

CD14 inhibition as a potential therapeutic for posttraumatic osteoarthritis.

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INTRODUCTION: Osteoarthritis (OA) is the most common joint disorder, and growing evidence has identified inflammation as a major driver of disease progression. During progression, the synovium serves both as a source and reservoir for inflammatory mediators and immune cells, including monocyte/macrophages.¹ Though *temporary* pain relief is offered by non-steroidal anti-inflammatory therapeutics, no therapies have been able to halt or delay disease progression. One potential therapeutic target, soluble CD14, a co-receptor of inflammatory toll-like receptor signaling, produced primarily by activated macrophages, is present in synovial fluid in patients with OA and is positively associated with joint space narrowing and pain.² We previously reported that global genetic CD14 deficiency in mice protects against OA-associated bone-remodeling and pain-related joint dysfunction.³ **Towards translation, we hypothesize that an anti-CD14 therapeutic will attenuate inflammatory activation in the synovium during OA and mitigate disease progression and pain.**

METHODS: *OA model (n=12-14):* We performed destabilization of the medial meniscus (DMM) surgery to induce OA in skeletally mature (10-12 wk old) C57BL/6 mice.⁴ *Intervention:* Mice were treated intra-articularly with either an anti-CD14 monoclonal antibody (mAb, clone biG53) or an IgG2a control (both 0.5mg/kg). Two dosing strategies were tested: *1) Prevention strategy:* mice received anti-CD14 or IgG control 3 weekly doses, starting 48 hrs post DMM. *2) Treatment strategy:* mice received 3 weekly injections beginning 4 wks post DMM. *Behavioral analyses:* At 4- and 8 wks post DMM, evaluation of spontaneous cage behaviors was performed using the Laboratory Animal Behavior Observation Registration and Analysis System (LABORASTM, Metris).⁵ Additionally, paw weight bearing distribution was measured via the Advanced Dynamic Weight Bearing (ADWB, Bioseb) system.⁵ *Histopathology analysis (n=5):* All mice were sacrificed at 8-wks post DMM, and knee joints were fixed, decalcified, paraffin embedded, and sectioned. Synovitis scoring was performed on H&E-stained coronal sections to assess lining hyperplasia (0-3), sub-lining cellularity (0-3), and fibrosis (0-1) across 4 synovial regions (medial-femoral and -tibial, lateral-femoral and -tibial gutters). Scores were averaged across 3 graders after determining acceptable inter-rater reliability. Cartilage degeneration scoring was performed by a board-certified veterinary pathologist on Toluidine blue stained coronal sections using the modified Osteoarthritis Research Society International (OARSI) score.⁶ Scores (0-5) were summed across regions (medial and lateral tibial plateau or femoral condyle). *Immunohistochemistry (n=5, prevention strategy group):* To evaluate innervation, coronal sections underwent antigen retrieval and overnight incubation with a primary antibody against PGP9.5, followed by incubation with fluorescent secondary antibody, and mounting medium containing DAPI nuclear dye, followed by imaging on a Zeiss Axio Scan.Z1. Immunofluorescent images were thresholded and expression of targets reported as percent fluorescent area across the entire knee joint (medial and lateral synovium, meniscus, intercondylar region, and cartilage). *Statistical analysis:* Student's t-test or one-way ANOVA with Šidák post-hoc were used with p<0.05 considered significant, as indicated in figures.

RESULTS: *Prevention strategy:* Early CD14 blockade significantly increased total distance traveled and rearing time at 4- and 8-wks post DMM, compared to control mice (p<0.05) (**Fig. 1A**). There was a decreasing trend (p=0.057) in weight shifting from the rear to the front paws (front to rear paw weight ratio, **Fig. 1A**) 8-wks post DMM in the anti-CD14 treated mice compared to controls. At 8-wks post DMM differences were observed in synovial cellularity (p<0.0001) and fibrosis (p=0.0078) between control-treated DMM-operated knees compared to unoperated knees, however no significant differences in synovial pathology were observed between CD14 blockade- and control-treated DMM knees (**Fig. 2C**). There was also no significant difference in cartilage pathology scoring in DMM-operated knees after early CD14 blockade compared to controls (**Fig. 2C**). *Treatment strategy:* When treatment was delayed until 4-wks post DMM, no significant behavior or weight-bearing changes were observed between groups (**Fig. 1B**). 8-wks post DMM, synovial cellularity and fibrosis scores increased compared to unoperated knees similarly in both anti-CD14 and IgG treated mice (**Fig. 2D**). Lining hyperplasia was significantly increased post DMM only in the IgG control group (**Fig. 2D**). There was a trend (p=0.054) towards increasing OARSI cartilage score in the anti-CD14 treated vs. IgG-treated DMM groups (**Fig. 2D**), but no significant differences between early or late treatment groups (IgG vs. CD14 blockade) (**Fig. 2C,D**). Immunofluorescent analysis of innervation in the early-dosed groups revealed significant increases in PGP9.5 expression in DMM-operated knees only in the IgG-control group, and a trend toward decreased staining in anti-CD14 treated DMM knees (**Fig. 3**).

