The Human Specific CCR1 Antagonist CP-481,715 Inhibits Cell Infiltration and Inflammatory Responses in Human CCR1 Transgenic Mice


J Immunol 2006; 176:3141-3148; doi: 10.4049/jimmunol.176.5.3141
http://www.jimmunol.org/content/176/5/3141

References This article cites 31 articles, 17 of which you can access for free at: http://www.jimmunol.org/content/176/5/3141.full#ref-list-1

Subscription Information about subscribing to The Journal of Immunology is online at: http://jimmunol.org/subscription

Permissions Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
The Human Specific CCR1 Antagonist CP-481,715 Inhibits Cell Infiltration and Inflammatory Responses in Human CCR1 Transgenic Mice


We previously described the in vitro characteristics of the potent and selective CCR1 antagonist, CP-481,715. In addition to being selective for CCR1 vs other chemokine receptors, CP-481,715 is also specific for human CCR1 (hCCR1), preventing its evaluation in classical animal models. To address this, we generated mice whereby murine CCR1 was replaced by hCCR1 (knockin) and used these animals to assess the anti-inflammatory properties of CP-481,715. Cells isolated from hCCR1 knockin mice were shown to express hCCR1 and migrate in response to both murine CCR1 and hCCR1 ligands. Furthermore, this migration is inhibited by CP-481,715 at dose levels comparable to those obtained with human cells. In animal models of cell infiltration, CP-481,715 inhibited CCL3-induced neutrophil infiltration into skin or into an air pouch with an ED50 of 0.2 mg/kg. CP-481,715 did not inhibit cell infiltration in wild-type animals expressing murine CCR1. In a more generalized model of inflammation, delayed-type hypersensitivity, CP-481,715 significantly inhibited footpad swelling and decreased the amount of IFN-γ and IL-2 produced by isolated spleen cells from sensitized animals. It did not, however, induce tolerance to a subsequent challenge. These studies illustrate the utility of hCCR1 knockin animals to assess the activity of human specific CCR1 antagonists; demonstrate the ability of the CCR1 antagonist CP-481,715 to inhibit cell infiltration, inflammation, and Th1 cytokine responses in these animals; and suggest that CP-481,715 may be useful to modulate inflammatory responses in human disease. The Journal of Immunology, 2006, 176: 3141–3148.

Lymphocyte infiltration into inflammatory sites is believed to be regulated by 8- to 10-kDa proteins known as chemokines. These chemokines are classified into four groups, depending on the spacing between two N-terminal cysteine residues, and are designated CC, CXC, XC, and CX3C chemokines. The therapeutic potential of inhibiting chemokines or their receptors is supported by their enhanced expression in human disease, numerous studies in animal models, and, in some instances, genetic association studies (1–4). These reports have prompted the identification and characterization of chemokine receptor antagonists, several of which are currently undergoing clinical trials (5).

One chemokine receptor thought to play a crucial role in several diseases is CCR1. CCR1 is expressed on monocytes, T cells, dendritic cells, and, in some cases, neutrophils (6–9), and interacts with at least eight different ligands, including CCL3 (MIP-1α), CCL5 (RANTES), CCL7 (MCP-3), CCL14 (hemofiltrate C-C chemokine-1), CCL8 (MCP-2), CCL15 (leukotactin-1), CCL23 (myeloid progenitor inhibitory factor-1), and hemofiltrate C-C chemokine-4 (CCL16) (10–12). These ligands have been shown to have potent chemotactic activity in vitro (9), and in some cases in vivo in which intradermal injection of CCL3 or CCL5 into human subjects induced a robust cell infiltration (8, 13). In addition to mediating cell migration, CCR1 signaling has been shown to up-regulate integrins such as Mac-1 (CD11b), thus causing the firm adherence of leukocytes to the endothelium (14). CCR1 signaling may also contribute to tissue damage and inflammation through the enhancement of T cell activation (15), regulation of Th1/Th2 cytokine polarization (16, 17), and stimulation of macrophage function (18) and protease secretion (14, 19, 20). Taken together, these properties support CCR1 as an attractive therapeutic target to modulate leukocyte infiltration and decrease the associated tissue damage common to many autoimmune diseases.

