Changes in Macrophage Phenotype and Induction of Epithelial-to-Mesenchymal-Related Genes Following Acute Achilles Tenotomy and Repair

Kristoffer B. Sugg, MD, Jovan Lubardic, MS, Jonathan P. Gumucio, BS, Christopher L. Mendias, PhD, ATC. University of Michigan, Ann Arbor, MI, USA.

Disclosures: K.B. Sugg: None. J. Lubardic: None. J.P. Gumucio: None. C.L. Mendias: None.

Introduction: Injuries to and diseases of tendons produce significant morbidity in patients, including debilitating pain and an overall decrease in functional capacity. Tendon injuries account for approximately 50% of musculoskeletal injuries treated in the United States annually (1), but the current understanding of the cellular and molecular factors that drive tendon healing is quite limited. In many injured tissues, the repair process is orchestrated by two types of cells, macrophages and fibroblasts. Macrophages have two general phenotypes -- classically activated proinflammatory macrophages (M1) that promote extracellular matrix (ECM) breakdown, inflammation and apoptosis, and alternatively activated anti-inflammatory macrophages (M2) that coordinate ECM deposition and tissue repair. M1 and M2 macrophage subpopulations are known to exist in injured tendon tissue (2), but their temporal relationship to each other has yet to be established in a clinically relevant tendon injury model. Furthermore, previous work has demonstrated that the epitenon, a loose epithelial-like tissue layer that surrounds the tendon, may serve as a source of fibroblasts contributing to tendon growth and repair (3,4). In other organ systems, reactivation of one or more members of the Snail family of transcriptional repressors under pathological conditions has recently been shown to promote fibrotic programs within the local epithelium through a process known as epithelial-mesenchymal transition (EMT) (5). However, expression of these EMT transcription factors in tendon has not been previously described. In order to improve the treatment of tendon injuries, a greater understanding of the changes between macrophage phenotypes is essential as well as identification of genes that may regulate cell function during tendon repair. We hypothesized that in response to a full-thickness tear of the Achilles tendon there would be an early accumulation of M1 macrophages within the first week followed by a later transition to the M2 phenotype, and that tendon repair would correlate with increased expression of EMT-related genes. Methods: This study was approved by our IACUC. Bilateral mid-substance tears of Achilles tendons were created in six-month-old male Sprague-Dawley rats. Using the Bunnell technique, immediate two-strand repair was performed and the paratenon was reapproximated (Fig. 1A). Ad libitum weightbearing and cage activity were allowed. Rats were sacrificed at 3, 7, 14, and 28 days (N=6 rats at each time point) following surgical tear and repair, and Achilles tendons were harvested for either gene expression analysis or immunohistochemistry. Achilles tendons were also collected from rats that did not undergo tenotomy and served as controls (N=6 rats). The tendons were snap frozen in OCT, cryosectioned through the callus, and then incubated with WGA-Lectin-AF488 to mark the ECM, DAPI to identify nuclei, and antibodies against CCR7 and CD163 to label M1 and M2 macrophages, respectively. RNA was isolated from tendons using a miRNeasy kit, reversed transcribed into cDNA, and qPCR was conducted using standard techniques. Target gene expression was normalized to the stable housekeeping gene, beta-2 microglobulin, and then further normalized to control tendons. A one-way ANOVA (p<0.05) followed by Tukey’s post-hoc sorting was performed to determine significance between groups. Results: Control tendons demonstrated the presence of a few M1 and M2 macrophages in the endotenon layers, but no macrophages in the tendon fibers (Fig. 1B). Three days following tear and repair, M1 macrophages accumulated in regions of newly formed tendon tissue and remained present throughout the study period. M2 macrophages slowly accumulated at sites of organizing tendon ECM and became the predominant macrophage phenotype by 28 days. Using gene expression analysis, the pan-macrophage marker F4/80 was elevated by 3 days (Fig. 2A). After an initial decline at 7 days, it remained elevated compared to controls thereafter. CCL2, which plays an important role in macrophage recruitment, was elevated by 3 days and then steadily declined over the next few weeks. Markers of M1 macrophages such as CD68, CCR7, and CD11b were dramatically elevated following tear and repair. CD68 and CD11b declined between 3 and 7 days. CD168, a marker for M2 macrophages, remained similar to controls until day 28 at which time it became significantly elevated. For EMT-related genes, the transcription factors Snail1 and Slug were elevated by 3 days and then declined by either 14 or 28 days, respectively (Fig. 2B). Goosecoid, a homeobox protein that induces EMT, was elevated at 7 and 14 days compared to controls. Twist1, a bHLH transcription factor that also induces EMT, was elevated by 7 days and remained elevated until 28 days at which time it returned to levels similar to controls.
Figure 2: Expression of IL-10 monocytes-derived and pVEGF activated genes at each time point in the newly-implanted Achilles tendon. Post-hoc section difference.
Discussion: Shortly following acute Achilles tenotomy and repair there is a dramatic accumulation of proinflammatory (M1) macrophages that slowly taper over the first month postoperatively. Anti-inflammatory (M2) macrophages do not appear to accumulate in large amounts until 28 days. While the findings from this study support our hypothesis that there is a sequential transition from the M1 to the M2 phenotype over time, the temporal relationship between the two phenotypes differs compared to previous studies that injure the Achilles tendon with collagenase (2). In the collagenase model, M1 macrophages return to control values by 14 days and M2 macrophages only display a tendency to increase by 28 days, while in our model M1 macrophages remain elevated throughout the study period and M2 macrophages were significantly elevated by 28 days. These differences are possibly explained by the mechanism of injury, whereby a full-thickness surgical tear is a much greater insult than local collagenase injection, and perhaps more representative of clinical injury. Furthermore, fibrotic programs in tendon appear to correlate with the expression of EMT-related genes, which to our knowledge has not been previously demonstrated in tendon. Further studies are required to determine both the specific roles of M1 and M2 macrophages in tendon healing, as well as the effects of EMT-related genes on cell function during the repair process.

Significance: This study demonstrates that M2 macrophages do not accumulate in large amounts in injured tendon tissue until 28 days, which supports the clinical observation that tendons are slow to heal. Expression of EMT-related genes following tendon repair may provide future areas of investigation for the development of new targeted therapies in the treatment of tendon disorders.

Acknowledgments: This work was supported by NIH grants R01-AR063649, T32-GM008616, and T32-GM008322.


ORS 2014 Annual Meeting
Poster No: 0011