Synovial Regulatory T Cells Mitigate Posttraumatic Osteoarthritis Progression After ACL Rupture in Mice

Xueyou ZHANG[1], Mingde Cao[1], Bruma Sai-Chuen Fu[1], Michael Tim-Yun Ong[1,2], Patrick Shu-Hang YUNG[1,2]
[1]Department of Orthopaedics & Traumatology, Faculty of Medicine, The Chinese University of Hong Kong
[2] CUHK medical center, department of Orthopaedics & Traumatology, Faculty of Medicine, The Chinese University of Hong Kong.
xueyou@link.cuhk.edu.hk

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INTRODUCTION: The prolonged inflammatory response following joint trauma is critical for the progression of posttraumatic osteoarthritis (PTOA). The injury provides a distinct time point for early intervention to prevent or slow disease progression. Regulatory T cells (Tregs) are known to modulate inflammation and maintain immune homeostasis. Studies have identified Tregs in synovium in OA-progressed patients. However, the role of Tregs in the early stages following knee joint trauma, before the onset of OA, remains unclear. To address this gap, we investigated how Tregs engaged within the synovium following trauma and contributed to PTOA development.

METHODS: 1) 10-week-old male C57BL/6 mice underwent non-invasive ACL rupture or sham operation (n=5~7/timepoint/group) at the right knee. Synovium was collected once from the uninjured sham group, and at day 3, week 1, 2, 4 post-ACL rupture. Flow cytometry and immunofluorescence staining were used to investigate the presence, quantity, and functionality of Tregs. 2) To selectively expand synovial Tregs following injury, adeno-associated virus (AAV) vector expressing CMV-IL-2-mcherry or the AAV-CMV-mcherry control vector was of intra-articular (I.A.) administration (5×10^8 vector genomes/10 µL/mouse, n=10/group) one week before injury. Joint samples from both groups were harvested 2 weeks and 4 weeks post-injury. Synovial inflammation and PTOA severity were assessed through H&E and Safranin O/Fast Green staining. All procedures were in accordance with Animal Experimentation Ethics Committee.

RESULTS SECTION: Tregs accumulated in the joint synovium early after ACL rupture (**Fig. 1a-d**). By three days post-injury, Tregs accounted for 44.95% ± 13.60% within the CD4 T cell compartment in the synovium, indicating a rapid response to the injury (**Fig. 1e**). Synovial Treg accumulation was associated with a significant reduction in joint inflammation (**Fig. 1f**). Selective expansion and enhanced expression of functional markers in synovial Tregs, induced by CMV-IL-2, were observed one-week post-injury (**Fig. 2b-d**), with 77.20% of expanded Tregs exhibiting an activated tissue-resident phenotype, characterized by $CD25^+CD44^+ST2^{hi}CD103^{hi}Ki67^+$ (**Fig. 2e**). Selective expansion of synovial Tregs early post-injury significantly reduced synovial inflammation (Control *vs.* Treat, 2 weeks: 7.25 ± 0.92 *vs.* 6.15 ± 0.63, p = 0.006) and mitigated PTOA severity (2 weeks: 4.45 ± 1.38 *vs.* 3.20 ± 1.38, p = 0.041; 4 weeks: 4.25 ± 1.72 *vs.* 2.50 ± 1.17, p = 0.029) (**Fig. 3**), underscoring their protective role in modulating inflammation and PTOA progression.

DISCUSSION: Our study found that Tregs accumulated in the synovium early in response to joint trauma. Furthermore, selectively expanded synovial Tregs provided protection against synovial inflammation and mitigated PTOA progression. Future research aims to identify the specific targets of synovial Tregs in regulating inflammation and providing PTOA protection through loss-of-function experiments and single-cell sequencing.

SIGNIFICANCE/CLINICAL RELEVANCE: Our study highlights Tregs as a promising potential target for disease modification in PTOA. Tregs can regulate various cells involved in inflammatory responses and maintain immune homeostasis, making them a potential option for addressing the complex inflammatory environment in PTOA. This Treg-mediated approach to controlling inflammation is particularly promising, especially given the limited success of targeting individual disease-associated factors such as IL-1β and ADAMTS5.

