for Medicare and Medicaid Services (CMS) Expert Work Group
- Safety Officer, NIH/NIAMS Cohen Project Data and Safety Monitoring Board; Member, multiple NIH/NINDS grant review panels.
- Past President, American Academy of Pain Medicine
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Learning Objectives

- Describe new mechanistic understanding of nociceptive sensitization through neuroimmune interactions
- Describe state-of-the-art understanding of the mechanisms of regenerative medicine
- Present key pre-clinical evidence of regenerative medicine in pain management

REVIEW

The Journal of Clinical Investigation

Neuroimmune modulation of pain and regenerative pain medicine

Thomas Buchheit,[1,2] Yul Huh,[1,3] William Maixner,[1] Jianguo Cheng,[4] and Ru-Rong Ji[1,3,5]

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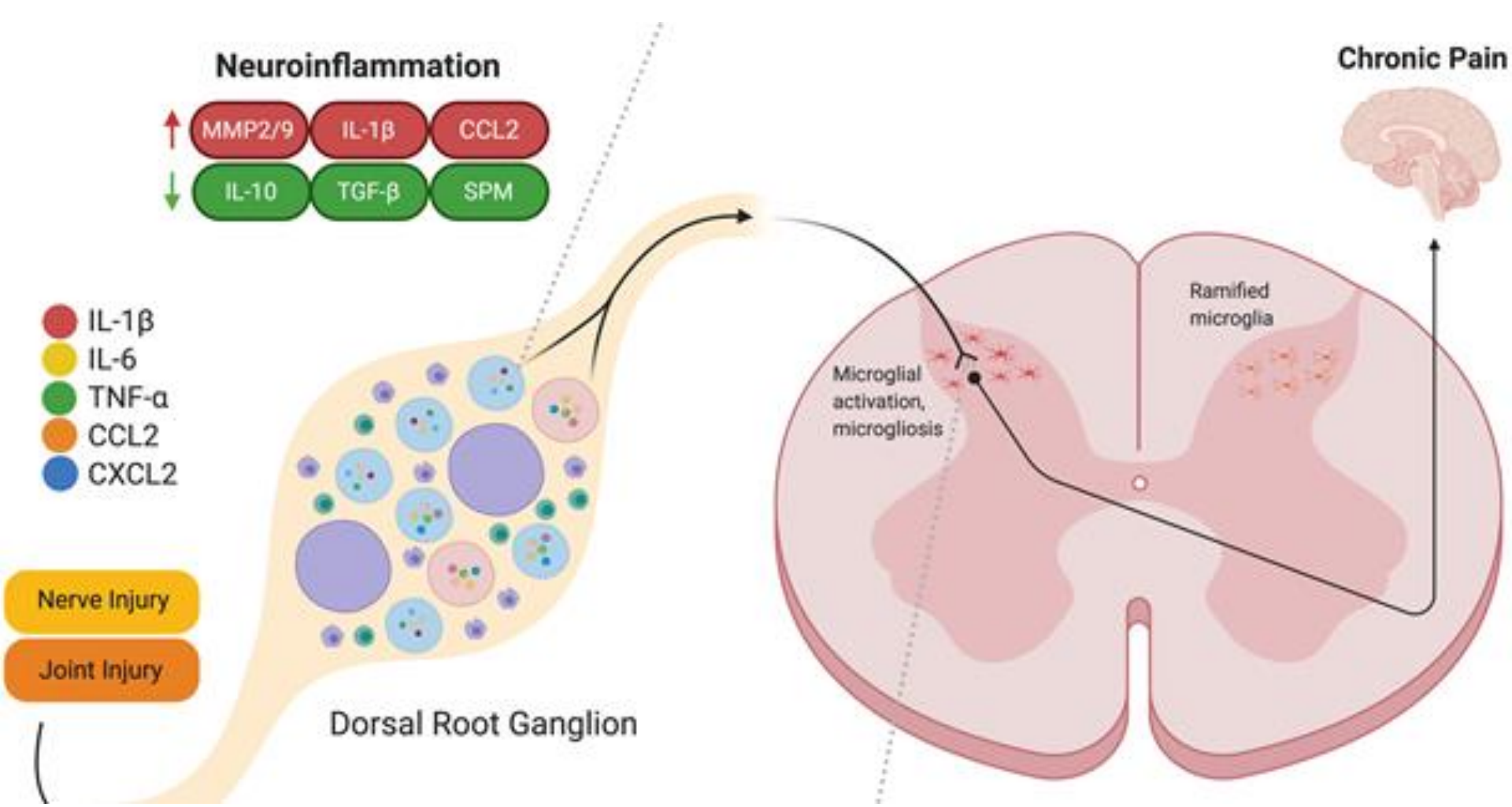
Regenerative pain medicine, which seeks to harness the body's own reparative capacity, is rapidly emerging as a field within pain medicine and orthopedics. It is increasingly appreciated that common analgesic mechanisms for these treatments depend on neuroimmune modulation. In this Review, we discuss recent progress in mechanistic understanding of nociceptive sensitization in chronic pain with a focus on neuroimmune modulation. We also examine the spectrum of regenerative outcomes, including preclinical and clinical outcomes. We further distinguish the analgesic mechanisms of regenerative therapies from those of cellular replacement, creating a conceptual and mechanistic framework to evaluate future research on regenerative medicine.

State-of-the-Art, Mechanism-guided Pain Medicine

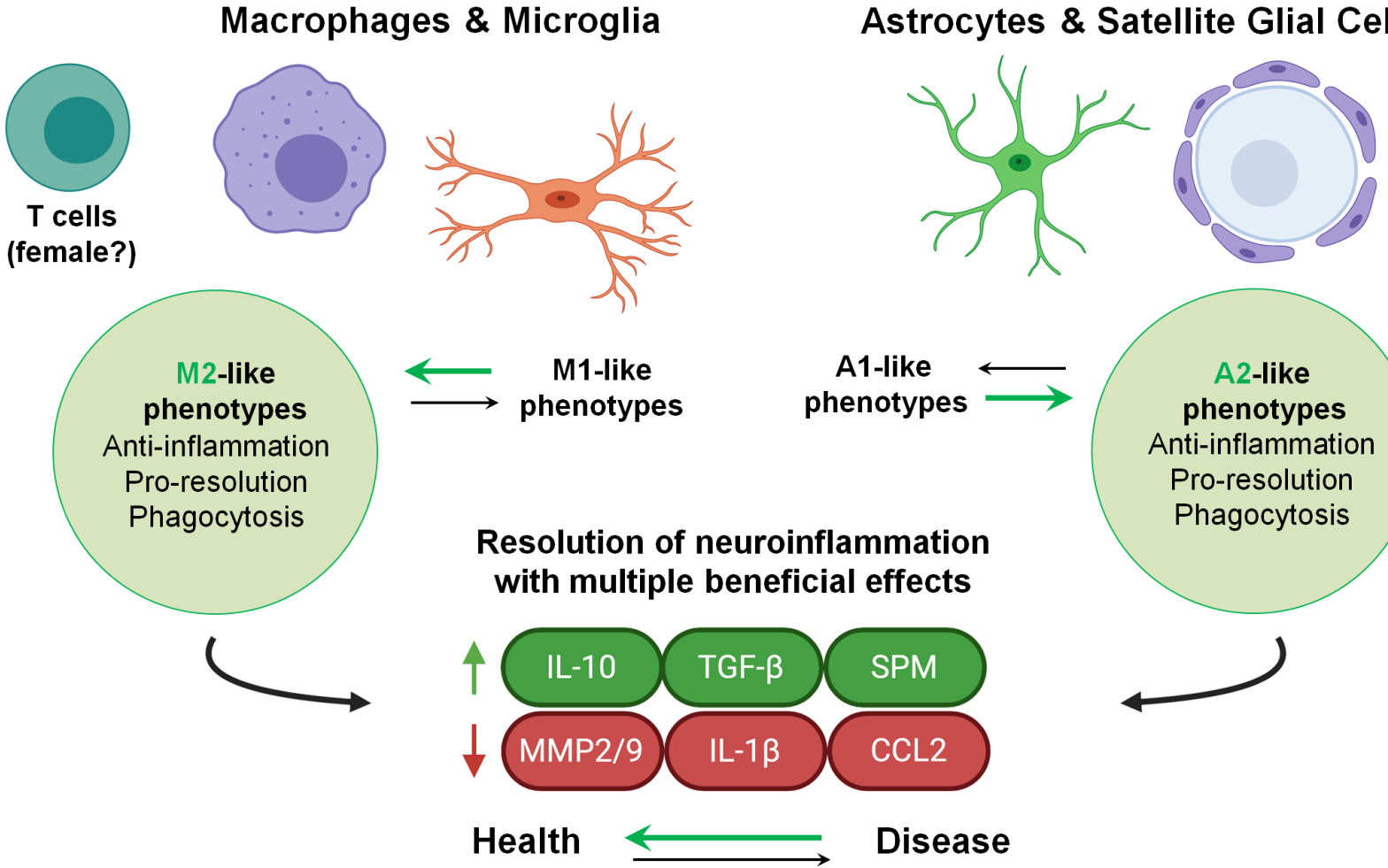
Cheng J, Huh Y, Ji RR. (2022). Mechanism-Based Treatment and Precision Medicine, ***Practical Management of Pain, 6th Edition,*** (Benson et al. Eds), Mosby/Elsevier, Philadelphia, USA.

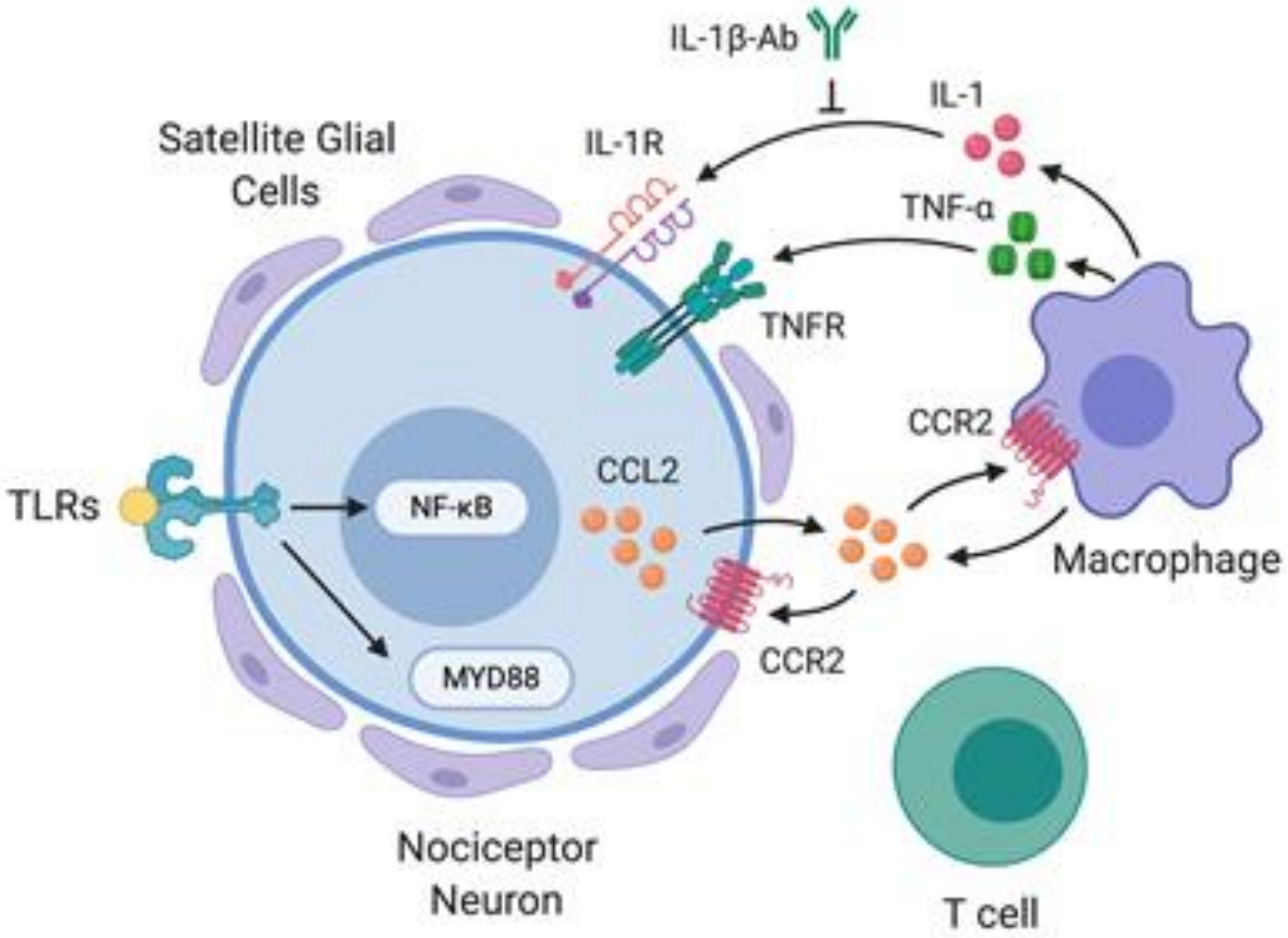
Nociceptive sensitization through neuroimmune interactions

