Draining lymph nodes orchestrate immune modulation and bone regeneration after fracture

Ann-Kathrin Mess[1,2], Christian H. Bucher[1,2], Agnes Ellinghaus[1], Merle Kochan[1], Georg N. Duda[1,2], Katharina Schmidt-Bleek[1,2]

[1]Julius Wolff Institut, Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Germany, [2] BIH Center for Regenerative Therapies, Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Germany

Background and Research Questions

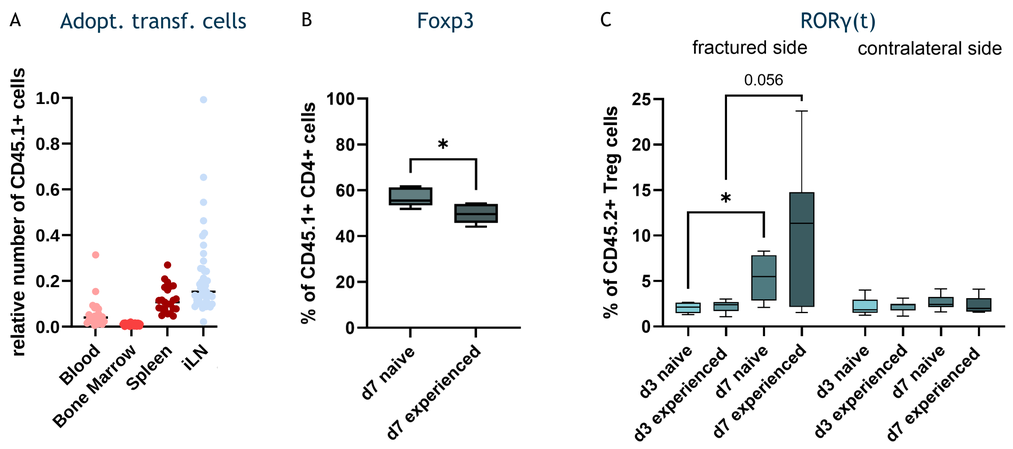
- Up to 30% of bone fracture patients face delayed healing/non-union[1]
- Patient-specific immune status impacts local immune reaction post-fracture and healing outcome, increased risk for delayed healing in elderly and „immune-experienced“ patients
- Adoptive transfer of regulatory T cells (Treg) leads to 50/50 healing outcome in „immune-experienced“ mice

1. Why are adoptively transferred Tregs only partially successful?
2. What are immune responses in lymph nodes post-fracture and their impact on regeneration?
3. Does a removal of the draining lymph node impact fracture repair?
4. Does a lymph node-bone axis play a crucial part in bone healing?

1

Adoptively transferred Tregs migrate to draining lymph node and are affected my immune experience





Firgure 1: A: Adopt. transf. Tregs predominantly migrate to the draining LN after 7d. B: Immune experience impacts Foxp3 expression - C: -and co-expression of RORγ(t) in draining LN (n=6), Welch‘s t-test *p<0.05 **p<0.01 ***p<0.001

2

Draining lymph nodes are marked by an inflammatory gene expression landscape post-fracture

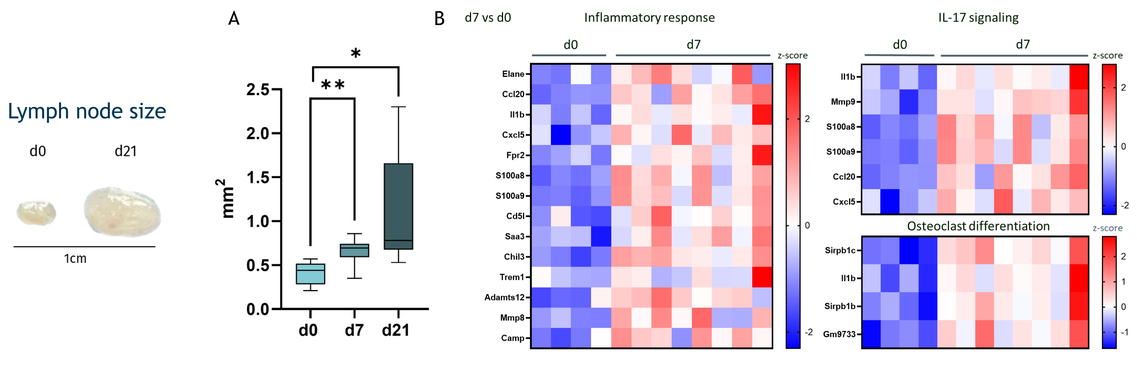


Figure 2: Lymph nodes in fracture settings. A: Lymph node size 7 and 21 days post-osteotomy (n=8), Welch‘s t-test *p<0.05 **p<0.01 ***p<0.001. B: Heatmaps of bulk RNA-sequencing data showing genes associated with selected signaling pathways, identified by KEGG pathway enrichment analysis, comparing d7 vs d0 in naive mice.

