B Lymphocyte Regulation in Fracture Healing

Katherine Lefferts[1], Jessica Cottrell[1]
[1]Seton Hall University, South Orange, NJ
katherine.lefferts@student.shu.edu, jessica.cottrell@shu.edu

Disclosures: The authors have nothing to disclose

INTRODUCTION: B lymphocytes act as key regulators of bone fracture healing outcomes by modulating the inflammatory response and subsequent tissue repair during fracture healing [1]. Bone fractures are a worldwide public health issue that affects millions of people a year. In addition to the detrimental physical effects of a fracture to an individual, bone fractures also cause considerable national economic burdens. Fractures are a prevalent injury and one of the most common causes of non-fatal burden, expressed as years live with disability [2]. Approximately 5-10% of fractures do not heal, leading to long-term disability [3]. Bone is maintained through modeling and remodeling, and these processes are also central to fracture healing [4]. Under homeostatic conditions, new bone is formed prior to resorption. During fracture healing, bone remodeling only occurs after bone resorption. B lymphocyte interplay with other immune cells and signaling pathways orchestrates the balance between inflammation and tissue regeneration. The exact mechanisms in B lymphocyte-driven fracture healing are unclear. Our primary objective is the characterization of the mechanisms through which B lymphocytes influence bone healing. We aim to elucidate how B lymphocyte signaling impacts osteoclasts, the cells responsible for bone resorption, during the bone remodeling process. To achieve this, we have employed a combination of in vitro 3D models and in vivo murine models, providing insight into how B cell-derived factors modulate immune responses and bone remodeling processes. Our in vitro research focuses on understanding the impact of B cell-derived factors on bone cell interactions. We have previously developed an in vitro 3D bone model [5], which will be utilized to study the effects of different B cell subtypes and associated signaling molecules on osteoblast and osteoclast coupling. This model enables us to explore changes in the 3D homeostatic environment over time. In vivo studies using an established mouse fracture model will focus on examining temporal fluctuations and localization of B lymphocyte subsets within the fracture site. By integrating in vitro and in vivo approaches, our research aims to provide a comprehensive understanding of the role of B lymphocytes in bone fracture healing, potentially paving the way for targeted therapeutic interventions in the future.

METHODS: This study utilized an established closed femur fracture model. 13-14 week old (at arrival) female Swiss-Webster WT mice (Charles River) were used for this study. Experimental protocols were approved by the Rutgers-New Jersey Medical School Institutional Animal Care and Use Committee (IACUC). A transverse mid diaphyseal fracture was made in the right, pinned femur using a 3-point bending apparatus and femurs were harvested at days 0,1, 2, 4, 7, and 10 post-fracture (n=7). The collected femurs were embedded, sectioned, and stained for antibody markers for B lymphocytes (CD19), T lymphocytes (CD3e), macrophages (F4/80), and neutrophils (EPR). For a subset of femurs, the intramedullary canals were aspirated and the aspirate was analyzed for cytokine and gene expression by qPCR and ELISA. Raw 264.7 and MC3T3 fluorescing murine cell lines were cultured according our established 3D models [5,6]. This 3D bone organoid model consists of MC3T3-E1 cells embedded into a hydrogel matrix that are induced to differentiate into osteoblasts and deposit a mineralized matrix. RAW 264.7 are added to the model and induced into osteoclast differentiation. To assess changes in osteogenic and osteoclastogenic proliferation, differentiation, and activity, the cell cultures were stained. In vitro samples were analyzed for cytokine and gene expression by qPCR and ELISA assays. Immunohistochemistry results were analyzed and quantified using QuPath software. Statistical analysis for all results was performed using GraphPad Prism software.

RESULTS SECTION: B lymphocyte CD19+ vs. macrophage F4/80+ fluctuations demonstrate a statistically significant correlation (P=0.0490). This correlation was not observed in CD19+ cells compared to CD3e+ or EPR+ cells.

DISCUSSION: Our results show a decrease in B lymphocytes during the initial phase of fracture healing. Contemporary research has demonstrated that B cells decrease in the early phases of fracture healing. Our research indicates a correlation between fluxes in the B lymphocyte population within bone marrow during fracture healing and the macrophage population. Research has established that there is significant bidirectional cross-talk between these cells.

SIGNIFICANCE/CLINICAL RELEVANCE: By integrating in vitro and in vivo approaches, our research aims to provide a comprehensive understanding of the role of B lymphocytes in bone fracture healing, potentially paving the way for targeted therapeutic interventions in the future. The comparison of in vivo and in vitro allows insights into B lymphocyte regulation of osteoclasts in an organism with metabolic and systemic effects and how these effects differ in a closed system like the 3D model. Further, our 3D model allows uses a cost-effective 3D model of bone homeostasis, which is a resourceful and ethical approach to studying relevant physiological systems while reducing reliance solely on in vivo models.

REFERENCES:
1. Rosser, E.C., P.A. Blair, and C. Mauri, *Cellular targets of regulatory B cell-mediated suppression.* Molecular Immunology, 2014. **62**(2): p. 296-304.
2. Wu, A.-M., et al., Global, regional, and national burden of bone fractures in 204 countries and territories, 1990-2019: a systematic analysis from the Global Burden of Disease Study 2019. The Lancet Healthy Longevity, 2021. 2(9): p. e580-e592.
3. Holmes, D., Closing the gap. Nature, 2017. 550(7677): p. S194-S195.
4. Wang, W. and K.W.K. Yeung, *Bone grafts and biomaterials substitutes for bone defect repair: A review.* Bioact Mater, 2017. **2**(4): p. 224-247.
5. Fuller, J., Lefferts, K. S., Shah, P., & Cottrell, J. A. (2024). Methodology and Characterization of a 3D Bone Organoid Model Derived from Murine Cells. *International journal of molecular sciences*, *25*(8), 4225. https://doi.org/10.3390/ijms25084225
6. Adams, M., & Cottrell, J. (2024). Development and characterization of an *in vitro* fluorescently tagged 3D bone-cartilage interface model. *Frontiers in endocrinology*, *15*, 1484912. https://doi.org/10.3389/fendo.2024.1484912

ACKNKOWLEDGEMENTS: We would like to thank J. Patrick O'Connor and Maya Scott of Rutgers-New Jersey Medical School for their ongoing collaboration.

IMAGES AND TABLES: