An indoleamine 2,3-dioxygenase galectin-3 fusion protein shifts joint metabolism in osteoarthritis

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INTRODUCTION: Inflammation from osteoarthritis (OA) contributes to chronic joint pain and disability. Current steroidal and non-steroidal treatments largely focus on suppressing immune responses and fail to provide long-term relief. Indoleamine 2,3-dioxygenase (IDO) is an enzyme that shifts tryptophan metabolism toward kynurenine production, which can drive the differentiation and polarization of immune cells to anti-inflammatory phenotypes. We have developed a fusion of IDO to galectin-3 (Gal3), an extracellular matrix anchoring protein to improve joint retention and extend the local anti-inflammatory effects of IDO. We previously showed that intra-articular IDO-Gal3 is retained for four weeks in the joint of rats with established OA, and treatment significantly alters gait compensations [1]. Here, our objective was to evaluate the ability of prophylactic IDO-Gal3 to shift local joint metabolism in OA.
METHODS: All animal experiments were approved by the University of Florida IACUC. OA was induced in 40 three-month-old male Lewis rats with a medial meniscus transection and medial collateral ligament transection in the stifle joint. Three days after surgery, 11.25 µg of IDO-Gal3 in 25 µL of PBS was delivered by intra-articular injection (n=20, MMT+IG). Twenty animals received surgery, but no treatment (MMT). Naïve animals (n=20) were used as controls. Eight animals per group were euthanized one week after surgery and 12 animals per group were euthanized at six weeks. Synovial fluid (SF) was wicked using Whatman filter paper for LC-MS metabolomics analysis. In MetaboAnalyst, partial least-squares discriminant analysis (PLS-DA) was used to compare the SF metabolome of each group within each timepoint. Variable importance of projection (VIP) was used to rank metabolite features by importance in distinguishing groups at each timepoint. Functional analysis with the *mummichog* algorithm was used to map features onto metabolic pathways and provide putative identities for metabolites. P-values from the gamma distribution for the identified pathways were adjusted for false discovery rate.
RESULTS: PLS-DA showed that at each timepoint, naïve, MMT, and MMT+IG had distinct metabolic profiles in the SF (Fig. 1). At each timepoint, there were unique and shared pathways between each group (Fig. 2). There were fewer shared pathways between all three groups at six weeks than at one week (19.5% vs. 32.7%), indicating divergence of the SF metabolic profile of each group as OA develops (Fig. 2). Among the top ten metabolic pathways differentiating MMT and MMT+IG, five pathways were shared across timepoints (Fig. 3). Notably, tryptophan metabolism was the highest-ranked pathway at one week (Fig. 3) but was not among the 17 total identified pathways at six weeks.
DISCUSSION: In a rat model of OA, intra-articular IDO-Gal3 treatment shifted the synovial fluid metabolic profile. Interestingly, treatment did not shift joint metabolism to a naïve-like profile, but to a profile distinct from that of naïve and untreated animals. As IDO specifically targets tryptophan metabolism, comparisons to the naïve groups were dominated by the numerous metabolic alterations associated with the MMT injury. Tryptophan metabolism was a significant pathway identified at one week but was not identified at six weeks, indicating that acute shifts in tryptophan metabolism in early injury are sufficient for long-term overall metabolic shifts in the OA joint. These data indicate that early metabolic shifts may alter the long-term health of the joint. At both timepoints, most of the top metabolic pathways differentiating treated and untreated animals were related to amino acid metabolism and energy metabolism. Because metabolites in SF can originate from different tissues in the joint, future work should evaluate metabolic shifts in the synovium, cartilage, and bone of the OA joint.
SIGNIFICANCE/CLINICAL RELEVANCE: Enzyme therapies can shift joint metabolism and synovial fluid metabolomics can assist in the evaluation of enzyme-based therapeutics for OA. This work demonstrates the ability of IDO-Gal3 to shift local metabolism in the joint.
REFERENCES: [1] Partain et al., 2023, Arthritis Research and Therapy.
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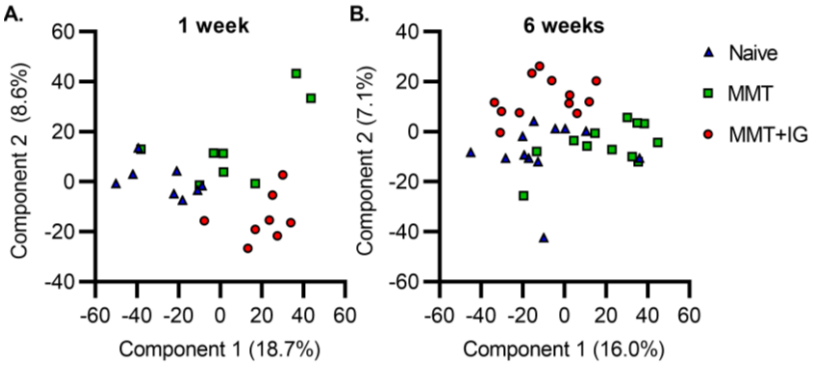

Fig. 1. PLS-DA results for A) 1 week and B) 6 weeks metabolites.

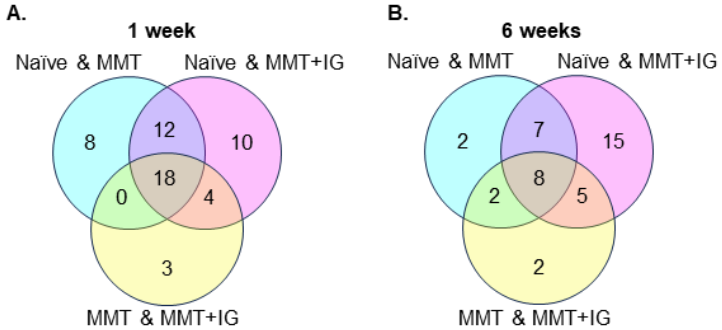

Fig. 2. Venn diagram of shared and unique metabolic pathways at A) 1 week and B) 6 weeks.

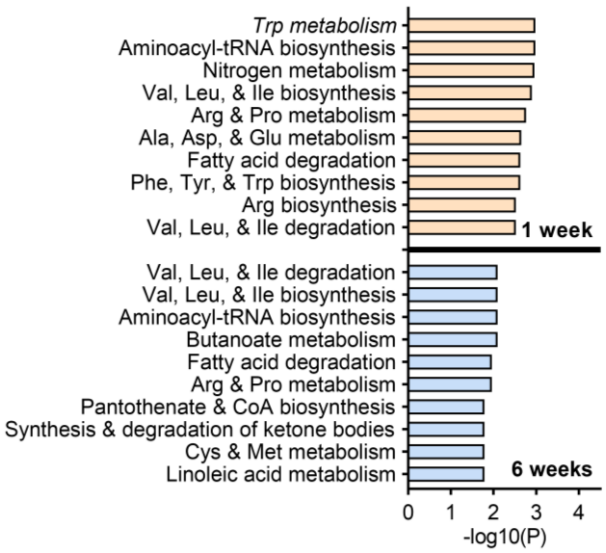

Fig. 3. Top ten metabolic pathways differentiating MMT and MMT+IG groups at 1 and 6 weeks.