3

Removal of draining lymph nodes post-fracture improves healing

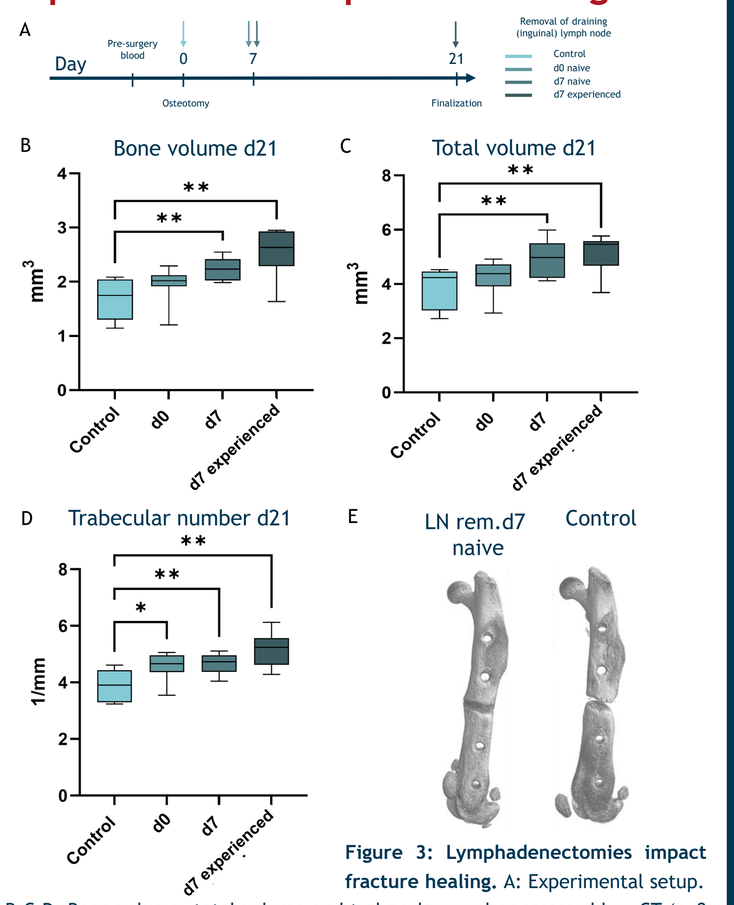


Figure 3: Lymphadenectomies impact fracture healing. A: Experimental setup. B,C,D: Bone volume, total volume and trabecular number assessed by µCT (n=8 per group), Welch‘s t-test *p<0.05 **p<0.01 ***p<0.001. E: µCT-rendered images of femurs of lymphadenectomized mouse and control.

4

Lymphatic vessels infiltrate fracture sites and bone marrow in delayed healing settings

