

Pro-inflammatory Cytokines Inhibit Chondrogenesis by Human Mesenchymal Stem Cells through NF- κ B-dependent Pathways

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INTRODUCTION: Cartilage loss through trauma or arthritis presents a major clinical challenge. The differentiation of mesenchymal stem cells (MSCs) into chondrocytes provides an attractive basis for cartilage regeneration. However, chondrogenesis will often need to occur in the presence of inflammatory mediators produced in response to injury or disease, including interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α). Among the intracellular mediators of IL-1/TNF activity are the nuclear factor- κ B (NF- κ B) family of transcription factors, which play important roles in the pathogenesis of osteoarthritis. Here we examined how these factors regulate the chondrogenic behavior of human bone marrow-derived MSCs.

METHODS: Vectors. Recombinant adenovirus encoding the cDNA for a 'super-repressor' inhibitor of NF- κ B (srI κ B) and driven by the CMV promoter [1] was provided by Dr. Paul Robbins (Pittsburgh, PA). Similar vectors encoding for green fluorescent protein (Ad.GFP) and firefly luciferase (Ad.CMV-Luc) were constructed previously [2]. Ad.NF- κ B-Luc was acquired commercially.

hMSC Culture. MSCs were recovered from the intramedullary canal of patients undergoing hip hemiarthroplasty [3]. For chondrogenesis, passage-2 monolayers were transduced with Ad.srI κ B or Ad.GFP. The next day, MSCs were formed into cell aggregates [4] and cultured with 10 ng/mL transforming growth factor- β 1 (TGF- β 1) and/or IL-1 β or TNF- α (0-10 ng/mL each). After six weeks, aggregates were collected for biochemical analysis, histology, immunohistochemistry, and quantitative RT-PCR.

NF- κ B Activity. hMSC monolayers were transduced with either Ad.NF- κ B-Luc or Ad.CMV-Luc. The next day, cells were transduced again with Ad.srI κ B or Ad.GFP. MSCs were then formed into aggregates and cultured with TGF- β 1. After five days, a portion of aggregates were stimulated with IL-1 β (10 ng/mL), and luciferase activities were measured after an additional five hours.

RESULTS: Both IL-1 β and TNF α dose-dependently suppressed hMSC aggregate size and proteoglycan synthesis in response to TGF-

β 1. These effects were associated with a marked activation of NF- κ B. Adenoviral delivery of srI κ B dose-dependently blocked NF- κ B-driven luciferase activity in response to IL-1 β . Using this vector in the chondrogenesis model, we found that srI κ B rescued proteoglycan (Fig. 1) and type II collagen synthesis within IL-1-stimulated aggregates. Although type X collagen followed this pattern, other markers of hypertrophic differentiation (ALP, MMP-13) responded differently.

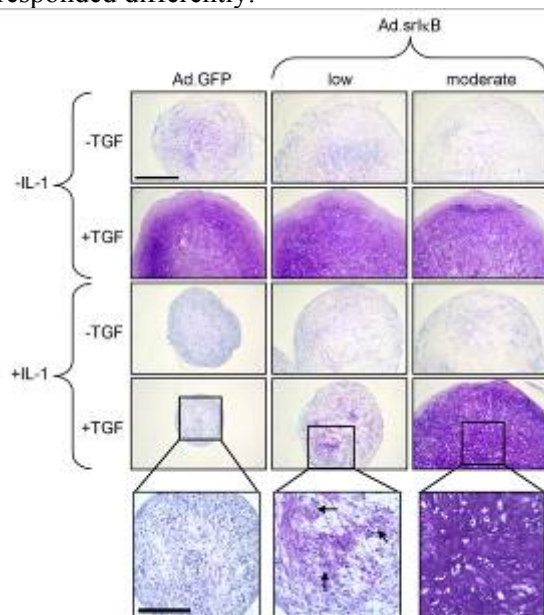


Figure 1: Toluidine Blue staining of 6-week hMSC aggregates. Scalebar = 0.5 mm. Magnified regions: scalebar = 0.2 mm.

CONCLUSIONS: Cell-based repair of lesions in articular cartilage will be compromised in inflamed joints. Strategies for enabling repair under these conditions include using specific antagonists against individual pyrogens, such as IL-1 and TNF, or the targeting of important intracellular mediators, such as NF- κ B.

REFERENCES: 1. Imuro *et al.* (1998) *J Clin Invest* 101(4): 802-11. 2. Palmer *et al.* (2003) *Methods Mol Biol* 215: 235-46. 3. Porter *et al.* (in press) *J Ortho Res* 4. Penick *et al.* (2005) *Biotechniques* 39(5): 687-91.

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