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Can we target CCR2 to treat osteoarthritis? The trick is in the timing!

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C-C motif chemokines, including CCL2, CCL7, and CCL12, affect cells that express the C-C chemokine receptor type 2, CCR2, a seven-transmembrane domain G-protein coupled receptor (GPCR) [1]. Signaling through CCR2 has been implicated in many pathologies, including autoimmune disorders, tumor metastasis, atherosclerosis, stroke, neurodegenerative disorders, and neurological complications of HIV [2]. As a GPCR, CCR2 is eminently druggable and many small-molecule antagonists have been tested in clinical trials for different conditions [2, 3].

The rheumatology research community has had a longstanding interest in the CCR2 signaling pathway, due to its manifest pro-inflammatory and chemo-attractant actions. As the main chemotactic pathway for monocytes, the CCL2/CCR2 axis is key for recruitment of CCR2-expressing circulating monocytes to sites of inflammation [3]. In addition, CCR2 is expressed by other immune cells such as CD4+ T_H1 cells [3]. Even though osteoarthritis (OA) is not considered a primarily inflammatory process, there is an increasing awareness that innate immune pathways and chronic low-grade inflammation may critically contribute to the pathogenesis of OA. For instance, chemokines, including CCL2, are clearly elevated in osteoarthritic compared to healthy joints [4]. Also, both in human disease and in mouse models, emerging evidence suggests that synovial monocyte/macrophage lineage cells contribute to driving structural damage in OA and that CD4+ T cells may be found in the OA joint [5, 6]. Such observations have led to an interest in CCR2 as a target for joint protection in OA. However, studies in *Ccr2* null mice have been largely discouraging (Table 1). When a small number of *Ccr2* null mice were first tested in a surgical model of OA induced by destabilization of the medial meniscus (DMM), these mutant mice developed similar cartilage damage as wild-types over an 8-week period [7]. In the largest study yet, two independent laboratories recently confirmed an absence of protection from cartilage damage in *Ccr2* null mice 8 weeks after DMM, while there was a slight trend toward chondroprotection at 12,16, and 20 weeks [8]. In contrast, a recent study reported partial protection from cartilage damage and synovitis, 20 weeks after DMM [9]; notably, this study was conducted in 20-week old mice, which resulted in more severe joint damage in wild-type mice by week 20 compared to surgery in 10-week old mice [8, 9]. While the protective

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effect was statistically significant, *Ccr2* null mice still developed significant joint damage in this study [9]. Thus, depending on the duration and the severity of the model, joint protection in *Ccr2* null mice is either absent or mild-to-moderate, and the observed degree of protection may not be sufficient to identify CCR2 as a viable target for joint preservation.

Pharmacologically, CCR2 can be blocked by a selective small-molecule CCR2 receptor antagonist (CCR2-RA). In the current issue of *Osteoarthritis and Cartilage*, Longobardi and colleagues report the first studies with prolonged therapeutic administration of a CCR2-RA (RS-504393) in the murine DMM model [10]. They found that sustained pharmacological blockade of CCR2 mimics the findings in *Ccr2* null mice. Thus, when treatment with CCR2-RA started 1 week after DMM surgery and continued until week 12, this sustained treatment protocol did not result in a significant chondroprotective effect at the end of the study (Table 2). The exciting and potentially translationally significant discovery in this study, however, was the observation that the effects of CCR2 blockade on joint damage demonstrated marked temporal effects (Table 2). No significant chondroprotective effect was observed when CCR2-RA was transiently administered from week 1 to week 4 only, or from week 8 to week 12 only. In contrast, Longobardi and colleagues reported a robust protection from joint damage (cartilage damage and osteophyte size) with transient CCR2 blockade from week 4 to 8.

