Assessment of macrophage presence and phenotypes at different time points in a rat model of disc-associated pain

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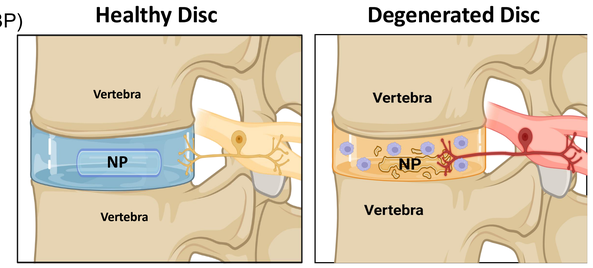
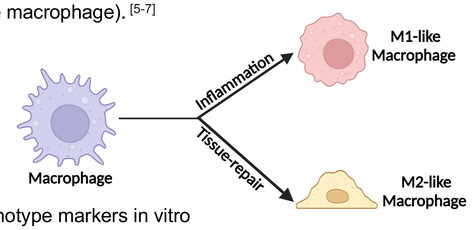
BACKGROUND

- Chronic low back pain (cLBP) is a leading contributor to disability worldwide with limited treatments.[1]
- Patients with disc-associated pain often have discs characterized by degeneration, inflammation, and abnormal innervation.[2]



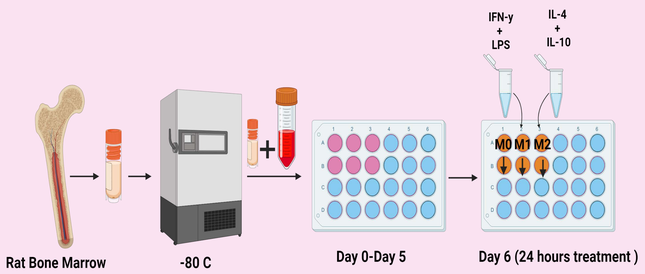
- Immune cells like macrophages infiltrate into the disc through vascularization of surrounding tissues, nerve pathways, disc herniation, and annulus tearing, resulting in disc inflammation.[3]
- Macrophages are a type of immune cell that can undergo functional polarization based on the microenvironment they encounter.[4]
- M1-like macrophages are pro-inflammatory and activated in response to injury[4]
- M2-like macrophages are anti-inflammatory and play a role in tissue repair and resolution of inflammation.[4]
- The most common markers used to determine the macrophage phenotype using immunofluorescence assays are CD11b and CD68 (General macrophage), CD86 (M1-like macrophage), and CD206 (M2-like macrophage). [5-7]



- **Aim of this work:**
  1. Validate macrophage phenotype markers in vitro
  2. Assess at what time points macrophages are present in our cLBP animal model
  3. What phenotype are macrophages expressing

METHODS

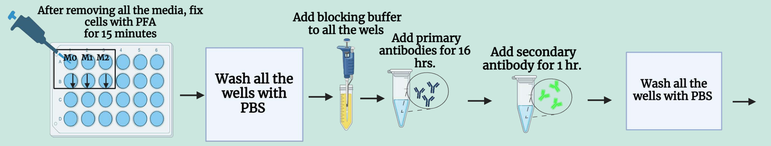
Macrophage Culture and Polarization



Medium Formula	Treatment
• DMEM-F12: 88% • Fetal Bovine Serum:10% • Penicillin Streptomycin: 1% • GlutaMax:1% • Rat-MCSF 50ng/mL	• **M1:** Interferon-γ (IFN- γ) + Lipopolysaccharide (LPS): 24 hrs. • **M2:** Interleukin 4 (IL-4) + Interleukin 10 (IL-10): 24 hrs.

