The impact of regulatory T cell plasticity on bone fracture healing

Ann-Kathrin Mess[1,3], Christian H. Bucher[1,3], Agnes Ellinghaus[1,3], Hans-Dieter Volk[2,3], Georg Duda[1,3], Katharina Schmidt-Bleek[1,3]
[1]Julius Wolff Institute, Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Germany, [2]Institute of Medical Immunology, Charité - Universitätsmedizin Berlin, Germany, [3] Berlin Center for Regenerative Therapies, Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Germany
ann-kathrin.mess@bih-charite.de

Disclosures: None

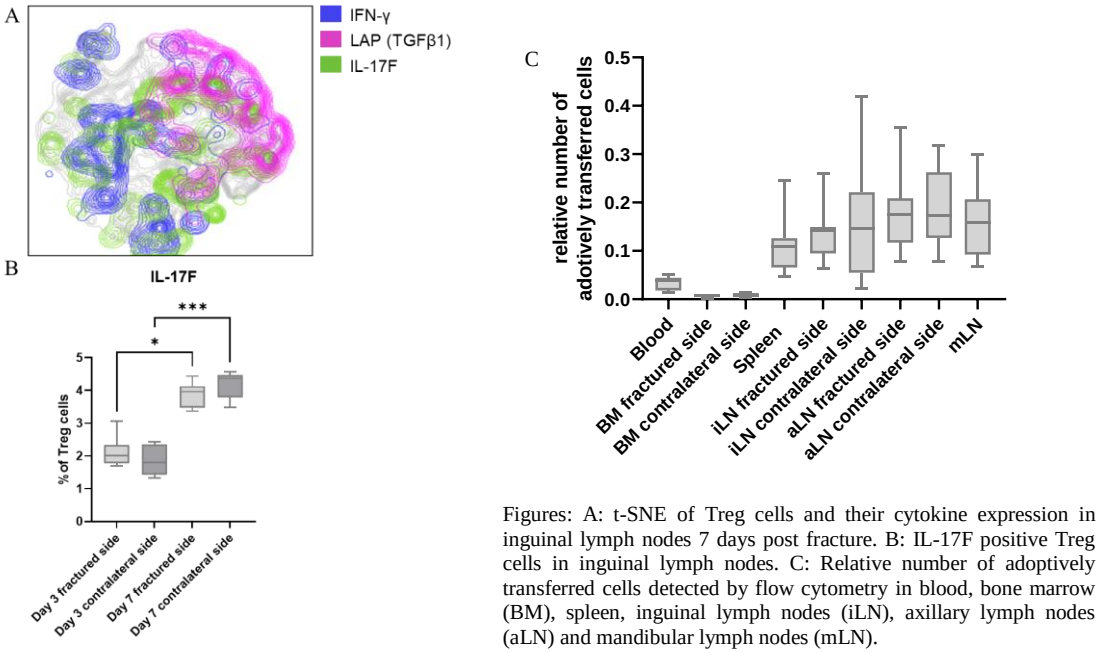
INTRODUCTION: Bone fracture repair displays a unique healing process, resulting in newly formed scar free bone. However, healing progression may be hampered and result in delayed healing or nonunion formation. Currently, there is a lack of therapies treating these patients. Regulatory T cells (Treg) are specifically involved in the early tissue repair phases and can help to promote healing. This makes Treg cells a promising target for novel regenerative therapy developments. We have shown that enriching Treg cells in a mouse osteotomy model boosts healing. More specifically, the systemic ratio of CD8+ effector memory T cells (Tefm) to Treg cells appears to be essential. Elevated Treg cell numbers improve bone healing while higher levels of Tefm cells can even lead to a detrimental healing outcome. We hypothesize that an increased systemic level of Tefm cells affects the phenotype and functionality of Treg cells during bone healing and therefore dampens their positive effect on tissue repair. Thus, the objective of this study is to investigate the role of Treg cell plasticity in fracture repair, especially with regards to their activation an immunoregulatory functions in dependency on the presence of Tefm cells.

METHODS: This study was approved by the State Office for Health and Social Affairs – LAGeSo. Treg cells of a congenic mouse line were adoptively transferred to C57BL/6N mice prior to an osteotomy. 3 and 7 days post osteotomy, donor and host cells were characterized by flow cytometry in blood as well as lymphoid organs and bones. Popliteal lymph nodes were cryo-embedded and histological sections were stained to identify adoptively transferred Treg cells and their potential loss of Foxp3 expression. Inguinal lymph nodes on the fractured and contralateral side were analyzed in their cytokine expression, including IFN-γ, IL-17F and LAP (TGFβ1), on day 3 and 7 post osteotomy. Statistical analyses were performed with GraphPad Prism including Mann-Whitney T-test, Kruskal-Wallis test, Wilcoxon rank sum test and ANOVA ($p<0.05$).

RESULTS: Unlike previously assumed, we found that adoptively transferred cells predominantly migrate to lymphoid organs and especially lymph nodes, but barely to the bone marrow itself. While congenic cell numbers were relatively coherent in mandibular and axillary lymph nodes, the highest absolute numbers were detected in inguinal lymph nodes on the fractured side. Adoptively transferred Treg cells and their proliferation was also identified in popliteal lymph nodes by immunofluorescence staining. We observed morphological changes and cell proliferation but most striking were significant differences in the secretion of IL-17F, IFN-γ and TGFβ1 in inguinal lymph nodes on the fractured and contralateral side 3 and 7 days post osteotomy. In addition, the expression of activation markers, such as CD69 was increased. This aligned with an elevated occurrence of peripheral Tefm cells. Also, the expression of PD-1 and co-expression of Foxp3 and RORγ(t) in Treg cells increased with a systemically higher percentage of Tefm cells.

DISCUSSION: Collectively, our data illustrate a strong interplay of Treg and Tefm cells during bone regeneration. Our findings give insights on the molecular mechanisms of Treg cell therapies to enhance bone repair. Unlike previously assumed, adoptively transferred Treg cells do not migrate to the bone marrow of a fractured bone, but migrate to and proliferate in lymphoid organs, especially lymph nodes close to the fracture. Our results indicate the key role of neighboring lymph nodes in fracture scenarios and the pro-regenerative effects of Treg cells from systemic sources rather than from the bone marrow. High levels of Tefm cells and with that a rather pro-inflammatory milieu impacts the immunomodulatory properties of Treg cells in a fracture setting. Correlating phenotypical instability indicated by an increased co-expression of Foxp3 and RORγ(t) is likely to promote an inflammatory milieu even further, resulting in hindered healing. In accordance with that, these Treg cells showed signs of exhaustion. Our findings illustrate the relevance of the T cell interplay and call for further analysis of the phenotypical stability of Treg cells to pave the ground for future clinical application of such strategies to treat fracture patients.

SIGNIFICANCE/CLINICAL RELEVANCE: Regulatory T cell therapies may also be a promising tool to treat complicated nonunion settings in patients, yet the phenotypical stability of Treg cells needs to be ensured. This study discussed the interplay of Treg cells and Tefm cells, targeting a key point for safe and efficient immune modulatory treatment strategies.



Figures: A: t-SNE of Treg cells and their cytokine expression in inguinal lymph nodes 7 days post fracture. B: IL-17F positive Treg cells in inguinal lymph nodes. C: Relative number of adoptively transferred cells detected by flow cytometry in blood, bone marrow (BM), spleen, inguinal lymph nodes (iLN), axillary lymph nodes (aLN) and mandibular lymph nodes (mLN).