The role of Wnt5a in T cell balance in Osteoarthritis

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INTRODUCTION: Osteoarthritis (OA) is nowadays regarded as a chronic inflammatory joint disease characterized by cartilage destruction, pain, and synovitis. Macrophages and T cells are the major constituents of OA synovial immune infiltrates[1]. An increased ratio of Th17 (a pro-inflammatory T cell subtype) vs. Treg (an anti-inflammatory T cell subtype) was found in OA patients and contributed to OA progression[2,3]. However, the mechanisms that control the balance of Th17 vs. Treg in OA remain largely unknown. Here, we demonstrated that synovial macrophage derived Wnt5a plays a pivotal role in OA development by disrupting the balance of Th17 vs. Treg in the OA joint, thus promoting local inflammation and joint damage.

METHODS: Animal models: All animal experimental procedures are approved by IACUC at Tufts University. 12-week-old male C57BL/6 wild type mice were subjected to anterior cruciate ligament transection (ACLT) or Sham surgery. 6 weeks later, the knee joint draining lymph nodes (LNs), popliteal lymph nodes and inguinal lymph nodes, and the whole knee joints were harvested. To determine the role of macrophage derived Wnt5a *in vivo*, lentivirus mediated Wnt5a overexpressing bone marrow-derived macrophages (BMDMs, Mφ-Wnt5a) were intraarticularly injected into the knee joints ($5x10^5$cells/joint) at 5 weeks after surgery (Fig. 3A). BMDMs overexpressing GFP (Mφ-GFP) were injected as a control. 1 week after injections, LNs and knee joints were harvested. Total number of T cells and the proportion of Th17 and Treg cells in the LNs (n=5/group) were assessed by flow cytometry. Saf.O staining, F4/80 (macrophage marker) and Wnt5a Immunohistochemistry (IHC) and Immunofluorescent (IF) staining were performed on the joint tissue sections. Numbers of macrophages and Wnt5a positive cells in the synovium were counted using Image J (n=3-4/group). ***In vitro* analysis:** All human materials and experiments are approved by IRB at Tufts University. Human peripheral T cells were subjected to *in vitro* differentiation of Th17 or Treg cells, with or without the treatment of rhWnt5a (100 ng/mL). Marker genes (Rorγt for Th17, Foxp3 for Treg) were detected by RT-qPCR and normalized by the internal control, TBP. Differential expression proteins were obtained by Reverse Phase Protein Array (RPPA, MD Anderson Core Facility). **Statistics:** Student's t-tests or ANOVA modeling with planned contrasts were done in Prism. Data are shown as mean ± standard error. *: p<0.05, **: p<0.01, ***: p<0.005, and ****: p<0.001, ns: not significant.

RESULTS: 1. T cell number and the ratio of Th17/Treg increased in ACLT-OA. Representative Saf. O staining shows that the ACLT-OA group has obvious cartilage destruction and synovitis 6 weeks after ACLT surgery (Fig. 1A&1B). Compared to the sham group, the ACLT-OA group has significantly more activated T cells ($CD4^+CD44^{hi}CD62L^{lo}$ effector T cells, Fig. 1C) and markedly higher Th17/Treg ratio (Fig. 1D) in the knee joint draining lymph nodes, consistent with the studies from human OA serum[3].

2. Wnt5a is increased in OA synovium and expressed mainly in macrophages. By analyzing DNA array databases and conducting RT-qPCR in OA patient samples, we found Wnt5a increased the most among all Wnts in OA (data not shown). This was confirmed by IHC staining in ACLT-OA joint sections, where the average number of Wnt5a positive cells was significantly higher in the ACLT-OA group than the Sham group. In addition, macrophages became more abundant in the ACLT-OA joints, showing a similar trend as Wnt5a positive cells (Fig. 2A). Immunofluorescence staining demonstrated that in ACLT-OA synovium, Wnt5a predominantly expressed in synovial macrophages (Fig. 2B).