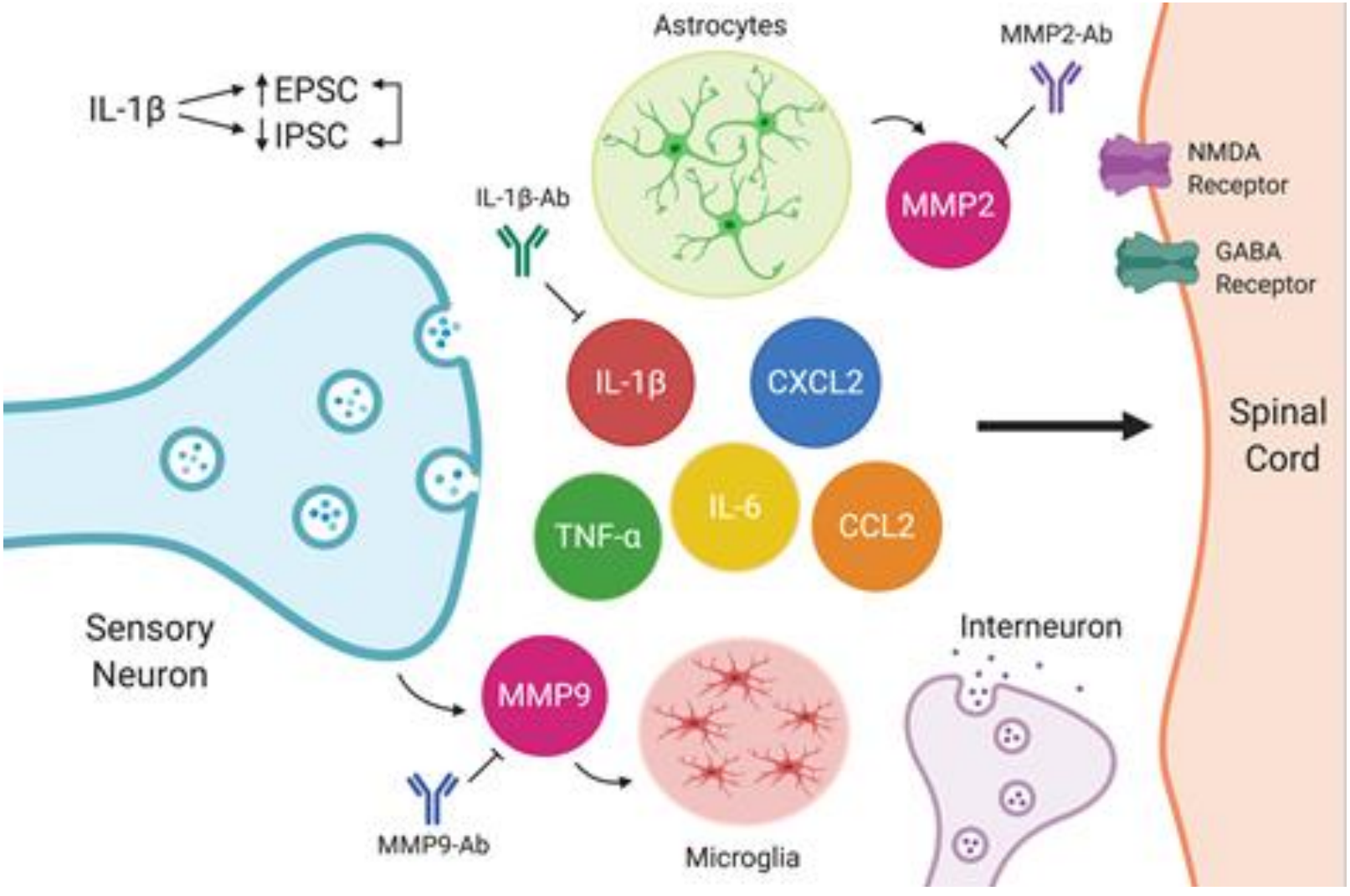
Buchheit T, Huh Y, Maixner W, Cheng J, Ji RR. Neuroimmune modulation of pain and Regenerative Pain Medicine. ***Journal of Clinical Investigation.*** 2020;130(5):2164-2176.


Neuroinflammation
MMP2/9
IL-1β
CCL2
IL-10
TGF-β
SPM
Chronic Pain
IL-1β
IL-6
TNF-α
CCL2
CXCL2
Ramified microglia
Microglial activation, microgliosis
Nerve Injury
Joint Injury
Dorsal Root Ganglion

Modulation of neuroinflammation in the PNS and CNS



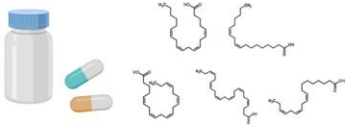

IL-1β-Ab
IL-1
IL-1R
Satellite Glial Cells
TNF-α
TNFR
CCR2
TLRs
NF-κB
CCL2
Macrophage
CCR2
MYD88
Nociceptor Neuron
T cell


IL-1β
EPSC
IPSC
Astrocytes
MMP2-Ab
MMP2
NMDA Receptor
GABA Receptor
IL-1β-Ab
IL-1β
CXCL2
TNF-α
IL-6
CCL2
Spinal Cord
Sensory Neuron
MMP9
MMP9-Ab
Microglia
Interneuron

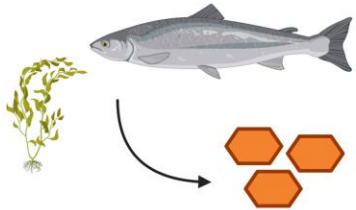
Intraarticular injections
Radiofrequency ablation
Neuromodulation (PNS)

