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The chemokine receptor CCR5 plays a role in post-traumatic cartilage loss in mice, but does not affect synovium and bone

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SUMMARY

Objective: C–C chemokine receptor type 5 (CCR5) has been implicated in rheumatoid arthritis and several inflammatory diseases, where its blockade resulted in reduced joint destruction. However, its role in modulating cartilage and bone changes in post-traumatic osteoarthritis (OA) has not yet been investigated. In this study, we investigated changes in articular cartilage, synovium and bone in a post-traumatic OA model using CCR5-deficient ($CCR5^{-/-}$) mice.

Method: Destabilization of the medial meniscus (DMM) was performed on the right knee of 10-week old $CCR5^{-/-}$ and C57BL/6J wild-type (WT) mice to induce post-traumatic OA. The contralateral left knee served as sham-operated control. Knee joints were analyzed at 4-, 8- and 12-weeks after surgery to evaluate cartilage degeneration and synovitis by histology, and bone changes *via* micro-CT.

Results: Our findings showed that $CCR5^{-/-}$ mice exhibited significantly less cartilage degeneration than WT mice at 8- and 12-weeks post-surgery. $CCR5^{-/-}$ mice showed some altered bone parameters 18- and 22-weeks of age, but body size and weight were not affected. The effect of CCR5-ablation was insignificant at all time points post-surgery for synovitis and for bone parameters such as bone volume/total volume, connectivity density index (CDI), structure model index (SMI), subchondral bone plate thickness, and trabecular bone number, thickness and spacing.

Conclusion: These findings suggest that $CCR5^{-/-}$ mice developed less cartilage degeneration, which may indicate a potential protective role of CCR5-ablation in cartilage homeostasis. There were no differences in bone or synovial response to surgery suggesting that CCR5 functions primarily in cartilage during the development of post-traumatic OA.

Introduction

Osteoarthritis (OA), the most common degenerative joint disease of the U.S. population, affects many joint tissue processes including articular cartilage, meniscus, and ligament degeneration, subchondral bone remodeling, osteophyte formation, and inflammation of the synovium (synovitis)[1–5]. Despite its widespread occurrence and consequences to society, the etiopathogenesis of OA remains largely elusive. It is thought that OA is dependent on multiple factors including degradative enzymes[5], inflammatory mediators[6], cytokines[3,7] and chemokines[8,9]. Chemokines are a family of small structurally-related proteins[8] that are involved in a wide-array of inflammatory and infectious diseases including OA[8–10]. Chemokines exert their biological functions through binding to specific cell membrane receptors[9,11].

We are interested in C–C chemokine receptor type 5 (CCR5) for the following specific reasons: (1) CCR5 has been identified to serve as a functional receptor for several inflammatory C–C-chemokines, including macrophage inflammatory protein 1α (MIP-1α, also called C–C motif ligand 3 or CCL3), MIP-1β (CCL4), and RANTES (regulated on activation, normal T cell expressed and secreted, also called CCL5)[9,11,12], (2) *in vitro* studies in our laboratory have shown that levels of many chemokines including CCL3, C–C motif ligand 3

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like 1 (CCL3L1) and CCL4 are elevated in human articular chondrocytes in response to the pro-inflammatory cytokine interleukin (IL)-1β and the adipokine resistin[13–15], (3) CCR5 and its ligands maintain the inflammatory process in rat adjuvant-induced arthritis whereas blocking of CCR5 has resulted in reduced joint destruction[16–18], (4) CCR5 has been reported to be expressed in normal and OA chondrocytes[14,19,20] and its expression is elevated in OA chondrocytes[14,21] as well as after RANTES stimulation[19], (5) CCR5 has been found in synovial fluid and synovial tissues of patients with rheumatoid arthritis[22–24] as well as accumulation of $CCR5^+$ T cells in the inflamed joint[25,26] and finally, (6) CCR5 plays an important role in the clearance of pro-inflammatory chemokines to resolve inflammation[27]. Despite the important role of chemokines and chemokine receptors in rheumatoid arthritis, direct evidence for the role of CCR5 in post-traumatic OA is not available.

In this study, we evaluated the progression of post-traumatic OA in CCR5-deficient ($CCR5^{-/-}$) and C57BL/6J wild-type (WT) mice. We used the destabilization of the medial meniscus (DMM) model to induce OA since this model provides practical ease and reproducibility and resembles slow-progressing human OA[28,29]. We hypothesized that removal of CCR5 *in vivo* would protect mice from developing post-traumatic OA by protecting from cartilage degeneration, synovial inflammation and OA-like bone phenotype.