DISCUSSION: Early intra-articular delivery of a CD14 blocking mAb after DMM injury was more effective at improving mobility, compared to delayed treatment, and reduced injury-related weight-bearing shifts towards the front paws. No significant impact of anti-CD14 treatment on cartilage degeneration or synovial histopathology was observed, despite the effects on weight bearing and mobility seen with early treatment. However, anti-CD14 attenuated post DMM increases in signal for the common nerve marker PGP9.5 across the joint, which may be one mechanism driving behavioral and weight-bearing differences. CD14 is known to facilitate inflammatory pathway activation via TLRs, which play important roles in both monocyte/macrophage differentiation and nociception. As such neuroinflammatory crosstalk has been implicated in OA, future work will focus on further elucidating effects of this treatment on the synovial neuroinflammatory milieu.⁷

SIGNIFICANCE: These results explore the optimal timing of delivery of an anti-CD14 therapeutic to influence OA pain-related behaviors, ultimately supporting future work in utilizing CD14 as a therapeutic target for post traumatic OA.

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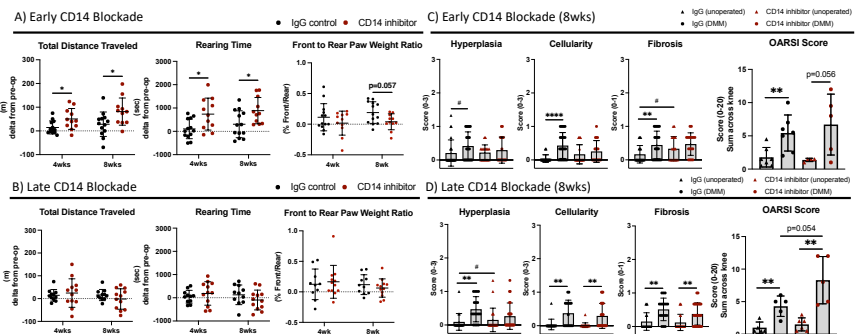


Figure 1: Spontaneous behavioral analysis. LABORAS behavioral analysis of the change from pre-op of Early (A) and Late (B) blockade groups. ADWB weight bearing analysis of the change from pre-op of front to rear paw & weight % ratio. *p<0.05 Student's T-test.

Figure 2: Histopathology analysis. Synovitis and OARSI scores across 4 knee compartments at 8-wks following DMM of Early (C) and Late (D) blockade groups. #p<0.1, *p<0.05, **p<0.01, ****p<0.0001 Student's T-test.

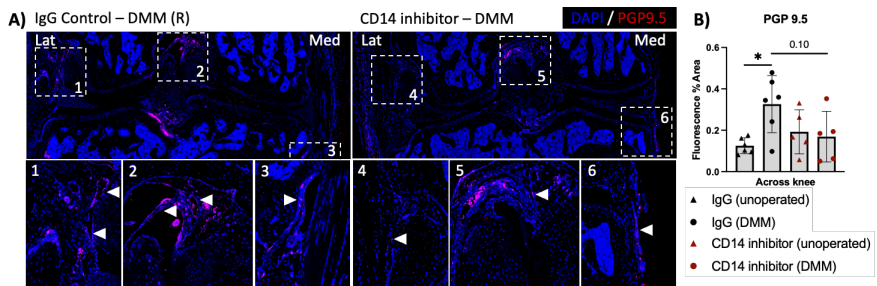


Figure 3: Innervation following DMM. (A) Fluorescent images of mice from IgG control and CD14 blockade treated mice (n=5) at 8 wks post DMM, stained for a general innervation marker (PGP9.5, white arrows). Medial and lateral regions are indicated. (B) PGP9.5 expression quantification via % fluorescent area across the knee (cartilage, meniscus, intercondylar region, medial- & lateral-synovium). *p<0.05 or as indicated, one-way ANOVA with Šidák's multiple comparison.