Obesity and Trauma Significantly Impact Synovial Immune Cell Landscape in OA Patients

Isabella Y. Goh[1,2]*, Ujjyani Ghosh[1,2,3]*, Baifeng Yu[1,2], Nikola N. Jaworska[1,2], Nurlatifa Zahra Mohd Fuad[1,2], Shivakumar R.Veerabhadraiah[1], Mick Jurynec[1], Josh Daryoush[1], Justin Haller[1], Natalia S. Harasymowicz[1,2,3#]

[1] Department of Orthopaedic, [2] Molecular Medicine Program, [3]Nutrition and Integrative Physiology, University of Utah, Salt Lake City, UT, USA, *co-first authors, #corresponding author

Email: natalia.harasymowicz@utah.edu **Disclosures**: IYG, UG, BY, NNJ, NZMF, SRV, MJ, JH (none), NSH (Immunis-1),

INTRODUCTION: Osteoarthritis **(OA)** is a group of diseases characterized by the degradation of articular cartilage[1], inflammation, and changes in bone structure[2]. One of the primary preventable risk factors for OA is **obesity**. Recent studies have shown that the increased joint stress associated with obesity, often referred to as "wear-and-tear," is not the only factor linking obesity to OA. Posttraumatic osteoarthritis (**PTOA**) is a type of OA that develops following a joint injury. Following the trauma, a complex interplay of biological and mechanical factors determines whether the articular surface is preserved or if cartilage degeneration occurs, leading to PTOA. While much research has focused on the mechanical aspects of these injuries, there is a significant gap in understanding the biological changes that take place after a joint trauma. Our previous animal studies revealed that injury and obesity act both synergistically and differentially in modulating synovial immune cell content during the progression of OA[3]. However, the impact of those two risk factors on the synovial immune cell profile in OA patients has not been previously analyzed. In this study, we evaluated the landscape as well as the molecular profile of immune cells in Normal-Weight and Obese OA patients with or without a previous history of trauma.

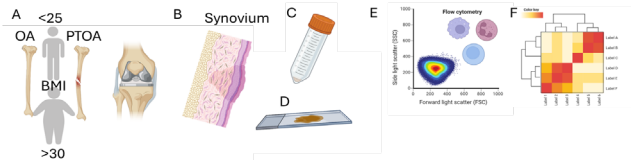

Fig.1. Study Design *(A) Patients undergoing Total Knee Arthroplasty (TKA) were selected based on their BMI and history of previous trauma. (B) Synovial tissues were collected. Tissues were either (C) digested, (D) or cryopreserved (E) synovial cells were analyzed by multiparameter flow cytometry. (F) Synovial Tissue Resident (TRM) and Monocyte-derived Macrophages (MDM) as well as T cells were sorted. RNA was isolated and bulk RNAseq was performed.*

METHODS: Here, we collected synovial tissue from n=40 patients (male and female) with end-stage knee OA who were indicated for total knee arthroplasty (TKA). **PTOA** patients had a known history of tibial plateau fracture, distal femur fracture, or anterior cruciate ligament tear prior to being indicated for TKA. Primary/idiopathic **OA** with end-stage knee degeneration had no previous history of traumatic injury, inflammatory arthritis, or connective tissue disorder. We divided the patient cohort depending on their BMI status during surgery (**Fig.1A**). Synovial tissue was collected during TKA and digested for multiparameter flow cytometry analysis[4] (**Fig.1B-C**). Synovial tissue biopsies were also cryopreserved and sectioned. Immunofluorescent (IF) staining was utilized to analyze the spatial presence of various immune cells within the synovium (**Fig.1D**). Finally, two subsets of macrophages, Tissue Resident Macrophages (TRMs) and Monocyte-derived Macrophages (MDMs), as well as T cells, were isolated by flow cytometry sorting. RNA was collected, and bulk RNAseq analysis was performed. One and Two-way ANOVA with Tukey's post hoc analysis were used for statistical analysis.

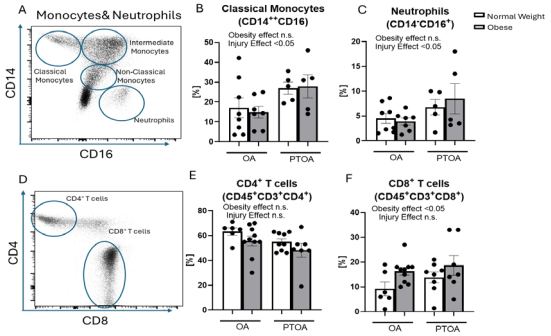

Fig.2. Synovial Immune Cells in Obese and Normal Weight OA and PTOA patients *(A) Flow cytometry gating strategy to assess the content of different subtypes of monocytes and neutrophils. (B) The content of classical monocytes and (C) neutrophils as established by flow cytometry. (D) Gating strategy used to analyze content of synovial T cells. (E) The content of $CD4^+$ and (F) $CD8^+$ T cells as established by flow cytometry. 2-way Anova, different letters indicate $p<0.05$ between groups. * $p<0.05$ indicates group effect, n=6-10 per group.*

