Investigation of gamma delta T cells and the JAK/STAT signaling pathway in tendon healing

Ella McGovern, Giulia Crosio, and Alice H. Huang
Columbia University, New York, NY
erm2204@columbia.edu

DISCLOSURES: Ella McGovern (N), Giulia Crosio (N), Alice H. Huang (N)

INTRODUCTION: Although it is well established that the immune response plays a critical role in tendon healing, the specific immune cells and molecular mechanisms that distinguish a regenerative immune response vs a fibrotic response are only beginning to be identified. Using a model of tendon regeneration in neonatal mice compared to scar formation in adult mice [1], we previously showed that functional regeneration depends on neonatal macrophages and regulatory T cells that rapidly polarize the immune environment toward an anti-inflammatory state [2, 3]. Using single cell RNA sequencing, we also identified a large population of CD4-/CD8- γδ T cells in neonates with no known role in tendon healing [2]. Since γδ T cells are often associated with the maintenance of tissue homeostasis [4, 5] and JAK/STAT signaling has been implicated in tenocyte migration and macrophage polarization in tendinopathy [6, 7], this study determined the temporal recruitment of γδ T cells after tendon injury and tested the role of the JAK/STAT pathway in poor adult tendon healing.

METHODS: Neonatal (P5) and adult (4–6-month-old) mice underwent Achilles tendon transection without repair on the right hindlimb and sham surgery on the left hindlimb. Flow cytometry was performed on cells isolated from tendon tissue at 5, 9, and 14 DPI. DAPI was used to detect all live cells, and antibodies against CD3, CD4, CD8, TCR β, and TCR γδ were used to detect T cell subsets. Real time qPCR was performed on tendon at 3 and 14 DPI to quantify RNA expression of JAK/STAT targets. To inhibit JAK signaling *in vivo*, 20 mg/kg of JAK 1/2/3 inhibitor, Tofacitinib citrate (MCE, HY-40354A), or DMSO vehicle control was administered to adult mice daily via IP injection from 3-14 DPI. Western Blot was performed on tendon tissue at 6 DPI with antibodies detecting phosphorylated JAK1 protein to confirm inhibition. At 14 DPI, gait was analyzed using Catwalk XT (Noldus). Equal number of males and females were used for all experiments. All animal studies were carried out under approved IACUC.

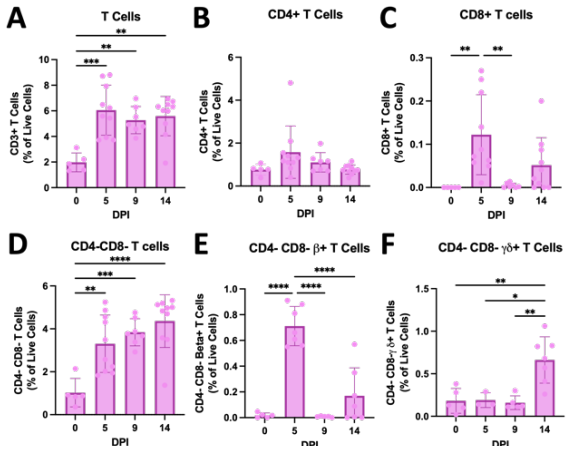

Fig 1: γδ T cells are enriched in neonatal tendons at 14 DPI. Flow cytometry profiling of T cells in neonatal tendons after injury (n=3-10, one-way ANOVA, Sidak's posthoc *p<0.05 **p<0.01 ***p<0.001 ****p<0.0001).

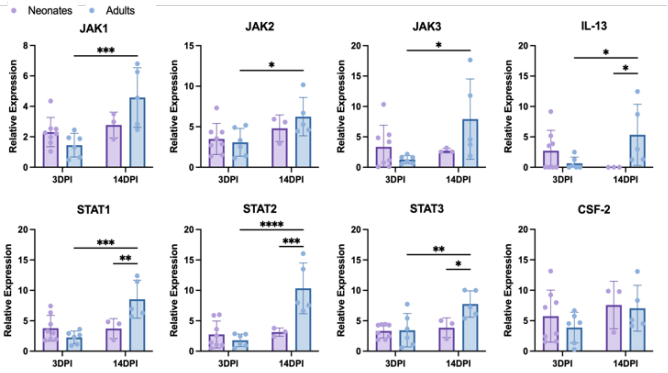

Fig 2: Gene Expression of JAK/STAT targets differs in neonates and adults after injury. RNA expression of JAK/STAT targets (RT-qPCR) at 3 DPI and 14 DPI in neonates and adults. (n = 3-8, two-way ANOVA, *p<0.05 **p<0.01 ***p<0.001 ****p<0.0001).

