

Osteoarthritis and Cartilage



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.

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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 enzymes⁵, inflammatory mediators⁶, cytokines^{3,7} and chemokines^{8,9}. Chemokines are a family of small structurally-related proteins⁸ 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 1 α (MIP-1 α , also called C–C motif ligand 3 or CCL3), MIP-1 β (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)-1 β 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 stimulation¹⁹, (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 inflammation²⁷. 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 (MML) 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 MML 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 MML. Mice were sacrificed by CO₂ asphyxiation at indicated time points. Knees were harvested and subjected to histological and micro-CT analyses.

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- μ m) were taken through the joint at eight levels with each level separated by 80- μ m 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 section²⁹. 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 system³¹ 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 μ m, energy = 45 kV, intensity = 177 μ A 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 parameters³³ 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 spheres³³. The bone parameters documented here are not only standard

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

osteoporosis measurements but have also been used to study bone changes in OA^{29,32,34}.

The subchondral bone thickness was outlined separately from trabecular bone at the tibia using the contouring methods on the cortical regions and measured the same algorithm as used for determining Tb. Th [Fig. 2(B)].

Statistical analysis

For each phenotype, we performed analysis of variance using the factors genotype (CCR5^{-/-}, WT) and procedure (sham, DMM) and the genotype-by-procedure interaction.

The interaction term controls for between genotype tissue differences present prior to surgery. *F* is the fundamental test statistic used in analysis of variance, and is calculated as the ratio of the mean squares of the factors (between group variation) and residual error (within group variation). We set the probability of significance of a factor at 0.05. When significant, the genotype-by-procedure interaction indicates differences between CCR5^{-/-} and WT in surgical response and thus the effect of CCR5-ablation on the OA phenotype. In these cases, we used Tukey's post-hoc adjustments of standard errors in pair-wise comparisons of sham- and DMM-operated knees of WT and CCR5^{-/-} mice, and report 95% confidence intervals for significant contrasts.

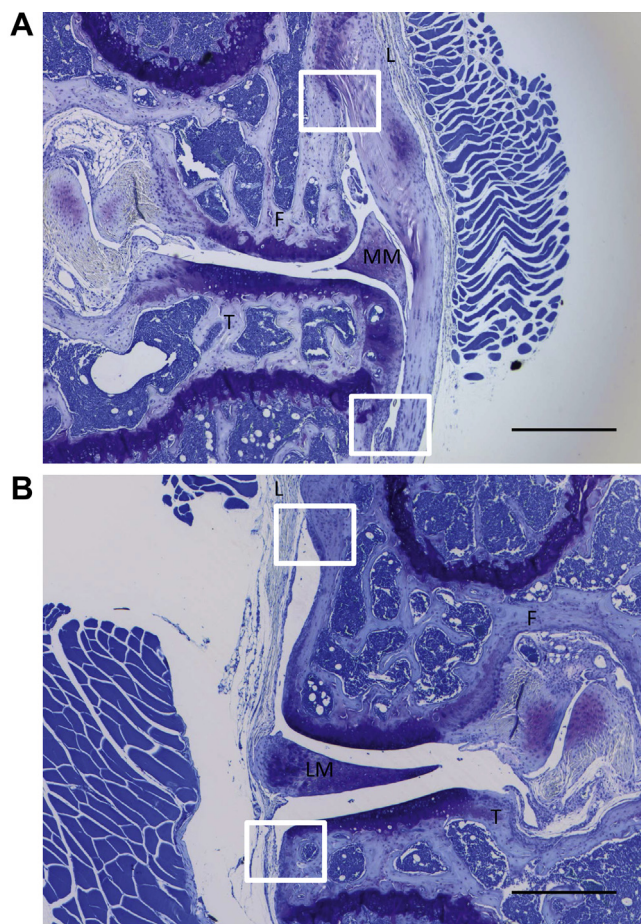


Fig. 1. Analysis of synovitis in CCR5^{-/-} and WT mice. Enlargement of synovial lining cell layer and density of the cells were obtained from all four quadrants (medial tibia, medial femur, lateral tibia, and lateral femur). The evaluation areas were white four-sided figures which were between the bone (femur or tibia) and ligament. (A) medial side of the knee (B) lateral side of the knee. Magnification 40× (Scale bar = 500 μm). F = femur; T = tibia; L = ligament; LM = lateral meniscus; MM = medial meniscus.

