Biochemical and Biophysical Research Communications 391 (2010) 1080–1086

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Pharmacological targeting reveals distinct roles for CXCR2/CXCR1 and CCR2 in a mouse model of arthritis

Soo-Hong Min, Yuanfan Wang, Waldemar Gonsiorek [1], Gopinadhan Anilkumar, Joseph Kozlowski, Daniel Lundell, Jay S. Fine [1], Ethan P. Grant *

Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA

ARTICLE INFO

Article history:
Received 3 December 2009
Available online 14 December 2009

Keywords:
CXCR2
CCR2
Arthritis
Pharmacology
Pharmacodynamics
Migration

ABSTRACT

Neutrophils and monocytes are abundantly represented in the synovial fluid and tissue in rheumatoid arthritis patients. We therefore explored the effects of small molecule chemokine receptor antagonists to block migration of these cells in anti-collagen antibody-induced arthritis. Targeting neutrophil migration with the CXCR2/CXCR1 antagonist SCH563705 led to a dose-dependent decrease in clinical disease scores and paw thickness measurements and clearly reduced inflammation and bone and cartilage degradation based on histopathology and paw cytokine analyses. In contrast, targeting monocyte migration with the CCR2 antagonist MK0812 had no effect on arthritis disease severity. The pharmacodynamic activities of both SCH563705 and MK0812 were verified by assessing their effects on the peripheral blood monocyte and neutrophil populations. SCH563705 selectively reduced the peripheral blood neutrophil frequency, and caused an elevation in the CXCR2 ligand CXCL1. MK0812 selectively reduced the peripheral blood monocyte frequency, and caused an elevation in the CCR2 ligand CCL2. The much greater impact of CXCR2/CXCR1 antagonism relative to CCR2 antagonism in this model of arthritis highlights the therapeutic potential for targeting CXCR2/CXCR1 in human arthritides.

Introduction

Targeting chemokine receptor-mediated cell migration has been an area of active research to treat autoimmune disorders including rheumatoid arthritis (RA), psoriasis, multiple sclerosis, and inflammatory bowel disease [1,2]. Neutrophils and monocytes are two major cell populations recruited to the joint in RA. Neutrophils are among the most abundant cells found in RA synovial fluid and, as a source of pro-inflammatory mediators and tissue-degrading enzymes, likely contribute to the disease pathology [3]. CXCR1 and CXCR2 are co-expressed on human neutrophils and are the principal mediators of neutrophil migration to sites of inflammation. RA synovial fluid contains elevated levels of a number of CXCR1 and CXCR2 ligands, including IL-8 (CXCL8), ENA-78 (CXCL5) and Groα (CXCL1), which contribute to the synovial infiltration by neutrophils [4,5]. To date, the potential impact of targeting neutrophils to treat RA has not been thoroughly explored in a clinical setting.

Neutrophils play a clear role in rodent models of arthritis. Neutrophil depletion using monoclonal antibodies reduced the severity of anti-collagen antibody-induced arthritis (ABIA), a model of the effector phases of the disease in which cells of the innate immune system orchestrate joint inflammation leading to cartilage and bone erosion [6,7]. Blockade of neutrophil recruitment using DF2162, a small molecule allosteric antagonist of CXCR1/CXCR2 reduced disease severity in methylated BSA-induced arthritis in mice and adjuvant arthritis in rats [8–10]. Whether CXCR1/CXCR2 are likewise critical for neutrophil recruitment in the ABIA model remains untested. Here, we evaluated the effects of a recently described CXCR1/CXCR2 antagonist SCH563705 [11] in the ABIA model and assessed the pharmacodynamic impact of CXCR1/CXCR2 antagonism on the circulating neutrophil compartment.

Monocytes, the precursors of macrophages, are another critical component of the inflammatory milieu of the RA synovium. These cells represent a major source of cytokines, including TNFα, IL-1β, and IL-6, that have proven to be clinically efficacious biological targets [12]. One strategy to prevent the infiltration of monocytes into the RA joint is through blockade of CCR2-dependent migration [2].

Abbreviations: ABIA, anti-collagen antibody-induced arthritis; AUC, area under the curve; b.i.d., twice per day; CIA, collagen-induced arthritis; MC, 0.4% hydroxypropyl methyl cellulose; p.o., oral gavage; RA, rheumatoid arthritis; SAA, serum amyloid A.

* Corresponding author. Fax: +1 908 740 7305.
E-mail addresses: soo.hong.min@spcorp.com (S.-H. Min), yuanfan.wang@spcorp.com (Y. Wang), waldemar.gonsiorek@roche.com (W. Gonsiorek), gopinadhan.anilkumar@spcorp.com (G. Anilkumar), joseph.kozlowski@spcorp.com (J. Kozlowski), daniel.lundell@spcorp.com (D. Lundell), jay.fine@roche.com (J.S. Fine), ethan.grant@spcorp.com (E.P. Grant).
[1] Present address: Hoffmann-La Roche, Inc., 340 Kingsland St., Nutley, NJ 07110, USA.

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.12.025

Elevated levels of CCL2, the principal ligand for this receptor, have been detected in RA synovial fluid [4,5]. However, small molecule and biologic antagonists of CCR2 and CCL2 have been evaluated in RA clinical trials, with no convincing evidence of efficacy [2,13,14]. Evidence of a role for CCR2 in animal models of arthritis has been mixed. CCR2-deficient mice displayed exacerbated severity in both the ABIA model and in T and B lymphocyte-dependent collagen-induced arthritis (CIA) [15]. In contrast, the CCR2 blocking antibody MC-21 had variable effects in the CIA model depending on when it was administered [16]. Here, we evaluated the effect of a potent and selective small molecule CCR2 antagonist MK0812 in the lymphocyte-indeopendent ABIA model, where its impact would be linked to prevention of monocyte migration to the joint. We likewise used MK0812 to evaluate the pharmacodynamic impact of CCR2 antagonism on the circulating monocyte population.

