Inflammatory Challenge Alters Cytoskeletal Networks and Transiently Inhibits Meniscus Cell Migration

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Introduction

- The meniscus is commonly injured, and subject to surgical intervention to restore pain-free function
- Pro-inflammatory cytokines, IL1b and TNFa, are found at higher levels post-injury, promote catabolic meniscal remodeling, and inhibit tissue repair[1]
- Inflammatory factors inhibit the 2D migration of meniscus fibrochondrocytes (MFCs)[2]
- Cellular accumulation at the injury site is limited under inflammatory conditions, leading to a failure in integrative repair[3,4]
- Little is known, however, regarding the underlying mechanisms by which these factors prevent cell migration, or how long such deficits persist after inflammation is resolved
- **We evaluated the effects of IL1b and TNFa on MFC migration in 2D and 3D, under both acute inflammatory challenge and with time after their removal, and tested whether these factors impact cell spreading and adhesion, which may regulate their migratory capacity[5]**

Methods

Micro-wound assay: Bovine MFCs were cultured in basal media (BM) on tissue culture plastic (TCP) until confluent and maintained as controls or exposed to 0.1, 5, 10, or 50 ng/ml of either TNFa or IL1b. Cytokines were introduced at the same time that the monolayer was scratched with a 200 µL pipet tip (Fig. 1).

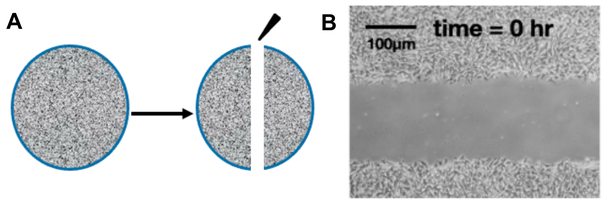


Figure 1: Micro-wound assay depiction (A) and (B) 10x image of micro-wound through MFCs on tissue culture plastic.

Micro-wound washout assay: MFCs were pre-treated with cytokines (0.1 and 10 ng/ml) for 24hr. At time zero, cells were cultured in fresh BM with no cytokines, and the scratch assay carried out on days 0, 1, 3, and 7 (with evaluation 8 hours post-scratch) after exposure to inflammation (Fig. 2).

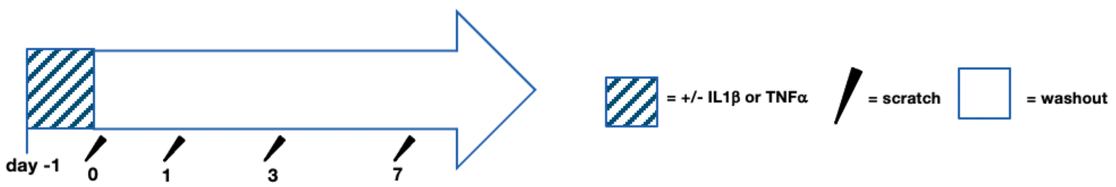


Figure 2: Micro-wound washout assay. MFCs exposed to inflammatory cytokines or control basal media for 24 hrs. Micro-wound made at time 0, or at day 1, 3, or 7 after inflammatory stimulus.

3D migration Assay: Living explants from juvenile menisci (5 mm diameter) were cultured in BM (n=3/group) for 3 weeks and labeled with CellTracker Green (Thermo). Explants were placed atop devitalized meniscal sections, which served as a tissue substrate onto which living cells could emerge and invade[7,8] (Fig. 3A). These samples were maintained in BM, IL1b, or TNFa (10ng/ml) for 48 hours. Samples were fixed, stained with DAPI, and confocal z-stacks (20x) were used to quantify cell egress onto and infiltration into the substrate (Fig 3B-C).