Results

Gross appearance and body weight

CCR5-ablation did not result in any overt phenotype or developmental abnormalities since both CCR5^{-/-} and WT mice grossly appeared alike as has also been reported by The Jackson Laboratory datasheet (<http://jaxmice.jax.org/strain/005427.html>). The average bodyweight of CCR5^{-/-} (22.53 ± 0.51 g) and WT (22.57 ± 0.39 g) mice at 10-weeks of age was equal.

Cartilage analysis in CCR5^{-/-} and WT mice

Representative sections from CCR5^{-/-} and WT sham joints at 8-weeks post-surgery are shown in Figs. 3(A) and 1(B). There was decreased cartilage degeneration in CCR5^{-/-} compared to WT mice at 8- [Fig. 3(C and D)] and 12-weeks after induction of DMM.

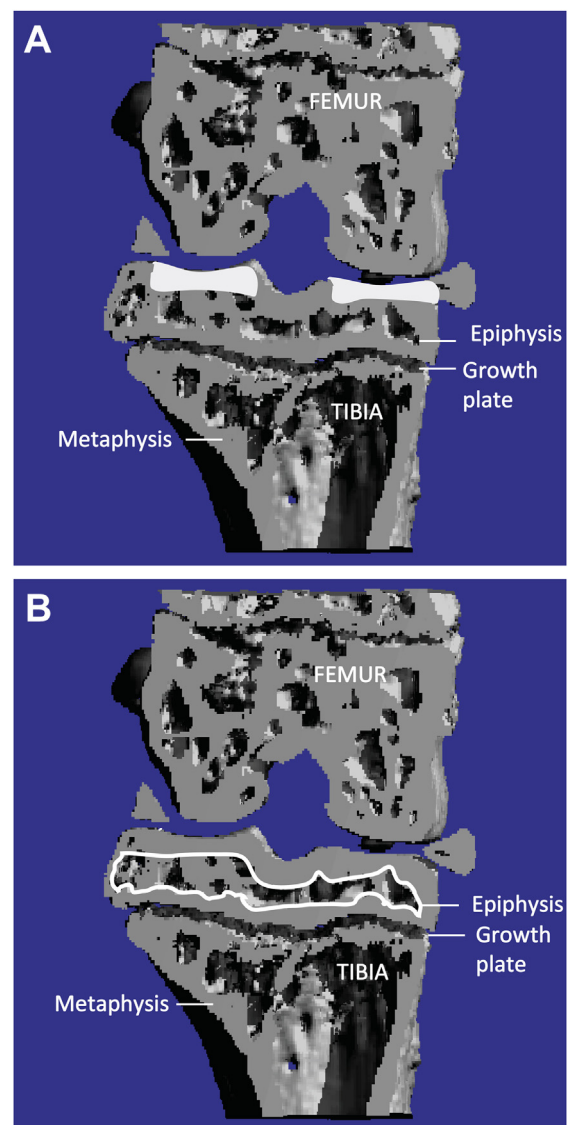


Fig. 2. Micro-CT analysis of bone changes. (A) The subchondral bone plate thickness was outlined (white shaded areas) separately from trabecular bone of the tibia using the contouring methods on the cortical regions and measured the same algorithm as used for determining Tb. Th. (B) The epiphysis of proximal tibia was selected as region of interest (outlined by white line). We were only interested in bone changes occurring in the epiphysis, i.e., area between the articular cartilage and the growth plate.