DMARDs: RA

Dietary supplements



Polyunsaturated fatty acids



DHA and EPA

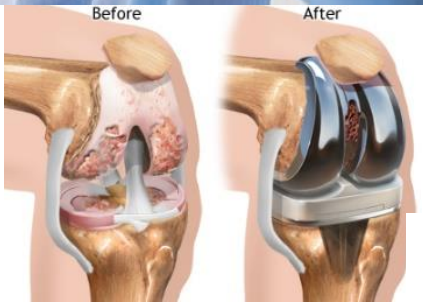
Exercise & Meditation

Regenerative Therapies

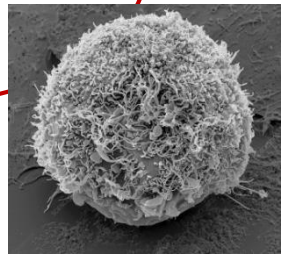
Platelet
Rich
Plasma

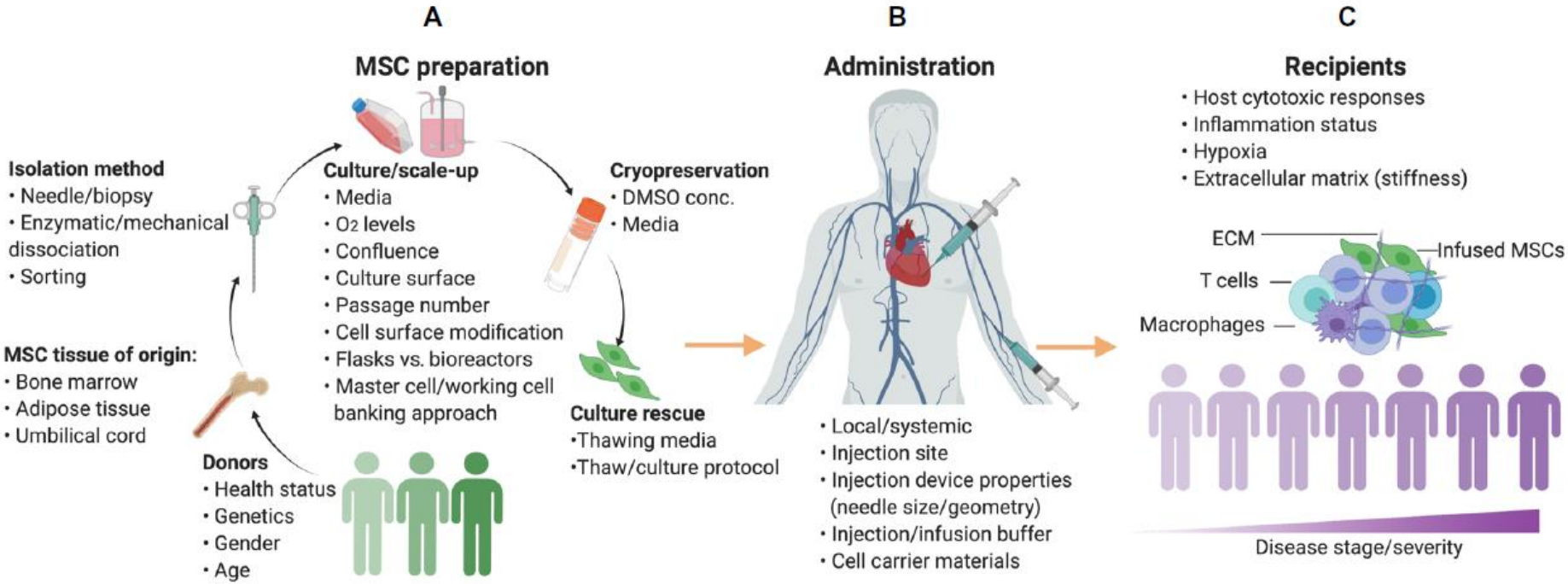
Autologous
Conditioned
Serum

Mesenchymal
Stem/Stromal
Cells



Joint replacement

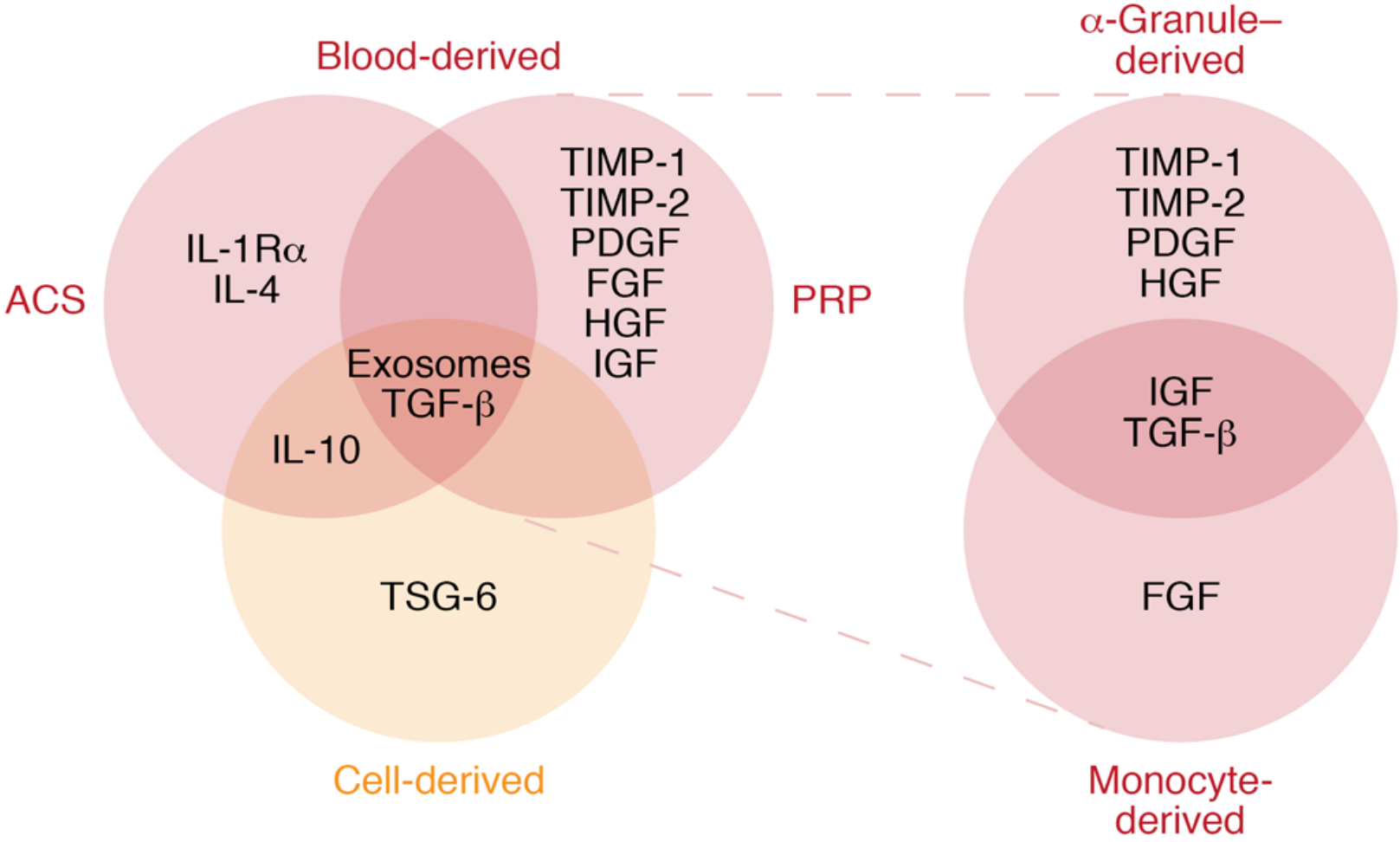


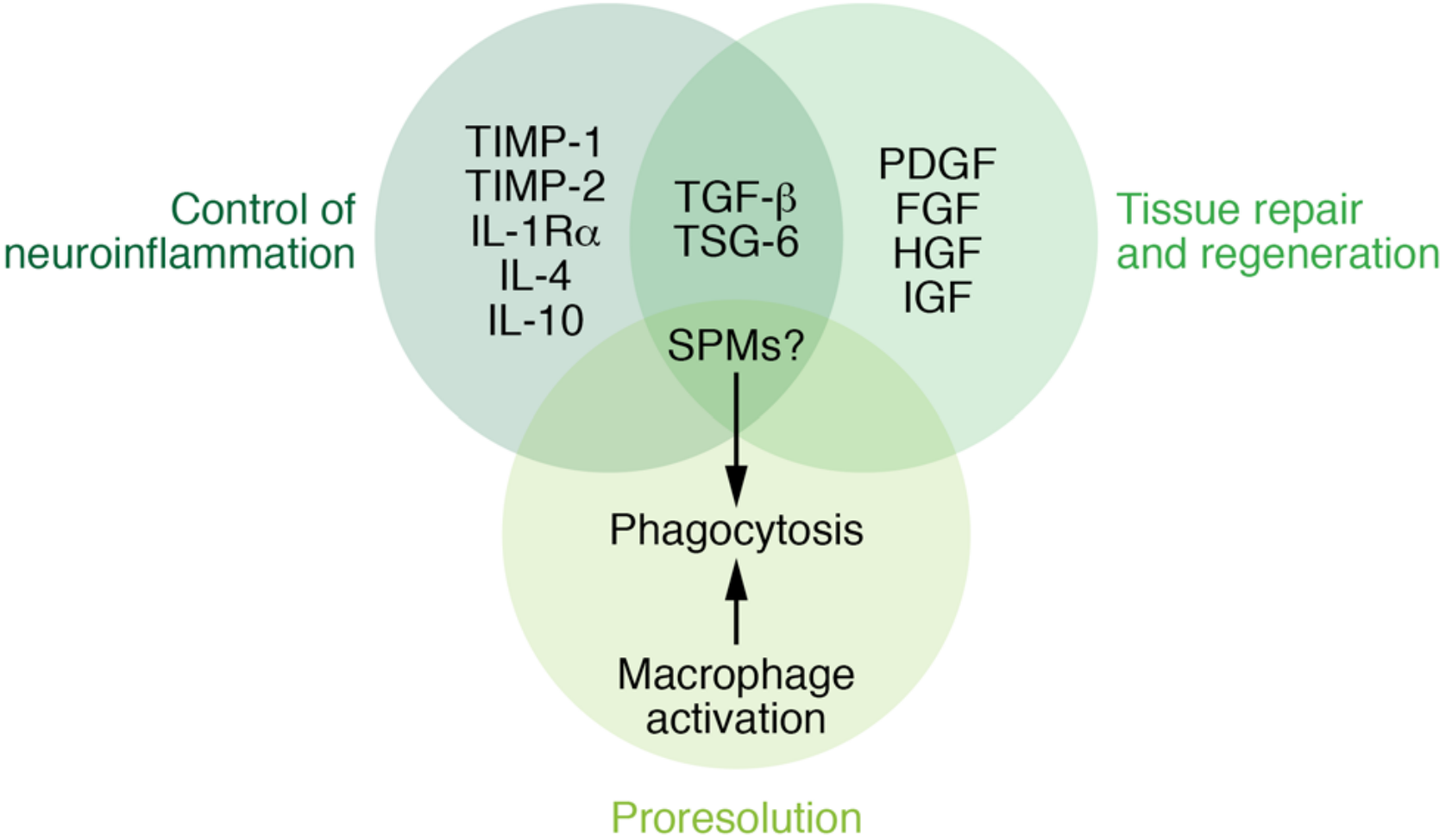

A
MSC preparation
Isolation method
• Needle/biopsy
• Enzymatic/mechanical dissociation
• Sorting
Culture/scale-up
• Media
• O2 levels
• Confluence
• Culture surface
• Passage number
• Cell surface modification
• Flasks vs. bioreactors
• Master cell/working cell banking approach
Cryopreservation
• DMSO conc.
• Media
Culture rescue
•Thawing media
•Thaw/culture protocol
MSC tissue of origin:
• Bone marrow
• Adipose tissue
• Umbilical cord
Donors
• Health status
• Genetics
• Gender
• Age
B
Administration
• Local/systemic
• Injection site
• Injection device properties (needle size/geometry)
• Injection/infusion buffer
• Cell carrier materials
C
Recipients
• Host cytotoxic responses
• Inflammation status
• Hypoxia
• Extracellular matrix (stiffness)
ECM
T cells
Macrophages
Infused MSCs
Disease stage/severity

Mechanisms of Regenerative Medicine

Buchheit T, Huh Y, Maixner W, **Cheng J**, Ji RR. Neuroimmune modulation of pain and Regenerative Pain Medicine. ***Journal of Clinical Investigation.*** 2020;130(5):2164-2176.

Cheng J, Huh Y, Ji RR. (2020). Mechanism-Based Treatment and Precision Medicine, ***Practical Management of Pain, 6th Edition***, (Benson et al. Eds), Mosby/Elsevier, Philadelphia, USA.


Blood-derived
α-Granule–derived
ACS
IL-1Rα
IL-4
TIMP-1
TIMP-2
PDGF
FGF
HGF
IGF
PRP
TIMP-1
TIMP-2
PDGF
HGF
Exosomes
TGF-β
IL-10
IGF
TGF-β
TSG-6
FGF
Cell-derived
Monocyte-derived

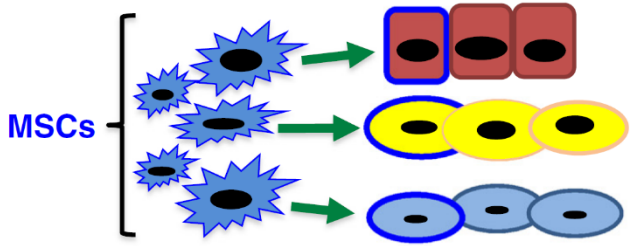

Control of neuroinflammation
TIMP-1
TIMP-2
IL-1Rα
IL-4
IL-10
TGF-β
TSG-6
PDGF
FGF
HGF
IGF
Tissue repair and regeneration
SPMs?
Phagocytosis
Macrophage activation
Proresolution

Mechanisms of stem cell therapy

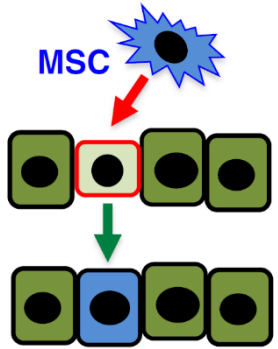
1. MSCs replace, rescue, and/or repair injured cells and tissues by diverse mechanisms
2. Paracrine function, immunomodulatory and antiinflammatory mechanisms

Spees JL, Lee RH, Gregory CA. Mechanisms of mesenchymal stem/stromal cell function. Stem Cell Res Ther. 2016;7:125.

a. Differentiation of MSCs to replace cells.



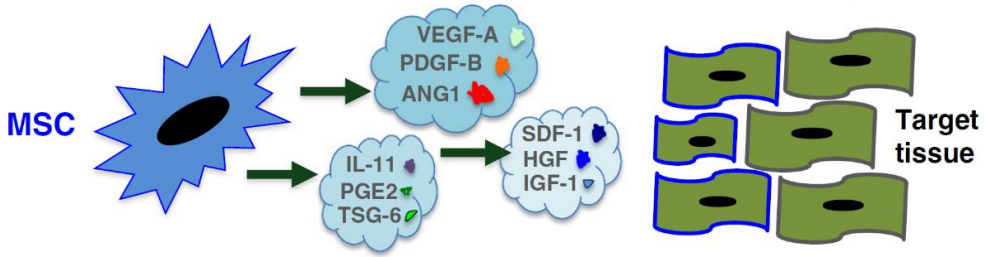
b. MSC/cell fusion.



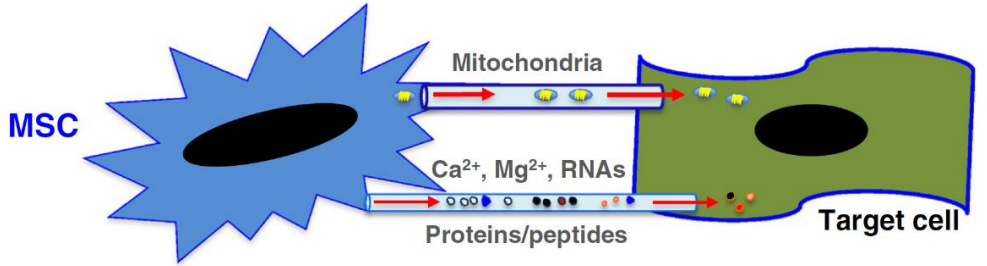
VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; ANG1, angiopoietin-1; IL-11, interleukin-11; PGE2, prostaglandin E2; TSG-6, TNF-stimulated gene-6; SDF-1, stromal-derived factor-1; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor-1; IDO, indoleamine 2,3-dioxygenase

Spees JL, Lee RH, Gregory CA.. Stem Cell Res Ther. 2016;7:125.

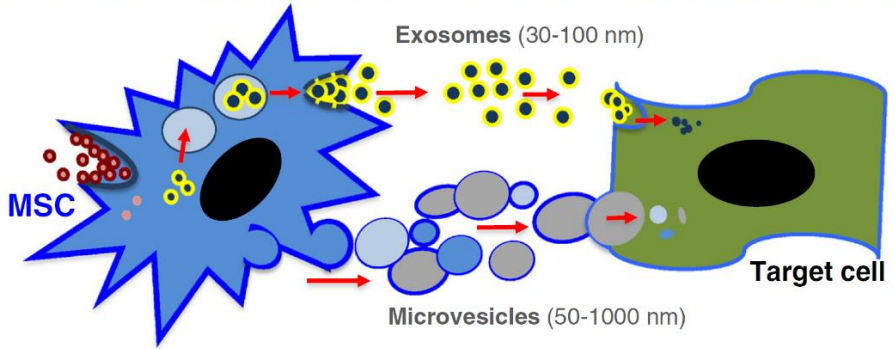
c. Paracrine activity of MSCs that promotes tissue rescue/repair.

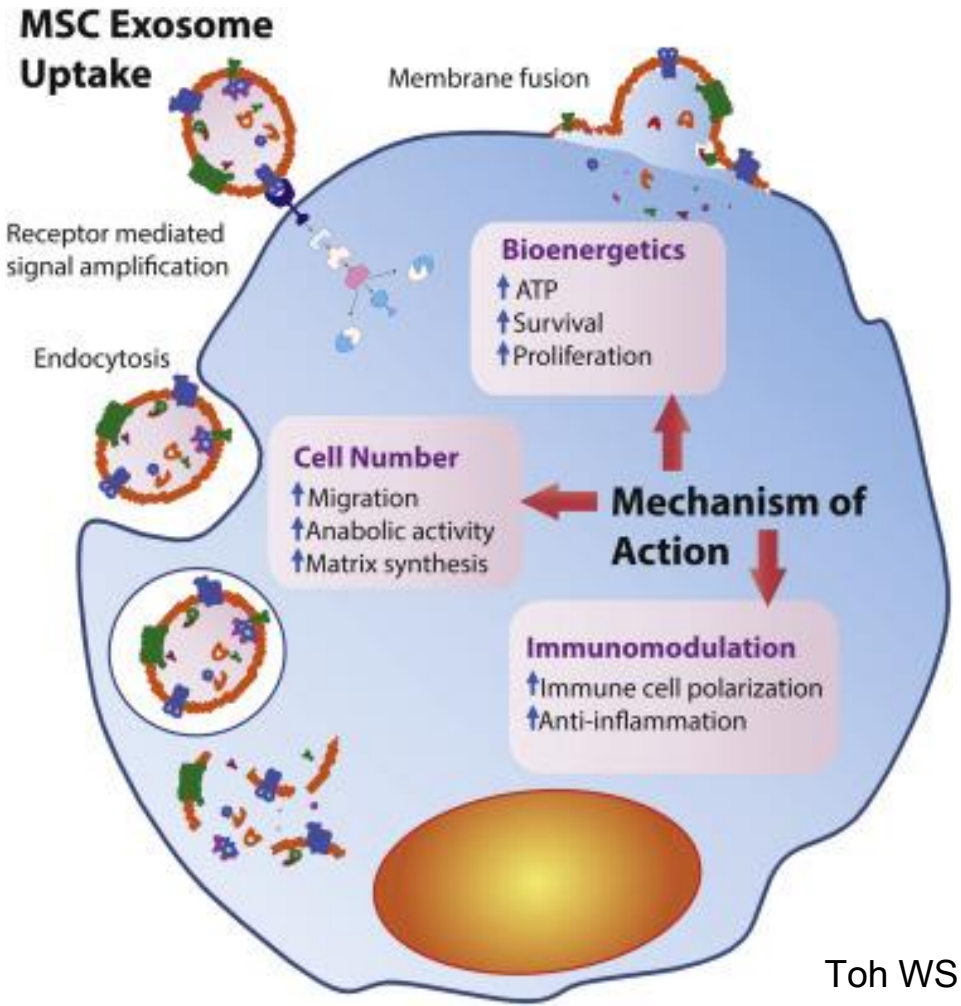


d. MSC-mediated transfer of organelles and/or molecules by TNTs.

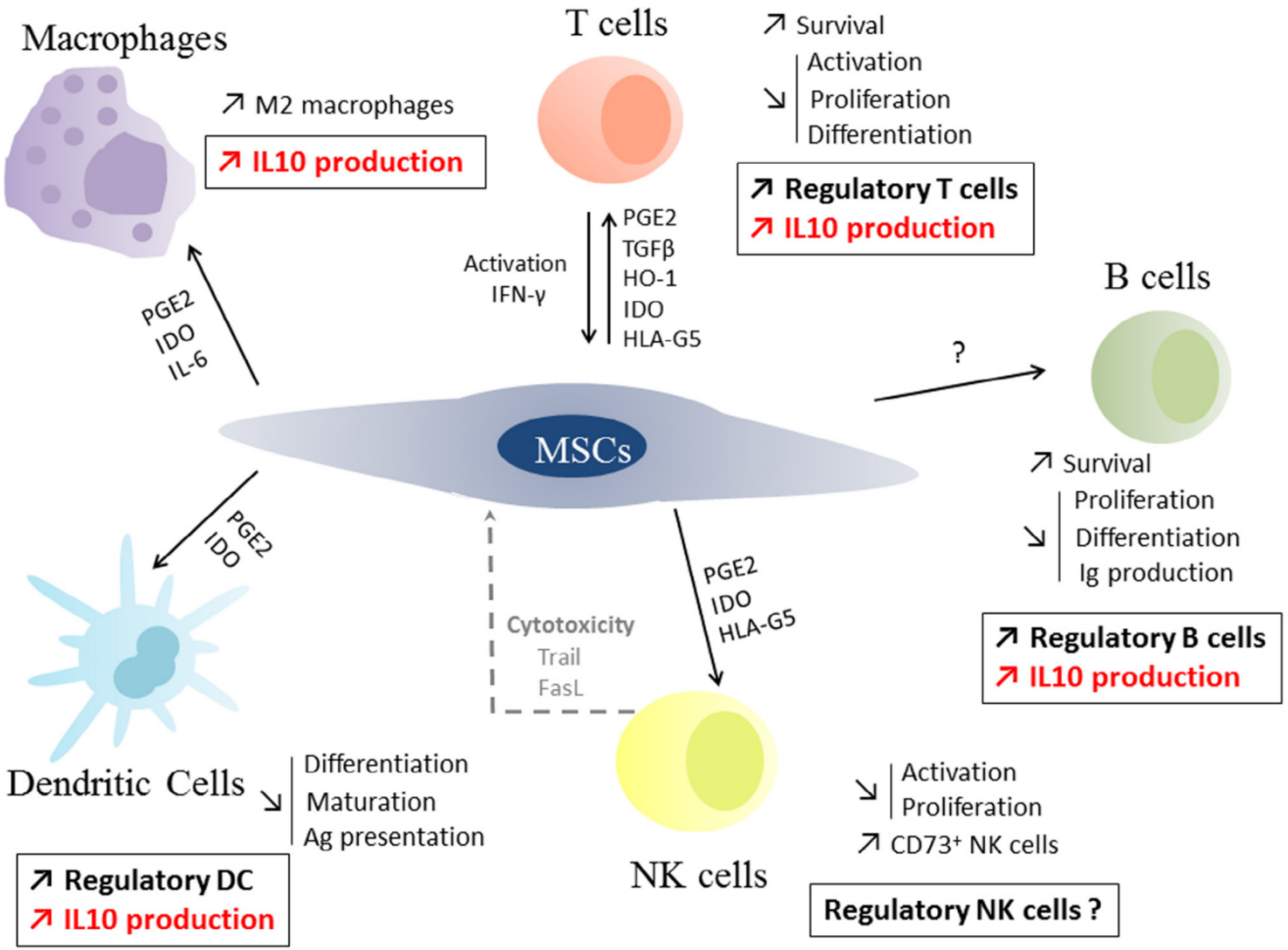


e. Transfer of molecules from MSC-derived exosomes or microvesicles.