Numerous animal disease models have shown that inhibition of CCR1 or its ligands abrogates disease. These data prompted discovery efforts to identify small molecular weight mass CCR1 antagonists and led to the identification of CP-481,715 (14). CP-481,715 is a potent CCR1 antagonist that retains activity in human whole blood. In addition to being selective for CCR1 as compared with other G protein-coupled receptors, CP-481,715 is also selective for the human CCR1 (hCCR1)1 receptor, preventing its assessment in classical animal models (14). To overcome this obstacle, we generated mice that had murine CCR1 replaced by hCCR1 and demonstrate the ability of CP-481,715 to inhibit in vivo inflammatory responses in these animals at clinically achievable dose levels.

Materials and Methods

Materials

The CCR1 antagonist, CP-481,715 (quinoxaline-2-carboxylic acid [4(R)-carbamoyl-1(5)-(3-fluorobenzyl)-2(5),7-dihydroxy-7-methyl-octyl]amide),

1 Address correspondence and reprints requests to Dr. Ronald P. Gladue, Associate Research Fellow, Pfizer Global Research and Development, Department of Immunology, MS 8220-2410, Eastern Point Road, Groton, CT 06340. E-mail address: Ronald.P.Gladue@Pfizer.com

2 Abbreviations used in this paper: hCCR, human CCR; ES, embryonic stem; KI, knockin; MPO, myeloperoxidase; ORF, open reading frame; WT, wild type.
was prepared by the Pfizer Medicinal Chemistry group, as previously described (21). All chemokines were obtained from PeproTech, unless otherwise indicated, checked for purity by HPLC, and verified to be free from endotoxin using the limulus amebocyte lysate assay (Associates of Cape Cod).

Animals

DBA/1 mice were obtained from The Jackson Laboratory. All animals were certified to be free from viral pathogens and were allowed food and water ad libitum. All experimental protocols were reviewed and approved by the Pfizer Institutional Animal Care and Use Committee.

Reagents

BSA was purchased from Sigma-Aldrich. Heparin was purchased from American Pharmaceutical Partners. Dulbecco’s PBS without calcium chloride and magnesium chloride (PBS), HBSS, and genetin were obtained from Invitrogen Life Technologies. FBS was purchased from HyClone. RPMI 1640, HEPES, glutamine, and penicillin/streptomycin were all obtained from BioWhittaker. Tissue culture medium for cell cultures consisted of RPMI 1640 containing FBS (10%), L-glutamine (2 mM), HEPES (10 mM), penicillin (100 U/ml), and streptomycin (50 μg/ml).

Design of the CCR1 knockin (KI) construct

The CCR1 replacement construct was generated using genomic DNA from a mouse (strain 129) DNA library. The CCR1 open reading frame (ORF) was subcloned into the expression vector, pCDNA3.1 (Invitrogen Life Technologies). From this vector, pMIP34, a hCCR1/Bgh pA fragment, was excised and spliced into the CCR1 replacement construct 3′ to the initiating ATG of the mouse CCR1 (Fig. 1). The CCR1 KI construct comprised 4.3-kb 5′ and 1.0-kb 3′ homology arms from the mouse genomic region, flanking both the hCCR1/Bgh pA and the pgk-neo resistance cassette running in the opposite orientation. This construct was subcloned into pBlueScript (Stratagene). For negative selection, the herpes simplex virus thymidine kinase gene was inserted outside of the 5′ homology arm in the opposite orientation. Targeting into mouse embryonic stem (ES) cells by homologous recombination replaced the mouse ORF of the CCR1 gene with the hCCR1 ORF.