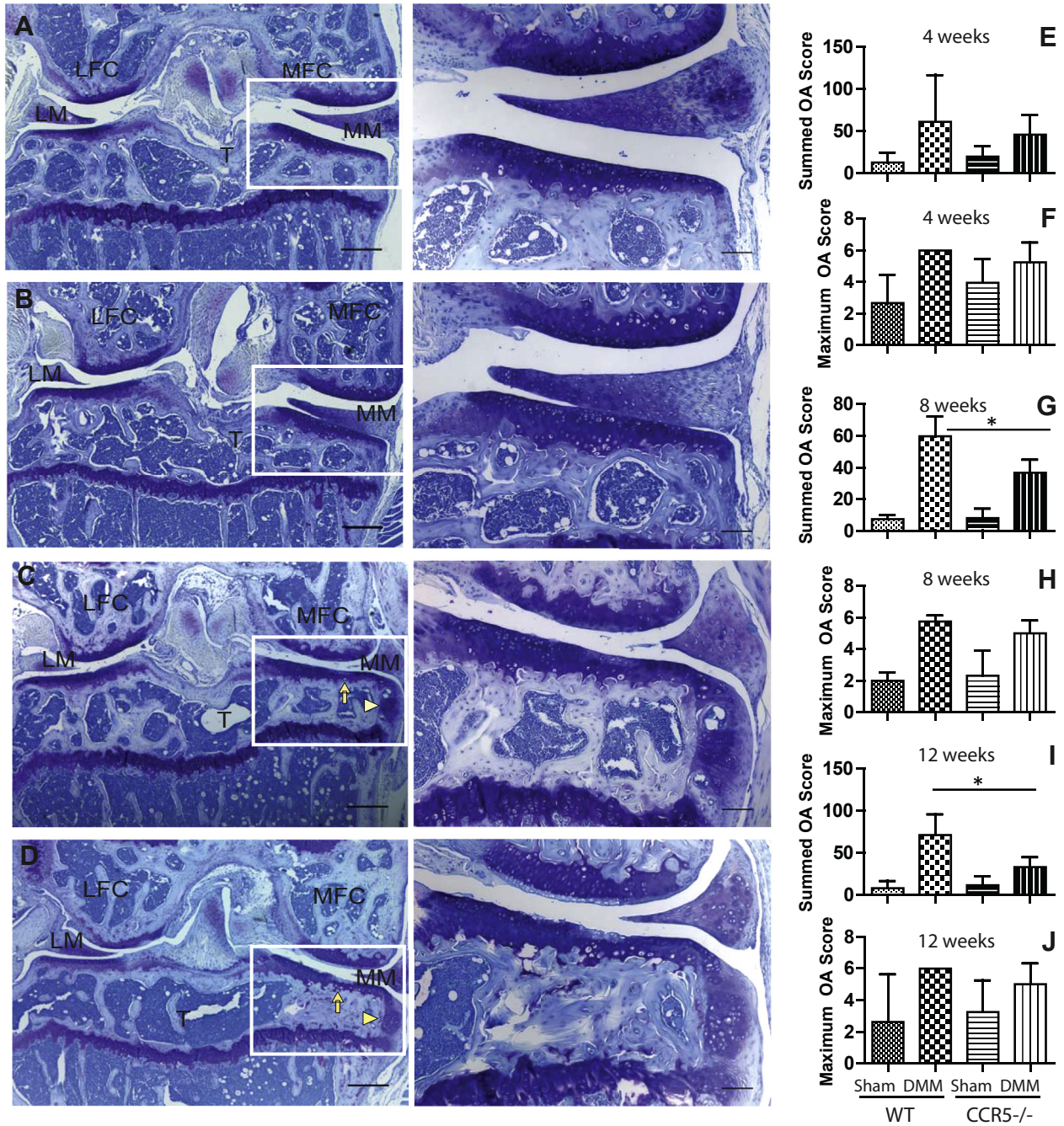


Fig. 3. Histological evaluation for cartilage degeneration in CCR5^{-/-} and WT mice. The sham-operated side of both CCR5^{-/-} (A) and WT (B) mice after DMM showed normal cartilage with no osteophyte formation at 8-weeks after DMM. Following DMM at 8-weeks, decreased articular cartilage degeneration (complete arrow) in CCR5^{-/-} (C) compared to WT mice (D) was observed. Osteophyte formation was also observed on the medial side in both CCR5^{-/-} and WT mice (arrowhead). The sum OA score from all four quadrants of five sections from each level with eight levels from each knee was based on cartilage damage and shows that CCR5^{-/-} mice develop less OA compared to WT mice after surgery only at 8-weeks (N = 14 for WT, N = 16 for CCR5^{-/-}) (G) and 12-weeks (N = 6 each genotype) (I) but not at 4-weeks post-surgery (N = 16 for WT, N = 14 for CCR5^{-/-}) (E). Pair-wise comparison showed that the summed OA score was found to be significantly lower in DMM knees of CCR5^{-/-} compared to WT mice at 8- (P < 0.01; 95% CI = 5.52–20.02 in CCR5^{-/-}) and 12-weeks (P < 0.01; 95% CI = 1.10–1.86 in CCR5^{-/-}) post-surgery. A maximum score (F, H, J) representing highest score from all sectioned levels for a given knee indicates that DMM knees have significant more OA score in both CCR5^{-/-} and WT mice compared to sham knees. It was also shown that there was no significant difference between CCR5^{-/-} mice and WT mice for maximum OA score at any time point post-surgery. MFC = medial femoral condyle; LFC = lateral femoral condyle; MM = medial meniscus; LM = lateral meniscus; T = Tibia. Images in the left panel have magnification of 40× and scale bar = 300 μm while images in the right panel have magnification of 100× and scale bar = 100 μm. Higher magnification images of the selected box showed right to the lower magnification images. Asterisks (*) indicate P < 0.05. Graphs represent mean ± 95% CI.

Whereas WT mice showed a loss of proteoglycan staining along with cartilage fibrillation in the superficial zone [Fig. 1(D)], CCR5^{-/-} mice demonstrated a focal loss of proteoglycan staining without severe loss in cartilage post-surgery [Fig. 3(C)]. Osteophyte formation was observed on the medial side in both CCR5^{-/-} and WT mice.

Statistical analysis of summed OA scores (across all four quadrants) showed that there is no significant genotype-by-procedure interaction effect at 4-weeks after surgery. However, this effect reached a borderline significance (P = 0.058) at the 8-week and formal significance (P = 0.039) at the 12-week time point (Table II).