RESULTS: In this IRB-approved study, we have immune-profiled human OA synovial tissue. Several immune cell populations were found in synovial tissue, including monocytes, macrophages, neutrophils, as well as B and T cells. We have shown that the history of previous injury had a significant effect on increasing the content of classical monocytes (**Fig.2A,B**) and neutrophils (**Fig.2C**) in the synovium of TKA patients, regardless of the BMI; additionally, obesity had a significant effect on increasing the content of Cytotoxic $CD8^+$ (**Fig.2D,E**) but not $CD4^+$ T cells (**Fig.2F**) in the synovium. We have also shown that there is a significant decrease in the content of protective TRMs defined as ($CD45^+CD64^+MERTK^+CD206^+$) (**Fig.3A,B**) as well as an increase in the content of MDMs defined as ($CD45^+CD64^+MERTK^-CD48^+$) in obese patients with a previous history of trauma (**Fig.3 F,G**). Our IF analysis also revealed significant disturbance of the content and localization of TRMs (defined as $MERTK^+$cells) in the synovial membrane both with obesity and trauma (**Fig.3D**). Furthermore, we have shown that both obesity and injury significantly impact the transcriptomic profile of synovial TRMs, MDMs, as well as T cells. For instance, obesity significantly modulated genes related to tight junctions in TRMs (**Fig.3C**), whereas trauma affected fatty acid biosynthesis pathways in these cells (**Fig.3E**). Interestingly, trauma also significantly induced expression of pro-inflammatory genes in MDMs (**Fig.3H**).

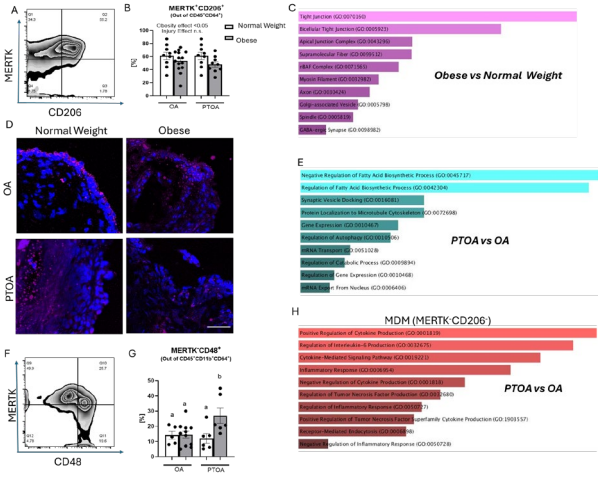

Fig.3. Synovial Macrophages in Obese and Normal Weight OA and PTOA patients *(A) Flow cytometry gating strategy to assess the content of tissue resident macrophages (TRMs) (B) The content of TRMs as established by flow cytometry (C) Gene ontology analysis of enriched pathways associated with Obesity status in TRMs (D) Representative IF staining of $MERTK^+$cells in synovial membrane (E) Gene ontology analysis of enriched pathways associated with history of previous trauma. (F) Flow cytometry gating strategy to assess the content of monocyte-derived macrophages (MDMs). (G) The content of MDMs as established by flow cytometry (H) Gene ontology analysis of enriched pathways associated with previous trauma in MDMs 2-way Anova, different letters indicate $p<0.05$ between groups. * $p<0.05$ indicates group effect, n=6-10 per group. Scale bar is 50μm.*

DISCUSSION: A variety of immune cells, including recently described novel macrophage populations, have been postulated to regulate the progression of joint damage following injury and obesity. Yet, the precise function and role of those cells in facilitating PTOA in obese subjects remain unclear. Here, in our in-depth cellular and transcriptomic profiling of human synovium, we have confirmed the inflammatory nature of the synovial landscape in OA patients that is significantly impacted by BMI and history of previous trauma. Such findings suggest that the processes governing the significant reduction of protective TRMs combined with homing and retention of inflammatory cells, such as MDMs and Cytotoxic $CD8^+$ T cells, in the synovium are crucial for the more rapid development of PTOA in obese patients.

SIGNIFICANCE: Focusing on novel immune cell populations enhances our understanding of OA pathogenesis in human subjects and leads to improved therapeutic options that address both mechanical and biological factors in joint injuries.

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