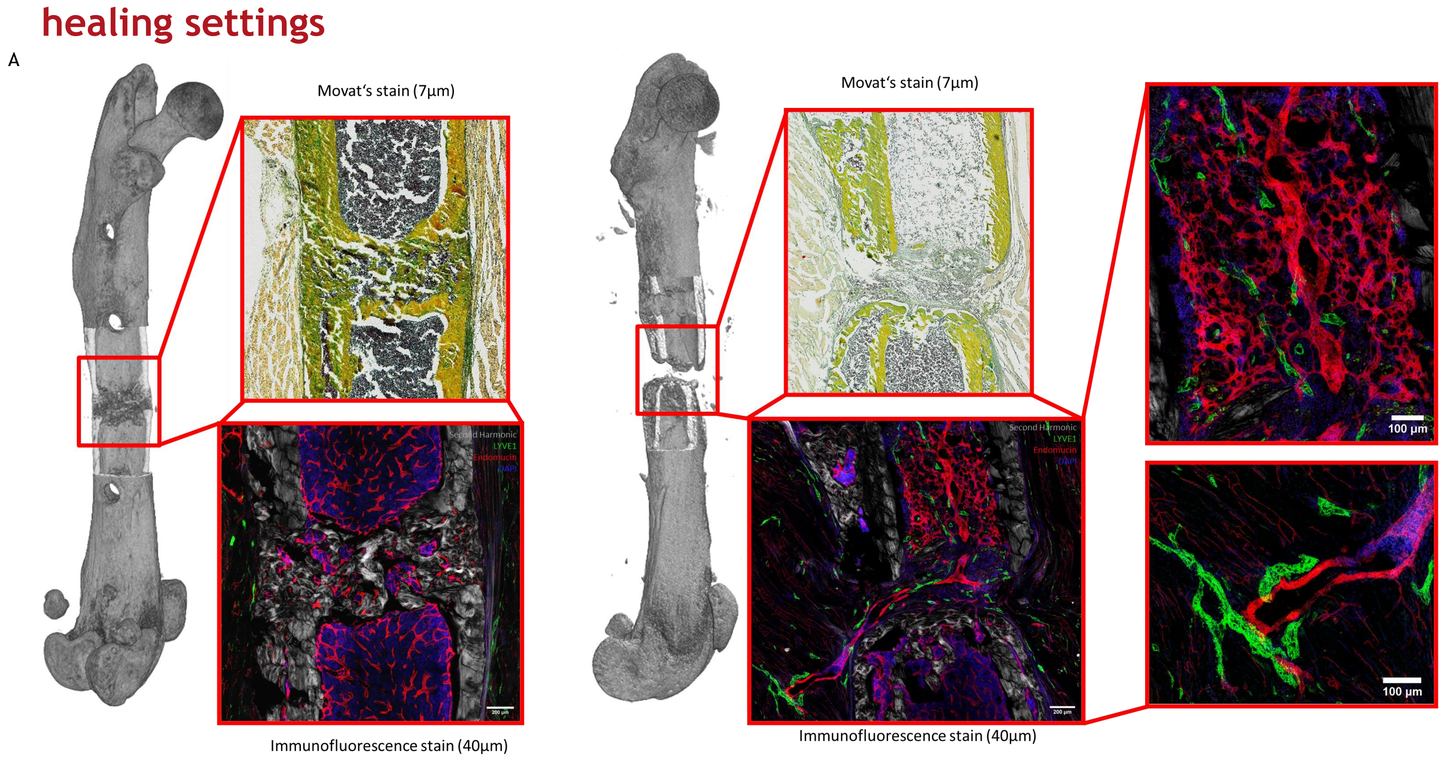
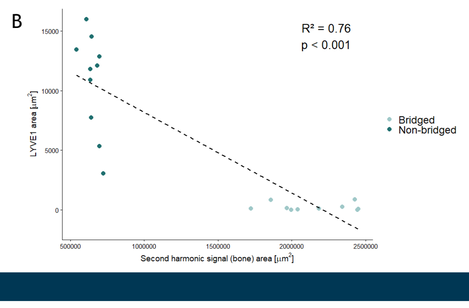


Figure 4: Lymphatic vessels in fracture settings. A: µCT-rendered images and histological stainings of the respective bones. Shown are Movat‘s stains on 7µm thick sections showing mineralized bone (yellow), nuclei (black), loose connective tissue (light blue), cartilage (green to blue), bone marrow (purple) and muscle (light yellow-orange). Immunofluorescent stainings on 40µm thick sections show DAPI (blue), second harmonic signal of bone (grey), LYVE-1 of lymphatic vessels (green) and endomucin of blood vessels (red) 21 days post-osteotomy. B: Pearson correlation of LYVE-1 and second harmonic signal area in bridged and unbridged bones 21 days post-osteotomy. N=8 in total with min. two 40µm sections stained and analyzed per bone.



Conclusion

- Adopt. transf. Tregs migrate to draining lymph nodes and are affected by systemic immune experience
- Lymph nodes harbour a great inflammatory milieu post-fracture
- Lymphadenectomies can improve healing
- Delayed healing scenarios revealed lymphatic vessel infiltration

The lymph node-bone axis plays a crucial part in fracture healing.

[1]In house results of a prospective multicenter clinical study with over 600 patients with long bone fractures

www.bihhealth.org www.jwi.charite.de

Contact:
Ann-Kathrin Mess
ann-kathrin.mess@bih-charite.de

CENTRUM FÜR MUSKULOSKELETALE CHIRURGIE

CHARITÉ

JULIUS WOLFF INSTITUT
BIH Center for Musculoskeletal Biomechanics and Regeneration