INTRODUCTION: Chemokines produced by synoviocytes of the subacromial bursa are up-regulated in subacromial inflammation (bursitis) and rotator cuff disease. Stromal cell-derived factor 1 (SDF-1α; CXCL12) is an important chemotactic factor in the subacromial bursa that stimulates recruitment of inflammatory cells, however its mechanism of induction and regulation in the subacromial bursa are unknown. Interleukin-1β (IL-1β) and interleukin 6 (IL-6) are important chemokine regulators of inflammation, and have been shown to stimulate SDF-1α expression in rheumatoid synovium. We hypothesized that SDF-1α production in bursal synoviocytes may be induced by local pro-inflammatory cytokines, IL-1β and IL-6.

METHODS:

Bursal cell cultures: Subacromial bursal tissue was obtained intra-operatively from patients undergoing shoulder surgery (Columbia University IRB #6012, Rhode Island Hospital IRB #4047-08). Specimens were minced and enzymatically digested in Dulbecco’s minimum essential medium (DMEM) containing 1 mg/ml collagenase, 0.15 mg/ml DNase, and 0.15 mg/ml hyaluronidase for 1 hour at 37°C. The suspension was then passed through sterile gauze to remove any undigested fragments and the cells were seeded in 75 cm² flasks with 0.15 μg/ml streptomycin, and 0.1% fungizone (amphotericin B). Cells were grown at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. In order to verify that isolated cells included bursal synoviocyte cell populations, cultured cells were first labeled with fluorescent probes. Subsequently, the cells were magnetically labeled and separated using a MACS separator (Miltenyi Biotec, Auburn, CA) and analyzed by flow cytometry to determine cell lineage. (Figure 1) Cultured bursal cells were characterized as fibroblastic synoviocyte lineage (>80%) by positive CD44 expression.

Gene Expression: Early passaged cells were plated in 6 well plates and grown to 85% confluence. Wells were then treated with 10ng/mL of IL-1β for 0, 6, 24, and 48hrs. Cells were harvested by trypsinization and resuspended in lysis buffer. Total RNA was isolated from the cells using Qiagen RNeasy Mini Kit (Qiagen, Valencia, CA). First stranded cDNA was synthesized from 1 μg of total RNA using SuperScript TM Firststrand synthesis system for RT-PCR (Invitrogen, Carlsbad, CA) and the Thermo Pfx2 thermal cycler. Real time RT-PCR was performed for SDF-1α (Opticon 2, MJ Research) and results were standardized to GAPDH housekeeping gene. Statistical analysis was performed using the student’s t-test.

Protein Expression: After the first passage, cells were plated into 6-well plates and grown to 85% confluence. For dose response experiments, cells were then treated (in triplicate) with IL-1β 20ng/mL, IL-1β 10ng/mL, IL-6 20ng/mL, or BSA. After 24 hours, supernatants were collected and assayed for SDF-1α expression using Quantikine® Human SDF-1α enzyme immunoassay kit (R & D Systems, Minneapolis, MN) according to the manufacturer’s instructions. For time course experiments, supernatants were collected at 6, 24, and 48 hours post-stimulation with cytokines. Standard curves were reconstituted using SDF-1α protein. The optical density of each well was determined using a microplate reader set to 450nm wavelength. In order to control for variability, testing was performed on three different bursal cell lines. Statistical analysis was performed using the Student’s t test. Statistical significance was present when p<0.05.

RESULTS: SDF-1α expression was increased in bursal cells treated with IL-1β compared to controls (p<0.05). Maximal stimulation was seen after 48 hours (p<0.05) of induction with IL-1β, showing a nearly five-fold increase in SDF-1α expression (p<0.05). (Figure 2). An increase in expression was first observed after 6 hours and continued to increase thereafter (Figure 3). IL-6 caused a minimal but not statistically significant increase in SDF-1α expression.

DISCUSSION: These data demonstrate that SDF-1α production in human bursal synoviocytes may be induced by local pro-inflammatory cytokines, IL-1β and IL-6. IL-1β was a potent inducer of SDF-1α, causing a more than five-fold increase in SDF-1α gene and protein expression. This induction occurred early (SDF-1α gene expression increased at six hours), and the resultant levels of SDF-1α levels were at or above physiologic levels. Based on these results, one potential pathway of inflammation in rotator cuff disease may involve local expression of cytokines by resident bursal cells. IL-1β produced in response to rotator cuff injury induces SDF-1α expression, and may contribute to chronic inflammation in the subacromial bursa.