ES cell transfection and generation of hCCR1 KI mice

The culture procedures for ES cells have been previously described (22). The CCR1 construct was linearized by digestion at a unique 5′ site from the hCCR1 GCTGTA-3′ hybridizing at bases 69–87, and antisense primer, 5′-GAGGAGGAAAATAGAAGAATGA-3′ hybridizing at bases 732–750. Thirty cycles of PCR were performed with an annealing temperature of 55°C using PerkinElmer AmpliTaq polymerase and the following primers to produce a 1.0-kb PCR product: sense primer, 5′-GAGGAGGAAAATAGAAGAATGA; antisense primer, 5′-GAGGAGGAAAATAGAAGAATG

Chemotaxis assays

Chemotaxis was conducted in 48-well chemotaxis chambers purchased from NeuroProbe, as previously described (14). Briefly, agonists were dissolved in RPMI 1640 containing 0.1% BSA, then added to the bottom wells of the chamber. Mouse-specific primers used were: sense primer, 5′-ATGCCCAAAAGACTGCTGTA-3′ hybridizing at bases 69–87, and antisense primer, 5′-GAGGAGGAAAATAGAAGAATGA-3′ hybridizing at bases 732–750. Thirty cycles of PCR were performed with an annealing temperature of 55°C using PerkinElmer AmpliTaq polymerase and the following primers to produce a 1.0-kb PCR product: sense primer, 5′-GAGGAGGAAAATAGAAGAATGA; antisense primer, 5′-GAGGAGGAAAATAGAAGAATG

Whole blood actin polymerization

Mouse blood, collected in EDTA, was incubated with various dilutions of CP-481,715 or diluent for 5 min at room temperature. CCL3 (10 nM) was then added, and after 50 s the reaction was terminated by adding FACS lysis solution (BD Biosciences) containing paraformaldehyde (Electron Microscopy Sciences). After 10 min, the cells were collected by centrifugation, washed with PBS, and stained for 1 h at room temperature in the

FIGURE 1. Engineering the hCCR1 KI gene into ES cells. The hCCR1 KI construct (A) replaced the endogenous mouse CCR1 ORF (B) via homologous recombination. This resulted in a complete KI of the human ORF while simultaneously deleting mouse CCR1 and introducing the pgk-neo cassette for positive selection (C). Upon this event, a unique PstI site from the hCCR1 was introduced. This resulted in a 6.3-kb mutant fragment as opposed to the 9.0-kb WT (D). Abbreviations: P, PstI; H, HindIII; X, XbaI; R, EcoRI; S, SmaI.

Analysis of mouse CCR1 and hCCR1 expression

Cells were collected from hCCR1 KI and WT animals and analyzed for hCCR1 expression by mRNA and cell surface receptor expression by FACS analysis. Neutrophils were collected from the peritoneum cavity 18 h after injection of 1.0 ml of 6% casein, whereby elicited macrophages were collected 3–4 days after casein injection. Lymphocytes were collected from the spleen or lymph nodes. RNA was isolated using the RNeasy purification method (Invitrogen Life Technologies) with DNase treatment. RT-PCR was done by reverse transcription using both random hexamers and oligo(dT) primers and avian myeloblastosis virus reverse transcriptase (Roche Diagnostics) and 5 μg of RNA. Mouse-specific primers used were: sense primer, 5′-ATGCCCAAAAGACTGCTGTA-3′ hybridizing at bases 69–87, and antisense primer, 5′-GAGGAGGAAAATAGAAGAATGA-3′ hybridizing at bases 732–750. Thirty cycles of PCR were performed with an annealing temperature of 55°C using PerkinElmer AmpliTaq polymerase and the following primers to produce a 1.0-kb PCR product: sense primer, 5′-GAGGAGGAAAATAGAAGAATGA; antisense primer, 5′-GAGGAGGAAAATAGAAGAATG

Downloaded from http://www.jimmunol.org/ by guest on April 20, 2017
dark with a solution containing lysophosphatidylcholine (Sigma-Aldrich), paraformaldehyde, and nitrobenzoxadiazole phallacidin (Molecular Probes). The cells were then washed with PBS containing 2% FBS, and the fluorescence was quantitated using a FACScan (BD Biosciences).

**Skin challenge study**

Mice were injected intradermally at 0 and 2 h with 1 μg of CCL3 or vehicle (0.5% BSA). After 4 h, skin was excised and frozen. An 8-mm skin punch was made from the frozen skin, and each section was placed into 1 ml of 50 mM K2PO4 (pH 6.0) buffer. The skin was homogenized, freeze thawed twice, and centrifuged, and the supernatants were collected for analysis of myeloperoxidase (MPO) levels.