Pair-wise comparison showed that the summed OA score was found to be significantly lower in DMM knees of CCR5^{-/-} compared to WT mice at 8- ($P < 0.01$; 95% CI = 5.52–20.02 for CCR5^{-/-}) and 12-weeks ($P < 0.01$; 95% CI = 1.10–1.86 for CCR5^{-/-}) post-surgery [Fig. 1(G–I)]. The maximum OA score that represents the highest score in any of the knee cartilage compartments was not significantly different between the two genotypes [Fig. 1(F, H and J)].

Synovium analysis in CCR5^{-/-} and WT mice

No significant differences were detected for synovial score (Table II) at any time point post-surgery. Synovitis scores for WT and CCR5^{-/-} DMM-operated knees were not significantly influenced by variation in genotype [Fig. 4(A–G)]. Post-surgical inflammation of synovium (i.e., synovitis) appeared to decrease in shams for 8- and 12-week time points compared to 4-week time point, whereas that of DMM limb persisted. This was to be expected given that post-surgical inflammation was higher at earlier time points. We failed to find any significant difference in degree of synovitis between the two genotypes/procedure combinations at any of the time points studied.

Analysis of bone phenotype

We examined bone of the distal tibia by comparing values for these parameters: BV/TV, connectivity density index (CDI), SMI, subchondral bone plate thickness, Tb.N., Tb.Th., and Tb.Sp. We used analysis of variance to find significant genotype-by-procedure interactions that would indicate CCR5-ablation causes a different surgical response compared to wild type. By this analysis, we found very few significant differences by genotype (BV/TV, SMI, subchondral bone thickness, and Tb.Th.) and by procedure (subchondral bone thickness, CDI, Tb.Th., BV/TV, and SMI). We did observe a significant interaction at 4 weeks for trabecular thickness (Table II), but it disappeared by 8- and 12-weeks. These results suggest no differences in bone quality between CCR5^{-/-} and WT in response to traumatic injury (DMM-surgery).

Discussion

Chemokines exert their effects through binding to specific cell membrane receptors^{9,11}. Functionally, they can be divided into

inflammatory, homeostatic, and angiogenic/angiostatic chemokines⁸. Several chemokines are expressed at elevated levels in OA chondrocytes, proposed to be due to an abnormal mechanical stress^{8,35}. The role of chemokines in post-traumatic OA has not been investigated. Molecular studies demonstrate that cartilage chondrocytes produce substantial quantities and varieties of CCR ligands and chemokines in response to cytokines^{14,15,19,20}, and the genes for CCL3 and CCL4 are regulated by the transcription factors NF- κ B and C/EBP β known to be involved in OA¹³. Therefore, we took the approach to study post-traumatic OA in mice deficient in CCR5.