Materials and methods

Animals. Female BALB/c mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA) and used between 8 and 10 weeks of age. All animal studies were conducted with prior approval by the Institutional Animal Care and Use Committee at the Schering-Plough Research Institute (Kenilworth, NJ).

Induction of anti-collagen antibody-induced arthritis. ABIA was induced in BALB/c mice ($n = 8$ mice per treatment group) as follows. On day 0, mice were injected intraperitoneally with 4 mg ArthritoMAB Arthritis-inducing Antibody Cocktail (MD Biosciences, St. Paul, MN). On day 3, mice were boosted intraperitoneally with 50 μg of lipopolysaccharide from *Escherichia coli* 055:B5 (MD Biosciences) in 200 μl sterile PBS. In all studies, SCH563705 was administered in a vehicle consisting of 0.4% METHOCEL E15 premium hydroxypropyl methylcellulose (MC) (Dow Chemical Co., Midland, MI). Clinical scores were determined daily as follows. Each paw was assigned a score of 0–4 based on the following criteria: asymptomatic, 0; slight redness, 1; one or more swollen digits in addition to redness, 2; swelling of entire paw, 3; ankylosing of joints and residing of swelling, 4. The sum of the four paw scores for each mouse (0–16) were plotted against time to calculate the area under the curve (AUC) of disease activity. Paw thickness measurements were made daily using a micrometer caliper over the metatarsals of the paw. The percent change in paw thickness relative to baseline (day 0) measurements was then calculated.

Cytokine and chemokine analysis in paw tissues. Excised mouse paws were frozen in liquid nitrogen and pulverized using the BioPulverizer (BioSpec Products Inc., Bartlesville, OK). Paws were placed in 2 ml of protease inhibitor buffer made with COMPLETE Protease Inhibitor Cocktail Tablets (Roche Applied Sciences, Indianapolis, IN) and homogenized using a PowerGen 125 Tissue Homogenizer (Fisher Scientific) and PowerGen Generator (Fisher Scientific). Samples were vortexed and centrifuged at 10,000*g* for 4 min at 4 °C in the Sorvall Legend Micro21R Centrifuge (Thermo Electron Corp). Supernatants were collected and stored at −80 °C. Cytokine and chemokine levels in the extracts were measured using a mouse Pro-Inflammatory 7-Plex Tissue Culture Kit (Meso Scale Diagnostics, LLC, Gaithersburg, MD). Total protein content was determined using a Coomassie Protein Assay (Thermo Scientific, Rockford, IL, USA). Serum amyloid A (SAA) levels in the plasma were measured by ELISA (Life Diagnostics, Inc., West Chester, PA).

Histology. Histopathology studies were conducted on hind paw sections stained with H&E or Safranin O and scored by a pathologist in a blinded fashion (Premier Laboratory, LLC, Longmont, CA, USA).

Chemokine-induced effects on blood cell populations in vivo. SCH563705 or MK0812 were administered in a 0.4% MC solution by oral gavage (p.o.). Two hours later, the frequency of $CD11b^{+}Ly6G^{-}Ly6C^{hi}$ monocytes and $CD11b^{+}Ly6G^{+}Ly6C^{+}$ neutrophils was determined by flow cytometry. Briefly, blood was collected from euthanized mice into EDTA-coated tubes and aliquoted into 96-well V-bottom plates. To block nonspecific binding, normal rat serum was added to a final concentration of 20% and incubated for 10 min at room temperature. Blood was then incubated with PerCP-Cy5.5-anti-CD11b, PE-antiLy6G, and FITC-anti-Ly6C antibodies (BD Biosciences, Franklin Lakes, NJ) for 20 min at RT. The cells were pelleted by centrifugation and erythrocytes were lysed using FACS Lysing Solution (BD Biosciences) according to the manufacturer's instructions. Cells were washed and analyzed on a FACS Calibur® instrument (BD Biosciences).

Statistical analyse. Data are expressed as mean ± SEM and statistics were calculated using ANOVA (Prism 4 GraphPad Software). Area under the curve (AUC) of the ABIA disease scores were calculated using Graphpad Prism 4 software.

Results

SCH563705 is a potent small molecule antagonist previously described to inhibit chemokine binding to human CXCR2 ($IC_{50} = 1.3$ nM) and CXCR1 ($IC_{50} = 7.3$ nM), (Exemplified as compound 16) [11]. We determined that SCH563705 is also a potent inhibitor of mouse CXCR2 ($IC_{50} = 5.2$ nM, data not shown), and was therefore a suitable tool to evaluate the effect of CXCR2 antagonism in mouse disease models.