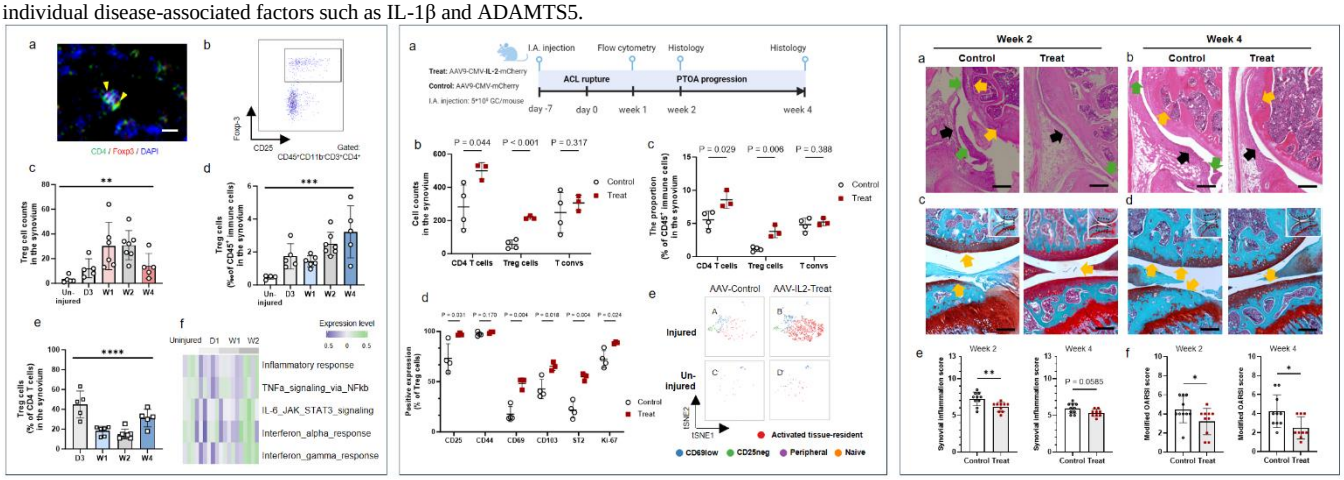

Fig. 1. Tregs accumulated in the joint synovium after ACL rupture in the PTOA mouse model. a) Representative immunostaining image of Tregs in the synovium of mice 1 week after ACL rupture. Scale bar: 100 µm. b) Gating strategy for Foxp3+ Tregs. c) Treg counts in the Synovium after ACL rupture injury. d) The proportions of Tregs in $CD45^+$ immune cells in the synovium after ACL rupture. e) The proportions of Tregs in CD4 T cells in the synovium after ACL rupture. f) Gene set variation analysis (GSVA) inflammatory response-related pathway scoring at different time points of injured joints after ACL rupture (GSE112641, C57BL/6 mice). n = 5~7 per group/timepoint.One-way ANOVA test through c-e. * * p < 0.01, * * * p < 0.001, * * * * p < 0.0001.

Fig. 2. AAV I.A. injection selectively expanded synovial Tregs. a) Experimental design for synovial Treg expansion in ACL rupture mode. b-c) Treg selective expansion was observed in the synovium. d) Significant improvements of synovial Treg function markers. e) Synovial Tregs from injured and uninjured groups, each treated with AAV-control or IL-2. t-distributed stochastic neighbor embedding (t-SNE) of Tregs built on key markers (CD69, CD25, CD44, ST2, CD103, Ki67, Nrp1). Activated tissue resident, $CD69^{hi}CD25^+CD44^+ST2^{hi}CD103^{hi}Ki67^+$; CD69low, $CD25^+CD44^+ST2^{hi}CD103^{hi}Ki67^+$; CD25neg, $CD44^+ST2^{hi}CD103^{hi}Ki67^+$; Peripheral, $Nrp1^+CD69^{hi}$; Naïve, $CD44^{lo}CD69^-$. n = 3~4. Multiple unpaired t-tests with FDR multiple comparisons through b-e.

Fig. 3. Selective expansion of synovial Tregs early post-trauma provided protection against synovial inflammation and ameliorated PTOA progression. a-b): Representative H&E-stained sections of the control and treat group in 2 weeks (a) and 4 weeks (b), with emphasis on the anterior synovium superior to the meniscal remnant. Black arrowheads, green arrowheads, and yellow arrowheads denote synovial hyperplasia, pannus, and bone erosion, respectively. c-d) Representative Safranin O/Fast Green staining sections of the control and treat group in 2 weeks (c) and 4 weeks (d). Yellow arrowheads denote cartilage erosion and damage. e-f) Synovial inflammation score (e) and PTOA severity scoring (f) in 2 weeks and 4 weeks. Scale bar: 200 µm. n=9~10. Mann-Whitney test. * p < 0.05, * * p < 0.01.