

CD14 Contributes to Joint Pain in a Murine Model of Osteoarthritis

Natalie S. Adamczyk¹, Shingo Ishihara¹, Matthew J. Wood¹, Anne-Marie Malfait¹, Carla R. Scanzello², Rachel E. Miller¹
¹Rush University Medical Center, Chicago, Illinois, ²University Of Pennsylvania, Philadelphia, Pennsylvania

Email of Presenting Author: natalie_adamczyk@rush.edu

Disclosures: NSA, SI, MJW, REM have nothing to disclose. AMM: consulted for 23andMe, Orion. CRS: provisional patent apps #63/479,605; #63/484,099

INTRODUCTION: Five hundred million people worldwide are affected by osteoarthritis (OA), yet many patients still have unmanaged pain due to our incomplete understanding of the pathways driving OA pain. Clinically, aspects of inflammation such as synovitis and effusion have been associated with pain sensitization in OA¹, and current evidence suggests that innate immune pathways play an important role in OA inflammation and pain². Toll like receptors (TLRs) help initiate first response inflammatory cascades in response to tissue injury or infection. There is evidence that knocking out TLR2 or 4 in mice is protective against pain development but not in protecting against cartilage damage. In addition, mice in which the TLR co-receptor CD14 was knocked-out were shown to have late stage protection against cartilage damage and preserved locomotor activity in the DMM mouse model³. This CD14 receptor has both a soluble and a membrane bound form. Soluble CD14 remains biologically active and can trigger responsiveness to TLR ligands in cells that do not normally express CD14. In OA, a correlation between synovial fluid levels of soluble CD14 and pain severity has been observed. To further understand the role of CD14 in OA joint pain, the goals of this study were to investigate whether CD14 contributes to pain-related behaviors in the partial medial meniscectomy (PMX) model of OA as well as whether CD14 contributes to knee hyperalgesia in response to intra-articular injection of TLR ligands.

METHODS: All animal procedures were approved by an IACUC committee. *Experiment one:* At 12 weeks of age, *Cd14*^{-/-} and C57BL/6 WT male mice underwent sham or PMX surgery of the right knee. A blinded observer assessed knee hyperalgesia (pressure application measurement, Ugo Basile) and hind paw mechanical allodynia (von Frey fibers) every four weeks; weight bearing (static incapacitance meter, Bioseb) was assessed at baseline, 6 and 10 weeks post PMX. The difference in force (right hind limb – left hind limb in grams) was calculated for each trial and averaged over three trials. *Experiment 2:* Naïve male *Cd14*^{-/-} mice and WT mice were used to determine whether CD14 is protective against knee hyperalgesia induced by intra-articular injection (i.a.) of TLR agonists. Mice were injected i.a. with either saline (3 µL), LPS (3 µg in 3 µL), or Pam3CSK4 (3 µg in 3 µL). Knee hyperalgesia was assessed prior to injection, two hours and four hours post injection. At 6 h post injection mice were taken down and ipsilateral L3-L5 dorsal root ganglia (DRG) were removed, placed in Trizol, and RNA was extracted by Qiagen RNeasy micro kit. qPCR was carried out using Qiagen SYBR green qPCR master mix and RT² primers for *Gadph* and *Ccl2*. *Gadph* was used as an internal control and was used to quantify *Ccl2* levels using the 2^{-ΔCt} method. *Experiment 3:* *In vitro* DRG cultures were performed using L3-L5 DRGs collected from either 2 *Cd14*^{-/-} or from 2 WT mice. DRGs from each mouse were harvested, dissociated and cultured on poly-L-lysine and laminin coated coverslips. On day three, cells were stimulated for 24 h with either vehicle, LPS, or Pam3CSK4 and supernatants were removed the following day and used for a CCL2 ELISA following manufacturer instructions.

RESULTS: *Experiment one:* Mice receiving PMX surgery (n=5 *Cd14*^{-/-}, n=5 WT) and sham (n=4 *Cd14*^{-/-}, n=5 WT) were assessed for knee hyperalgesia and hind paw mechanical allodynia until eight weeks post-surgery. At baseline there was no significant difference between groups, but 4 and 8 weeks post PMX, *Cd14*^{-/-} mice had significantly less knee hyperalgesia in comparison to WT mice (p=0.0009, 2-way ANOVA, Fig 1A). A similar trend was found when assessing mechanical allodynia. *Cd14*^{-/-} mice had less mechanical allodynia at 4 (p=0.0035) and 8 weeks (p=0.0394) than WT mice (Fig 1B). Finally, WT PMX mice developed weight-bearing deficits in the operated limb by 10 weeks post-surgery compared to WT sham mice (p=0.0164), while *Cd14*^{-/-} mice did not (p=0.9194) (Fig 1C). *Experiment two:* Naïve mice were intra-articularly injected with either saline, LPS, or Pam3CSK4. Intra-articular injection of saline did not induce knee hyperalgesia in either WT (n=7) or *Cd14*^{-/-} mice (n=5) (Fig 2A). In contrast, LPS-induced knee hyperalgesia developed over the course of 4 hours in WT mice (n=8), but to a lesser extent in *Cd14*^{-/-} mice (n=6) (p=0.0002, 2-way ANOVA) (Fig 2B). A similar pattern was observed upon injection of the TLR2 ligand, Pam3CSK4 – by 4 hours post injection, WT mice (n=8) had greater knee hyperalgesia compared to the *Cd14*^{-/-} mice (n=7) (p=0.0005) (Fig 2C). We have previously shown that LPS and Pam3CSK4 can cause increases in the production of the chemokine CCL2 by DRG cells. Here, by 6 hours post injection of LPS, WT DRGs had higher levels of *Ccl2* transcript levels compared to *Cd14*^{-/-} mice (p=0.00542-way ANOVA) (Fig 2D). To confirm qPCR results, a separate experiment was performed by culturing L3-L5 DRG cells obtained from WT or *Cd14*^{-/-} mice. In this case, stimulation with both LPS and Pam3CSK4 induced increased protein production of CCL2 in WT mice compared to vehicle (p<0.0001, p<0.0001), while *Cd14*^{-/-} cells produced lower amounts of CCL2 compared to WT in response to both LPS (p=0.0003) and to Pam3CSK4 (p=0.01) (Fig 2E).

DISCUSSION: This study supports previous work suggesting that *Cd14*^{-/-} mice were partially protective against development of locomotion deficits. Here we added to this work by showing that these mice also develop less mechanical sensitization in an additional model of OA and in response to direct injection of TLR ligands into the knee joint. This protection might be partially mediated by reducing the amount of CCL2 (a pro-algesic chemokine) produced. Future work will determine whether CD14 acts directly on neurons or whether these effects are due to indirect actions on other cells such as macrophages.

SIGNIFICANCE/CLINICAL RELEVANCE: This study suggests that CD14 plays a role in mechanical sensitization, pain development and subsequent TLR induced cytokine production in murine models of OA. Targeting CD14 may be useful for reducing OA symptoms.

REFERENCES: 1. Neogi et al, Arthritis Rheumatol. 2016;68(3):654-61; 2. Miller RJ et al, Osteoarthritis Cartilage. 2020;28(5):562-571; 3. Sambamurthy et al, PLoS One. 2018;13(11):e0206217. **ACKNOWLEDGEMENTS:** R01AR077019 (REM), R01AR064251, R01AR060364, P30AR079206 (AM), F31AR083277 (NA), R01AR075737, VA I01BX004912 (CRS).