We first assessed the activity of SCH563705 in an ABIA model of arthritis. To initiate disease activity, a cocktail of anti-collagen antibodies was administered to BALB/c mice on day 0 followed by an LPS boost on day 3. Clinical scores and paw thickness were assessed daily as described in Materials and methods. SCH563705 dissolved in the vehicle 0.4% hydroxypropyl methyl cellulose (MC), or vehicle alone was dosed twice daily (b.i.d.) by oral gavage (p.o.) from days 0 to 7 of the model. SCH563705 dose-dependently inhibited disease activity, plotted as the area under the curve (AUC) of the disease scores over time, by up to 98% relative to the MC group (Fig. 1A). Likewise, paw swelling was completely inhibited at all doses tested relative to the MC group (Fig. 1B). The difference in disease activity that was evident by clinical assessment of paw swelling and redness (see Fig. 1C, top row, for representative photomicrographs) was also reflected in reduced inflammation, pannus formation, bone, and cartilage erosion determined by histological analysis of paw sections (Fig. 1C, middle row, Table 1). Likewise, proteoglycan content at the cartilage surface, as revealed by Safranin O staining, was also protected in animals dosed with SCH563705 (Fig. 1C, bottom row).

To assess whether the activity of this compound impacted proinflammatory mediator production, we prepared total protein extracts from paw tissues and measured TNFα, IL-1β, IL-6, and CXCL1 levels. Higher levels of IL-1β, IL-6, and CXCL1 were detected in paw extracts from arthritic relative to naïve animals (Fig. 2A–C). Levels of these mediators were significantly reduced in the SCH563705-treated group to levels comparable to what was present in paw extracts from naïve animals. Finally, the acute phase protein serum amyloid A (SAA) was also reduced in the plasma of animals treated with SCH563705 relative to MC (Fig. 2D), reflecting a reduced systemic inflammatory response.

Previous reports have implicated neutrophils as a primary target for CXCR1 and CXCR2-mediated migration in the context of arthritis. We therefore assessed the impact of SCH563705 on the circulating neutrophil compartment in the dose range used for the studies described above. Naïve BALB/c mice were dosed with SCH563705 at 50 mg/kg, and blood neutrophil and monocytes were quantified by flow cytometry using the cell surface markers

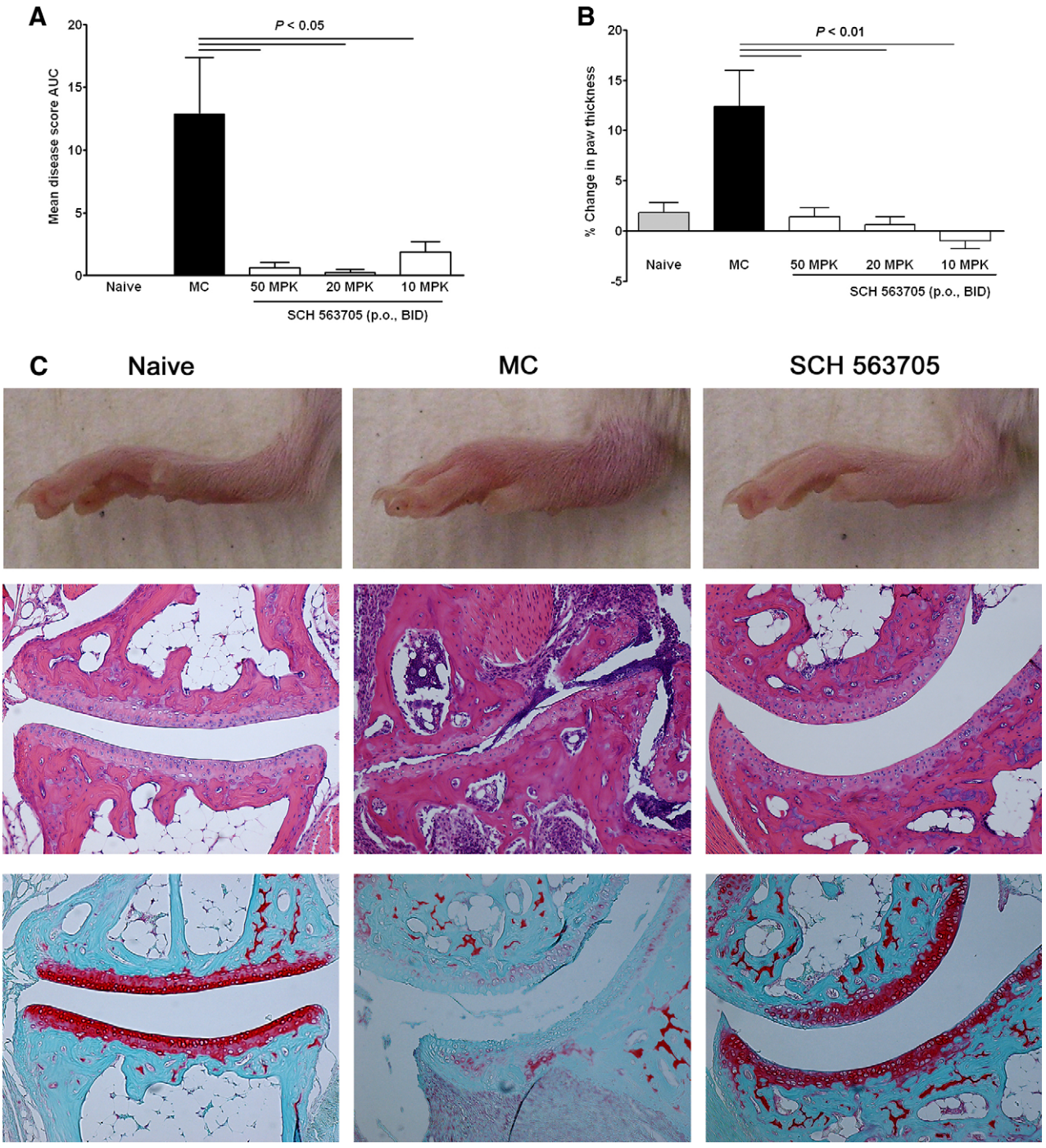

Fig. 1. CXCR2/CXCR1 antagonism reduces ABIA severity. ABIA was induced in BALB/c mice and monitored daily for clinical scores and paw thickness. Disease score AUC (A) and the percent change in paw thickness (B) between days 0 and 7 were plotted. Animals were dosed with vehicle (0.4% MC) or SCH563705 at the indicated doses p.o., b.i.d. from days 0 to 7 of the study. On day 7, hind paws were collected and processed for histology (C). Representative gross paw appearance (top row), H&E (middle row) and Safranin O (bottom row) photomicrographs are presented. The data presented are the mean ± SEM and are representative of two independent experiments.