This study shows that mice lacking CCR5 are less susceptible to post-traumatic OA as evidenced by a significant reduction in cartilage degeneration in CCR5^{-/-} mice after DMM surgery compared to WT control mice. There were no significant differences in the degree of synovitis and bone metabolic parameters between CCR5^{-/-} and WT mice in response to DMM surgery. In addition, there was no difference in osteophyte formation between CCR5^{-/-} and WT mice, which reflects a normal anabolic process in both genotypes.

We observed that CCR5^{-/-} mice exhibit significantly less cartilage degeneration than WT mice, strongly suggesting involvement of CCR5 in cartilage degeneration. While differences in articular cartilage degeneration were not seen between CCR5^{-/-} and WT mice at the early 4-week time point, they were observed at the later 8- and 12-week time-points, a time line that has been shown previously for other mouse strains^{29,32}. While degeneration of articular cartilage is considered a hallmark of end-stage OA^{36,37}, observing less degeneration in CCR5^{-/-} mice suggests some degree of protection from developing post-traumatic OA.

We found a significant genotype-by-procedure interaction effect for the summed OA score but not for maximum OA score. The absence of significance for maximum OA score is attributable to the nature of scoring system: any out of range score in summed OA scoring system is covered by the calculation of means across all the sectioned levels. In contrast, for maximum OA scoring, one has to take into consideration the maximum score in any one of the sections without calculating means across all sections. Thus, the CCR5^{-/-} mice showed less widespread areas of cartilage destruction compared to WT control mice.

Under normal physiological conditions the synovial lining is made up of a thin layer of cells³⁵, while in OA the synovium shows

Table II
F ratios and associated *P* values for various parameters studies for CCR5^{-/-} and WT mice by time points

