Evaluation of the biomechanical environment on rheumatoid arthritis pathogenesis using multiplex cytokine analysis

K.L. Wagoner, R.A. Bader

Department of Biomedical & Chemical Engineering, Syracuse University, Syracuse, NY 13244

Statement of Purpose: Rheumatoid arthritis is a systemic inflammatory disease characterized by an imbalance in pro-inflammatory and anti-inflammatory cytokines, concomitant with infiltration of chronic inflammatory cells into the typically hypocellular synovial membrane. Although progress has been made in recent years, contributors to the pathogenesis of the disease are not fully understood, particularly as pertains to biomechanical signals. Despite evidence that joint mobilization can alter the progression of arthritic diseases,[1] the molecular mechanism behind the observed phenomena remains largely unknown. Our goal is to elucidate the impact of biomechanical stimulation on the complex network of cytokines that underlie rheumatoid arthritis pathogenesis.

Methods: Primary rheumatoid arthritis synovial fibroblasts (RASFs) and normal synovial fibroblasts (NSFs) were obtained from Cell Applications, Inc. (San Diego, CA) and incubated in synoviocyte basal medium at 37°C and 5% CO2. At passages 3 and 4 respectively, the NSFs and RASFs were seeded onto untreated 6-well BioFlex® culture plates and grown for 72-96 hours such that the cells were 70-80% confluent. The media was replaced, and the cells were exposed to dynamic equibiaxial tensile strain at a magnitude of 10% and a frequency of 0.5 Hz (sinusoidal waveform) in the FlexCell® Tension Plus™ System. At selected intervals, the supernatant was removed (N=3 wells for each time point), and the concentrations of the following cytokines were determined using multiplex immunoassay on the Luminex® platform: IL-1α, IL-1β,IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12(p70), IL-13, IL-15, IL-17, FGF-2,GM-CSF, IFN-γ, MIP-1β, RANTES, TNF-α, and VEGF. For comparison, the above procedure was repeated for RASFs and NSFs seeded onto untreated 6-well plates to which no mechanical regimen was applied.

Results: Negligible amounts of IL-1α, IL-1β, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, IL-17, MIP-1β, and TNF-α were detected within the supernatant for all experimental conditions, at all time points. As shown in Fig. 1 for the 24 hour time point, the application of dynamic tensile strain generally led to reductions in the levels of cytokines associated with the recruitment of inflammatory cells by synovial fibroblasts, i.e. IL-8, RANTES, GM-CSF, VEGF, and FGF-2 (not shown). Asterisks (*) are used to indicate a significant difference in cytokine concentration from cells to which a mechanical regimen was applied ($p < 0.05$). Similar trends were observed at 1, 3, 6, and 12 hours. The levels of GM-CSF and VEGF observed for RASFs were significantly greater than those of NSFs at nearly all time points, regardless of mechanical stimulation.

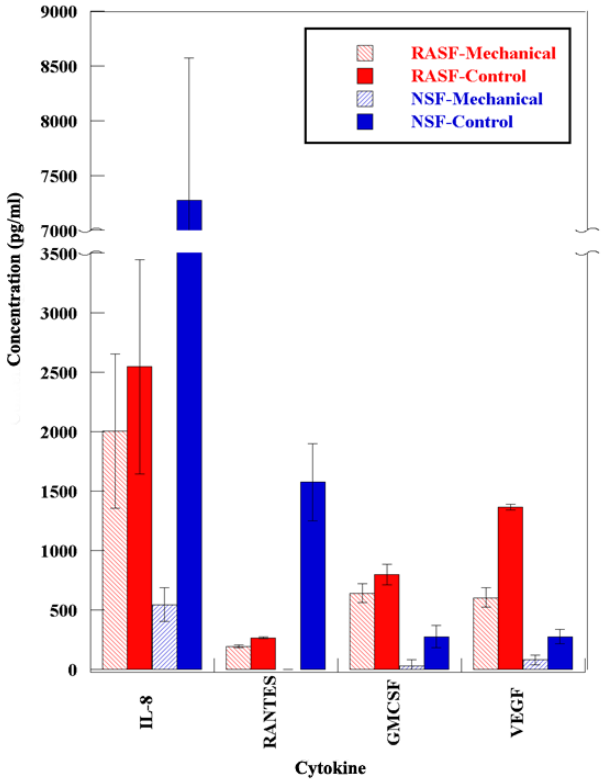

Figure 1

Conclusions: The current study demonstrates that the biomechanical environment has a profound impact upon the response of both diseased and non-diseased synovial fibroblasts. Specifically, the application of dynamic tensile strain led to a reduction in the concentration of cytokines associated with the recruitment of leukocytes. The results correlate with the alteration in disease progression observed with joint mobilization[1] and offer some insight into the mechanism by which manual therapy can be used to manage rheumatoid arthritis.[2] Elevated GM-CSF and VEGF levels for RASFs relative to NSFs are consistent with previous observations.[3,4] Future work will be aimed at incorporating the synovial fibroblasts into a more physiologically relevant *in vitro* model that includes a three dimensional matrix, as well as other relevant cell types, such macrophages and T-cells, that are associated with synovial fibroblast activation.

References:

1. Kim H. J Rheumatol. 1995;22:1714-1721.
2. Van den Ende CH. Br J Rheumatol. 1998;37:677-687.
3. Fiehn C. Z Rheumatol. 1992;51:121-126.
4. Sone H. Life Sci. 2001;69:1861-1869.