RESULTS: Analysis of T cells in neonatal tendon after injury revealed that CD3+ T cells increased at 5 DPI and persisted to 14 DPI. While the presence of CD4+ helper T cells remained relatively constant, CD8+ cytotoxic T cells peaked at 5 DPI. Analysis of the double negative CD4-CD8- T cell population showed significant recruitment at 5 DPI that was maintained to14 DPI (**Fig 1**). Within this double negative population, β+ T cells showed a similar pattern to cytotoxic T cells while γδ+ T cells were not significantly recruited until 14 DPI (**Fig 1**). Gene expression analysis of JAK/STAT signaling molecules showed that generally there were no differences in JAK/STAT targets in the neonatal tendons from 3 to 14 DPI. (**Fig 2**). At 14 DPI, expression of *Il-13*, *Stat1*, *Stat2*, and *Stat3* was higher in adults compared to neonates (**Fig 2**). To determine whether this heightened activation of JAK/STAT is responsible for directing poor healing in adults, an inhibitor of JAK1/2/3 was administered daily from 3-14 DPI (**Fig 3A**). Western Blot at 6 DPI confirmed a 39.34% reduction in pJAK1 in injured tendons of inhibitor-treated mice compared to carrier-treated mice (**Fig 3B**). Gait analysis at 14 DPI revealed that injured hindlimbs of inhibitor-treated mice had impaired gait parameters compared to the contralateral sham hindlimb; while max intensity was not recovered for both conditions (consistent with our previous studies), parameters that were recovered in the carrier-treated mice remained impaired with JAK inhibition (**Fig 3C**).

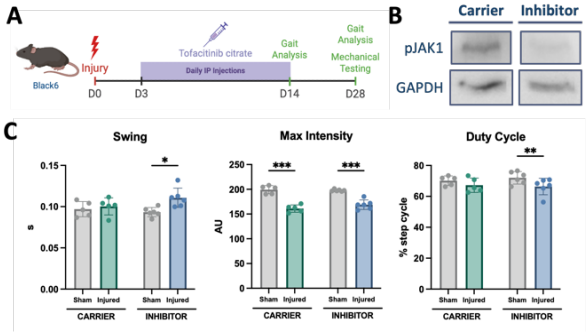

Fig 3: Inhibition of JAK/STAT pathway impairs gait at 14 DPI. A. JAK1/2/3 inhibitor regimen **B.** Western Blot for pJAK1 (130 kDa) at 6 DPI after JAK inhibitor injections 3-6 DPI, and housekeeping control GAPDH (36 kDa) **C.** Gait analysis at 14 DPI (n=5-6, multiple paired t tests, *p<0.05 **p<0.01 ***p<0.001 ****p<0.0001).

DISCUSSION: These studies demonstrated that there is significant recruitment of CD4-CD8- T cells after neonatal injury that may contributed to immune polarization. β and γδ T cells only partially accounted for this population, with distinct temporal dynamics in recruitment. Interestingly, while we hypothesized that γδ T cells were involved in the inflammatory response, these cells were only enriched in the neonate at 14 DPI, when polarization toward an anti-inflammatory type 2 response has already occurred. Future studies will define the role of these cells, using cytokine analysis of FACS cells and *in vivo* depletion studies. Future studies will also compare γδ T cells after injury in adults to determine any differences compared to neonates. This study also investigated the role of the JAK/STAT signaling pathway in tendon healing. Consistent with our prior studies suggesting that adults maintained a chronic inflammatory phenotype after injury, we found upregulation of most JAK/STAT targets at 14 DPI compared to neonate. Surprisingly, preliminary studies inhibiting JAK signaling during adult tendon healing showed worsened gait compared to control. Ongoing studies will determine whether tensile mechanical properties of the tendon are similarly affected and whether immune polarization is altered with JAK inhibition. Overall, these studies indicate complex interactions between multiple immune cell types and signaling pathways that orchestrate immune response during tendon healing.

SIGNIFICANCE: Elucidating the key immune cells and their mechanisms in tendon healing is crucial to promote an environment permissive for regeneration.

REFERENCES: [1] Howell+, Sci Rep, 2016. [2] Arvind+, Sci Adv, 2025. [3] Howell+, FASEB, 2021. [4] Ribot+, Nat Rev Immunol, 2021. [5] Paul+, Int Rev Immunol, 2014. [6] Crosio+, Trans. ORS, 2025. [7] Zhang+, Int Immunopharm, 2025.

ACKNOWLEDGEMENTS: This work was funded by NIH/NIAMS R01 AR081674.