Absence of CC - Chemokine Receptor 7 (CCR7) is Linked to Reduced Structural and Functional Manifestations of Knee Osteoarthritis in a Murine Model

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Introduction: Synovial inflammation, or synovitis, is a common but variable feature of knee osteoarthritis (OA) associated with more severe joint symptoms (1), development (2) and progression (3) of cartilage erosion. However, it is still unclear whether prevention or treatment of synovitis will diminish development of OA or related joint disability after a pre-disposing injury, and the key molecular targets for treatment of synovitis in OA are not yet established. To identify potential therapeutic targets, we previously used microarray analysis to identify a set of chemokine genes upregulated in synovial membrane and associated with synovial inflammation in patients with meniscal tears and early-stage OA. This set of transcripts included the chemokine receptor CCR7 and its two ligands CCL19 and CCL21 (4). This receptor plays a central role in homeostatic trafficking of leukocyte populations, including lymphocytes and dendritic cells, and also impacts lymphocyte activation. The current study was undertaken to determine if CCR7 plays a mechanistic role in development of OA-related structural and functional manifestations using a murine model of knee OA.

Methods: Knee synovial tissues from 15 patients with degenerative meniscal tears undergoing arthroscopic meniscectomy, 13 patients with advanced knee OA undergoing total knee arthroplasty, and 9 organ donors without a history of chronic knee symptoms (asymptomatic donors) were collected through IRB-approved biorepositories. CCR7 expression in the synovial tissues was examined by immunoperoxidase staining using rabbit anti-human CCR7 (ab32527). Staining was quantified by automated analysis (NIS-Elements AR software, Nikon Inc.) of 10X images, and reported as % total area analyzed (1.25 x 106 mm2).

Genetically modified mice lacking expression of CCR7 (B6.129P2(C)−Ccr7tm1Rfor/J, referred to as CCR7−/−) (24), backcrossed onto the C57BL/6 background, were obtained from Jackson Laboratory (Bar Harbor, Maine). C57BL/6 were used as wild-type controls. Both strains were subjected to DMM surgery. CCR7 expression in the synovial tissues was examined by immunoperoxidase staining using rabbit anti-human CCR7 (ab32527). Staining was quantified by automated analysis (NIS-Elements AR software, Nikon Inc.) of 10X images, and reported as % total area analyzed (1.25 x 106 mm2).

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patterns are classified into different behaviors (locomotion, climbing, eating, drinking, grooming, and rearing) using validated software algorithms (5). Spontaneous murine behavior was measured in 16-hour sessions overnight. Baseline analysis was done 2 to 5 days prior to surgery, then post-operatively at 4, 8, and 12 weeks. Percentage of total time spent climbing, and distance traveled (in 16 hours) was recorded.

**Results:** Synovial tissue of patients with meniscal tears and advanced OA patients demonstrated CCR7-positive staining in the synovial lining layer, endothelium, and perivascular inflammatory infiltrates (Figure 1a). Although CCR7-positive staining was also present in the synovial lining layer of asymptomatic donors, quantitative image analysis revealed a significantly higher percentage area stained for CCR7 in patients with meniscal tears (Mean % area +/- SEM = 12.26 +/- 2.4) compared to asymptomatic donors (3.55 +/- 2.7, p=0.01 Figure 1b). Patients with advanced knee OA exhibited intermediate levels of CCR7 staining (6.77 +/- 1.45).

Six-weeks after DMM surgery, CCR7 -/- mice exhibited significantly lower scores for cartilage degeneration (Mean +/- SEM = 1.60 +/- 0.81) compared to wild-type controls (5.20 +/- 1.06, p<0.0001 compared to CCR7-/-, Figure 2). There was no cartilage degeneration observed in age-matched naive or sham-operated mice of either strain.

Activity was measured as described at baseline (pre-operative) and monthly up to twelve weeks post-operatively in WT (n=4) and CCR7 -/- mice (n=9) subjected to DMM surgery (Figure 3). Changes in climbing activity and locomotion (measured as meters traveled per 16 hours) from pre-operative baseline levels were calculated at each post-operative time point. Decreases in climbing activity post-operatively were observed in WT mice by 4 weeks post-DMM, and were sustained up to 12 weeks. Decreases in meters traveled were also seen at 4 weeks post-operatively, but returned to pre-operative levels thereafter. In contrast, CCR7 -/- mice maintained pre-operative levels of climbing activity and locomotion at all post-DMM time points.

**Discussion:** CCR7 expressing cells were enriched in the synovial membrane of patients with degenerative meniscal tears as well as patients with advanced knee OA. In a well-established OA animal model, deficiency of CCR7 was associated with less severe cartilage erosion six weeks after DMM surgery. Wild-type (C57BL/6) mice subjected to DMM surgery decreased climbing activity by 4 weeks post-DMM, and these decreases were sustained up to 12 weeks. In contrast, mice deficient in CCR7 maintained pre-operative levels of climbing activity over the entire 12 weeks post-DMM surgery. Thus, deficiency of CCR7 reduced both early structural development of OA, and ameliorated signs of chronic joint dysfunction and pain in this murine model of OA-like pathology. Whether these effects are mediated by reductions in synovitis will be investigated. Our results suggest that targeted anti-inflammatory treatments aimed at blockade of CCR7 or its ligands should be tested for both disease- and symptom-modifying effects in future studies.

**Significance:** Inflammation of the synovium is clearly a feature of human OA, although variable, and our group identified CCR7 as a potential marker of synovitis and symptoms. In this study the genetic absence of CCR7 lessened the severity of OA in a murine model of disease. This is the first study to identify a role for CCR7 in a functional disease model of OA using specialized behavioral monitoring technology.
Figure 1: Staining of CCR7 in synovium from patients with meniscal tears and advanced knee OA compared to asymptomatic donors.

a: Representative sections from each group.

b: Quantitation of staining by automated image analysis.

Figure 2: Cartilage degeneration score 6 weeks after DMM surgery in C57BL/6 wild-type (black squares) and CCR7 deficient (blue circles) mice.
Figure 3: Changes in climbing activity (a) and distance traveled (b) over 12 weeks post-DMM surgery in CCR7 -/- and C57BL/6 wild-type mice. Multiplicity-adjusted p-values *<0.05, **≤0.001, ***≤0.0001 CCR7 -/- vs. WT (repeated measures ANOVA followed by Sidak’s post-test).

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