**MPO assay**

Test samples were placed into wells of a 96-well flat-bottom plate containing 150 μl of substrate (750 μl of N,N-dimethylformamide (Sigma-Aldrich), 49.25 ml of buffer, 10 mg of α-dianisidine (Sigma-Aldrich), and 11 μl of 3% H2O2 (Sigma-Aldrich)). The plate was incubated at 37°C for 15 min, and the reaction was stopped by adding 100 μl of 0.4 M glycine (pH 10.4). The absorbance was read at 450 nm, and the amount of MPO was determined from a standard curve.

**Air pouch model of cell infiltration**

Subcutaneous air pouches were formed on the back of animals, as previously described (24). Briefly, 3 ml of air was injected s.c. on day 1 and then re injected again 3 days later in the same area. On the fourth day, animals received a single i.p. injection of CP-481,715, followed by two injections of CCL3 (1 μg/ml) administered directly into the air pouch at time 0 and 2 h. The pouches were washed with 3 ml of PBS containing 10 mM EDTA 2 h after the last injection of CCL3. The number of cells was counted microscopically.

**Delayed-type hypersensitivity model**

Delayed-type hypersensitivity was assessed in SRBC-sensitized mice. Briefly, defibrinated SRBC (REMEL) were washed, and 1 × 106 cells were injected i.v. into animals to sensitize them. Six days later, mice were injected into the footpad with 106 SRBCs in 25 μl. Footpad swelling was measured with calipers 24 h after rechallenge. In some animals, CP-481,715 was administered as a single injection (i.p.) at the time of rechallenge. In other studies, CP-481,715 was administered i.p. daily beginning at the time of sensitization.

Cytokine analysis in sensitized animals was determined on splenic lymphocytes. Spleens were collected from hCCR1 KI and WT animals 6 days after sensitization, and a single cell suspension was made. Lymphocytes were isolated over Ficoll (Sigma-Aldrich), and cells were cultured in the presence of 15% fetal bovine serum. Cytokine concentrations were determined by ELISA (R&D Systems).

**Statistical analysis**

Statistical comparisons between groups were performed using Student’s t test. A p < 0.05 was considered significant.

**Results**

**Characterization of hCCR1 in KI animals**

Gene targeting was confirmed by Southern blot analysis initially in ES cells and subsequently using DNA from hCCR1 KI animals (Fig. 2). External probes (5'/SE0.8) and 3’ (EX0.2) were used in combination with the appropriate restriction enzyme digests to yield restriction fragment-length polymorphisms indicative of 9-kb WT and 6.3-kb hCCR1 KI alleles (Fig. 2, A and B). Animals were born with the expected ratios and were viable and healthy. RT-PCR analysis of neutrophils, macrophages, and lymphocytes from WT and hCCR1 KI animals revealed that CCR1 was expressed in all three cell types, with murine CCR1 expressed only in cells from WT animals and hCCR1 expressed only in cells from hCCR1 KI animals (Fig. 2C). Cell surface expression of hCCR1 was assessed by FACS analysis (Fig. 3) and demonstrated that hCCR1 was present on neutrophils, monocytes, and lymphocytes from hCCR1 KI mice.

**Functional responses of cells**

To ensure that hCCR1 expressed on leukocytes was functional and responsive to murine CCL3, an essential attribute to use these animals to evaluate the effects of CP-481,715 on inflammatory responses, we isolated neutrophils from the peritoneal cavity following casein elicitation and assessed their ability to migrate in response to human and murine CCL3 in vitro. As shown in Fig. 4, cells isolated from hCCR1 KI animals migrate in response to both human and murine CCL3 at levels comparable to cells isolated from WT animals. This cross-reactivity of murine CCL3 on hCCR1 was also confirmed in chemotaxis assays using the human monocyte cell line THP-1 (data not shown). These results demonstrate that both murine and human CCL3 are active on hCCR1, and confirm that hCCR1 expressed on cells from KI animals is functional.