Ly6G, a neutrophil-specific marker, and Ly6C, which is expressed on neutrophils and at high levels on the $Ly6G^-$ monocyte population. All animals dosed with SCH563705 had a clear reduction in blood $Ly6G^+Ly6C^+$ neutrophil frequency and unchanged levels of $Ly6G^-Ly6C^{hi}$ monocytes (Fig. 3A, right panels) relative to animals that were dosed with MC alone (Fig. 3A, left panels). We further assessed the impact of SCH563705 over a dose range covering the doses tested in the arthritis studies and observed a significant, dose-dependent effect of the compound on circulating neutrophils, with no effect on blood monocytes (Fig. 3B). A principal effect of this compound *in vivo* is therefore related to its impact on neutrophil migration. Chemokine receptor inhibition with small molecule antagonists has been observed to lead to an accumulation of the cognate chemokine ligands in the plasma [17]. We therefore examined the effect of SCH563705 on plasma levels of one of the major CXCR2 ligands, CXCL1. SCH563705 treatment caused a dose-dependent elevation in plasma levels of CXCL1 (Fig. 3B). This effect was specific to CXCR2 ligand, since no effect on the CCR2 ligand CCL2 was observed (Fig. 3B).

CCR2-mediated migration of monocytes has been postulated to play an important role in arthritis and other inflammatory diseases. We therefore extended our analyses of the ABIA model using

Table 1
ABIA histopathology scores. H&E and Safranin O-stained tissue sections were prepared from the hind paws of five animals from each of the following groups: Naïve; arthritic/MC, arthritic/SCH563705 50 mg/kg from the ABIA study depicted in Fig. 1. Inflammation, pannus, cartilage, and bone erosion scores were assigned by a blinded pathologist. Data are mean ± SEM. The score for each parameter was scored on a scale from 0 (normal) to 5 (severe).

Group	Inflammation	Pannus	Cartilage	Bone	Total
Naive	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
MC	2.5 ± 0.5	2.3 ± 0.6	2.2 ± 0.6	2.3 ± 0.7	9.2 ± 2.3
SCH563705	0.5 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	1.0 ± 0.6

a potent and selective CCR2 antagonist, MK0812. We first assessed the pharmacodynamic effects of MK0812 in naive BALB/c mice. Administration of MK0812 at 30 mg/kg, p.o. reduced the frequency of $Ly6G^{-}Ly6C^{hi}$ monocytes in the peripheral blood, while no impact on circulating $Ly6G^{+}Ly6C^{+}$ neutrophil frequency was observed (Fig. 3A, middle panels). In addition, MK0812 treatment caused a dose-dependent reduction in circulating $Ly6C^{hi}$ monocytes and a corresponding elevation in the CCR2 ligand CCL2 (Fig. 3C). These effects were selective, since no effect was observed on $Ly6G^{+}Ly6C^{+}$ neutrophil frequency or on plasma levels of CXCL1 (Fig. 3C).

Given that MK0812 proved to be a suitable pharmacological tool for *in vivo* studies in mice, we tested the effect of this compound in the ABIA model in comparison to SCH563705. While SCH563705 again had a dramatic impact on arthritis disease score severity and paw swelling, MK0812 had no discernable effect (Fig. 4A and B) on either parameter. In fact, there was a trend toward increased disease severity in MK0812-treated animals relative to the MC group.

One criticism of the ABIA model is that an LPS challenge is used to drive joint inflammation and the physiologic relevance of this trigger is questionable in the context of human arthritis. It has been reported that the LPS injection can be substituted with local footpad injections of recombinant TNFα [18]. As a potentially closer mimic to the effector phase of human disease, we therefore assessed whether SCH563705 and MK0812 would impact this TNFα-driven ABIA. As before, SCH563705 dramatically inhibited disease progression, while MK0812 had no effect on this model. The mean plasma drug concentrations at 12 h post the final dose (representing the minimum drug concentration reached throughout the 7-day study) were 40 nM for SCH563705 (8× the IC_{50} value against CXCR2) and 327 nM for MK0812 (17× the IC_{50} value against CCR2). Therefore, the lack of efficacy of MK0812 in these models was not due to inadequate CCR2 receptor coverage.

Discussion

A central mechanism that underlies inflammation is the process by which monocytes, neutrophils, lymphocytes, and other cells are recruited from the circulating blood to the site of inflammation. The biochemical basis for this process is a complex system of controlled expression of a family of more than 40 chemokines that attract cells expressing different patterns of more than 20 chemokine receptors. Thus, on the one hand, the induced expression of chemokines at a site of inflammation results in the formation of a gradient that facilitates the recruitment of cells bearing the appropriate receptors. On the other hand, the distinct expression pattern and combination of chemokine receptors expressed on individual cell types determines which cells are capable of responding to a given chemokine gradient. In this way, the pattern of chemokines expressed in a particular setting dictates at a molecular level which cell types will be recruited.