3. Ectopic Wnt5a elevates the ratio of Th17/Treg both *in vitro* and *in vivo*. 5 weeks after ACLT surgery, Mφ-Wnt5a were injected into the joints to test whether Wnt5a expression from macrophages was sufficient to regulate the synovial T cell balance (Fig. 3A). Compared with the Mφ-GFP group, Mφ-Wnt5a increased synovitis and the ratio of Th17/Treg in the ACLT-OA group (Fig. 3B). To determine how Wnt5a affects the T cell ratio, human peripheral T cells were subjected to *in vitro* differentiation of Th17 and Treg cells. With the treatment of rhWnt5a, Rorγt (Th17 marker) was induced and Foxp3 (Treg marker) was reduced, resulting an elevated ratio of Th17 and Treg (Fig. 3C). These data suggest that Wnt5a promoted Th17 and inhibited Treg differentiation. Interestingly, Wnt5a ectopic expression in macrophages did not overtly alter macrophage signature gene expression (data not shown), suggesting the effects of Wnt5a on T cells were not due to changing macrophage phenotypes. To gain mechanistic insights, the differentiated Th17 and Treg cells (with or without Wnt5a treatment) were subject to proteomic analysis via RPPA. More than 400 proteins were measured, and the representative heatmap shows that 68 proteins exhibited significantly differential expression (Fig. 3D, Treg Ctrl vs. Treg+Wnt5a), suggesting that a complex Wnt5a downstream signaling contributed to the altered Th17/Treg ratio.

Fig.1 T cell number and Th17/Treg ratio are increased in ACLT-OA.

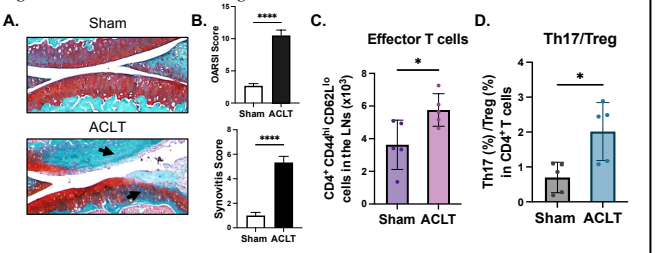


Fig.2 Wnt5a is increased in OA synovium and expressed in macrophages.

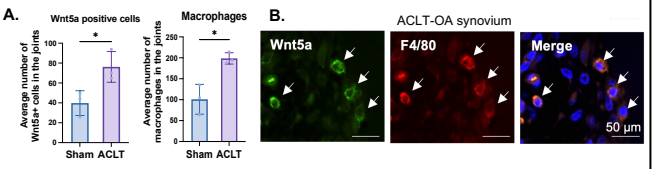
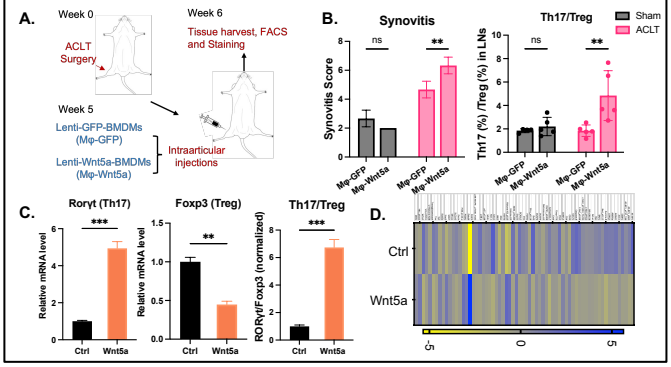


Fig.3 Ectopic Wnt5a elevates the ratio of Th17/Treg both in vitro and in vivo.



DISCUSSION: Our data suggests that macrophage derived Wnt5a rearranges the balance of Th17/Treg by promoting Th17 and suppressing Treg in OA circumstances, thus enhancing OA joint inflammation. However, not all the synovial macrophages express Wnt5a, indicating the heterogeneity of macrophages. It will be interesting to explore the diversity of macrophages, their interactions with other immune cells, and how they contribute to OA development. In addition, which pathway Wnt5a goes through and what signaling Wnt5a perturbs to regulate the Th17/Treg balance in OA still need to be elucidated.

SIGNIFICANCE: This study is the first to investigate the regulating mechanisms of T cell subtype balance in OA unique inflammatory microenvironment and the first to determine the function of Wnt5a on altering such balance. The success of this work will provide important insights into OA treatment strategies.

REFERENCES: [1] Li YS, Front Immunol. 2017. [2] Penatti A. Arthritis Res Ther. 2017. [3] Ye X. Mol Med. 2021