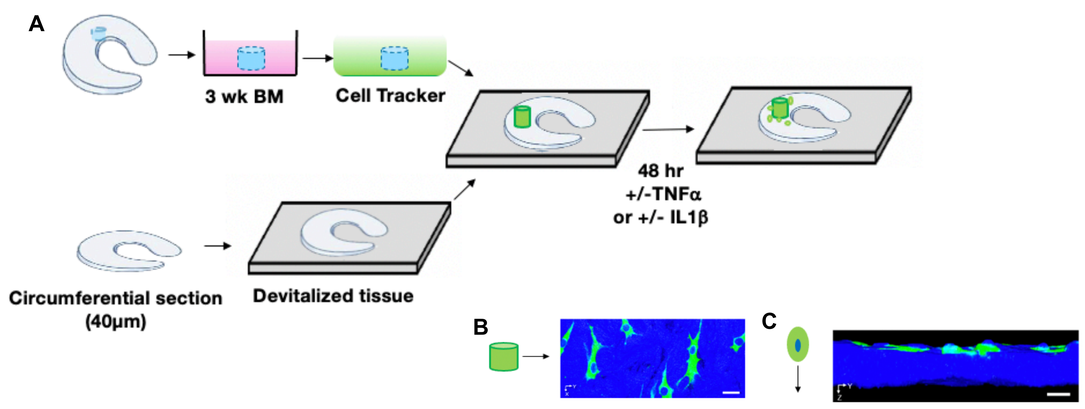


Figure 3: 3D migration assay. (A) study schematic depicting meniscus explants cultured and placed on devitalized meniscus tissue, (B) cells egressing onto the devitalized tissue, and (C) cells infiltrating into the devitalized meniscus tissue.

Focal Adhesion Staining & Analysis: Glass slides were seeded with MFCs and maintained as no cytokine controls or were exposed to 10 ng/ml TNFa or IL1b. After 24hr, cells were fixed and stained for paxillin to identify focal adhesions (FA), with a DAPI nuclear counterstain (Prolong Gold, Invitrogen). Confocal z-stacks were obtained at 60x (n=8/group), and FA number and area was measured[6].

Results

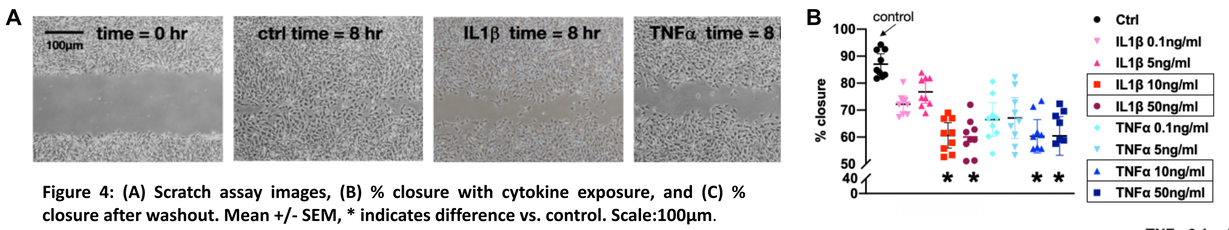
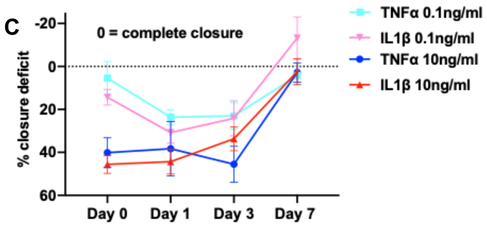


Figure 4: (A) Scratch assay images, (B) % closure with cytokine exposure, and (C) % closure after washout. Mean +/- SEM, * indicates difference vs. control. Scale:100µm.

2D migration: For both IL1b and TNFa, 10 and 50 ng/ml resulted in greater inhibition than 5 or 0.1 ng/ml (Fig. 4A-B). Cytokine exposure despite removal and washout on day 0, resulted in persistent attenuation of 2D migration through day 3. By day 7, MFCs had returned to control migration levels (Fig. 4C).