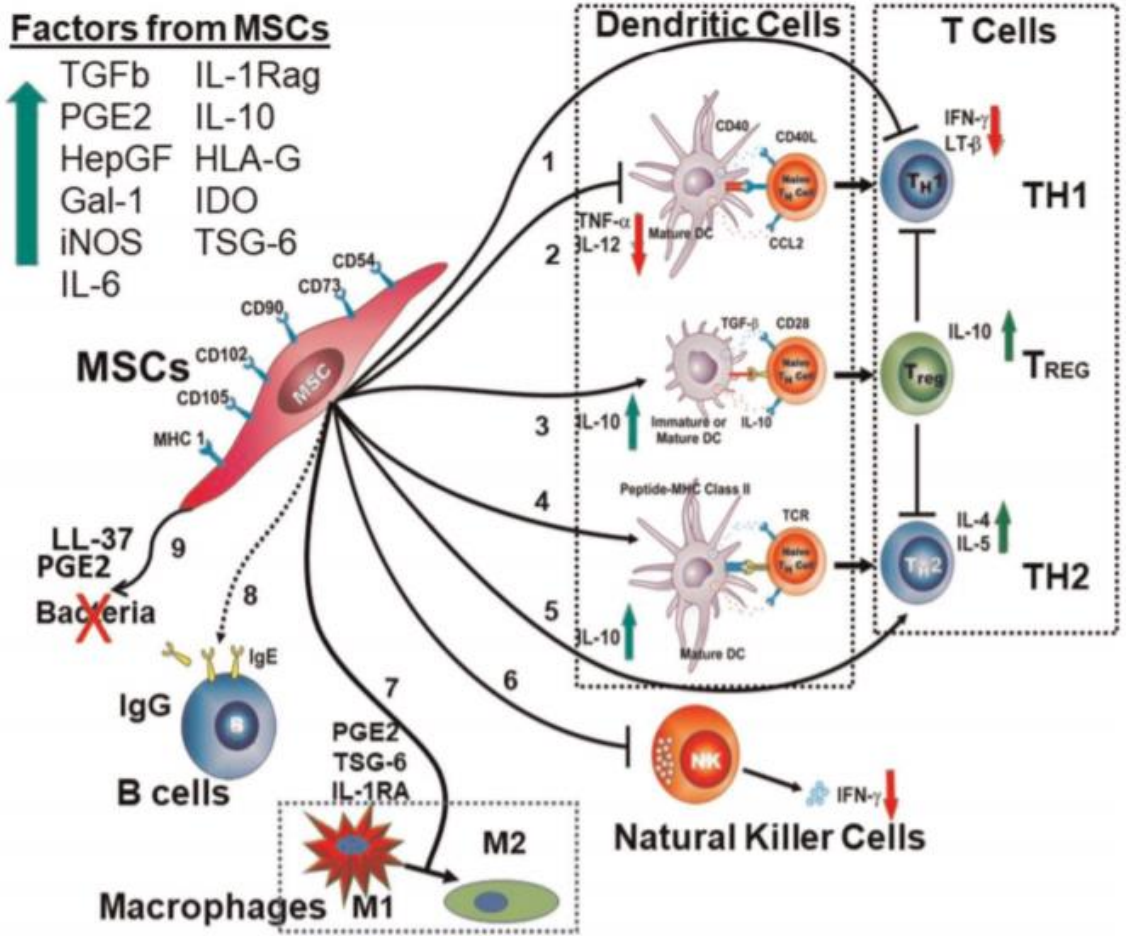




Toh WS, et al. Semin Cell Dev Biol. 2016



Najar M, et al. Cytotherapy. 2016;18:160-71.



Pittenger MF, Discher DE, Péault BM, Phinney DG, Hare JM, Caplan AI. Mesenchymal stem cell perspective: cell biology to clinical progress. NPJ Regen Med. 2019;4:22.

Preclinical Evidence for Stem Cell Therapy

- Degenerative joint pain
- Neuropathic pain
- Opioid tolerance

Buchheit T, Huh Y, Maixner W, **Cheng J**, Ji RR. Neuroimmune modulation of pain and Regenerative Pain Medicine. ***Journal of Clinical Investigation.*** 2020;130(5):2164-2176.

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RESEARCH ARTICLE

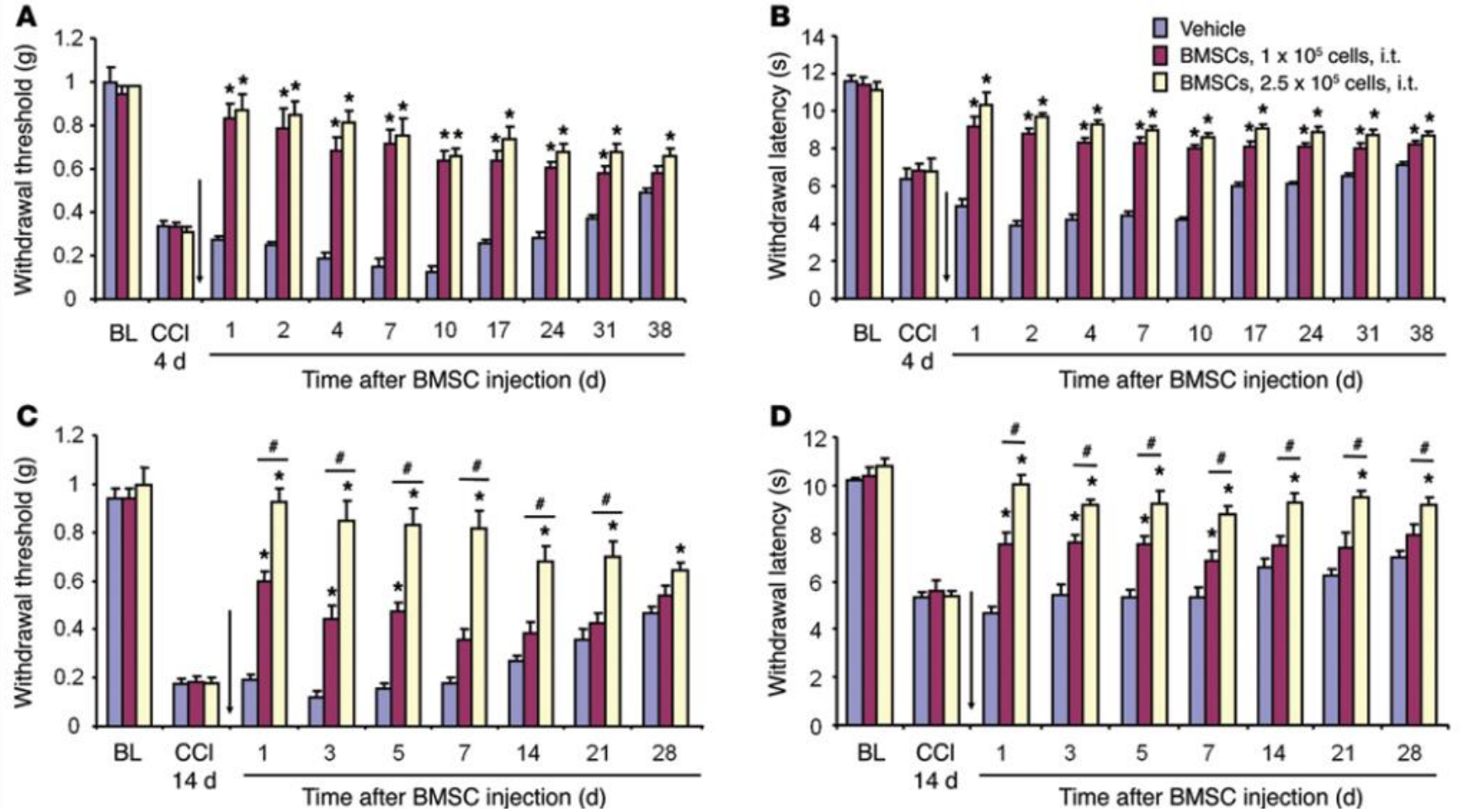
The Journal of Clinical Investigation

Intrathecal bone marrow stromal cells inhibit neuropathic pain via TGF-β secretion

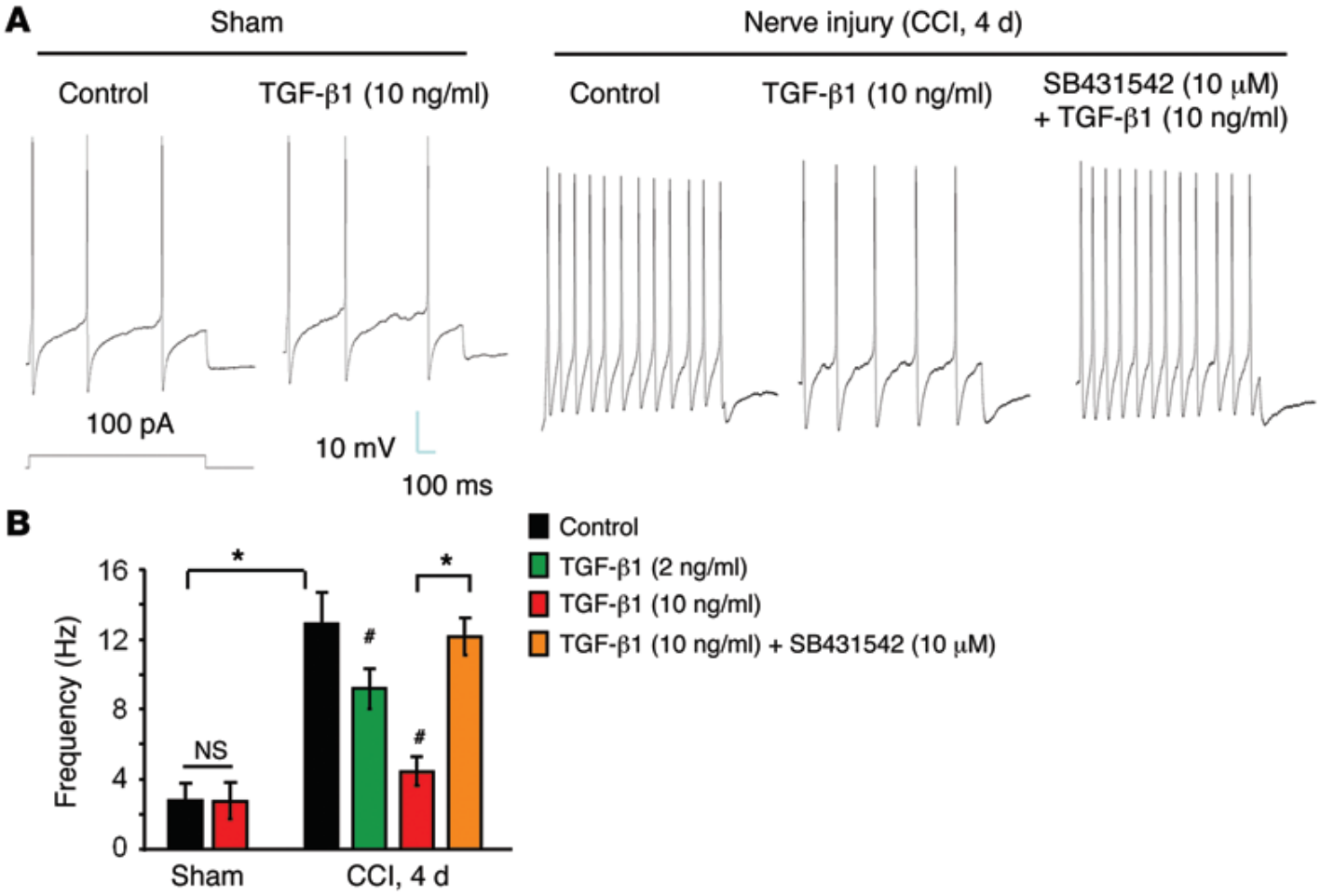
Gang Chen,[1,2] Chul-Kyu Park,[1,3] Rou-Gang Xie,[1,4,5] and Ru-Rong Ji[1,6]

[1]Department of Anesthesiology, Duke University Medical Center, Durham, North Carolina, USA. [2]Jiangsu Key Laboratory of Neuroregeneration, Co-Innovation Center of Neuroregeneration, Nantong University, Nantong, China. [3]Department of Physiology, College of Medicine, Gachon University, Incheon, South Korea. [4]Department of Anesthesiology and Pain Management, Xijing Hospital, and [5]Institute of Neuroscience, Fourth Military Medical University, Xian, China. [6]Department of Neurobiology, Duke University Medical Center, Durham, North Carolina, USA.

Neuropathic pain remains a pressing clinical problem. Here, we demonstrate that a local, intrathecal (i.t.) injection of bone marrow stromal cells (BMSCs) following lumbar puncture alleviates early- and late-phase neuropathic pain symptoms, such as allodynia and hyperalgesia, for several weeks in murine chronic constriction injury (CCI) and spared nerve injury models. Moreover, i.t. BMSCs reduced CCI-induced spontaneous pain and axonal injury of dorsal root ganglion (DRG) neurons and inhibited CCI-evoked neuroinflammation in DRGs and spinal cord tissues. BMSCs secreted TGF-β1 into the cerebrospinal fluid, and neutralization of TGF-β1, but not IL-10, reversed the analgesic effect of BMSCs. Conversely, i.t. administration of TGF-β1 potently inhibited neuropathic pain. TGF-β1 acted as a powerful neuromodulator and rapidly (within minutes) suppressed CCI-evoked spinal synaptic plasticity and DRG neuronal hyperexcitability via TGF-β receptor 1–mediated noncanonical signaling. Finally, nerve injury upregulated CXCL12 in lumbar L4–L6 DRGs, and this upregulation caused migration of i.t.-injected BMSCs to DRGs through the CXCL12 receptor CXCR4, which was expressed on BMSCs. BMSCs that migrated from the injection site survived at the border of DRGs for more than 2 months. Our findings support a paracrine mechanism by which i.t. BMSCs target CXCL12-producing DRGs to elicit neuroprotection and sustained neuropathic pain relief via TGF-β1 secretion.