A central challenge over the past few decades has been to decipher which of these many chemokine:receptor pairs are central to inflammation in the context of complex diseases such as arthritis. Here we have used selective small molecule antagonists to CCR2 and CXCR2/CXCR1 to define the roles of these receptors in a mouse model of inflammatory arthritis. Such antagonists provide a means to precisely disrupt chemokine:receptor interactions during an inflammatory disease *in vivo* to define which molecular interactions are drivers of the disease process. Our data support a role for neutrophils and CXCR2-dependent cell migration in a rodent model of arthritis. SCH563705 reduced all clinical signs of disease

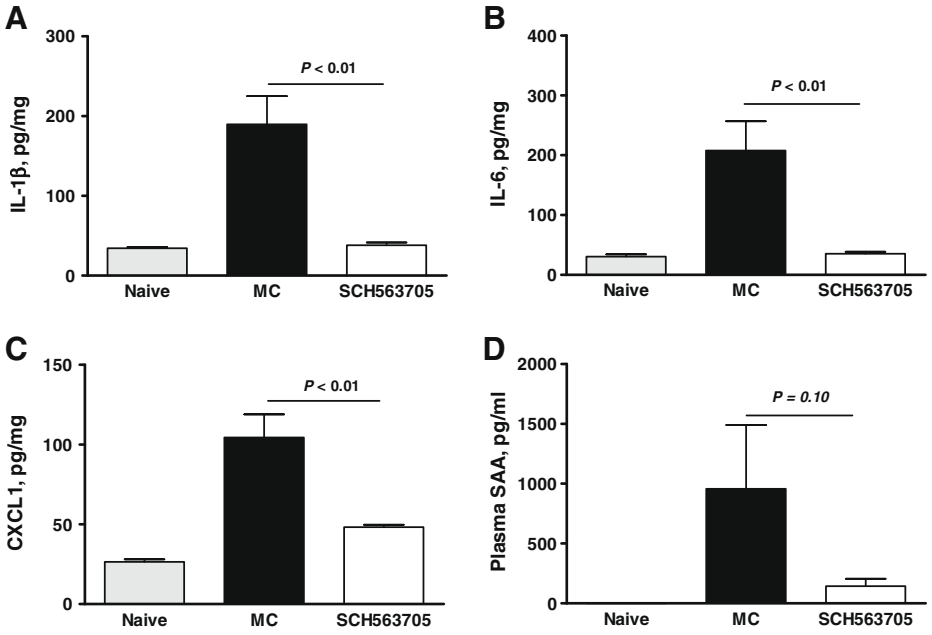

Fig. 2. CXCR2/CXCR1 antagonism reduces inflammatory mediator production. Paw tissue collected from an ABIA study as depicted in Fig. 1 were homogenized and cytokine levels in the extract were determined. IL-1β (A) IL-6 (B), and CXCL1 (C) levels were three cytokines determined to be consistently elevated in arthritic (MC) paw extracts. (D) Plasma from the same animals was assayed for levels of the acute phase protein SAA. The data presented are the mean ± SEM and are representative of two independent experiments.