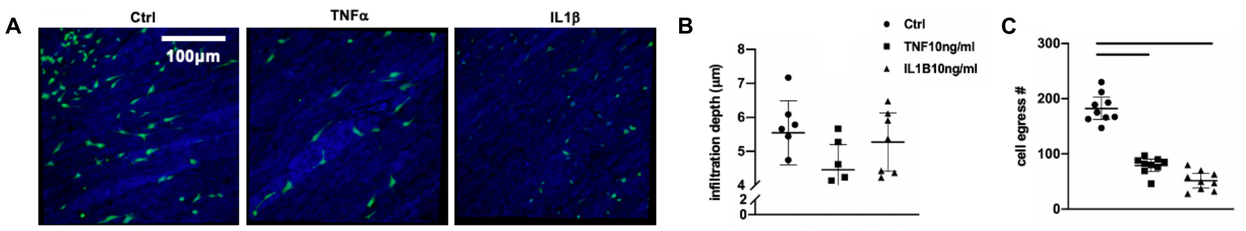


Figure 5: (A) representative tissue substrates 48 hrs after explant incubation with TNFa or IL1b, (B) infiltration depth, and (C) number of cells migrated from explant to tissue substrate. Mean +/- SEM, bars indicate differences. Scale: 60µm.

3D migration: TNFa or IL1b did not alter infiltration depth (Fig. 5B), but both factors reduced the number of cells that had migrated from the explant onto the substrate (Fig 5C).

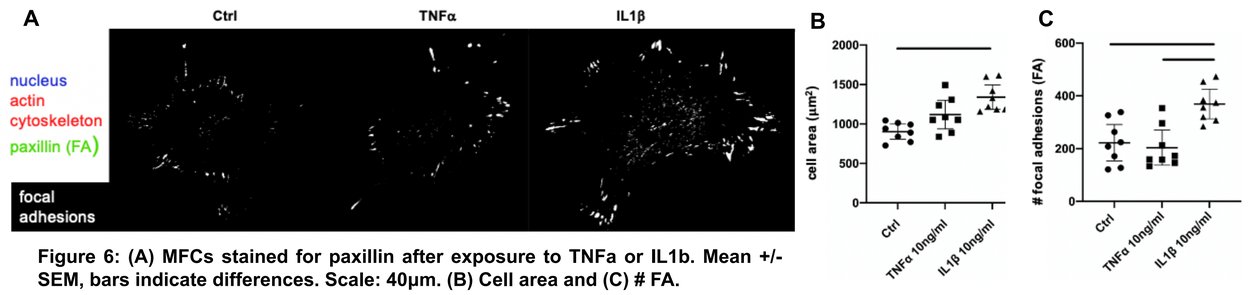


Figure 6: (A) MFCs stained for paxillin after exposure to TNFa or IL1b. Mean +/- SEM, bars indicate differences. Scale: 40µm. (B) Cell area and (C) # FA.

Cell analysis: MFCs treated with IL1b were larger (Fig. 6B) and had an increased number of FAs compared to controls (Fig. 6C).

Discussion

- Cells remain compromised for at least 3 days after exposure to even very low levels of pro-inflammatory cytokines
- In a 3D culture system, either cytokine reduced colonization of the surface of the tissue substrate, but neither factor impacted the ability of those few cells that entered the tissue to infiltrate, compared to controls
- IL1b increases cell size and FA number, indicating that meniscus cells exposed to inflammatory conditions are more firmly attached to the substrate (and so perhaps less mobile[5]) than control cells
- Future work
  - mechanism of FA changes
  - inflammation +/- stiffness
  - release of anti-inflammatory factors at the injury site (Fig. 7)

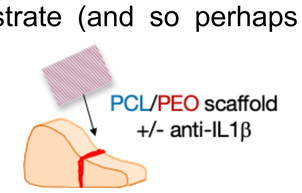


Figure 7: Schematic of a scaffold with anti-inflammatory components delivered directly to a meniscus tear.

References

1. Fernandes+ Biorheol, 2002; 2. Riera+ Arthr Res Ther, 2011; 3. Hennerbichler+ OA&C, 2007; 4. Wilusz+ JOR, 2008; 5. Peyton & Putnam, J Cell Phys, 2005; 6. Berginski+ F1000Res, 2013; 7. Qu+ Sci Rep, 2018; 8. Heo+ Trans ORS, 2019.

Acknowledgements

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