Adipose-Derived Mesenchymal Stem Cells Modulate Tendon Fibroblast Responses to Macrophage-Induced Inflammation

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Disclosures:

Introduction: Recent evidence suggests that M1 macrophages may contribute to the poor healing response of tendon via the secretion of various pro-inflammatory factors (i.e., IL-1β, TNFα), and that modulation of M1 macrophages may be advantageous for tendon healing [1,2]. Recent studies indicate that mesenchymal stem cells can modulate the innate immune response by promoting differentiation of monocytes into anti-inflammatory macrophages (i.e., M2 or alternatively-activated macrophages) as opposed to classically activated, pro-inflammatory macrophages (i.e., M1 macrophages) [3,4]. The purpose of this study was to investigate the effects of pro-inflammatory factors secreted by macrophages on tendon fibroblasts (TFs) and the ability of adipose-derived mesenchymal stem cells (ASCs) to modulate those effects.

Methods: TFs, ASCs, and macrophages were isolated from 6 week old male C57BL/6J mice (n=5). TFs were isolated from tail tendon and ASCs were isolated from abdominal adipose tissue. Macrophages were derived from total bone marrow using L929-conditioned media as a source of macrophage colony stimulating factor. An in vitro model of inflammation was established via co-culture of TFs (2.6x10^4 cells/cm²) with macrophages of varying phenotypes (M0, non-polarized; M1, classically activated/pro-inflammatory; or M2, alternatively activated/anti-inflammatory) for 1 and 5 days. The effect of soluble factors produced by macrophages on TFs was determined by monitoring TF gene expression of factors related to inflammation (TNFα, IL1β), degradation (MMP1a, MMP13), matrix production (COL1, COL3), and TF differentiation (SCX, TNMD). ASCs were then incorporated into the system (1.2x10^4 cells/cm²) and their ability to modulate the inflammatory environment was examined by assessing changes in gene expression (compared to the co-culture groups, which lacked ASCs). Gene expression data was analyzed using the delta delta Ct method (compared to GAPDH) and Ct values were compared using a 2-way ANOVA (for macrophage type and presence of ASCs), followed by a Fisher’s post-hoc test. The macrophage/ASC populations were further analyzed using flow cytometry to determine whether co-culture with ASCs led to a phenotypic switch in the macrophage population. Surface markers for all bone marrow-derived macrophages (CD11b+, F480+) and specifically for M2 macrophages (CD206+, CD301+) were assessed. Statistical differences were assessed using a multi-factor ANOVA (for the effects of macrophage type, ASCs, and TFs) followed by a Fisher’s post-hoc test. Significance was set to p < 0.05.

Results: All three macrophage phenotypes induce up-regulation of pro-inflammatory factors by TFs relative to untreated TFs (Figure 1). Furthermore, exposure of TFs to an inflammatory environment for a single day led to significant up-regulation of factors related to matrix degradation and down-regulation of factors related to extracellular matrix formation (Figure 1 and data not shown). Of the three phenotypes, however, the M1 macrophages were significantly more harmful to the co-cultured tendon fibroblasts than M0 or M2 macrophages (Figure 1). ASC co-culture for 1 and 5 days with M1 macrophages successfully suppressed the negative effects of the M1 macrophages on the tendon fibroblasts (Figure 2 and data not shown). Flow cytometry analysis revealed that ASCs induce a phenotypic switch toward an anti-inflammatory macrophage phenotype (i.e., M2) (Figure 3). M0 macrophages co-cultured with ASCs for 5 days expressed significantly greater levels of both M2 macrophage-specific markers (i.e., CD206+ CD301+), while M1 macrophages exhibited a 2.3-fold increase in CD206 expression.

Discussion: This study supports the premise that M1 macrophages may be detrimental to tendon healing through the secretion of pro-inflammatory factors. Our results further suggest that ASCs may be able to protect native TFs from the negative effects of M1 macrophages even if ASC treatment is delayed until after monocytes have differentiated into M1 macrophages. Moreover, if ASC treatment is performed prior to monocyte polarization into M1 macrophages, ASCs may be able to further modulate the inflammatory environment by pushing the non-polarized (M0) macrophages towards an anti-inflammatory activation phenotype (M2). Future studies will aim to investigate whether ASCs delivered at the time of tendon repair can enhance tendon healing via modulation of the early inflammatory phase.

Significance: Excessive inflammation during the early period following tendon repairs may be a key factor leading to poor clinical outcomes. Modulation of the naturally occurring inflammatory response may be beneficial for tendon healing [1, 2].

Acknowledgments: This study was funded by the National Institutes of Health (NIH R01 AR062947).

Figure 1: Macrophages induced up-regulation of matrix degradation-related genes by T3s and down-regulation of matrix production-related genes by T3s. Fold changes in TF gene expression after 1 day of co-culture with M0, M1, and M2 macrophages are presented relative to control (i.e., untreated T3s). *p < 0.05. Abbrev, significant effect of macrophage type for all genes, *p < 0.05. Fisher's post-hoc comparisons. * by the error labels signifies a significant difference compared to untreated T3s (N=5).
Figure 2: ASCs suppress the effects of M0 and M1 macrophages on TFs after 5 days of co-culture. TF gene expression after 5 days of co-culture with macrophages (M0, M1, M2) or tri-culture with macrophages and ASCs (M0+ASC, M1+ASC). Data are normalized to normal (i.e., untreated TFs) and presented as group means ± SD. * p < 0.05, multi-factor ANOVA with Fisher’s post-hoc test. Line above pair of bars signifies Fisher’s post-hoc comparisons. * above bars signifies a significant difference from the untreated TFs control.
Figure 3: IS0 co-culture shifts macrophages toward an M2 phenotype. Mean fluorescent intensity of M2 macrophage-specific surface markers, CD206 and CD301, after 5 days of co-culture with M0 or M1 macrophages. Data are presented as group means ± SD. * p < 0.05, 2-way ANOVA with Fisher's post-hoc test. Bars signify Fisher's post-hoc comparison; * by the y-axis labels signifies a significant difference compared to control ITS (n=4).

ORS 2014 Annual Meeting
Poster No: 1375