Chen G, Park CK, Xie RG, Ji RR. Intrathecal bone marrow stromal cells inhibit neuropathic pain via TGF-β secretion. J Clin Invest. 2015;125(8):3226-40.



Chen G, Park CK, Xie RG, Ji RR. Intrathecal bone marrow stromal cells inhibit neuropathic pain via TGF-β secretion. J Clin Invest. 2015;125(8):3226-40.

PLOS ONE

RESEARCH ARTICLE

Intravenous administration of human mesenchymal stem cells derived from adipose tissue and umbilical cord improves neuropathic pain via suppression of neuronal damage and anti-inflammatory actions in rats

Kanako Miyano[1]*, Minori Ikehata[2], Kaori Ohshima[1,3], Yuki Yoshida[4], Yasuhiro Nose[2], Sei-ichi Yoshihara[2], Katsuyuki Oki[2], Seiji Shiraishi[5], Miaki Uzu[6], Miki Nonaka[1], Yoshikazu Higami[4], Yasuhito Uezono[1]

1 Department of Pain Control Research, The Jikei University School of Medicine, Nishishimbashi, Minato-ku, Tokyo, Japan, **2** R&D Department, Biomimetics Sympathies Inc., Aomi, Koto-ku, Tokyo, Japan, **3** Pathology, Immunology and Microbiology, Graduate School of Medicine, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo, Japan, **4** Laboratory of Molecular Pathology and Metabolic Disease, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Yamazaki, Noda, Chiba, Japan, **5** Division of Cancer Pathophysiology, National Hospital Organization Kure Medical, Kure, Hiroshima, Japan, **6** Vitrigel Project, Institute of Agrobiological Sciences, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan

* k.miyano@jikei.ac.jp

Citation: Miyano K, Ikehata M, Ohshima K, Yoshida Y, Nose Y, Yoshihara S-i, et al. (2022) Intravenous administration of human mesenchymal stem cells derived from adipose tissue and umbilical cord improves neuropathic pain via suppression of neuronal damage and anti-inflammatory actions in rats. PLoS ONE 17(2): e0262892. https://doi.org/10.1371/journal.pone.0262892

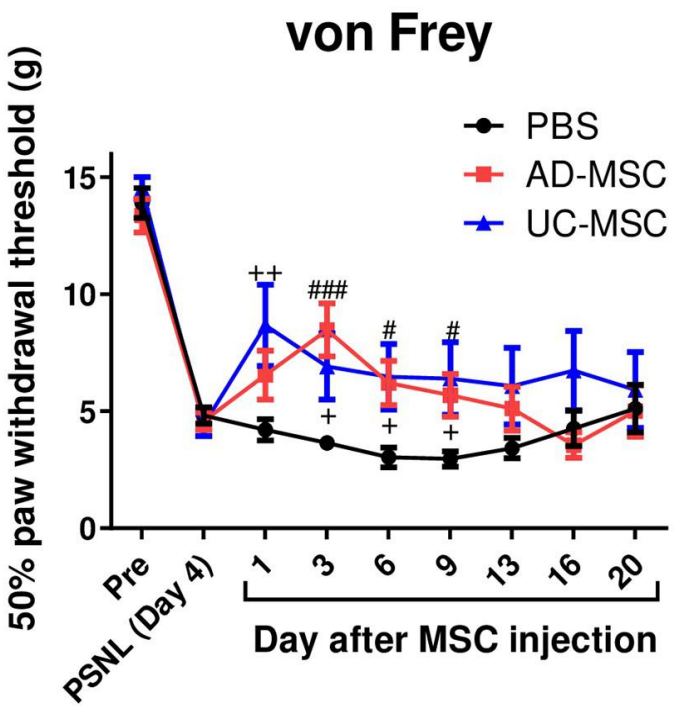
Editor: Masabumi Minami, Hokkaido Daigaku, JAPAN

Abstract

Mesenchymal stem cells (MSCs), which are isolated from adipose tissue (AD-MSCs), umbilical cord (UC-MSCs), or bone marrow, have therapeutic potential including anti-inflammatory and immunomodulatory activities. It was recently reported that MSCs are also effective as a therapeutic treatment for neuropathic pain, although the underlying mechanisms

Human AD- and UC-MSCs significantly improved mechanical pain threshold and weight distribution of the hind paws

A



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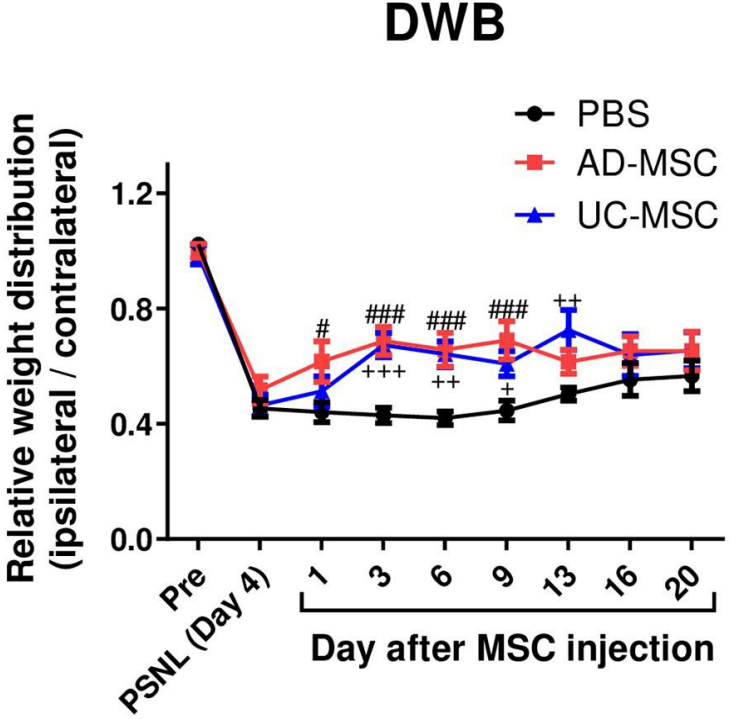
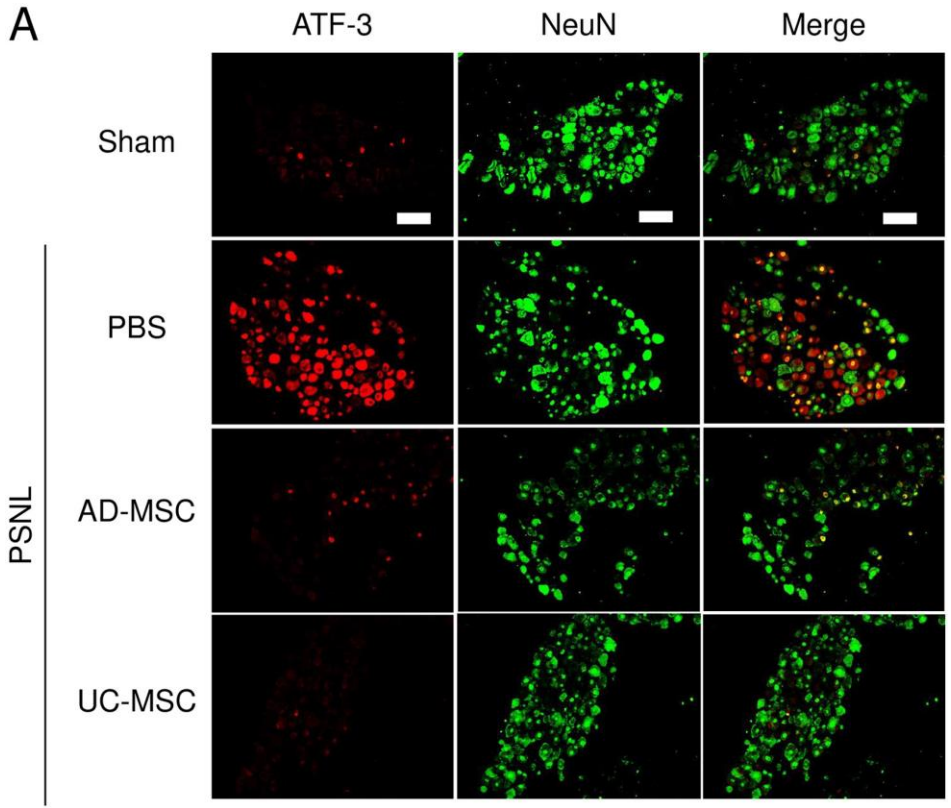
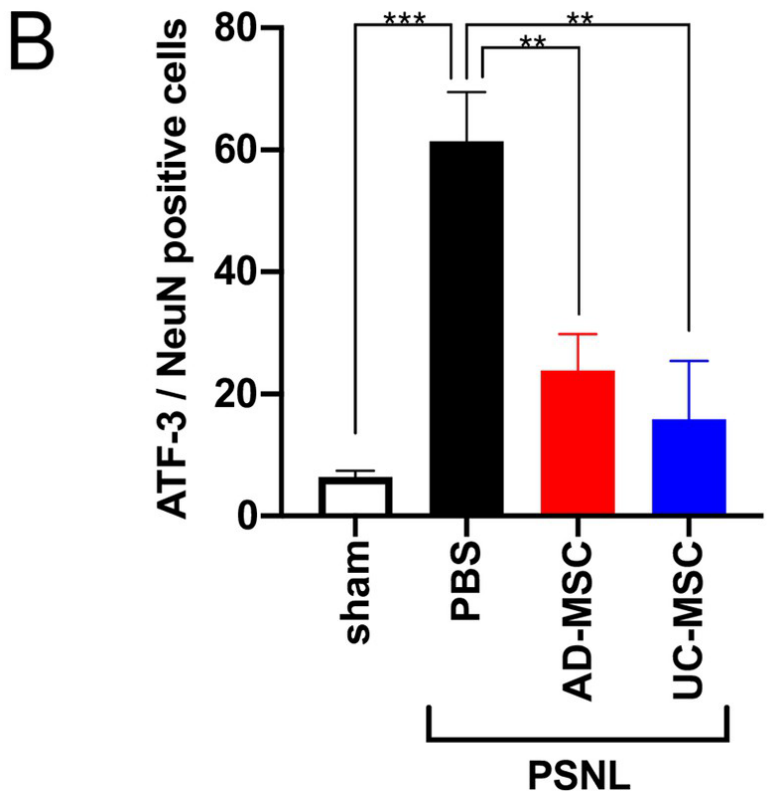
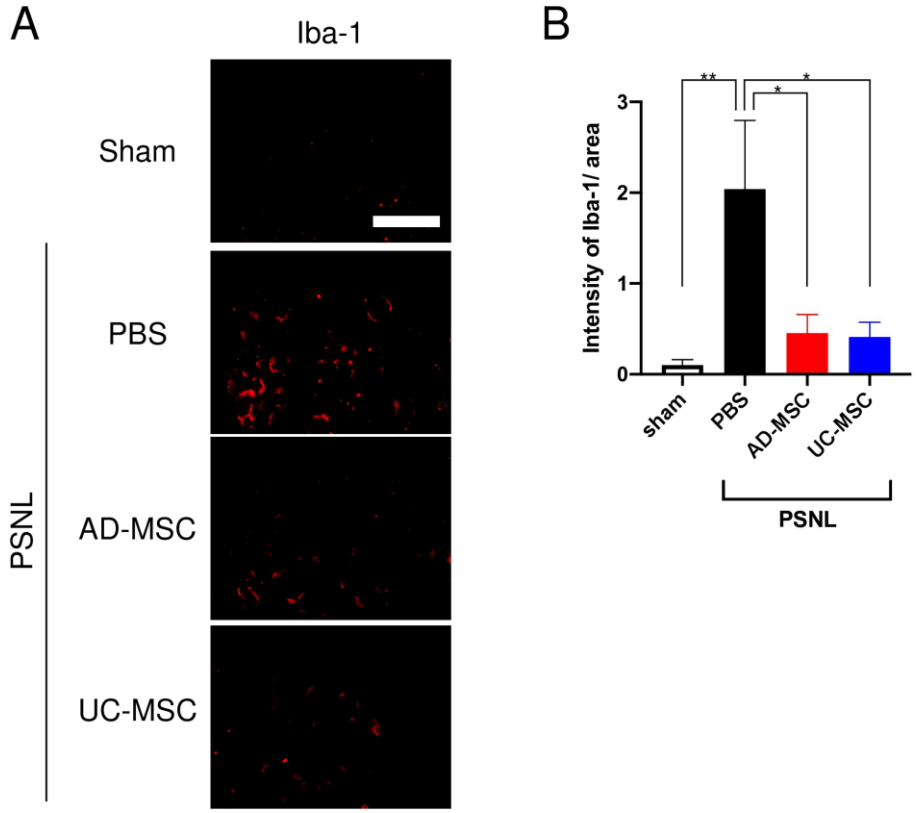


AD- and UC-MSCs significantly suppresses the increase in ATF-3-positive neurons induced by PSNL in the DRG