METHODS

Immunohistochemistry



Phenotype	Primary	Secondary
M0	CD11b & CD68	Alexa Fluor 647 & Alexa Fluor 555
M1	CD86	Alexa Fluor 488
M2	CD206	Alexa Fluor 555

RESULTS

Macrophage Polarization

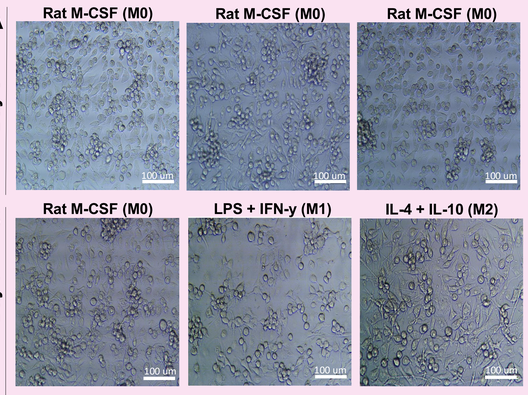


Figure 1. Morphology of rat primary macrophages. (A) Primary bone marrow cells were cultured for 6 days and induced into macrophage cells using Rat M-CSF at 50 ng/mL. Untreated primary M0-like macrophages exhibit an oval shape. **(B)** M1-like macrophages that were treated with IFN-γ and LPS for 24 hrs. are large and round compared to M0-like cells. M2-like macrophages treated for 24 hours with IL-4 and IL-10 are smaller and longer in shape compared to M1-like cells. Images taken with 10X magnification.

Immunohistochemistry

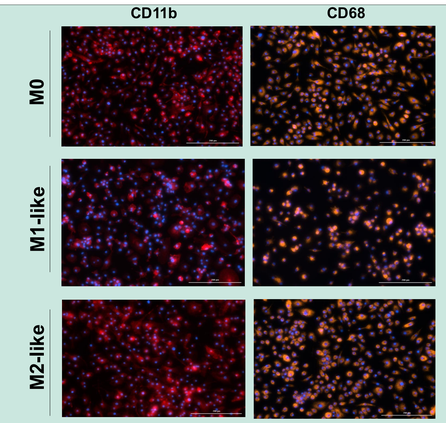


Figure 2. Immunocytochemistry results of general macrophages. The first row of the fixed cells in a 24-well plate was stained with CD11b and CD68 to validate general macrophage markers. CD11b expression was observed in all macrophage phenotypes, with a higher expression in M0 and M2-like macrophages. Similarly, CD68 was expressed across all macrophage phenotypes, showing elevated levels in M0 and M2-like macrophages.

RESULTS

Immunohistochemistry

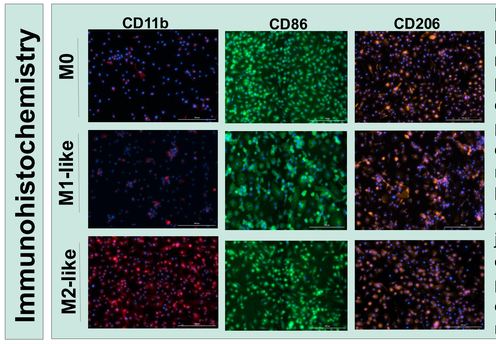


Figure 3. Immunocytochemistry results of macrophage phenotypes. CD11b was expressed in all macrophage phenotypes, with higher expression in M2-like macrophages. CD86 appears to be cell fluorescence and not true staining when compared to just fixed cells. CD206 was expressed in all macrophage phenotypes, showing higher expression in M2-like macrophages.

DISCUSSION AND FUTURE WORK

Discussion:

- CD11b and CD68 are known to be general macrophage cells markers
- CD11b and CD68 were present in M0, M1-like, and M2-like macrophages (**Fig.2**) making them good markers to be used to stain for general macrophages
- CD86 is an M1-like marker, it was expressed in all different macrophage phenotypes (**Fig.2**)
- CD206 is an M2-like marker, it was expressed in all different macrophage phenotypes (**Fig.2**)

Future Work:

- The final picked macrophage markers will be used on rats injured IVD tissues from different time points post-IVD injury
- The different time points are 4-,8-, and 10 weeks post IVD injury.

ACKNOWLEDGEMENTS

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MCNAIR SCHOLARS PROGRAM

REFERENCES

[1] James, et al, The Lancet, 2018
[2] Hadjipavlou, et al, The Journal of Bone and Joint Surgery, 2008
[3] Feng et al, 2017
[4] Chen et al, 2020
[5] Atsushi et al, 2021
[6] Chen et al, 2016
[7] Ayumu et al, 2020

Graphical illustrations: Biorender

QUESTIONS?

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