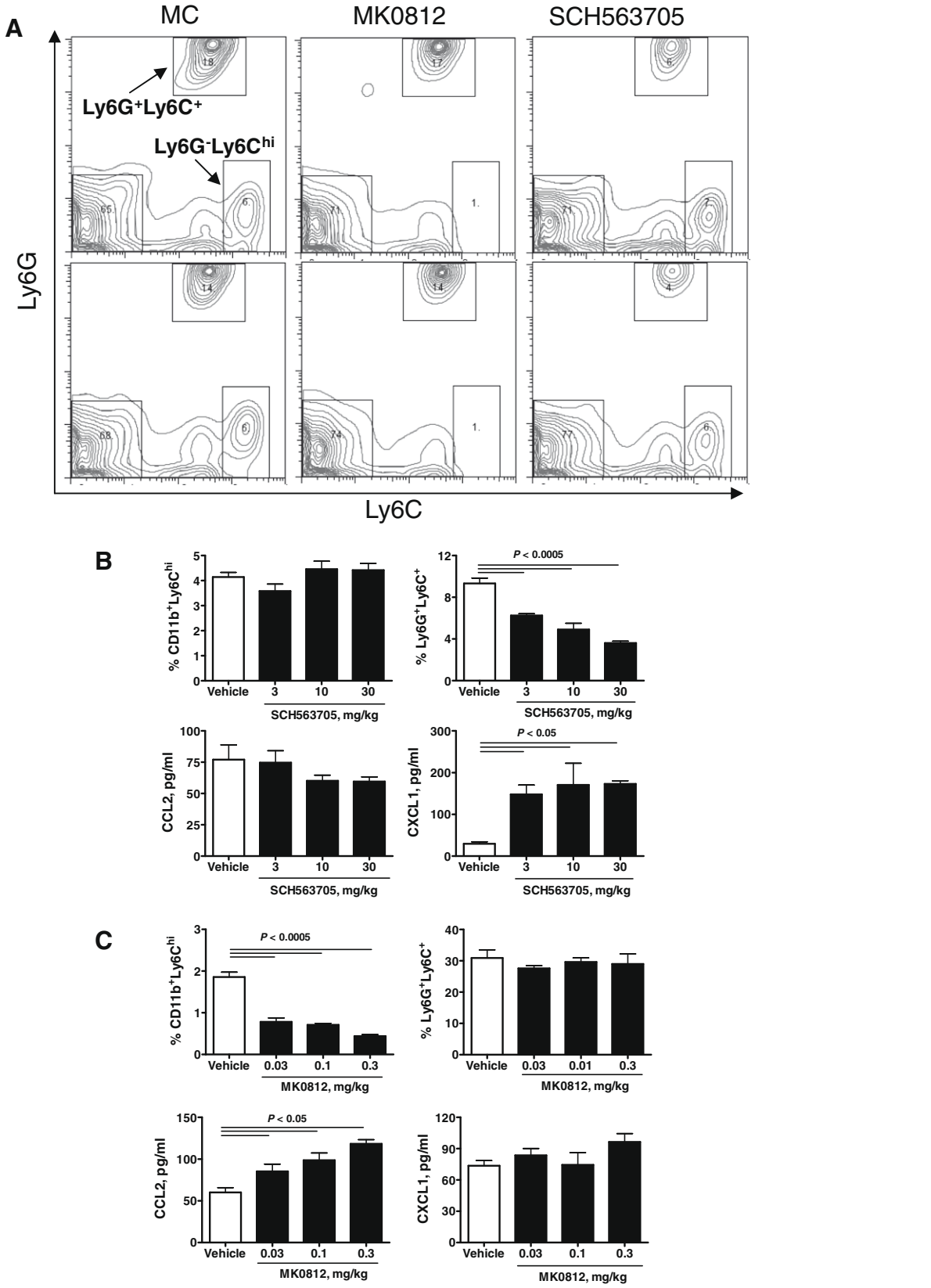

Fig. 3. Pharmacodynamic effects of CXCR2/CXCR1 and CCR2 antagonism. (A) BALB/c mice were administered MC (vehicle), MK0812 or SCH563705 at 30 mg/kg p.o. Two hours later, peripheral blood Ly6G$^-$Ly6C^{hi} monocyte (lower right boxed field) and Ly6G$^+$Ly6C$^+$ neutrophil (upper boxed field) frequencies were determined. Representative histograms from two mice from each group are depicted. (B, C). BALB/c mice were administered vehicle (MC) or the indicated dose of SCH563705 (B) or MK0812 (C) p.o. Two hours later, peripheral blood Ly6G$^-$CD11b$^+$Ly6C^{hi} monocyte and Ly6G$^+$Ly6C$^+$ neutrophil frequencies were determined by flow cytometry. Plasma CCL2 and CXCL1 levels were determined by ELISA. The data presented are the mean ± SEM and are representative of three independent experiments.

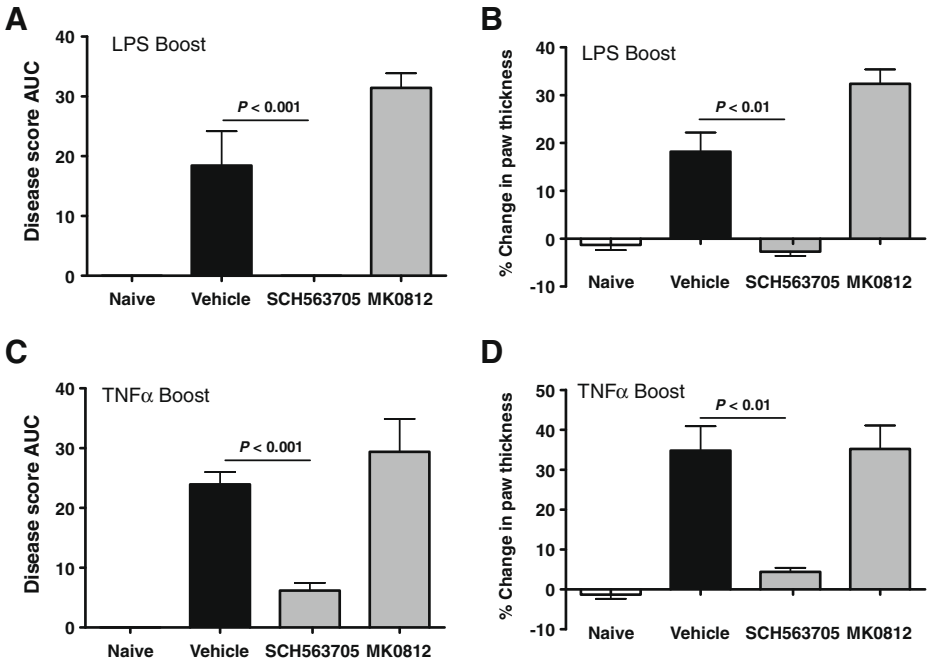

Fig. 4. CCR2 antagonism with MK0812 does not reduce ABIA severity. ABIA was induced in BALB/c mice and monitored daily for clinical scores and paw thickness. Anti-collagen antibodies were administered on day 0. On day 3, (A, B) LPS (50 μg, i.p.) or (C, D) TNFα (10 ng s.c. into each hind footpad) was administered. Disease score AUC (A, C) and the percent change in paw thickness (B, D) was calculated and plotted. Animals were dosed with vehicle (0.4% MC), SCH563705 or MK0812 at 30 mg/kg, p.o., b.i.d. from days 0 to 7 of the study. The data presented are the mean ± SEM and are representative of three independent experiments.

and markedly reduced the levels of key pro-inflammatory cytokines in the paw tissue. These findings suggest that an early event in the induction of joint inflammation is the recruitment of neutrophils through CXCR2. Given the dual inhibition of human CXCR1/CXCR2 by this compound, it is likely that it has some degree of potency against mouse CXCR1. Thus, the effects we observed with SCH563705 may reflect a combination of effects on CXCR1/CXCR2-dependent neutrophil migration. Although neutrophils are among the most abundant cells present in RA synovial fluid, little is known about their contribution to the chronic disease state. The clinical failure of a neutralizing antibody against CXCL8 dampened enthusiasm about targeting CXCR1/CXCR2 [5]. However, multiple chemokines that are upregulated in RA, including CXCL8, CXCL5, and CXCL1, can mediate CXCR1/CXCR2-dependent migration. Thus, therapeutic targeting of the receptors rather than individual chemokines may overcome potential redundancies in function and be a fruitful approach.

