The Role of TNF-α Signaling in the Healing of the Knee Following ACL Rupture and Reconstruction

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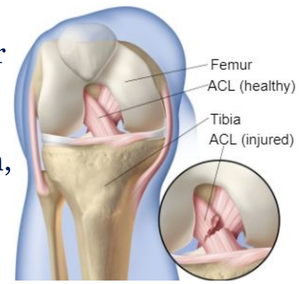
Penn Undergraduate Research Mentoring (PURM) Program 2020

Aim 1: Establish the effects of TNF-α signaling on healing following ACL rupture
Aim 2: Establish the effects of TNF-α signaling on healing following ACL reconstruction

Background

- Up to 87% of people who rupture their anterior cruciate ligaments (ACL) develop post-traumatic osteoarthritis (PTOA), characterized by chronic pain, stiffness, inflammation, and other progressively-worsening symptoms
- ACL re-injury following ACL reconstruction is becoming increasingly common
- Inflammation is one of the main reasons ACL reconstruction often happens several days to several weeks after rupture
- PTOA inflammation is caused in part by TNF-α, a proinflammatory cytokine that triggers signaling to release cartilage-destroying matrix metalloproteinases (MMPs) from synovial fibroblasts and downregulates transcription factors to inhibit chondrogenesis
- In situations in which TNF-α levels are naturally elevated, recovery after ACL reconstruction is prolonged



(Mayo Clinic 2019)

Hypothesis

Global Hypothesis: abnormal TNF-α signaling will worsen the ACL healing response

- Elevated TNF-α signaling will accelerate joint degradation by increasing levels of cartilage-destroying MMPs and suppressing chondrogenesis
- Depressed TNF-α signaling will slow healing due to the lack of clearance of the necrotic and grafted tissues to allow for repopulation by healthy tissues

Significance

- Since cases of ACL reinjury following a reconstructive surgery are becoming more frequent, studying how chronic inflammation affects the knee is important
- The mechanisms by which an inflammatory response triggered by TNF-α affects the healing of the ACL post-injury and post-reconstruction are not well understood

Approach

Three groups of mice (different TNF-α signaling levels)

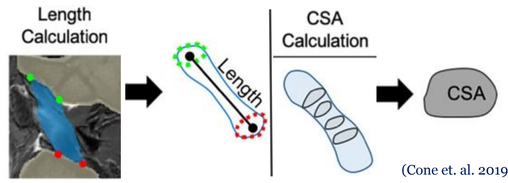
Wildtype	Overactive TNF-α	Depressed TNF-α
Normal TNF-α signaling → unmodified mouse	$Tace^{flox}$ x Ai9 x Col2CreERT transgenic mouse	$Sirt1^{loxP}$ x Ai9 x Col2CreERT transgenic mouse

Four treatment groups (performed at 16 weeks)

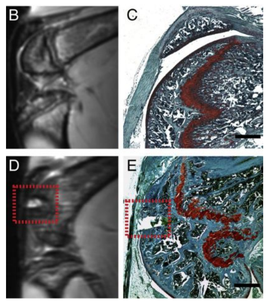
ACL rupture, surgical transection	ACL rupture, manual
ACL reconstruction (ACLR)	Control (uninjured)

Measurements
(*For all groups unless otherwise noted*)

- 9.4 T MRI
  - 15 (pre-op) and 20 weeks (post-op)
  - Assessment of articular cartilage →
  - Geometric assessment of the ACL ↴

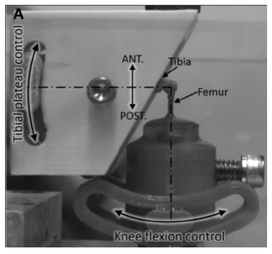


(Cone et. al. 2019)

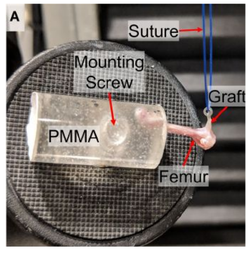


(Mak et. al. 2015)

- Anterior and Posterior Drawer Tests
  - Maximum anterior and posterior displacements following cyclic loading will be recorded →
  - Measurement of knee joint stability



(Kamalitdinov et. al. 2020)

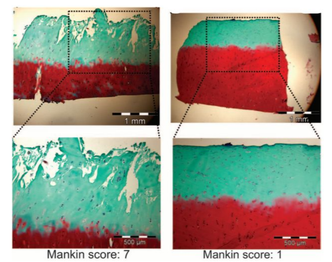


(Kamalitdinov et. al. 2020)

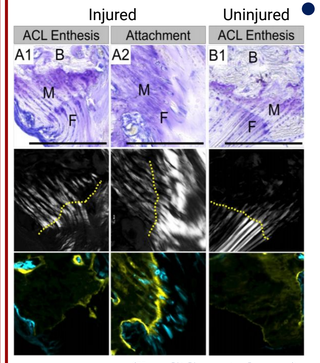
- Pullout Tests
  - ACL reconstruction group only
  - ← Records maximum loads required for graft failure
  - Will contribute to assessment of tunnel integration quality

Approach, *cont.*

- Histological Analysis of Cartilage
  - Cryosections stained with Saffarin-O (cartilage) and Fast Green (bone) →
  - Assess cartilage damage with modified Mankin scores



(Heinemeier et. al. 2016)



(Kamalitdinov et. al. 2020)

- Histological Analysis of Tunnels and Entheses
  - ← Cryosections of the graft attachments of the ACLR group and ACL entheses of the ACLR and control groups
  - Mineral deposition ⇒ demeclocycline (bottom)
  - Collagen fiber orientation ⇒ polarized light (middle)
  - Proteoglycan ⇒ toluidine blue (top)

Limitations & Potential Pitfalls

- Cells outside the cartilage also produce TNF-α → Solution: Add rheumatoid arthritis drug regimens to the depressed TNF-α protocol to further suppress signaling
- In humans, delays between ACL injury and reconstructive surgery vary → Solution: Run a separate experiment to map out TNF-α levels after injury and after reconstruction

Innovation

Establishing the effects of varying levels of TNF-α signaling in the knee and elucidating its mechanisms after ACL injury could inform future treatments

References

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