Time point	Effects	Summed OA score	Max OA score	Synovium	BV/TV	CDI	SMI	Tb.N.	Tb.Th.	Tb.Sp.	Subchondral bone thickness
4 weeks	Genotype	<i>F</i> = 0.016 <i>P</i> = 0.900	<i>F</i> = 0.618 <i>P</i> = 0.439	<i>F</i> = 0.716 <i>P</i> = 0.405	<i>F</i> = 0.036 <i>P</i> = 0.848	<i>F</i> = 0.141 <i>P</i> = 0.708	<i>F</i> = 0.249 <i>P</i> = 0.619	<i>F</i> = 0.385 <i>P</i> = 0.537	<i>F</i> = 0.840 <i>P</i> = 0.363	<i>F</i> = 0.026 <i>P</i> = 0.870	<i>F</i> = 1.870 <i>P</i> = 0.182
	Procedure	<i>F</i> = 17.124 <i>P</i> < 0.001	<i>F</i> = 8.584 <i>P</i> = 0.006	<i>F</i> = 0.373 <i>P</i> = 0.546	<i>F</i> = 0.022 <i>P</i> = 0.880	<i>F</i> = 2.504 <i>P</i> = 0.119	<i>F</i> = 2.866 <i>P</i> = 0.095	<i>F</i> = 0.003 <i>P</i> = 0.953	<i>F</i> = 1.518 <i>P</i> = 0.223	<i>F</i> = 0.034 <i>P</i> = 0.852	<i>F</i> = 6.112 <i>P</i> = 0.019
	Genotype*procedure	<i>F</i> = 1.689 <i>P</i> = 0.205	<i>F</i> = 1.939 <i>P</i> = 0.175	<i>F</i> = 0.247 <i>P</i> = 0.623	<i>F</i> = 1.057 <i>P</i> = 0.308	<i>F</i> = 2.267 <i>P</i> = 0.137	<i>F</i> = 0.083 <i>P</i> = 0.774	<i>F</i> = 0.179 <i>P</i> = 0.673	<i>F</i> = 5.241 <i>P</i> = 0.025	<i>F</i> = 0.233 <i>P</i> = 0.630	<i>F</i> = 0.015 <i>P</i> = 0.901
8 weeks	Genotype	<i>F</i> = 7.267 <i>P</i> = 0.011	<i>F</i> = 1.531 <i>P</i> = 0.224	<i>F</i> = 1.680 <i>P</i> = 0.203	<i>F</i> = 5.022 <i>P</i> = 0.029	<i>F</i> = 1.127 <i>P</i> = 0.292	<i>F</i> = 4.149 <i>P</i> = 0.046	<i>F</i> = 0.722 <i>P</i> = 0.398	<i>F</i> = 1.487 <i>P</i> = 0.227	<i>F</i> = 0.919 <i>P</i> = 0.341	<i>F</i> = 9.173 <i>P</i> = 0.005
	Procedure	<i>F</i> = 63.105 <i>P</i> < 0.001	<i>F</i> = 75.004 <i>P</i> < 0.001	<i>F</i> = 14.046 <i>P</i> = 0.001	<i>F</i> = 0.282 <i>P</i> = 0.597	<i>F</i> = 5.633 <i>P</i> = 0.021	<i>F</i> = 1.116 <i>P</i> = 0.295	<i>F</i> = 0.497 <i>P</i> = 0.483	<i>F</i> = 5.185 <i>P</i> = 0.026	<i>F</i> = 0.225 <i>P</i> = 0.636	<i>F</i> = 19.570 <i>P</i> < 0.001
	Genotype*procedure	<i>F</i> = 3.820 <i>P</i> = 0.058	<i>F</i> = 1.808 <i>P</i> = 0.187	<i>F</i> = 0.678 <i>P</i> = 0.415	<i>F</i> = 2.151 <i>P</i> = 0.148	<i>F</i> = 0.127 <i>P</i> = 0.722	<i>F</i> = 1.026 <i>P</i> = 0.315	<i>F</i> = 0.344 <i>P</i> = 0.559	<i>F</i> = 0.110 <i>P</i> = 0.740	<i>F</i> = 0.145 <i>P</i> = 0.704	<i>F</i> = 0.756 <i>P</i> = 0.391
12 weeks	Genotype	<i>F</i> = 1.662 <i>P</i> = 0.222	<i>F</i> = 0.030 <i>P</i> = 0.865	<i>F</i> = 0.584 <i>P</i> = 0.458	<i>F</i> = 5.959 <i>P</i> = 0.040	<i>F</i> = 0.002 <i>P</i> = 0.958	<i>F</i> = 12.731 <i>P</i> = 0.007	<i>F</i> = 0.141 <i>P</i> = 0.716	<i>F</i> = 8.047 <i>P</i> = 0.021	<i>F</i> = 0.151 <i>P</i> = 0.707	<i>F</i> = 0.596 <i>P</i> = 0.462
	Procedure	<i>F</i> = 5.306 <i>P</i> = 0.040	<i>F</i> = 5.435 <i>P</i> = 0.038	<i>F</i> = 14.428 <i>P</i> = 0.002	<i>F</i> = 0.092 <i>P</i> = 0.768	<i>F</i> = 6.802 <i>P</i> = 0.031	<i>F</i> = 3.337 <i>P</i> = 0.105	<i>F</i> = 2.907 <i>P</i> = 0.126	<i>F</i> = 7.316 <i>P</i> = 0.026	<i>F</i> = 1.648 <i>P</i> = 0.235	<i>F</i> = 25.507 <i>P</i> < 0.001
	Genotype*procedure	<i>F</i> = 5.304 <i>P</i> = 0.040	<i>F</i> = 1.453 <i>P</i> = 0.251	<i>F</i> = 3.180 <i>P</i> = 0.097	<i>F</i> = 0.403 <i>P</i> = 0.543	<i>F</i> = 0.023 <i>P</i> = 0.881	<i>F</i> = 0.000 <i>P</i> = 0.984	<i>F</i> = 0.133 <i>P</i> = 0.724	<i>F</i> = 2.302 <i>P</i> = 0.167	<i>F</i> = 0.077 <i>P</i> = 0.787	<i>F</i> = 0.001 <i>P</i> = 0.972

BV/TV = trabecular bone volume fraction, the ratio of trabecular bone volume over endocortical total volume; CDI = connectivity density index, SMI = structure model index, Tb.N. = trabecular number, Tb.Th. = trabecular thickness, Tb.Sp. = trabecular separation. Bold values indicate statistical significance at $P < 0$.

