MECHANICAL INJURY POTENTIATES THE COMBINED EFFECTS OF TNF-α AND IL-6/sIL-6R ON PROTEOGLYCAN CATABOLISM IN BOVINE CARTILAGE

INTRODUCTION: Acute traumatic joint injury increases the risk of developing osteoarthritis (OA) [1]. The mechanisms by which injury causes chronic cartilage degradation in vivo are not fully understood, but elevated levels of injury-induced pro-inflammatory cytokines [2], including TNF-α and IL-6 [3], may play pivotal roles in the pathogenesis of OA. TNF-α causes a synergistic loss of PG from mechanically-injured cartilage in vitro [4], but the pathways regulating this synergy are unclear. The objectives of this study were to (1) examine the combined effect of TNF-α and IL-6/sIL-6R on proteoglycan degradation in mechanically-injured cartilage, and (2) to determine the role of endogenous IL-6 in the cartilage catabolism induced by both TNF-α and mechanical injury.

METHODS: Cartilage disks (3 mm diam., 1 mm thick) were harvested from the middle zone of the femoropatellar groove of 2-week-old calves, and equilibrated for 2 days in normal medium (DMEM + 1% ITS) prior to treatment. Cytokine and Mechanical Injury Treatments: Location-matched disks were either injuriously compressed (50% strain, 100%/second strain rate, cultured in medium with rhTNF-α (25 ng/ml), treated with rhl-6 (50 ng/ml) plus soluble IL-6 receptor (sIL-6R, 250ng/ml), or treated with combinations of these three conditions (Fig.1). Culture was terminated after 6 days of treatment. In a separate experiment, half the cartilage disks (Fig. 3) were pre-equilibrated for 6 days with an IL-6 blocking Fab fragment (50 ug/ml, Centocor, J&J) prior to treatment; the remaining disks were incubated in normal medium during this period. Afterward, disks were either injuriously compressed, incubated with rhTNF-α (25 ng/ml), or treated with combined injury + TNF-α. Disks that were pre-treated with the IL-6 blocking Fab fragment continued to receive Fab fragments until the termination of the experiment. Aggrecan Western Blotting, GAG Content and Histology: Culture medium from each condition was collected on day 2, 4 and 6 after the initial injury and/or cytokine treatments. Concentrated medium used was to perform Western blot analysis using a monoclonal Ab specific to the G1- NITEGE fragment of aggrecan (kindly provided by C. Flannery, Wyeth). DMDB dye was used to quantify sGAG released into the medium. Selected cartilage samples were fixed in gluteraldehyde with RHT, paraaffin-embedded, sectioned, and stained with Toludine Blue. Additional disks were radiolabeled during days 4-6 with 5 µCi/ml 35SO42- to assess proteoglycan synthesis.

RESULTS: 3-way ANOVA analyses followed by post-hoc Tukey’s pairwise comparisons showed that TNF-α formed interactions with both IL-6/sIL-6R (p=0.001) and injurious compression (p=0.001) causing increased sGAG release (Fig. 1(i)) and decreased proteoglycan synthesis (data not shown). While IL-6/sIL-6R significantly augmented TNF-α-induced proteoglycan degradation (Fig.1(iii)B,G), the largest amount of GAG loss was caused by the combination of injury+TNF-α + IL-6/sIL-6R (Fig.1(i)D). Histology showed that GAG loss was not uniform across the disk cross-section, but instead was initiated at the disk periphery and progressed towards the disk center with time (Fig. 2). The most rapid, severe progression of GAG loss was observed in disks treated with the combination of injury + TNF-α + IL-6/sIL-6R (Fig. 2e-f). Analysis of conditioned medium for aggrecan fragments by Western blotting demonstrated that the dramatic release of aggrecanase-generated cleavage products occurred in response to treatment with TNF-α + IL-6/sIL-6R, both with and without injurious compression (Fig. 1(iii)B,D). The IL-6 blocking Fab fragment was effective in neutralizing exogenous rhl-6 in the medium, and was not toxic to cells (data not shown). In separate studies, TNF-α + mechanical injury caused greater GAG loss than either treatment alone (Fig.3). Importantly, the IL-6 blocking Fab fragment significantly reduced the combined catabolic effects of TNF-α + mechanical injury on GAG loss, with no exogenous IL-6 present (Fig.3D).

DISCUSSION: We found that the combined treatment with TNF-α and IL-6/sIL-6R induced significantly more GAG loss than either cytokine alone did, consistent with previous studies of TNF-α/IL-6 treatment [3], and suggesting this catabolic response was associated with aggrecanase (but not MMP) activity. Additionally, we now report that the catabolic effect of TNF-α, and the combined effect of TNF-α + IL-6/sIL-6R are both highly potentiated by mechanical injury. The degradative effects of injury + TNF-α appear to be due, in part, to the action of endogenous IL-6, as sGAG loss was partly abrogated by the IL-6 blocking Fab fragment. This result is also consistent with the increased loss of sGAG upon addition of exogenous IL-6 to the combination of TNF-α and mechanical injury. Histology observations (Fig. 2) suggest that the kinetics of cartilage degradation is not merely a consequence of the activities of proteolytic enzymes, but also depends strongly on the transport of cytokines, proteases, anti-IL-6 Fab and other cartilage biomolecules, which may be altered by overload injury. In conclusion, our study suggests that pro-inflammatory cytokines, whose productions are elevated by traumatic joint injury, can interact to potentiate cartilage catabolism. The mechanobiological (cell-mediated) responses to overload [5], as well as altered transport of cytokines and proteases in the damaged matrix, may both be affected by joint injury, making the damaged cartilage tissue more susceptible to further degradation by biochemical mediators.


Fig. 1. Effect of TNF ± Injury ± IL6/sIL-6R on (i) A-H GAG loss; (iii) A-H aggrecanase-generated fragments with NITEGE neoepitope.

Fig. 2. Toludine blue staining showing GAG loss with treatment and time; dotted line (in c,f) = explant boundary.

Fig. 3. Blocking endogenous IL-6 significantly decreased the GAG loss caused by the combination of TNF-α and mechanical injury.