We next assessed the ability of CP-481,715 to inhibit the chemotaxis of neutrophils, lymphocytes, and macrophages from hCCR1 KI and WT mice in response to CCL3. As shown in Fig. 5, A and B, CP-481,715 inhibited the chemotaxis of neutrophils and lymphocytes taken from hCCR1 KI mice (but not WT mice) in response to CCL3, demonstrating the specificity of CP-481,715 for the human receptor and confirming that the CCL3-induced chemotaxis of cells isolated from hCCR1 KI animals was hCCR1 mediated. As shown in Fig. 5C, the concentration of CP-481,715 necessary to inhibit the chemotaxis of both lymphocytes and neutrophils (IC50 = 114 and 93 nM, respectively) isolated from hCCR1 KI mice was similar to that necessary to inhibit the chemotaxis of human monocytes in response to CCL3 (IC50 =
97 nM). Interestingly, although hCCR1 was expressed on macrophages from KI animals, CP-481,715 only partially inhibited the chemotactic response (≤25%) to CCL3, suggesting alternative receptors other than CCR1 dominate this chemotactic activity.

**Whole blood actin polymerization**

To ensure that CP-481,715 would have activity in hCCR1 KI mouse whole blood, we assessed the ability of CP-481,715 to inhibit CCL3-induced actin polymerization in neutrophils by FACS analysis. As shown in Fig. 6, CCL3-induced neutrophil actin polymerization in hCCR1 KI mouse blood was inhibited by CP-481,715 with an IC50 of 33 nM. The concentration of CP-481,715 necessary to inhibit this response in hCCR1 KI blood was similar to that previously reported to inhibit monocyte actin polymerization in human whole blood in response to CCL3 (IC50 = 58 nM) (14). CP-481,715 did not inhibit CCL3-induced actin polymerization in blood taken from WT animals.

**CP-481,715 inhibits CCL3-induced cell infiltration in hCCR1 KI mice**

The ability of CP-481,715 to inhibit in vivo cell migration in hCCR1 KI animals was clearly demonstrated in two separate models. In the first model, CP-481,715 inhibited neutrophil infiltration in response to an intradermal injection of CCL3 as assessed in skin biopsies by MPO levels (Fig. 7A) with an ED50 of 0.23 mg/kg. In the second model, neutrophil infiltration into an air pouch was inhibited by CP-481,715 with an ED50 of 0.22 mg/kg (Fig. 7B).

---

**FIGURE 3.** FACS analysis for hCCR1 expression in cells isolated from hCCR1 KI animals. A, hCCR1 expression on neutrophils in the peripheral blood of hCCR1 KI mice. B, hCCR1 expression on peritoneal macrophages isolated from hCCR1 KI mice 3–4 days following casein elicitation. C, hCCR1 expression on lymphocytes isolated from the lymph nodes of hCCR1 KI mice. Background staining with the isotype control (Iso) is shown for comparison with staining with a human specific CCR1 Ab.
these models, CCL3 primarily induces a neutrophil infiltration as assessed microscopically. No inhibition was observed on CCL3-induced cell infiltration in WT animals. Furthermore, as shown in Fig. 7C, although CP-481,715 was able to inhibit cell infiltration in hCCR1 KI animals in response to CCL3, it did not inhibit neutrophil infiltration in response to the murine chemokine KC, a neutrophil chemotactic agent acting through CXCR2. The plasma levels of CP-481,715 necessary to inhibit 90% of the cell migration in response to CCL3 using the air pouch model are shown in Fig. 7D, and suggest that a trough level of 40 ng/ml, maintained for only 2 h, was sufficient to inhibit the inflammatory cascade in this model. These studies confirm the in vivo selectivity of CP-481,715 and illustrate its ability to inhibit CCL3-dependent cell migration at clinically achievable dose levels.

Effects of CP-481,715 on delayed-type hypersensitivity

Because lymphocyte chemotaxis in response to CCL3 was also CCR1 dependent and could be blocked by CP-481,715 using cells from hCCR1 KI animals, we next examined the ability of CP-481,715 to inhibit inflammation in a more classical lymphocyte-mediated inflammatory response. As shown in Fig. 8A, CP-481,715 significantly inhibited delayed-type hypersensitivity with an ED50 of 0.88 mg/kg. This inhibition was observed when CP-481,715 was administered as a single injection at the time of rechallenge. No inhibition of delayed-type hypersensitivity with CP-481,715 was observed in WT animals (data not shown), again confirming the selectivity of CP-481,715 and the dependence of this response on CCR1. Furthermore, this inhibition of delayed-type hypersensitivity in hCCR1 KI animals was also observed when treatment was delayed up to 6 h after rechallenge in these animals (data not shown).