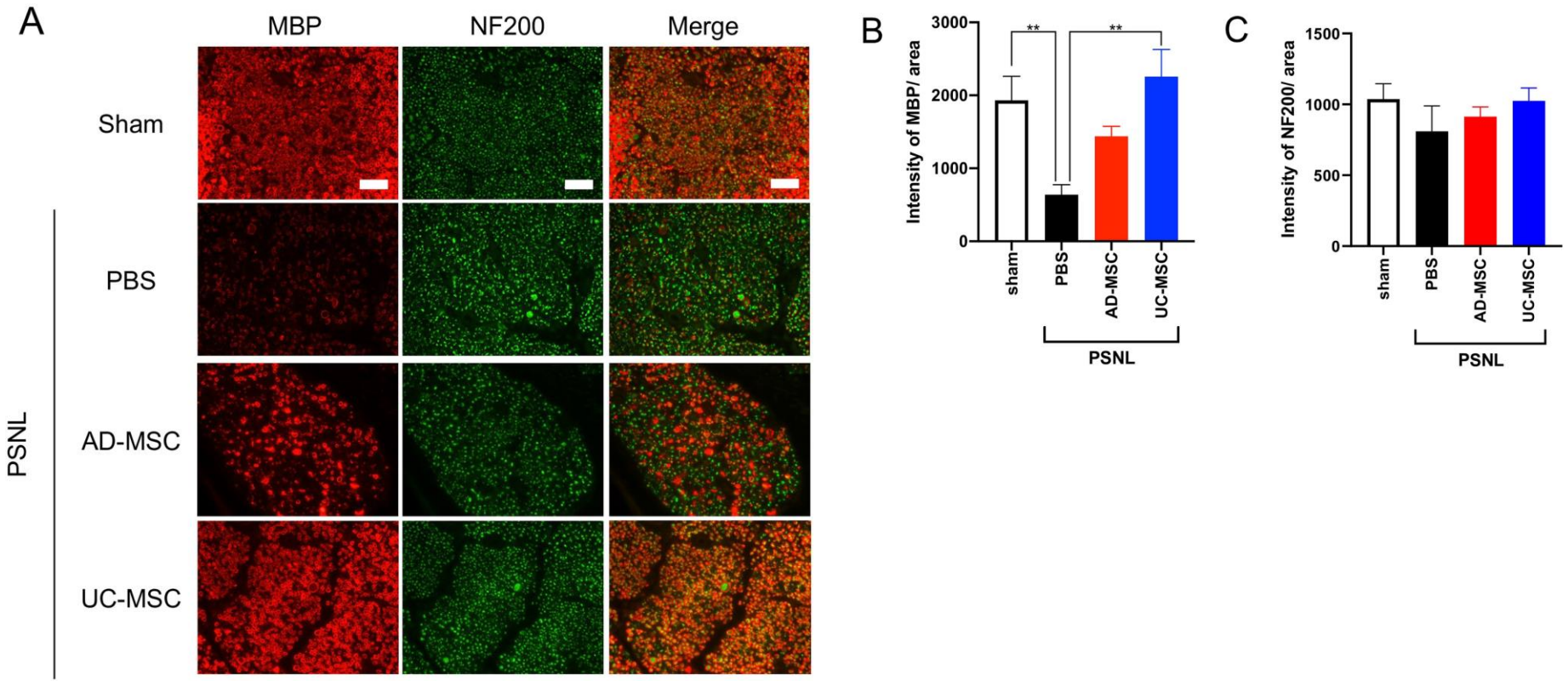




AD- and UC-MSCs significantly suppress the PSNL-induced accumulation of macrophages in the DRG



UC-MSCs improved demyelination of the sciatic nerves



Opioid Tolerance

- **Opioids** often lead to opioid tolerance (OT) and opioid-induced hyperalgesia (OIH).
- **OT**: the body adjusts to frequent opioid exposure and requires escalating doses to achieve the same effect.
- **OIH**: individuals taking opioids develop an increased sensitivity to noxious stimuli (hyperalgesia).
- **OT and OIH** limit the efficacy of opioid therapy, compromise safety, and often lead to drug overdose, abuse, and even death. Prescription Opioid overdose claimed >15,000 deaths in 2015 in the US (CDC).

OPEN

Mesenchymal Stem Cells Reversed Morphine Tolerance and Opioid-induced Hyperalgesia

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Zhen Hua[1,2,*], LiPing Liu[1,*], Jun Shen[1], Katherine Cheng[1], Aijun Liu[1], Jing Yang[1], Lina Wang[1], Tingyu Qu[3], HongNa Yang[3], Yan Li[3], Haiyan Wu[1], John Narouze[1], Yan Yin[1] & Jianguo Cheng[1]

More than 240 million opioid prescriptions are dispensed annually to treat pain in the US. The use of opioids is commonly associated with opioid tolerance (OT) and opioid-induced hyperalgesia (OIH), which limit efficacy and compromise safety. The dearth of effective way to prevent or treat OT and OIH is a major medical challenge. We hypothesized that mesenchymal stem cells (MSCs) attenuate OT and OIH in rats and mice based on the understanding that MSCs possess remarkable anti-inflammatory properties and that both OT and chronic pain are associated with neuroinflammation in the spinal

The Use of Stem Cell Therapy to Reverse Opioid Tolerance

Fei Li[1,2], LiPing Liu[2], Kathleen Cheng[2], Zhongbo Chen[1] and Jianguo Cheng[2]

Opioid tolerance (OT) and opioid-induced hyperalgesia (OIH) are major challenges in medicine. Here we report that transplantation of mesenchymal stem cells (MSCs) prevented and reversed OT and OIH in rats and mice. The preventive and therapeutic effects were long-lasting and consistent across different assessment schemes. Both intrathecal and intravenous transplantations were effective and safe. This emerging therapeutic strategy has thus shown promise to impact clinical practice and improve the efficacy and safety of opioid therapy.

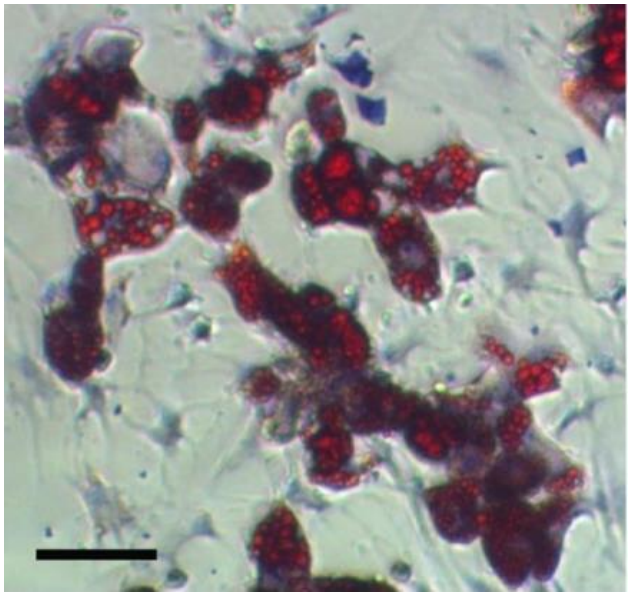
The use of opioids in pain management is often associated with chronic pain as a public health issue and the opioid epidemic as a national crisis.

MSC TRANSPLANTATION (MSC-TP) TO TREAT OT AND OIH

Neuroinflammation is closely associated with neuropathic pain, OT, and OIH. Discrete molecular mechanisms of neuroinflammation have been linked to the development of OT and OIH, which are two closely related but clinically different entities (**Figure 1**). Morphine can directly activate Toll-like receptor 4 on microglia and cause OT.[4] In contrast, OIH is mediated by μ-

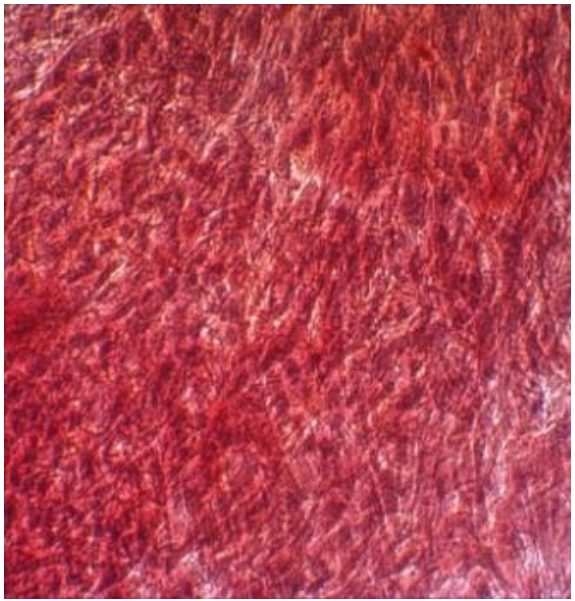
MSC isolation and characterization

Adipogenesis



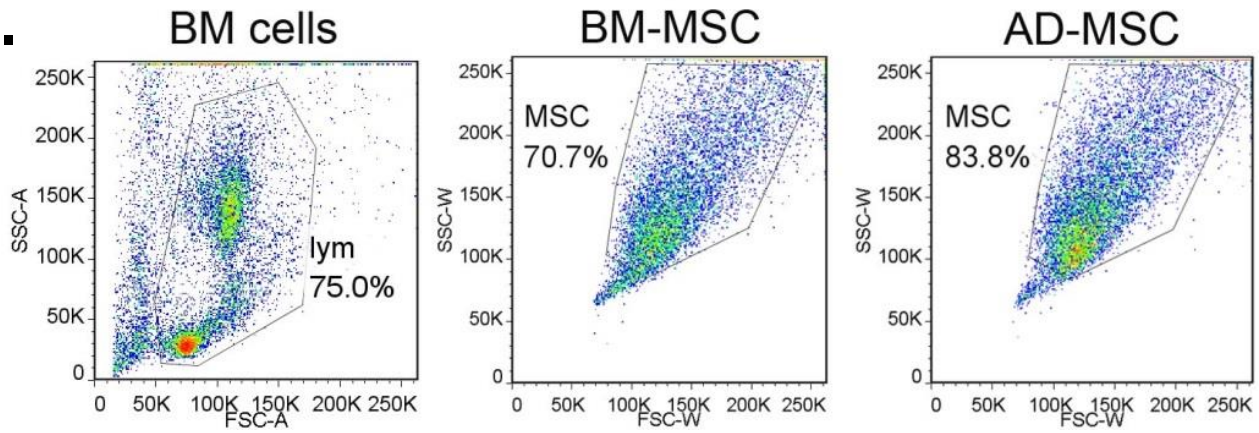
Oil red A

Osteogenesis

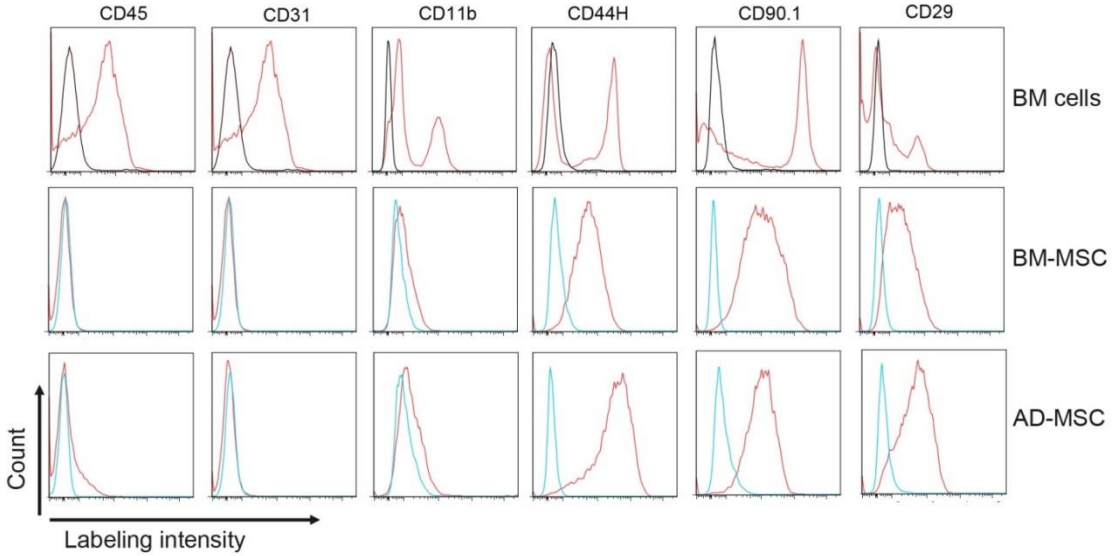


Alizarin red O

A.

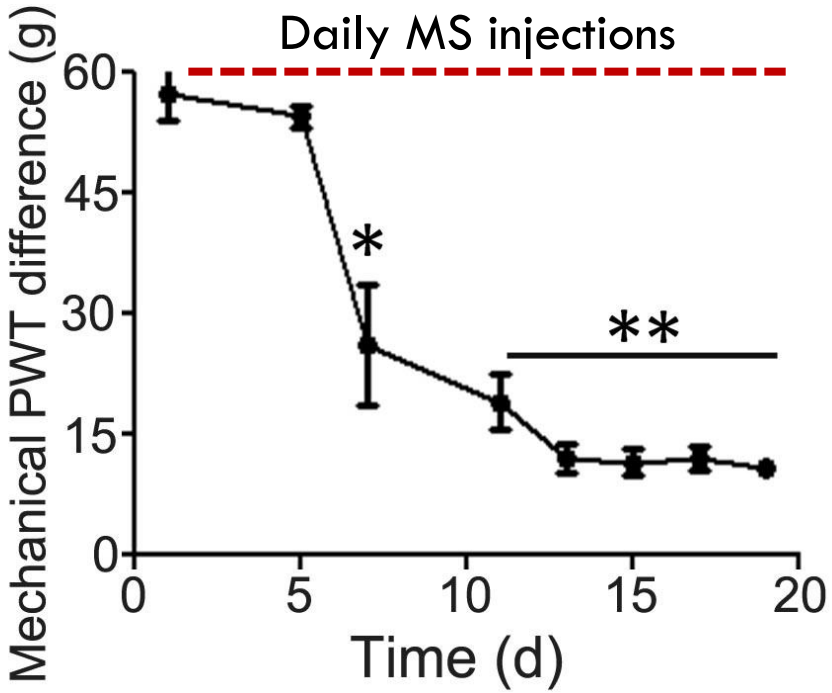

BM cells
BM-MSC
AD-MSC
SSC-A
SSC-W
FSC-A
FSC-W
250K
200K
150K
100K
50K
0
lym
75.0%
MSC
70.7%
MSC
83.8%

B.

