# CD14 inhibition as a potential therapeutic for posttraumatic osteoarthritis.

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## Disclosures

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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

A 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).[3] 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 ($p < 0.05$) (Figure 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,

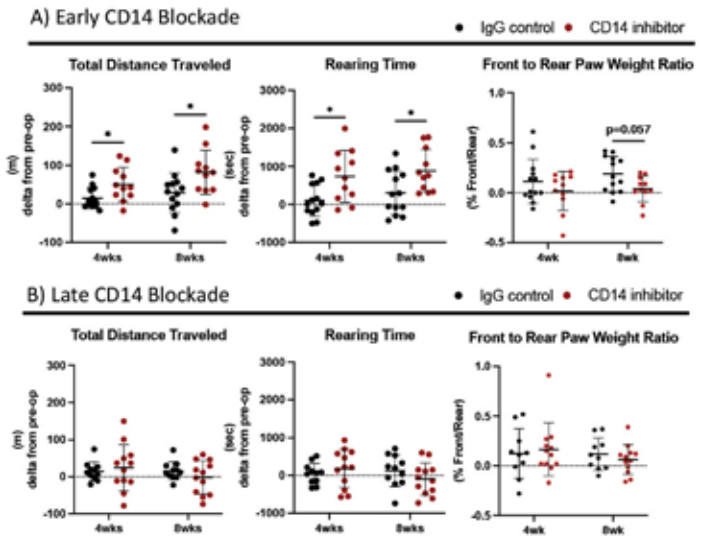


**Figure 1.** Spontaneous behavioral analysis. LABORAS behavioral analysis of the change from pre-op of Early **(A)** and Late **(B)** blockade groups. ADWB weight bearing analysis of the change from pre-op of front to rear paw & weight % ratio. *p<0.05 Student's T-test.

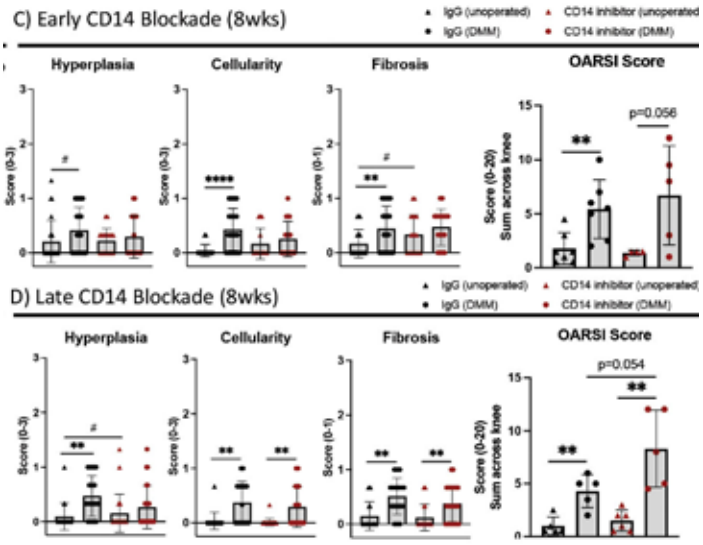


**Figure 2.** Histopathology analysis. Synovitis and OARSI scores across 4 knee compartments at 8-wks following DMM of Early **(C)** and Late **(D)** blockade groups. #p<0.1, *p<0.05, **p<0.01, ****p<0.0001 Student's T-test.

Figure 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 (Figure 2C). There was also no significant difference in cartilage pathology scoring in DMM-operated knees after early CD14 blockade compared to controls (Figure 2C). Treatment strategy: When treatment was delayed until 4-wks post DMM, no significant behavior or weight-bearing changes were observed between groups ((Figure 1B). 8-wks post DMM, synovial cellularity and fibrosis scores increased compared to unoperated knees similarly in both anti-CD14 and IgG treated mice (Figure 2D). Lining hyperplasia was significantly increased post DMM only in the IgG control group (Figure 2D). There was a trend ($p = 0.054$) towards increasing OARSI cartilage score in the anti-CD14 treated vs. IgG-treated DMM groups (Figure 2D), but no significant differences between early or late treatment groups (IgG vs. CD14 blockade) (Figure 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 (Figure 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

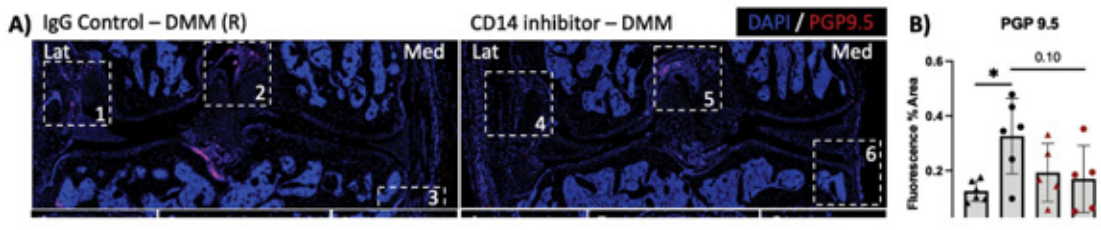


**Figure 3.** Innervation following DMM. **(A)** Fluorescent images of mice from IgG control and CD14 blockade treated mice (n=5) at 8 wks post DMM, stained for a general innervation marker (PGP9.5, white arrows). Medial and lateral regions are indicated; **(B)** PGP9.5 expression quantification via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

CD14 as a therapeutic target for post traumatic OA.

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