The estimated percent inhibition of joint damage when CCR2 blockade was sustained for the entire experiment, or was transient between weeks 1–4 or weeks 8–12 was between 29% and 57%, which is in line with other published studies in surgical rat and mouse models, where continuous pharmacological CCR2 blockade resulted in a similar modest degree of protection [9–11] (Table 2). The observation that transient *vs.* sustained CCR2 inhibition leads to different structural outcomes in the murine DMM model may provide important insights into the role of inflammation in pathogenesis of OA after joint injury. Inflammation plays a key role in healing after injury but, when inflammation is not self-limited and becomes chronic, it may perpetuate and amplify joint destruction and remodeling [4]. Therefore, modulating inflammation in the right place and only at the right time may be paramount for successful intervention.

Monocyte migration to site of injury is an important early event in the immune defense repertoire [12]. It has indeed been shown that monocyte/macrophage lineage cells infiltrate the synovium after DMM surgery [5, 9, 13], but there is a need for better characterization of the temporal characteristics of the infiltrate, as well as its exact composition – for instance, is there polarization toward a pro-inflammatory M1 *vs.* an anti-inflammatory M2 phenotype [14]? Thus, it could be hypothesized that CCR2 signaling may be crucial for recruiting cells that are involved in resolving acute post-injury inflammation and/or promoting repair, which may partly explain the absent or modest joint protective effects of *Ccr2* ablation or sustained pharmacological CCR2 blockade, as well as the dependency of the effect size on the severity of the model. In this respect, Miotla Zarebska *et al.* found distinctly altered inflammatory responses in whole-joint extracts from *Ccr2* null mice compared to wild-types, 6 hours and 7 days after destabilization [8]. Notably, in wild-type mice, one of the most strongly induced genes – besides the CCR2 ligand, *Ccl2* – was *Arg1*, the gene encoding arginase 1, which is expressed in macrophages and partly defines the M2 macrophage phenotype [14]. *Ccr2* null

joints showed significantly less expression of *Arg1*, 6 hours after surgery, suggesting that CCR2 promotes anti-inflammatory polarization. Raghu *et al.* found that sustained prophylactic CCR2 blockade resulted in reduced monocyte infiltration in the synovium by week 16, but they did not characterize the phenotypes [9]. Longobardi *et al.* did not analyze cellular infiltration as part of their study, but the fact that the most effective treatment strategy was from week 4 to 8 supports the idea that early CCR2-related macrophage polarization may be important for healing. At this time, it is not clear why the optimal window for CCR2 blockade would be during this particular time period, but these observations clearly warrant an in-depth characterization of the monocyte/macrophage (and other immune cells) infiltrate into the mouse joint after DMM (and sham) surgery, both with respect to timing and composition of the cellular infiltrate.

It should also be considered that CCR2 is a widely expressed molecule, present on monocytes, T cells, neurons, osteoclasts, and chondrocytes [4, 10]. In cartilage, a number of chemokines, including the three CCR2 ligands, are upregulated in rodent models of OA, but the role of chemokine signaling within cartilage in OA pathogenesis remains unclear [4, 10]. Therefore, the complex interplay between tissues and cells in the joint, and temporal changes in these interactions, may contribute to the observed phenotype in mice where CCR2 is blocked genetically or pharmacologically. Selective ablation of *Ccr2* in specific cells (*e.g.*, chondrocytes, macrophages or macrophage subsets) may help elucidate these interactions, and this approach may guide treatment strategies for targeting CCR2.

