Legg-Calvé-Perthes Disease Produces Chronic Hip Synovitis and Elevation of Interleukin-6 in the Synovial Fluid

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Introduction: Legg-Calvé-Perthes disease (LCPD) is a childhood form of ischemic osteonecrosis of the femoral head which afflicts 1 in 740 boys and 1 in 3500 girls. The hip disorder can produce a permanent deformity of the femoral head and early osteoarthritis. Synovitis of the affected hip joint is a common feature of LCPD which produces pain, loss of hip joint motion, and poor clinical outcome. Very little research, however, has been performed to delineate the nature and the pathophysiology of the hip synovitis present in LCPD. The purposes of this study were to determine the chronicity of hip synovitis in LCPD, the inflammatory cytokine present in the synovial fluid at an active stage of LCPD, and a potential mechanism responsible for the elevation of interleukin-6 (IL-6), which was found to be significantly elevated.

Methods: The human subject and animal research were approved by the IRB and the IACUC, respectively, at the UT Southwestern Medical Center. Serial MRI was performed on 28 patients to assess the chronicity of the synovitis. T2-weighted and gadolinium-enhanced MR images were analyzed using Digimizer software by 2 independent observers. Synovial effusion and hyperemia were quantified as indicators of synovitis. Bio-Plex cytokine assay was used to determine the levels of 27 inflammatory factors present in the synovial fluid obtained at the time of surgery in 13 patients. Ischemic osteonecrosis of the femoral head was induced in immature pigs by applying a ligature tightly around the right femoral neck to disrupt the blood flow to the femoral head (osteonecrosis group). Un-operated contralateral femoral heads were used as controls. Histology, immunohistochemistry, and ELISA were performed to assess synovitis and IL-6 production in the hip tissues at 4 weeks post-ischemia. In vitro studies: Articular chondrocytes and synovial cells were isolated and cultured in normoxic (21% O2) or hypoxic (1% O2) condition to determine the effects of hypoxic stress on inflammatory cytokine expression (IL-1β, TNFα, IL-6) and IL-6 protein levels. The role of HIF-1 signaling on IL-6 up-regulation was investigated using HIF-1 siRNA (a loss of function experiment). Effects of hypoxic chondrocyte conditioned media on inflammatory cytokine response of synovial cells were investigated along with IL-6 receptor blockade using tocilizumab. Statistical analysis: Mann Whitney U test, student’s t-test, ANOVA and Tukey’s post hoc test (>2 groups) were used. A p value <0.05 was considered statistically significant.

Results: Serial MRI studies of 28 patients (mean age 7.6 ± 2.2 years) with active LCPD revealed chronic synovitis in LCPD (Fig 1A). MRI analysis showed 5.0 ± 3.3 fold and 3.1 ± 2.1 fold increase in the synovial effusion volume in the affected hip vs. the contralateral unaffected hip at the initial and the last follow up MRI, respectively, with the mean duration of 17.69 ± 8.3 months between the MRIs. The synovial...
enhancement on the contrast MRI was similarly increased indicating the chronic nature of the synovitis. In the synovial fluid of affected hip joint, IL-6 protein levels were significantly increased on the Bio-Plex Cytokine 27-plex Assay (LCPD: 509.26 ± 519.34pg/ml, non-LCPD: 18.8 ± 21.79pg/ml; p=0.002) (Fig 1B). Induction of ischemic osteonecrosis of the femoral head in piglets also produced increase synovitis, synovial effusion, and IL-6 elevation in the synovial fluid. Immunostaining and ELISA showed that articular chondrocytes of the femoral head had significantly increased IL-6 production. In vitro studies revealed that hypoxic culture conditions (1% oxygen) increased IL-6 production by articular chondrocytes via HIF-1α activation since HIF-1 activator, deferroxamine increased IL-6 expression in a dose dependent manner and HIF-1 inhibition by siHIF-1 reduced IL-6 expression by 94% (p<0.01), levels comparable to normoxic culture conditions (Figure 2A). Treatment of primary pig synovial cells with hypoxic chondrocyte conditioned medium increased gene expression of IL-1β and TNFα. The enhanced inflammatory cytokine response was mediated by IL-6 in the hypoxic conditioned medium since blockade of IL-6 receptor on synovial cells with tocilizumab significantly decreased the cytokine response (Figure 2B).

Discussion: Our study demonstrates that chronic hip synovitis is present in LCPD with significant elevation of IL-6 in the synovial fluid during the active stage of the disease. Hypoxic stress induces IL-6 production from articular chondrocytes which induces inflammatory cytokine response from synovial cells. IL-6 blockade mitigated the inflammatory cytokine response and may be a potential therapeutic strategy for treating synovitis in LCPD. These findings provide new insight and direction to treat LCPD. In pediatric inflammatory arthropathies, like systemic juvenile idiopathic arthritis, identifying the role of IL-6 has resulted in an effective treatment using IL-6 receptor blocker, tocilizumab³.

Significance: Our study establishes LCPD as having a chronic inflammatory component with a significant elevation of IL-6 in the synovial fluid. Given our findings, it would be important to further investigate the role of IL-6 in the pathophysiology of synovitis in LCPD and how it affects the bone healing.
Figure 1: Chronic synovitis and elevation of IL-6 protein levels in the synovial fluid in LCPD (A) Left panel: increased synovial fluid (yellow arrows) in non-contrast T2-weighted image. Right panel: Increased synovial enhancement (red arrows) in post-contrast subtraction image. (B) IL-6 protein levels in the synovial fluid of patients.
**Figure 2**

(A) IL-6 mRNA levels in articular chondrocytes cultured under hypoxic or normoxic conditions for 6h following transfection with siControl or siHIF-1α for 24h.

(B) mRNA levels in normal synovial cells treated with normoxic (NCM) or hypoxic (HCM) chondrocyte conditioned medium and IL-6 receptor blocker, Tocilizumab (100μg/ml) for 6h. * Vs NCM, # Vs (-) Tocilizumab. ANOVA and Tukey’s test * p=0.05, ** p<0.01.