CCR2-dependent migration of monocytes has long been considered a promising intervention point for RA. Recent clinical failures with antibodies against CCL2 or CCR2 as well as with the small molecule CCR2 antagonist MK0812 have diminished interest in this target [19]. Data from rodent arthritis models generated with $CCR2^{-/-}$ mice and with anti-CCR2 antibodies in fact are aligned with these human clinical data in that worsening of rodent arthritis severity was observed [15,16]. Here, we extend these findings in the ABIA model, where we demonstrate that despite robust pharmacological coverage of CCR2 receptors with MK0812, no reduction in disease severity was observed. One explanation for the lack of activity in both mouse ABIA and human RA is that redundant chemokine receptors function on monocytes (i.e. CCR1 and CCR2), which may preclude clinical efficacy when only one receptor is blocked. Dual antagonism of CCR1 and CCR2 may be a promising approach to overcome this hurdle. Another explanation that has been put forward for the lack of efficacy of MK0812 is that its activity against CCR5 may alter regulatory T cell function [20]. This latter mechanism is unlikely to be involved in the ABIA model given that there is a minimal role for lymphocytes in this disease model.

In addition to the role that induced chemokine expression plays in the recruitment of cells to inflammatory sites, the constitutive production of a subset of chemokines is an important biochemical mechanism to recruit newly-formed cells from the bone marrow to the peripheral circulation. Constitutive production of the CCR2 ligand CCL2 maintains homeostatic levels of $Ly6C^{hi}$ monocytes in the blood and constitutive production of the CXCR2 ligand CXCL1 recruits neutrophils from the bone marrow to the circulation. We demonstrated that the CCR2 antagonist MK0812 disrupts the constitutive $Ly6C^{hi}$ monocyte recruitment and leads to a corresponding elevation in circulating levels of CCL2. These data indicate that the homeostatic levels of CCL2 in the plasma are tightly controlled and are regulated by binding to CCR2, such that in the presence of a CCR2 antagonist, CCL2 levels accumulate. These finding are in accord with previous studies that showed that circulating $Ly6C^{hi}$ monocytes are found at reduced levels in CCR2 knockout mice relative to wild-type controls [21]. Likewise, the CXCR2/CXCR1 antagonist SCH563705 caused a dose-dependent reduction in the circulating blood neutrophil population. This reduction was accompanied by an increase in circulating levels of CXCL1, revealing a role for CXCR2 in regulating the plasma levels of its ligands. These pharmacodynamic changes highlight the selective impact of the antagonists used in these studies on the neutrophil and monocyte populations relative to one another. Further, these data reveal a general molecular mechanism by which blood levels of constitutively-produced chemokines is controlled.

Numerous rodent models of arthritis have been employed to evaluate new therapeutics for RA. Each has its own advantages and shortcomings based on the underlying mechanisms and cell type dependencies and the degree to which those mechanisms overlap with RA. Herein, we used an ABIA model that recapitulates the effector phase of RA driven by innate immune cell types such as neutrophils and monocytes, and the pro-inflammatory cytokines that they produce. We examined two variations of this ABIA model,

one with an LPS systemic injection and a second that employed local TNFα injections to drive the disease pathology. CXCR2/CXCR1 antagonism had a substantial impact on both forms of this ABIA model, whereas CCR2 antagonism had no effect on either model. Whether a similar impact of CXCR2/CXCR1 antagonism on human arthritides remains to be seen, but appears to warrant investigation.

Conclusions

The ABIA mouse model of the effector phase of arthritis was employed to evaluate chemokine receptor-targeted therapeutics. We find that CXCR2/CXCR1 antagonism potently suppressed disease activity and was accompanied by pharmacodynamic changes in circulating neutrophils. In contrast, CCR2 antagonism caused clear pharmacodynamic changes to the circulating monocyte pool, yet had no impact on ABIA disease severity. These data support further exploration of CXCR2/CXCR1 as a target for RA and reveal molecular interactions involved in monocyte and neutrophil migration under both inflammatory and homeostatic conditions.