In addition to the potential role of the CCR2 signaling pathway in joint damage, it is important to highlight that this pathway has been extensively characterized as a pain target and plays a well-documented role in the initiation and maintenance of neuropathic and inflammatory pain [15]. The nociceptive actions of CCR2 signaling include direct neuronal excitation through transactivation of transient receptor potential channel subfamily V member 1 (TRPV1) and other ion channels, as well as the recruitment of monocytes, which can release pro-algesic molecules [15]. Indeed, in the DMM model, chronic pain behaviours associated with experimental OA (persistent referred mechanical allodynia, weight bearing deficits, and activity-induced pain behaviours) are clearly attenuated in *Ccr2* null mice (Table 1), likely through a combination of neuronal and macrophage-driven mechanisms [7, 8]. In agreement with these studies in *Ccr2* null mice, Longobardi and colleagues found that pharmacological blockade of CCR2 resulted in reversal of weight bearing deficits, and this reversal was present in all treatment protocols tested, sustained and transient (Table 2). Interestingly, the analgesic effect was sustained even after the treatment had been suspended. Thus, preclinical findings to date suggest that CCR2 may be a target for OA pain. Here too, detailed studies on the mechanisms of action and potential temporal effects of CCR2 blockade on the pain pathway will likely be very helpful to aid clinical translation. Clinicaltrials.gov lists a randomized, double-blind, placebo-controlled phase 2 study in subjects with OA pain of the knee, using a selective CCR2 antagonist, but trial results have not been reported.

In conclusion, the study by Longobardi *et al.* is noteworthy for several reasons. First, it highlights that CCR2 may be a viable target for the treatment of osteoarthritis, particularly in mid-stage disease. Secondly, it confirms observations in *Ccr2* null mice that this pathway

may be an attractive target for OA pain. Finally, it also illustrates how important it is to do careful temporal studies in experimental models. If these observations can be reproduced and the mechanistic and cellular pathways involved can be elucidated, CCR2 may be a very appealing target in OA - not just for joint preservation, but at the same time for management of OA pain.

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Table 1

DMM surgery in wild type *vs. Ccr2* null mice

Study Design	Histology Results	Pain Assessments	Ref
8 weeks; n= 4 – 5/group; Surgery at age 10 wks	8 wks: WT and *Ccr2* null mice developed similar levels of joint damage	*Ccr2* null mice are protected from persistent secondary mechanical allodynia by week 16 *Ccr2* null mice are protected from locomotion deficits through week 16	Miller *et al*, 2012
8, 12, 16, 20 weeks; n = 8–39/time point; Surgery at age 10 wks	8 wks: WT and *Ccr2* null mice developed similar levels of cartilage damage 12–20 wks: Slight trend toward protection against cartilage damage in *Ccr2* null mice	*Ccr2* null mice developed delayed weight-bearing deficits (17 weeks post surgery) compared to WT mice (11 weeks post surgery)	Miotla Zarebska *et al*, 2016
16 and 20 weeks; n= 4–5/ group Surgery at age 20 wks	16 wks: *Ccr2* null mice were partially protected against macrophage infiltration 20 wks: *Ccr2* null mice were partially protected against cartilage damage, synovitis, osteophytes	Not done	Raghu *et al*, 2016

Table 2

Comparison CCR2-RA (RS504393) experimental OA studies

Model	Treatment Plan	Estimated percent inhibition of cartilage damage*	Ref
Rat ACLT/pMNX; n=5; Surgery at 11–12 weeks of age	Time of surgery – 10 weeks; Mini-pump; 2 mg/kg/day	**10 wks: 57% (p<0.05)**	Appleton *et al*, 2015
Mouse DMM; n=7–12; Surgery at 20 weeks of age	1 day post surgery – 12 weeks; 2x daily oral gavage; 4 mg/kg/day	**12 wks: 32% (p<0.05)**	Raghu *et al*, 2016
Mouse DMM; n=6 for each study; Surgery at 10 weeks of age	Weeks 1 – 12; drinking water; 4 mg/kg/day	12 wks: 50% (n.s.)	Longobardi *et al*, 2016
	Weeks 1 – 4; drinking water; 4 mg/kg/day	12 wks: 57% (n.s.)	
	Weeks 4 – 8; drinking water; 4 mg/kg/day	**12 wks: 85% (p=0.0013)**	
	Weeks 8 – 12; drinking water; 4 mg/kg/day	12 wks: 29% (n.s.)	

*This was calculated by RM and AM by using the estimated published means of the vehicle and RS504393-treated DMM groups: % inhibition = {(mean RS504393-treated) – (mean vehicle-treated)} / (mean vehicle-treated) × 100

n.s. = Not statistically significant