Interleukin-1-induced Subacromial Synovitis and Shoulder Pain in Rotator Cuff Disease

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ABSTRACT INTRODUCTION:

It is generally accepted that the subacromial bursa (SAB) is the main source of pain in rotator cuff disease. Histologically, the synovial tissue of the SAB tends to undergo proliferative or degenerative changes in rotator cuff disease, but the bursal inflammation does not include prominent inflammatory cell infiltration. This prompted us to examine the relationship between inflammatory cytokines which stimulate the peripheral nociceptors and shoulder pain in rotator cuff disease.

Interleukin-1β (IL-1β) and IL-1 receptor antagonists (IL-1ra) are considered to be an important reactive factor during inflammation. The ratio of IL-1β to IL-1ra is probably a critical factor in determining the severity of the inflammatory response. In rotator cuff disease, subacromial synovitis is responsible for the generation of the shoulder pain, and its severity may correlate with the intensity of pain. In the present study, we semi-quantitatively examined mRNA expression levels of IL-1β and two forms of IL-1ra (secreted IL-1ra (sIL-1ra) and intracellular IL-1ra (icIL-1ra)) and mRNAs using reverse transcriptase-polymerase chain reaction (RT-PCR) in rotator cuff disease. We also employed in situ RT-PCR to detect the cells producing these cytokine-mRNAs.

METHODS:

Synovial tissue specimens of the SAB were obtained from 39 patients with rotator cuff diseases during surgery. The average age was 56 yrs. The SAB specimens were harvested from the surrounding tissue of the greater tuberosity. The average duration of pain in the patients was 1 yr. Synovial tissue specimens were stored at -80°C for total cellular RNA isolation. Specimens were also fixed in 4% paraformaldehyde in phosphate buffer for 2 h at room temperature and embedded in paraffin. Control SAB specimens were obtained during surgery from 10 patients with anterior shoulder instability. These control groups had no resting pain at any time and showed no symptoms of subacromial impingement. All specimens were obtained with informed consent. The degree of shoulder pain was evaluated in each patient before the operation using the VAS (0 to 10 according to a subjective symptom scale: 0, no pain; 5, moderate; 10, severe pain). Patients' symptoms were recorded by hospital staff blind to this study.

Cytokine-specific cDNA fragments were amplified by the hot-start procedure with primers. PCR amplification of specific cDNA fragments of the cytokines was proportional to the amount of starting material under these conditions. PCR products separated from 3% agarose gels were blotted onto to membranes. The blots were hybridized with 32P-labelled oligonucleotide probes at 55°C for 16 h. Signal intensity was quantified with the Interactive Build Analysis System. The amounts of PCR product in each sample relative to the positive controls were calculated. β-actin was used as a control to ensure equivalent loading of RNA in each lane. Total cellular RNA prepared from the CHU-2 cell line was used as a positive control for IL-1β. RNA preparations from normal tissue were used as a positive control for sIL-1ra, and preparations from skin specimens were used for icIL-1ra.

Sections of 4μm thickness on silanized glass slides were used for in situ RT-PCR. The sections were deparaffinized, hydrated and air dried, and then fixed with 4% paraformaldehyde in phosphate buffer for 8 h at room temperature. RT and PCR were performed in chambers covered with concave silicon rubber diaphragms and steel cover clips in a sidecycler. The PCR products with DIG were incubated with rabbit anti-DIG antibody, and incubated with HRP-labelled sheep anti-rabbit antibody. Reaction products were visualized with 0.2% DAB for 5 min at room temperature. Successful PCR reactions were confirmed by treatment without DNase digestion or RT reaction.

Spearman’s rank correlation test was used for the analysis of possible relationships among the different parameters recorded in this study. The Mann-Whitney U-test was used for comparisons of parameters between controls and patients with rotator cuff diseases.

All specimens were obtained with informed consent and local ethics committee approval.

RESULTS SECTION:

The mRNA expression levels of the cytokines were significantly correlated with the degree of pain (IL-1β: r=0.782; secreted IL-1ra (sIL-1ra): r=0.756; intracellular IL-1ra (icIL-1ra): r=0.806, P<0.001, respectively). The relative ratio (sIL-1ra to icIL-1ra) mRNA significantly increased in proportion to the increase in IL-1β mRNA expression (r=0.767, P<0.001).

The in situ RT-PCR analysis indicated that both synovial lining and sublining cells produce IL-1β, while synovial lining cells predominantly produce icIL-1ra and sublining cells secrete sIL-1ra.

DISCUSSION:

This study demonstrated that mRNA expression levels of IL-1β, sIL-1ra and icIL-1ra in the SAB were correlated with the degree of shoulder pain in patients with rotator cuff diseases, and that the expression of icIL-1ra mRNA in synovial lining cells is relatively up-regulated compared with that of sIL-1ra in sublining cells, in proportion to the overexpression of IL-1β mRNA produced by both types of synovial cells. Based on these findings, we propose that the differential regulation of the two forms of IL-1ra in synovial cells in the SAB may play an important role in shoulder pain in patients with rotator cuff diseases.

The relative ratio (sIL-1ra:icIL-1ra) significantly increased with increased levels of IL-1β mRNA expression level. In human synovial fibroblasts and dermal fibroblasts, IL-1α mRNA is regulated in a time- and dose-dependent manner by lipopolysaccharide (LPS) and phorbol myristate acetate (PMA) (their relative IL-1α induction efficiency: PMA > LPS). In addition, LPS preferentially induces sIL-1ra mRNA, while PMA differentially induces icIL-1ra mRNA, suggesting that the regulation of the two forms of IL-1ra mRNA depends on the inflammatory response. In the present study, the relative production of sIL-1ra mRNA vs icIL-1ra mRNA in the SAB depended on the level of IL-1β mRNA expression at the site; the production of icIL-1ra mRNA was relatively higher compared with that of sIL-1ra, as the severity of subacromial synovitis worsened.

The differential localization of IL-1α mRNAs in this study is noteworthy. Our RT-PCR analysis demonstrated that relative to sIL-1ra mRNA, icIL-1ra mRNA is relatively up-regulated with higher expression levels of IL-1β mRNA. In situ RT-PCR demonstrated that synovial lining cells preferentially produce icIL-1α mRNA, while synovial sublining cells produce sIL-1α mRNA. Relative up-regulation of icIL-1α contributes to the inefficiency of counteracting IL-1α biological behavior. These results suggest a different inflammatory response between synovial lining and sublining cells in the SAB, and that up-regulation of icIL-1α mRNA production by synovial lining cells provides a disadvantage to counteract against IL-1-induced subacromial synovitis in rotator cuff diseases.

In conclusion, we suggest that the differential regulation of the two forms of IL-1α in synovial cells in the SAB may play an important role in shoulder pain in rotator cuff diseases, regulating IL-1-induced subacromial synovitis.