Effects of CP-481,715 on Th1 cytokine responses

Although CP-481,715 was able to inhibit delayed-type hypersensitivity when administered at the effector stage of the response, we next wanted to determine whether blockade of CCR1 altered cytokine production in sensitized animals, as previously reported in CCR1 knockout animals using other models (16, 17). As shown in Fig. 8, B and C, the level of both IFN-γ and IL-2 was reduced >50% in supernatants from cells obtained from CP-481,715-treated animals in response to Con A, suggesting that CP-481,715 altered general inflammatory responses and T cell activity at the spleen.

Discussion

We describe the generation of a transgenic mouse expressing hCCR1 in place of murine CCR1 and illustrate its utility to assess the anti-inflammatory properties of the human specific CCR1 antagonist CP-481,715. CCR1 KI mice express hCCR1 in cell types comparable to those expressing murine CCR1 in WT animals, and were demonstrated to be functional both in vitro and in vivo. In
addition, the concentration of CP-481,715 necessary to inhibit CCL3-induced chemotaxis or whole blood actin polymerization using cells from these animals was similar to those concentrations necessary to inhibit responses using human cells. As such, studies in hCCR1 KI animals should be useful to demonstrate the anti-inflammatory properties of CP-481,715 and help predict the plasma levels necessary to inhibit cell migration in clinic.

The functional expression of hCCR1 in these animals allowed us to assess the role of CCR1 in several models of inflammation. Blockade of hCCR1 with CP-481,715 inhibited neutrophil infiltration induced by CCL3 and prevented inflammatory responses in a model of delayed-type hypersensitivity. Furthermore, in agreement with data generated in CCR1−/− animals, CP-481,715 also modulated Th1 cytokine responses in immunized animals (16). The CCR1 dependence of these responses and the selectivity of CP-481,715 for hCCR1 were clearly demonstrated by the lack of effect of CP-481,715 in any of these models using WT animals that express murine CCR1. Collectively, these studies clearly illustrate the anti-inflammatory potential of a CCR1 antagonist.

The plasma trough level of CP-481,715 necessary to inhibit both delayed-type hypersensitivity and CCL3-induced cell infiltration at the 90% efficacy level was 40 ng/ml (achieved with a 1.0 mg/kg dose level). Interestingly, it was not necessary to continuously maintain these levels to observe activity. In fact, maintaining plasma levels for a 24-h period in the delayed-type hypersensitivity model by multiple injections did not result in improved efficacy (data not shown) as compared with a single injection at the time of rechallenge in which plasma levels were only detectable for only a few hours. One explanation for this might relate to interrupting

**FIGURE 6.** CP-481,715 inhibits CCL3-induced actin polymerization in whole blood taken from hCCR1 KI mice, but not WT mice. Blood was collected in EDTA from either hCCR1 KI or WT animals. CP-481,715 was added to the blood, followed by the addition of 10 nM CCL3. Actin polymerization was determined in the neutrophil population by FACS analysis. The data represent the percentage of inhibition of the median channel fluorescence induced by CCL3 ± SD from triplicate samples (median channel fluorescence = 150 for CCL3 in the absence of CP-481,715). The data are representative of four separate experiments. *, p < 0.05.