References

[1] C. Gerard, B.J. Rollins, Chemokines and disease, Nat. Immunol. 2 (2001) 108–115.
[2] R. Horuk, Chemokine receptor antagonists: overcoming developmental hurdles, Nat. Rev. Drug Discov. 8 (2009) 23–33.
[3] E. Kitsis, G. Weissmann, The role of the neutrophil in rheumatoid arthritis, Clin. Orthop. Relat. Res. (1991) 63–72.
[4] T. Iwamoto, H. Okamoto, Y. Toyama, S. Momohara, Molecular aspects of rheumatoid arthritis: chemokines in the joints of patients, FEBS J. 275 (2008) 4448–4455.
[5] A.E. Koch, Chemokines and their receptors in rheumatoid arthritis: future targets?, Arthritis Rheum 52 (2005) 710–721.
[6] K.S. Nandakumar, L. Svensson, R. Holmdahl, Collagen type II-specific monoclonal antibody-induced arthritis in mice: description of the disease and the influence of age, sex, and genes, Am. J. Pathol. 163 (2003) 1827–1837.
[7] D. Tanaka, T. Kagari, H. Doi, T. Shimozato, Essential role of neutrophils in anti-type II collagen antibody and lipopolysaccharide-induced arthritis, Immunology 119 (2006) 195–202.
[8] M.M. Barsante, T.M. Cunha, M. Allegretti, F. Cattani, F. Policani, C. Bizzarri, W.L. Tafuri, S. Poole, F.Q. Cunha, R. Bertini, M.M. Teixeira, Blockade of the chemokine receptor CXCR2 ameliorates adjuvant-induced arthritis in rats, Br. J. Pharmacol. 153 (2008) 992–1002.
[9] F.M. Coelho, V. Pinho, F.A. Amaral, D. Sachs, V.V. Costa, D.H. Rodrigues, A.T. Vieira, T.A. Silva, D.G. Souza, R. Bertini, A.L. Teixeira, M.M. Teixeira, The chemokine receptors CXCR1/CXCR2 modulate antigen-induced arthritis by regulating adhesion of neutrophils to the synovial microvasculature, Arthritis Rheum. 58 (2008) 2329–2337.
[10] R. Grespan, S.Y. Fukada, H.P. Lemos, S.M. Vieira, M.H. Napimoga, M.M. Teixeira, A.R. Fraser, F.Y. Liew, I.B. McInnes, F.Q. Cunha, CXCR2-specific chemokines mediate leukotriene B4-dependent recruitment of neutrophils to inflamed joints in mice with antigen-induced arthritis, Arthritis Rheum. 58 (2008) 2030–2040.
[11] J. Chao, A.G. Taveras, J. Chao, C. Aki, M. Dwyer, Y. Yu, B. Purakkattle, D. Rindgen, J. Jakway, W. Hipkin, J. Fosetta, X. Fan, D. Lundell, J. Fine, M. Minnicozzi, J. Phillips, J.R. Merritt, C(4)-alkyl substituted furanyl cyclobutenediones as potent, orally bioavailable CXCR2 and CXCR1 receptor antagonists, Bioorg. Med. Chem. Lett. 17 (2007) 3778–3783.
[12] P.V. Voulgari, Emerging drugs for rheumatoid arthritis, Expert Opin. Emerg. Drugs 13 (2008) 175–196.
[13] J.J. Haringman, D.M. Gerlag, T.J. Smeets, D. Baeten, F. van den Bosch, B. Bresnihan, F.C. Breedveld, H.J. Dinant, F. Legay, H. Gram, P. Loetscher, R. Schmouder, T. Woodworth, P.P. Tak, A randomized controlled trial with an anti-CCL2 (anti-monocyte chemotactic protein 1) monoclonal antibody in patients with rheumatoid arthritis, Arthritis Rheum. 54 (2006) 2387–2392.
[14] C.E. Vergunst, D.M. Gerlag, L. Lopatinskaya, L. Klareskog, M.D. Smith, F. van den Bosch, H.J. Dinant, Y. Lee, T. Wyant, E.W. Jacobson, D. Baeten, P.P. Tak, Modulation of CCR2 in rheumatoid arthritis: a double-blind, randomized, placebo-controlled clinical trial, Arthritis Rheum. 58 (2008) 1931–1939.
[15] M.P. Quinones, S.K. Ahuja, F. Jimenez, J. Schaefer, E. Garavito, A. Rao, G. Chenaux, R.L. Reddick, W.A. Kuziel, S.S. Ahuja, Experimental arthritis in CC chemokine receptor 2-null mice closely mimics severe human rheumatoid arthritis, J. Clin. Invest. 113 (2004) 856–866.
[16] H. Bruhl, J. Cihak, J. Plachy, L. Kunz-Schughart, M. Niedermeier, A. Denzel, M. Rodriguez Gomez, Y. Talke, B. Luckow, M. Stangassinger, M. Mack, Targeting of Gr-1+, CCR2+ monocytes in collagen-induced arthritis, Arthritis Rheum. 56 (2007) 2975–2985.
[17] A.E. Cardona, M.E. Sasse, L. Liu, S.M. Cardona, M. Mizutani, C. Savarin, T. Hu, R.M. Ransohoff, Scavenging roles of chemokine receptors: chemokine receptor deficiency is associated with increased levels of ligand in circulation and tissues, Blood 112 (2008) 256–263.
[18] T. Kagari, H. Doi, T. Shimozato, The importance of IL-1 beta and TNF-alpha, and the noninvolvement of IL-6, in the development of monoclonal antibody-induced arthritis, J. Immunol. 169 (2002) 1459–1466.
[19] A. Kalinowska, J. Losy, Investigational C–C chemokine receptor 2 antagonists for the treatment of autoimmune diseases, Expert Opin. Investig. Drugs 17 (2008) 1267–1279.
[20] A.E. Proudfoot, Is CCR2 the right chemokine receptor to target in rheumatoid arthritis?, Arthritis Rheum 58 (2008) 1889–1891.
[21] C.L. Tsou, W. Peters, Y. Si, S. Slaymaker, A.M. Aslanian, S.P. Weisberg, M. Mack, I.F. Charo, Critical roles for CCR2 and MCP-3 in monocyte mobilization from bone marrow and recruitment to inflammatory sites, J. Clin. Invest. 117 (2007) 902–909.