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. Though temporal pain relief is offered by non-stereoidal 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. We previously reported that global genetic CD14 deficiency in mice protects against OA-associated bone-remodeling and pain-related joint dysfunction. 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: O4 model (n=12-14): We performed destabilization of OA (DMM) surgery to generate OA in 5 male BALB/c (10-12 wk old) C57Bl/6 mice. 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 days 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 (LABORAS14, Metris). Additionally, paw weight bearing distribution was measured via the Advanced Dynamic Weight Bearing (ADWB, Bioseb) system.

Histopathology analysis (n=5): 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 femor and lateral synovium (meniscus, intercondylar region, and cartilage). Statistical analysis: Student’s t-test or one-way ANOVA with Sidák 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 between groups. Scores were summed across 4 graders to assess 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. Scores (0-5) were summed across regions (medial and lateral tibial plateau or femoral condyle).

Histopathology analysis (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 femoral and lateral synovium (meniscus, intercondylar region, and cartilage). Statistical analysis: Student’s t-test or one-way ANOVA with Sidák post-hoc were used with p<0.05 considered significant, as indicated in figures.

DISCUSSION: Early intra-articular delivery of a CD14 blocking mAb after DMM injury was more effective at improving mobility, compared to delayed treatment and no 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, 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, antiinflammatory crosstalk has been implicated in OA, future work will focus on further elucidating effects of this treatment on the synovial neuroinflammatory milieu.

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.


ACKNOWLEDGEMENTS: We would like to thank Inotiv Co for performing histopathology analysis for this study. Funding provided by VA BL&R&D (I01 BX004912) and NIAMS (CRS: R01 AR075737) (AMM: P30AR072906).