Immune Dysregulation as a Novel Pathogenetic Mechanism in Glucocorticoid-Induced Osteonecrosis

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INTRODUCTION: Osteonecrosis (ON) is a debilitating skeletal disorder that accounts for approximately 10% of all total hip replacement procedures. In systemic lupus erythematosus (SLE), the reported prevalence of osteonecrosis ranges from 1.7% to 52%, and high-dose glucocorticoid (GC) therapy is strongly associated with its development. Although the hips and knees are the most commonly affected areas, ON can involve multiple anatomical sites and frequently remains undetected until pain appears, by which time bone death is already advanced. At present, preventive strategies and disease-reversing therapies remain limited. GCs engage several pathways that can initiate and propagate osteonecrosis. Proposed mechanisms for this process include impaired angiogenesis, microvascular injury, hypercoagulability, apoptosis of osteocytes, osteoblasts, and endothelial cells, and a shift of marrow stromal cells toward adipogenesis with fat hypertrophy that elevates intraosseous pressure and compromises perfusion (1). While GC therapy is often used to treat inflammatory diseases, including SLE, only some patients who are treated with GC develop ON. Moreover, the underlying cellular and molecular mechanisms of ON pathogenesis remain poorly understood.

METHODS: A prospective, single-center study was conducted enrolling adults with or at-risk for ON based upon high-dose GC exposure. Herein, we present the results of a sub-group with SLE, grouped according to the presence or absence of osteonecrosis (2). Baseline assessments included age, sex, body mass index (BMI), disease duration, and the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K). Glucocorticoid exposure was evaluated based on patient report and medical record. Blood samples were collected. Serum cytokines were measured by ELISA. Peripheral blood mononuclear cells (PBMCs) were profiled by flow cytometry analysis. Osteoclastogenesis assays were performed by differentiating $CD14^+$ monocytes with macrophage colony-stimulating factor (M-CSF) and RANKL, in the presence or absence of dexamethasone. RNA sequencing was performed to characterize the gene expression signatures in osteoclasts. Tartrate-resistant acid phosphatase (TRAP)-positive multinucleated osteoclasts were imaged and counted. Co-culture experiments were conducted using activated T cells to assess their effect on osteoclast differentiation. This study was approved by our institutional IRB.

RESULTS: A total of 28 patients with systemic lupus erythematosus (SLE) were analyzed, of whom 18 had osteonecrosis (SLE+ON group) and 10 did not (SLE-ON). Demographic and clinical parameters between the two groups were comparable in terms of age, sex and body mass index (BMI), whereas disease duration was longer and total cumulative GC dose was higher in the ON group. The mean age was 41.2 ± 13.0 years in the SLE+ON and 39.9 ± 12.6 years in the SLE-ON ($p = 0.1647$). Women comprised the majority in both cohorts (SLE+ON: 16/18, 89%; SLE-ON: 8/10, 80%). Stimulation with glucocorticoids enhanced $TRAP^+$ multinucleated osteoclast formation of $CD14^+$ monocytes from SLE-ON. In contrast, $CD14^+$ monocytes from SLE+ON showed impaired enhancement of differentiation into $TRAP^+$ multinucleated osteoclasts by GCs stimulation. Transcriptomic profiling by bulk RNA sequencing showed that the interferon (IFN)-response gene set was enriched in osteoclasts derived from ON patients compared to those from control patients, suggesting the enrichment of inflammatory and IFN signatures in the ON group. Consistently, serum CXCL10, a well-known IFN target, significantly increased in ON patients compared to SLE-ON. Immunophenotyping analysis showed alterations in circulating monocyte subsets and a significantly lower CD4/CD8 T cell ratio in the ON group. Especially, cytotoxic CD8+ T cells, with increased frequencies of IFN-γ^+ and tumor necrosis factor $(TNF)^+$ cells, were higher in the ON group compared to the control group, while regulatory CD8 T cells did not differ between groups. CD8+ T cells in ON patients also express higher levels of IFN-γ and TNF. Furthermore, co-culture with activated T cells from ON group markedly depleted osteoclasts. Collectively, these findings indicate that ON patients exhibit impaired osteoclast differentiation and reduced GCs sensitivity in the setting of heightened CD8 T cell activation and IFN-driven inflammation.

DISCUSSION: Our research highlights the cellular dynamics and immune changes in SLE patients with ON. Our study revealed osteoclast-specific activation of IFN-response genes in ON patients, along with increased IFN-γ+TNF+ CD8+ T cells and elevated serum CXCL10. These findings suggest that osteonecrosis in SLE patients is linked to impaired osteoclast differentiation and altered glucocorticoid responsiveness. Activated CD8+ T cells were more common in patients with osteonecrosis, and the secretion of IFN-γ from CD8+ T cells leads to reprogramming differentiation of bone cells and exacerbation of inflammatory responses. Our study suggests that in ON patients, altered immune cell profiles, imbalanced serum cytokines, and changes in glucocorticoid response, along with increased chronic inflammatory signaling, disrupt glucocorticoid-mediated regulation in bone cells, contributing to the development of ON. Overall, these findings support a model in which activated CD8+ T cell–driven responses impair the osteoclastogenic program, which further interrupts the removal of dead bone and bone remodeling in GC-treated patients.

SIGNIFICANCE/CLINICAL RELEVANCE: These findings show that specific gene patterns in immune cells and a dysregulated profile of cytotoxic T cells is associated with ON and may serve as a predictive factor for those patients most at risk for glucocorticoid-induced ON. Targeting this immune-bone interaction may lead to new treatments to prevent or manage osteonecrosis in affected patients.

REFERENCES: (1) Kaneko K et al. *Clin Transl Med*. 2021 11(10):e526. (2) Krez A et al. *Osteoporosis Int.* 2021 32(10): 2095