Method

Mice

All procedures were approved by the Washington University Animal Studies Committee. $CCR5^{-/-}$ (in a C57BL/6J background) and C57BL/6J WT mice were procured from The Jackson Laboratory (Bar Harbor, ME) as homozygous pairs. All mice in a genotype were bred by brother-sister mating and raised at our mouse facility operating at constant temperature of 21°C and on a 12-h light/dark cycle at high standards of sanitation. Offspring were housed with their mothers until weaning at 3-weeks of age, and then separated into sex-specific cages of 4–5 mice/cage with each cage individually ventilated. The genotypes of offspring were confirmed by using a genotyping kit (KAPA Biosystems, Boston, MA). All mice were fed on irradiated rodent chow (Purina 5053, Purina Mills St. Louis, MO) with food and water provided *ad libitum*. Table I depicts the number of mice and time points used in this study.

Induction of post-traumatic OA

OA was induced through DMM surgery in which the medial meniscotibial ligament (MMTL) was transected in 10-week old mice as described elsewhere[28,29]. Briefly, mice were anesthetized using an intra-peritoneal injection of rodent cocktail (100 mg/kg ketamine, 20 mg/kg xylazine and 10 mg/kg acepromazine) before their right knee MMTL was resected to displace the medial meniscus. The contralateral left knee served as a sham, receiving the exact same surgery as DMM but without severing the MMTL. Mice were sacrificed by CO_2 asphyxiation at indicated time points. Knees were harvested and subjected to histological and micro-CT analyses.

Table I
Numbers of mice in each experimental group for each strain and time point

Genotype	Time point	*N*
WT	4 weeks	16
	8 weeks	14
	12 weeks	6
$CCR5^{-/-}$	4 weeks	14
	8 weeks	16
	12 weeks	6
Total		**72**

Histological analysis of cartilage

The harvested knees were fixed in 10% neutral-buffered-formalin, decalcified with 14% ethylenediaminetetraacetic acid for 6-days with constant shaking before embedding in paraffin. Coronal sections (5-μm) were taken through the joint at eight levels with each level separated by 80-μm intervals. From each level, three sections were stained with toluidine blue for histological assessment of cartilage and synovium. The changes in cartilage were semi-quantitatively scored[29,30] by two observers blinded to mouse identity and surgical procedure. Histological cartilage scores were assigned to four quadrants (medial tibial plateau, medial femoral condyle, lateral tibial plateau, and lateral femoral condyle) of each knee joint at all sectioned levels. For each individual, summed OA scores (representing whole joint changes) and maximum OA scores (representing the highest score within all sectioned levels of a given knee) were calculated from all four quadrants of each section[29]. The mean of the final scores from each time-point (4-, 8-, 12-weeks), genotype ($CCR5^{-/-}$, WT) and procedure (sham, DMM) were used for analysis.

Histological analysis of synovium

The synovial pathology (i.e., synovitis) was analyzed on all the toluidine blue stained sections from which summed and maximum OA scores were obtained. Degree of synovitis was scored using a published synovitis scoring system[31] that measured the enlargement of the synovial lining cell layer on a scale of 0–3 (0 = 1–2 cells, 1 = 2–4 cells, 2 = 4–9 cells and 3 = 10 or more cells) and cellular density in the synovial stroma on a scale of 0–3 (0 = normal cellularity, 1 = slightly increased cellularity, 2 = moderately increased cellularity and 3 = greatly increased cellularity). Synovitis scores obtained from all four quadrants (medial tibia, medial femur, lateral tibia, and lateral femur) [Fig. 1(A–B)] for both of the above parameters were averaged separately and then the sum of averages from both parameters was used for analysis (on a scale of 0–6).

Micro-CT analysis of bone

Prior to decalcification, knee joints were scanned using a vivaCT-40 micro-CT scanner (Scanco-Medical, Bassersdorf, Switzerland) for analysis of 3-dimensional structure of bone for several parameters as described previously[29,32] with the following setting: voxel size = 21 μm, energy = 45 kV, intensity = 177 μA and integration time = 300 ms. In order to analyze bone changes, the epiphysis of the proximal tibia was chosen as the region of interest. The region of interest was identified between the cartilage and the growth plate [Fig. 2(A)]. The outline of the epiphysis was carefully selected without inclusion of outgrowing osteophyte(s). The following morphometric parameters[33] of the tibial cancellous bone were calculated for trabecular compartments: trabecular bone volume fraction (BV/TV), i.e., the ratio of trabecular bone volume to endocortical total volume, trabecular thickness (Tb.Th.), trabecular separation (Tb.Sp.), trabecular number (Tb.N.) and structure model index (SMI). SMI, an indicator of structure of trabecular bone, is designed so that a 0 = parallel plate-like trabecular bone, 3 = cylindrical rod-like structure and 4 = perfect spheres[33]. The bone parameters documented here are not only standard