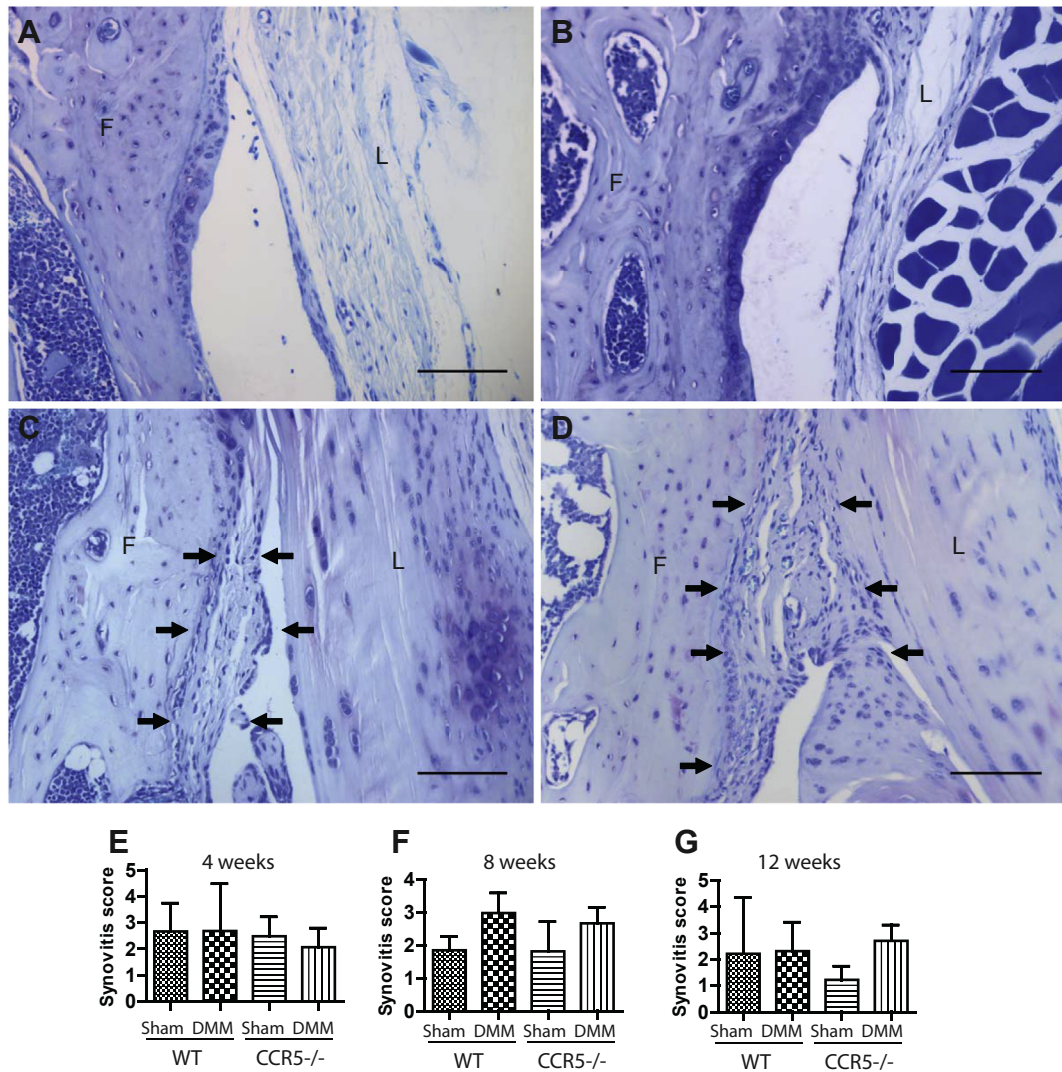


Fig. 4. Degree of synovitis in CCR5^{-/-} and WT mice. The sham-operated side of both CCR5^{-/-} (A) and WT (B) mice after DMM showed normal plasticity and cellularity at 8-weeks after DMM. The DMM sides in CCR5^{-/-} mice (C) and WT mice (D) showed more hyperplasia and increased cell density compared to sham-operated knees following induction of DMM at 8-weeks. The synovitis score at 4-weeks ($N = 16$ for WT, $N = 14$ for CCR5^{-/-}) (E), 8-weeks ($N = 14$ for WT, $N = 16$ for CCR5^{-/-}) (F) and at 12-weeks ($N = 6$ each genotype) (G) from all the four quadrants of three sections from each knee was based on the synovial lining thickness and the synovial stromal cell density. We observed no statistically significant differences in degree of synovitis between the two genotypes. Arrows show synovial hyperplasia and increased cell density. F = femur; L = ligament. Magnification 200 \times (Scale bar = 100 μ m). Graphs represent mean \pm 95% CI.

hypertrophy and hyperplasia with an increased number of lining cells³⁸. In addition, activated synovium may produce proteases, catabolic mediators, and cytokines that accelerate the progression of OA^{35,38}. It has been reported that CCR5 is expressed in OA synovial fibroblasts, therefore, we expected to see less synovitis in CCR5^{-/-} mice than WT. While we were unable to observe a statistically significant difference among the genotypes/procedure combinations for hyperplasia or synovial cell density, we did see a slightly higher overall synovitis in DMM knees of WT compared to CCR5^{-/-} at 4- and 8-weeks [Fig. 2(E and F)] that resolved at 12-weeks. Other studies^{17,39} in rats have shown blockade of CCR5 in an adjuvant-induced arthritis model resulted in reduced inflammation of synovium¹⁷. In addition, CCR5 antagonists have been reported to reduce joint destruction and synovitis in collagen-induced arthritis, both in monkeys³⁹ and mice⁴⁰. A possible explanation for the differences in the effect on synovitis between our findings and those reported above may reside in the nature of the arthritis models: collagenase-induced arthritis (as well as adjuvant-induced arthritis) is more inflammatory^{41,42} than the

slowly eroding OA induced by DMM surgery²⁸. Our findings suggest that cartilage degeneration can proceed without significant synovitis. Despite the evidence for the pro-inflammatory role of CCR5, several studies suggest its anti-inflammatory role^{27,43}. Therefore, we suggest that further mechanistic studies are needed to investigate the roles of CCR5 and its ligands in modulating changes in cartilage following a traumatic joint injury.