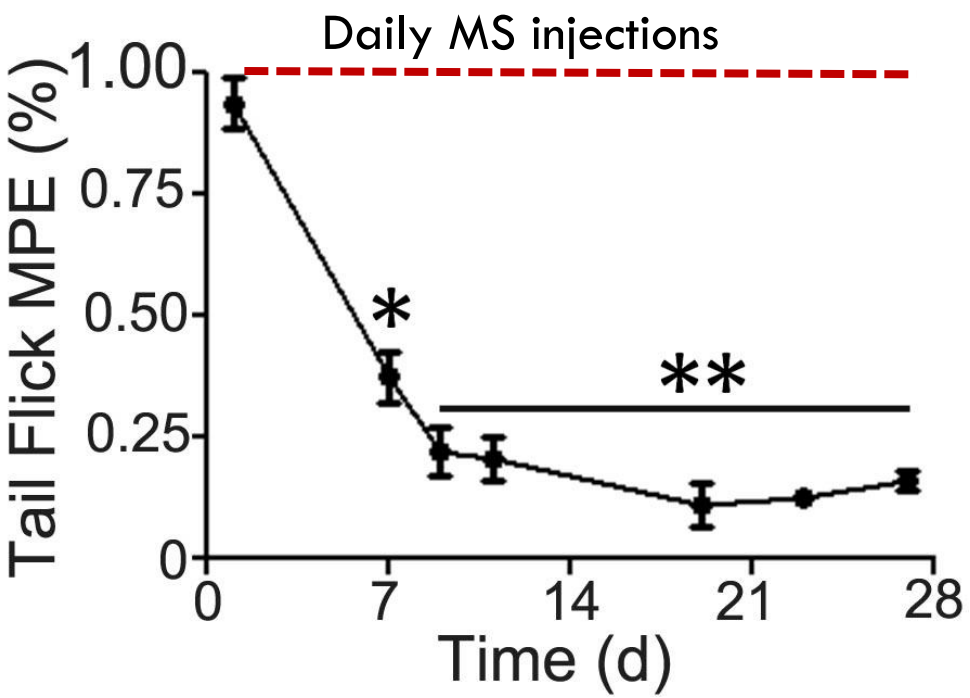

CD45
CD31
CD11b
CD44H
CD90.1
CD29
BM cells
BM-MSC
AD-MSC
Count
Labeling intensity

Development of OT

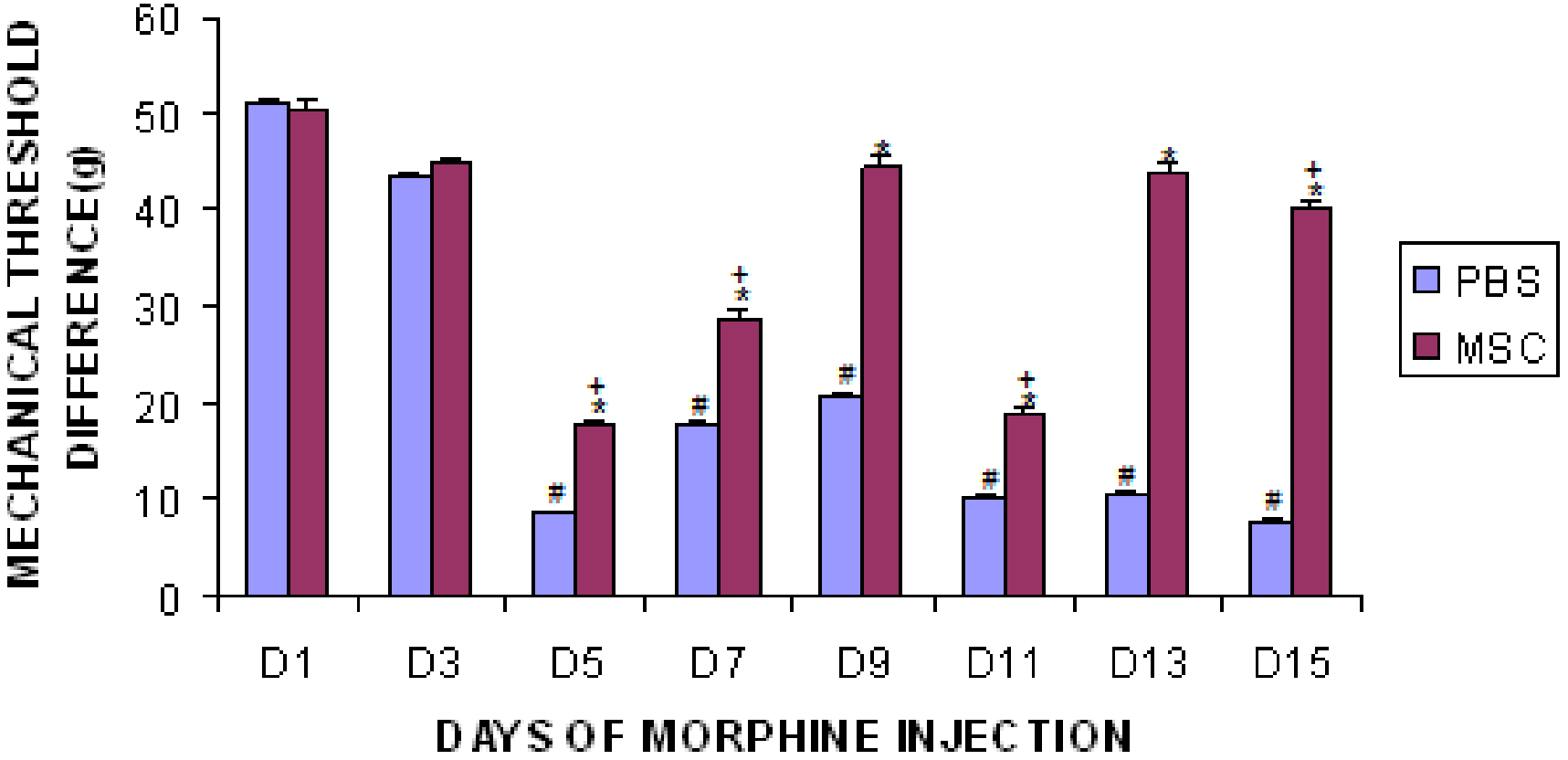
A.



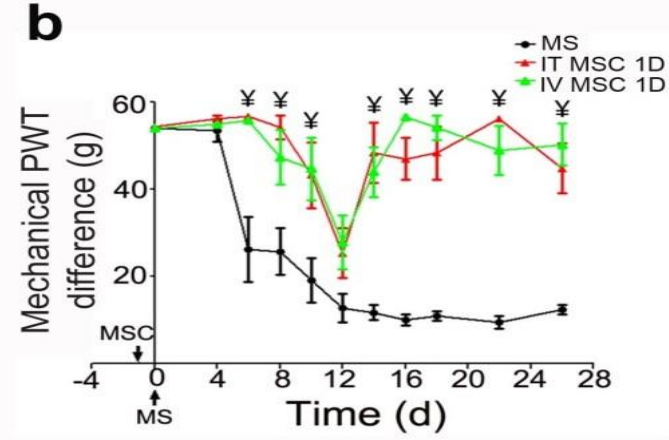
B.

