

# The role of Cx3cr1 in tendon development and response to loading

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**INTRODUCTION:** Tendons function to transfer load from muscle to bone to produce joint motion. Tendons are under high cyclic loads during movement and are prone to damage and injury [1]. Resident tendon cells remodel their extracellular matrix and continually repair minor damage caused by pathology or overloading. However, accumulation of damage can result in degeneration and eventual rupture. As sentinel cells, tissue resident macrophages detect damage and invasion from pathogens. Macrophages phagocytose the damaged cells and extracellular matrix and/or initiate inflammatory responses leading to repair [2]. Recent reports have indicated that macrophages [3,4] and the chemokine receptor CCR2 [5,6] play critical roles in tendon healing. However, there has been little focus on the necessity of macrophages for tendon development and on their responsiveness to tendon loading. Here, we focused on CX3CR1, a chemokine receptor that plays a crucial role in macrophage homeostasis, migration, and inflammatory responses. A CX3CR1 knockout mouse model [7] was used to examine how macrophages contribute to tendon formation and treadmill exercise was used to examine their role in tendon mechanoresponsiveness [8]. As sentinel cells, we hypothesized that cells expressing CX3CR1 are necessary for tendon development and for responding to homeostatic alternations caused by exercise loading.

**METHODS:** Animal work was approved by Columbia University's IACUC. Approximately equal numbers of 6 weeks old male and female mice were used for all assays. To validate that Cx3cr1<sup>GFP/GFP</sup> reporter mice had Cx3cr1 deleted, peripheral blood was stained with anti-CX3CR1 antibody and cells were counted using flow cytometry. Cx3cr1<sup>GFP/GFP</sup> mice did not express Cx3cr1 (Fig. 1A). Thus, Cx3cr1<sup>GFP/GFP</sup> mice were deficient in Cx3cr1, and labeled as Cx3cr1<sup>GFP/KO</sup> moving forward. To generate control mice, Cx3cr1<sup>GFP/KO</sup> mice were crossed with C57Bl/6J mice to produce Cx3cr1<sup>GFP/Het</sup> heterozygous mice that had Cx3cr1 restored. To examine responses to loading, both mouse models were subjected to treadmill running at a decline of -15°, at a speed of 15m/min, 40min/day, for 4 weeks [8]. To determine the number of CX3CR1+ macrophages in tendon, whole mount supraspinatus tendon-bone samples were visualized using a confocal microscope and GFP+ cells were counted (n=10-20 per group). To evaluate tendon mechanical properties, supraspinatus tendon-humeral bone samples were isolated from adult mice, microcomputed tomography imaging was completed to determine humeral head bone morphometry and tendon cross sectional area, and uniaxial tendon mechanical testing was performed (n=7-11 per group). For histologic analysis, supraspinatus tendon-humeral head bone samples were decalcified, embedded in OCT, sectioned at 10 μm, and stained with anti-CX3CR1 antibody (1:100). Flow cytometry was used to confirm the microscopy-based cell counts data: pooled supraspinatus and infraspinatus tendons (n=5 mice) were digested with liberase and cells were stained for Live/Dead, GFP, CD45-BV711, CD11b-BV510, and CX3CR1-APC. Cell gates were determined using spleen and bone marrow cells. Statistical significance was determined using a two factor ANOVA for activity level and genotype followed by LSD post hoc tests when appropriate.

**RESULTS:** Deletion of Cx3cr1 led to increased numbers of GFP+ cells (Fig. 1B,C) and inferior mechanical properties (Fig. 2; decreased yield force, work [area under load deformation curve], strength [maximum stress], and resilience [area under stress strain curve]). Treadmill exercise led to increased tendon cross sectional area and decreased resilience in wild type mice. Exercise did not lead to any apparent changes in knockout mouse tendon area or mechanical properties.

**DISCUSSION:** The number of CX3CR1+ cells was substantially increased in Cx3cr1<sup>GFP/KO</sup> tendons subjected to exercise. This result is contrary to our expectation that tendons lacking this critical receptor would be less responsive to loading. This may not be due to changes in the chemokine CX3CL1, the specific ligand for CX3CR1, as preliminary immunohistochemistry did not show apparent changes in the ligand after exercise. Rather, increased numbers of CX3CR1+ cells may be due to local proliferation or migration through other chemokines, such as CCR2. Despite increased cell numbers, deletion of Cx3cr1 had a significant effect on tendon development, with decreased mechanical properties in knockout mice. Furthermore, tendons lacking Cx3cr1 were less responsive to loading from the structural point of view. Further study is needed to elucidate the mechanisms driving Cx3cr1-dependent responses in tendon.

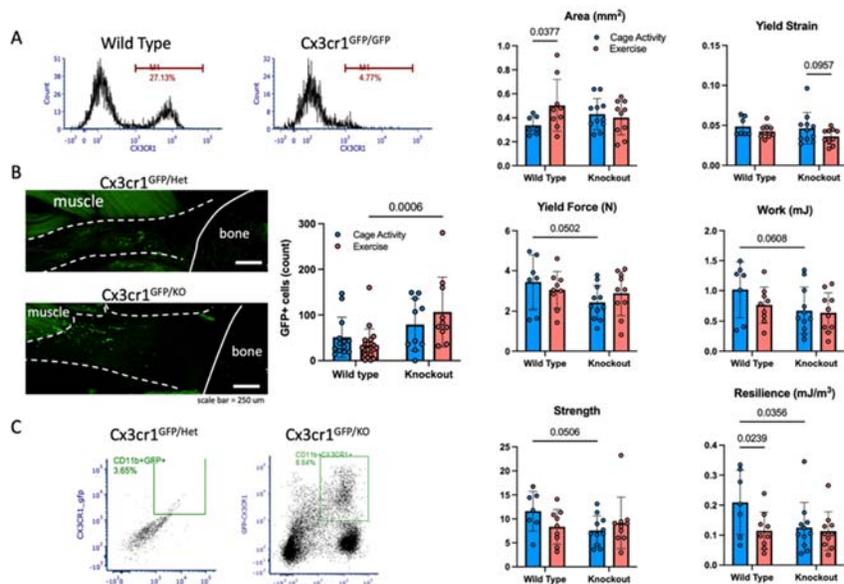
**SIGNIFICANCE:** We characterized tendon resident macrophages under exercise using knockout/reporter mice. These mouse models provide novel tools to study these cells under physiological and pathophysiological conditions.

**REFERENCES:** [1] Thomopoulos JOR (2015).

[2] Kurowska-Stolarska & Alivernini Nat Rev Rheumatol

(2022). [3] Howell KL et al FASEBJ (2023). [4] Best KT et al SciRep (2019). [5] Muscat S et al FASEB J (2022). [6] Elisberg CD JBJS (2023). [7] Chakarov, S et al Sci (2019). [8] Abraham, A et al JOR (2022).

**ACKNOWLEDEMENTS:** Supported by NIH R01 AR080717.



**Fig 1.** Increased macrophages in ST in Cx3cr1GFP/KO mice.

**Fig 2.** CX3CR1 KO led to impaired mechanical function of ST.