Our findings indicate that there are small baseline differences in few bone parameters between the two genotypes (but not in response to surgery). However, the biological significance of these differences is difficult to predict with these data. In this study, measurements from the lateral and medial compartments of the tibial plateau were combined. In others when lateral and medial compartments were analyzed separately, medial subchondral bone thickening was correlated with the degree of cartilage damage^{29,32}. The lack of significant genotype-by-procedure interaction effect suggests that the response of the bone to DMM in CCR5-deficient mice is not different from that of WT mice. This finding is in line with a previous study that has shown that expression of RANTES,

an inducer of CCR5¹⁹, is not upregulated as much in traumatic injury as is in OA and rheumatoid arthritis⁴⁴.

A limitation associated with the current study is the use of contralateral limb as sham-operated because this approach could pose issues such as gait modification and movement provoked pain. In spite of studies that report use of sham and operated knees in different mice^{32,45}, there are also reports using sham and surgery-operated knees within the same animal^{29,46}. Interestingly, in unpublished observations, we noted that the measurements of bone parameters of sham-operated knees differed depending on whether the contralateral knee was itself a sham or DMM-operated knee. Although debatable, combining sham and DMM operations in the same mouse provides a better control for the effect of the surgery up to but not including transection of the MMTL.

Taken together, our findings demonstrate a protective role of the CCR5-ablation in post-traumatic OA development through modulating changes in cartilage (less degeneration), without involving changes in bone and synovium. Therefore, CCR5 may be a target for intervention after traumatic insult to the articular joint.

Contributions

All authors contributed to the conception and design of the study, analysis, and interpretation of data, and preparation and approval of the manuscript. In addition, MFR, and KT contributed to data acquisition, MFR, KT, and EJS analyzed the data, MFR, KT, EJS and LJS wrote the manuscript, and MFR, KT, and LJS take responsibility for the integrity of the work.

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Conflict of interest

The authors have no conflict of interest.

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