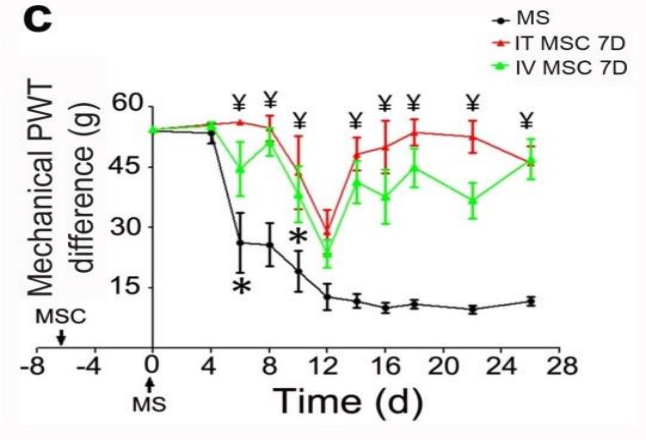


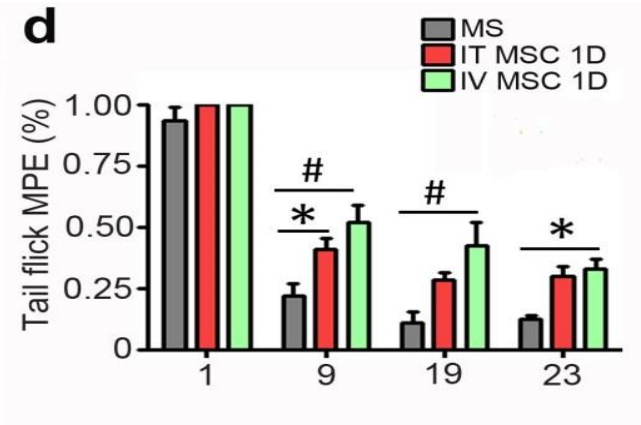
Opioid Tolerance

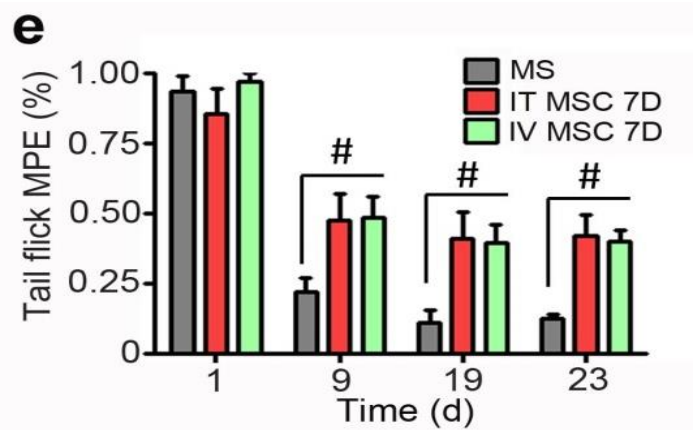


MSC-TP prevented OT

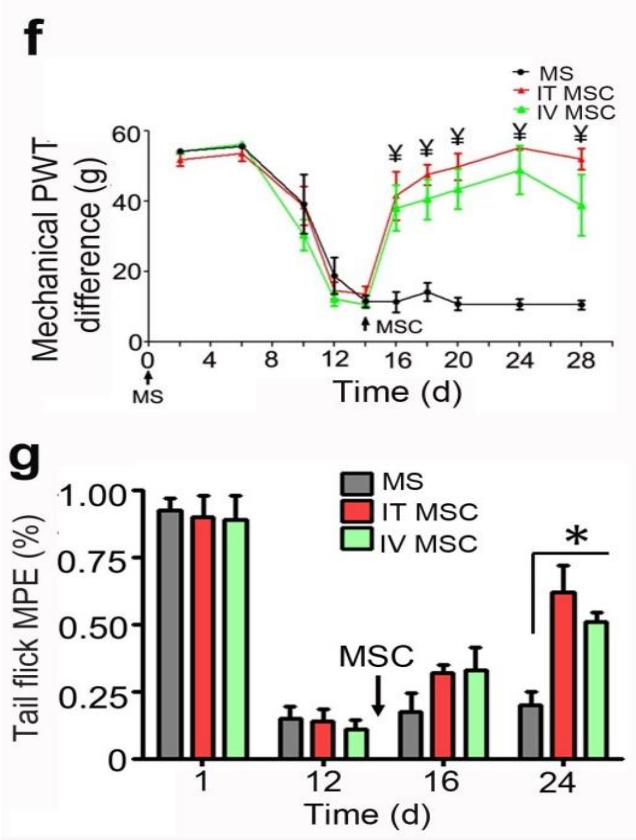


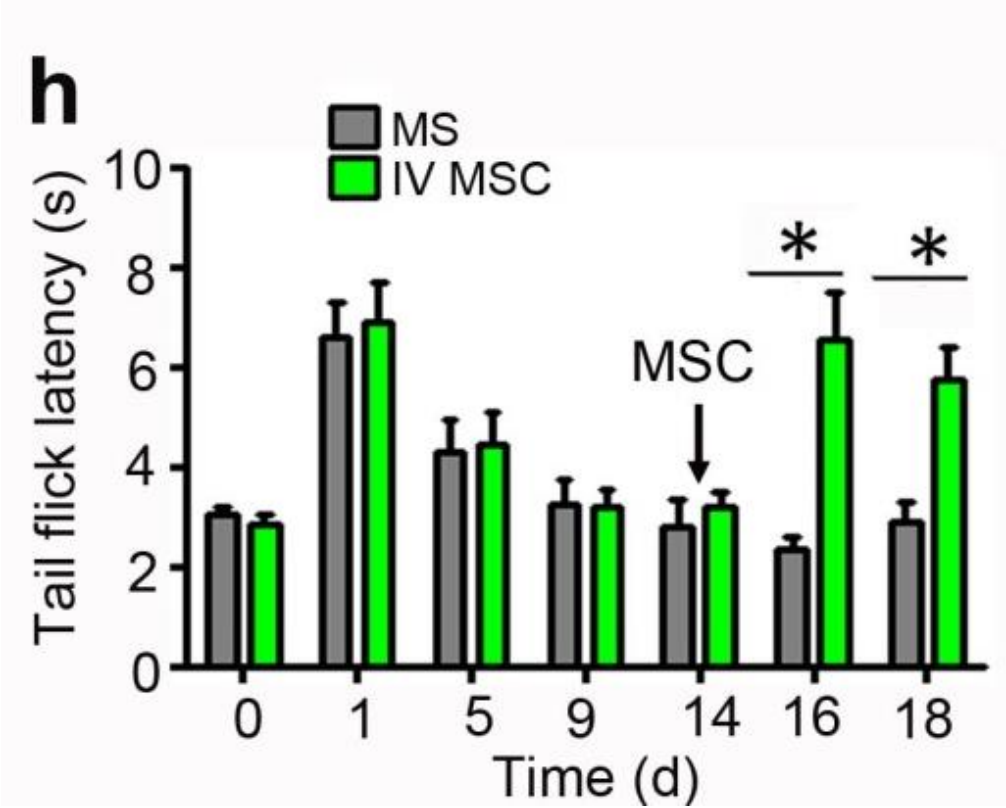




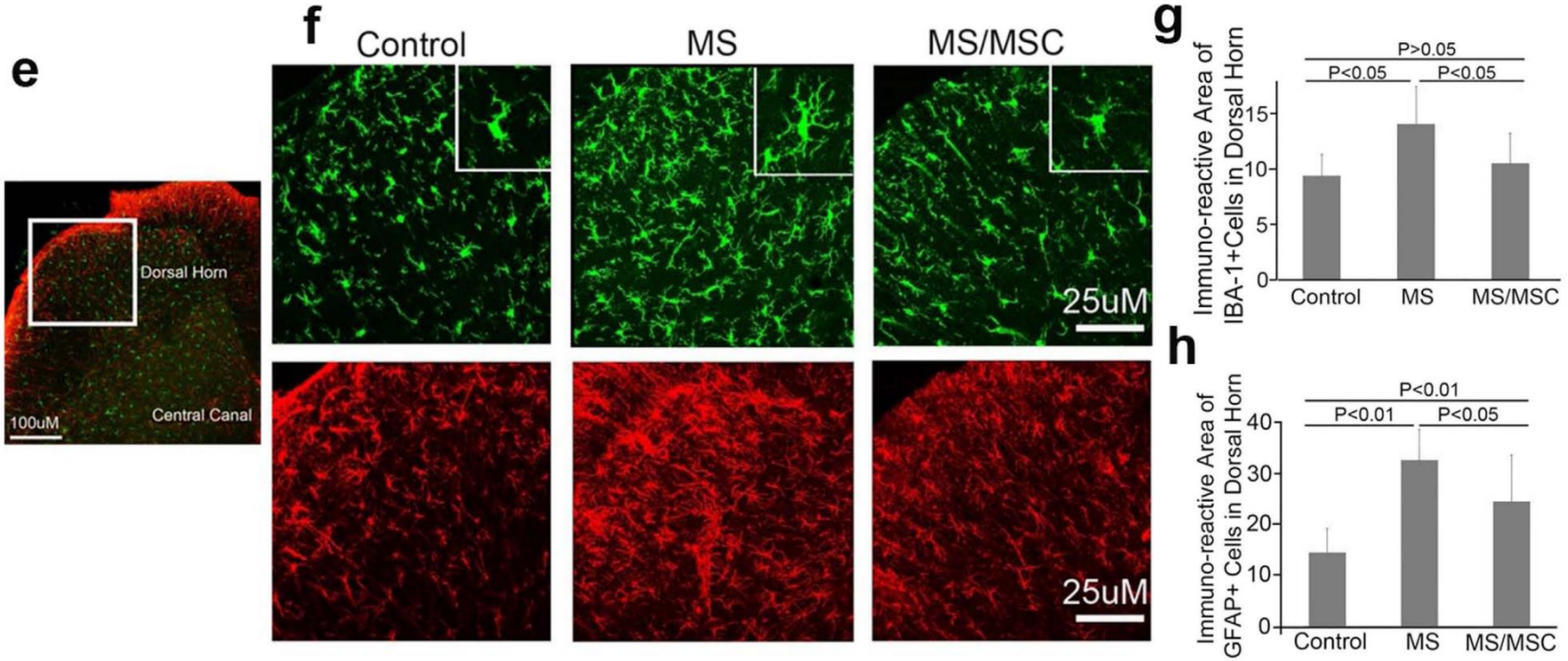


MSC-TP reversed OT

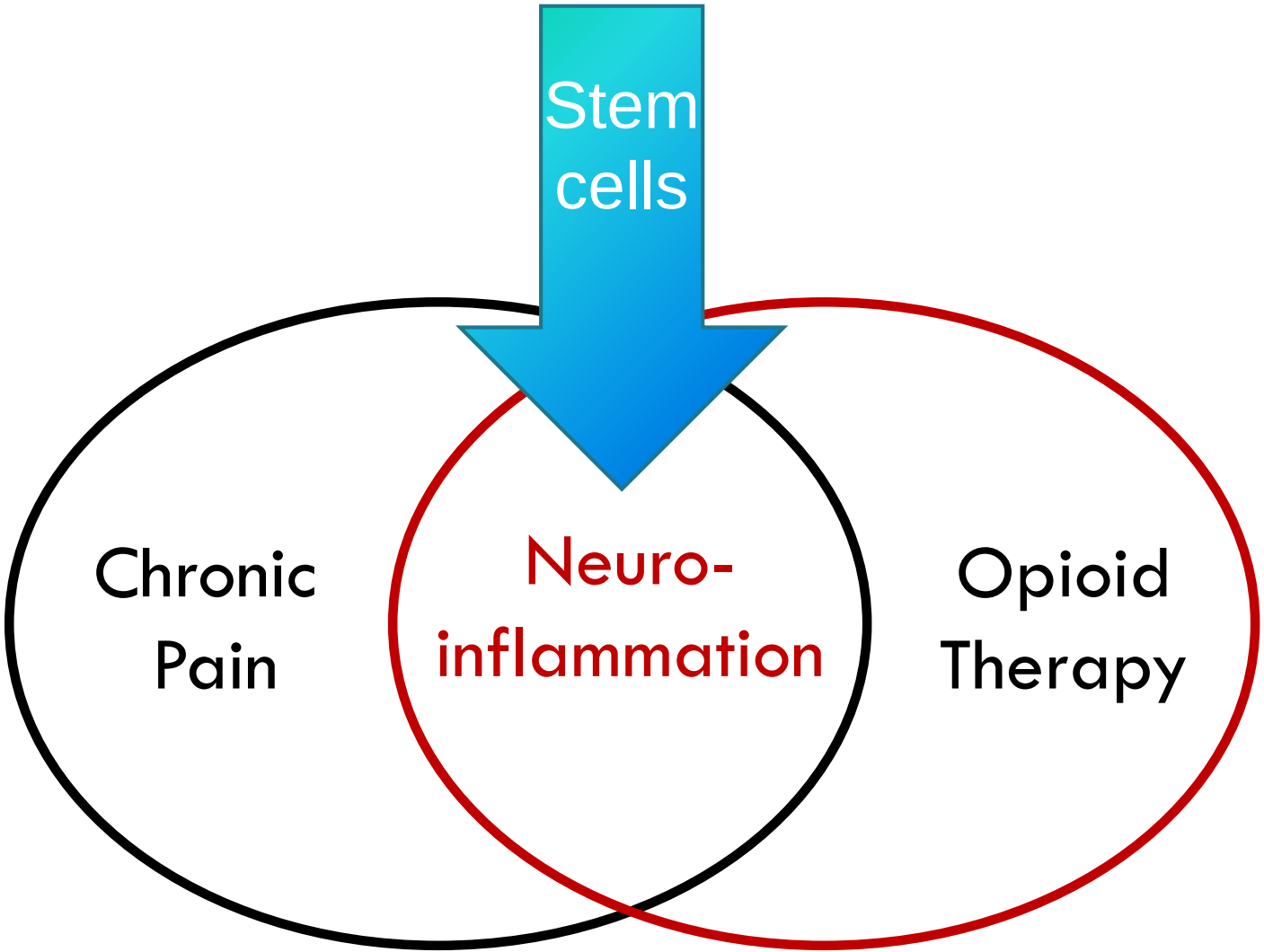




MSC-TP inhibited microglial and astrocyte activation



Iba1: Microglia GFAP: Astrocytes


Stem cells
Chronic Pain
Neuro-inflammation
Opioid Therapy

HEALTH AND MEDICINE

Shattering barriers toward clinically meaningful MSC therapies

Oren Levy[1]*, Rui Kuai[1,2]*, Erika M. J. Siren[1]*, Deepak Bhere[2,3], Yuka Milton[1], Nabeel Nissar[3], Michael De Biasio[1], Martina Heinelt[1], Brock Reeve[4], Reza Abdi[5], Meshael Alturki[6,7], Mohanad Fallatah[7], Abdulaziz Almalik[6,7], Ali H. Alhasan[6,7], Khalid Shah[2,3,4†], Jeffrey M. Karp[1,2,4,8†]

More than 1050 clinical trials are registered at FDA.gov that explore multipotent mesenchymal stromal cells (MSCs) for nearly every clinical application imaginable, including neurodegenerative and cardiac disorders, perianal fistulas, graft-versus-host disease, COVID-19, and cancer. Several companies have or are in the process of commercializing MSC-based therapies. However, most of the clinical-stage MSC therapies have been unable to meet primary efficacy end points. The innate therapeutic functions of MSCs administered to humans are not as robust as demonstrated in preclinical studies, and in general, the translation of cell-based therapy is impaired by a myriad of steps that introduce heterogeneity. In this review, we discuss the major clinical challenges with MSC therapies, the details of these challenges, and the potential bioengineering approaches that leverage the unique biology of MSCs to overcome the challenges and achieve more potent and versatile therapies.

THE LANDSCAPE OF MSC THERAPIES

Multipotent mesenchymal stromal cells (MSCs) have been extensively investigated as a cell therapy, showing promise in treating an array

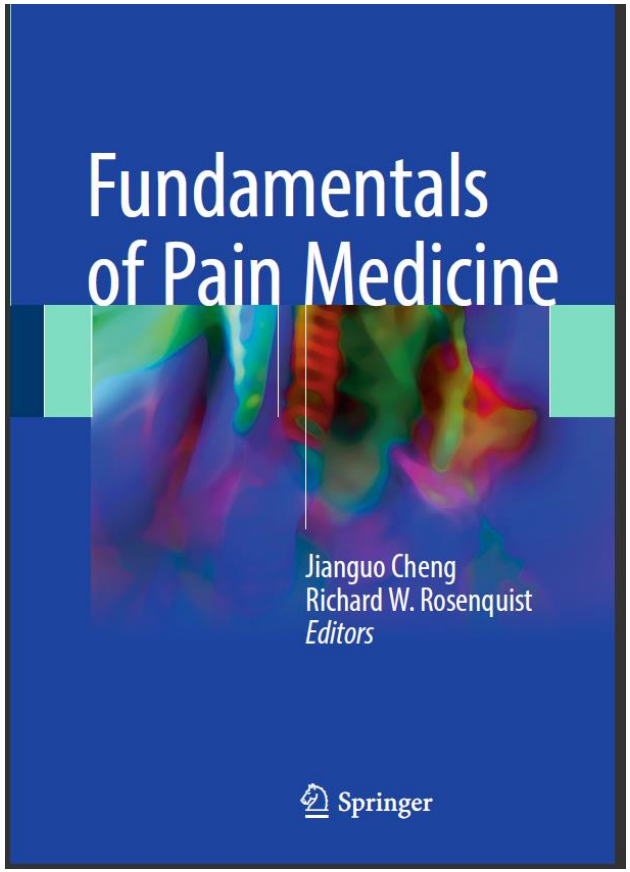
loaded submicrometer extracellular vesicles (EVs), and immune-mediated phagocytosis (*6*–*9*), which can lead to long-term effects. In line with this, many studies have shown that secreted biologics

Table 2. MSC products that have received regulatory approval.

Name	MSC type	Indication	Country of approval (year)	Company
Alofisel	Human AT-MSC	Complex perianal fistulas in CD	Europe (2018)	TiGenix NV/Takeda
Prochymal (remestemcel-L)	Human BM-MSC	GvHD	Canada (2012) New Zealand (2012)	Osiris Therapeutics Inc./ Mesoblast Ltd.
Temcell HS Inj	Human BM-MSC	GvHD	Japan (2015)	JCR Pharmaceuticals
Queencell	Human AT-MSC	Subcutaneous tissue defects	South Korea (2010)	Anterogen Co. Ltd.
Cupistem	Human AT-MSC	Crohn's fistula	South Korea (2012)	Anterogen Co. Ltd
Neuronata-R	Human BM-MSC	Amytrophic lateral sclerosis	South Korea (2014)	Corestem Inc.
Cartistem	Human UC-MSC	Knee articular cartilage defects	South Korea (2012)	Medipost Co. Ltd.
Stemirac	Human BM-MSC	Spinal cord injury	Japan (2018)	Nipro Corp.
Stempeucel	Human BM-MSC	Critical limb ischemia	India (2016)	Stempeutics Research PVT
Cellgram-AMI	Human BM-MSC	Acute MI	South Korea (2011)	Pharmicell Co. Ltd.

Summary

- Neuroimmune modulation is an active area of research that provides new insight into the mechanisms of pain and regenerative pain medicine.
- New treatments with biologics may reduce pain and modify disease process through neuroimmune modulation.
- Regenerative medicine has shown promise and evidence in pain management.
- MSC therapy is a current reality in many countries.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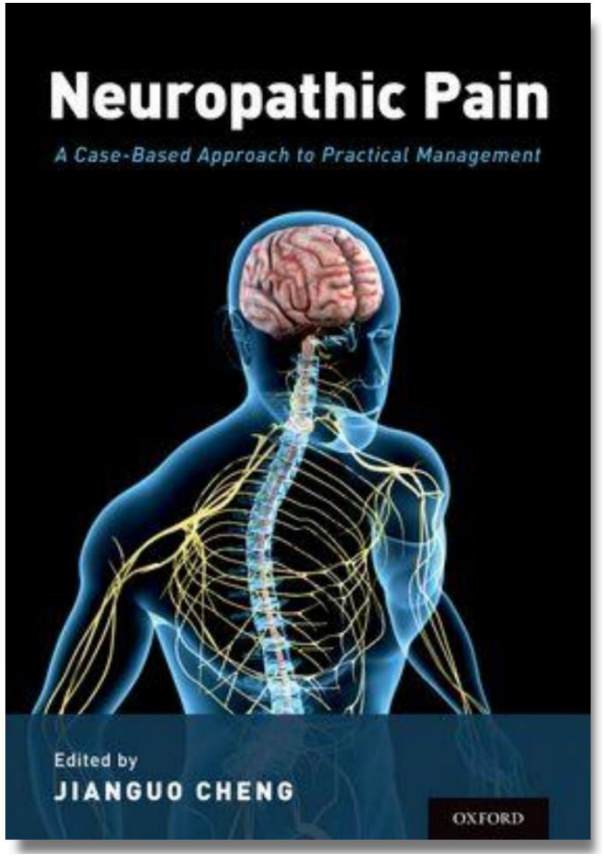
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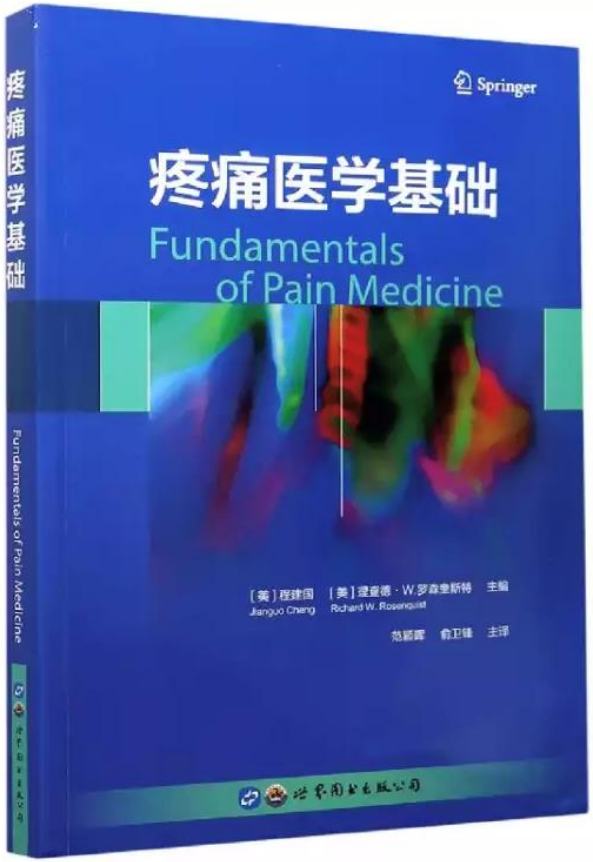
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