**FIGURE 7.** CP-481,715 inhibits cell infiltration in response to CCL3, but not KC in hCCR1 KI animals. A. Cell infiltration into skin following an intradermal injection of 1 μg of CCL3, as assessed by MPO levels in excised skin punches. The data represent the mean MPO level/ml in an 8-mm skin punch homogenized in 1.0 ml of buffer from a minimum of five animals per group. The data are representative of three separate experiments. *, p < 0.05. B. Neutrophil infiltration into an air pouch following two injections of 1 μg of CCL3. The number of cells migrating into the air pouch was 3.6 × 10⁶ in response to CCL3. The data represent the percentage of inhibition of this cell infiltration ± SD by various dose levels of CP-481,715 using a minimum of five animals per group. The data are representative of greater than 10 separate experiments. *, p < 0.05. C. Neutrophil infiltration into an air pouch following two injections of either KC or CCL3 and the level of inhibition by a 10 mg/kg dose of CP-481,715. The mean cell number per pouch ± SD from a minimum of five animals per group is shown. The data are representative of three separate experiments. *, p < 0.05. D. Serum levels of CP-481,715 following a 1.0 mg/kg dose (i.p.) associated with the inhibition of cell infiltration into air pouches in response to CCL3.
Although studies in hCCR1 KI animals are valuable to help assess the ability of a human specific CCR1 antagonist to prevent cell infiltration, extending these studies in animal disease models to help predict human disease indications must be done with caution because the dominant role of CCR1 in CCL3-induced chemotactic responses varies with cell type between human and mouse. As with human cells, the lymphocyte chemotactic responses induced by CCL3 in hCCR1 KI mice can be blocked by CP-481,715. This is not the case for monocytes. Whereas human monocyte chemotaxis in response to CCL3 was completely inhibited by CP-481,715 (14), monocytes taken from hCCR1 KI mice were only partially inhibited. Studies using CCR1\(^{-/-}\) mice have also indicated that CCR1 is important for neutrophil and lymphocyte migration, but not monocyte migration, as indicated by the lack of effects on monocyte infiltration into the peritoneal cavity in response to thioglycolate (16). Although this difference between mouse and human is potentially related to differences in CCR1 receptor expression on these cells, macrophages from mice did express high levels of CCR1. An alternative explanation is that specific differences in the expression of other chemokine receptors are responsible for the decreased role of CCR1 on monocytes in mice. A likely candidate is CCR5, which also uses CCL3 as a ligand. CCR5\(^{-/-}\) animals have been shown to have a defect in monocyte migration in response to thioglycolate elicitation (26) (unlike CCR1\(^{-/-}\) mice (16)), suggesting that CCR5 may serve a more important and dominant role on monocyte migration in the mouse. As such, it is possible that CCR5 may be the dominant receptor for CCL3 on monocytes in mice, which is in contrast to what we have observed on human monocytes (14).

Another important difference in CCR1 function between mice and humans that we observed in our studies is its role on neutrophil migration. In mice, CCR1 is an important neutrophil chemotactic factor, as illustrated both in vitro and in vivo by our studies and further supported by studies in CCR1\(^{-/-}\) mice in which neutrophil infiltration was suppressed in response to thioglycolate (16). In contrast, the role of CCR1 in human neutrophil responses has been controversial. For example, some reports have indicated that neutrophils isolated from human peripheral blood require stimulation by cytokines such as GM-CSF to express CCR1 (27), while others have claimed CCR1 is expressed on neutrophils, but the functional response is limited to certain CCR1 ligands such as leukotactin-1 (28). Interestingly, when CCL3 is injected intradermally into normal human subjects, a robust neutrophil infiltration was observed as early as 2 h after injection (8), raising questions as to whether isolation techniques used for human cells might down-regulate CCR1 expression and/or alter ligand-induced functional responses. Nonetheless, differences exist between humans and mice that have to be considered when assessing the disease potential of a CCR1 antagonist in which neutrophils are involved in the pathogenesis.

Studies to address the role of CCR1 using pharmacological agents in mice have been limited due to the human specificity of most agents. One exception is the CCR1 antagonist BX-471, which has been used to demonstrate activity in several animal disease models, including transplant rejection, renal fibrosis, arthritis, and multiple sclerosis (29). Although these studies support a role for CCR1 in modulating inflammation, a limitation is the high concentration of the agent needed to inhibit rodent CCR1 (~100-fold higher than that necessary for hCCR1) (30). As such, one concern is that at these high dose levels, other rodent G protein-coupled receptors (including chemokine receptors) may also have been inhibited, a selectivity issue that is difficult to address without cloning receptors and evaluating the compound at these concentrations on a series of rodent G protein-coupled receptors. More recently, a series of CCR1 antagonists have been described.

The inflammatory cascade, whereby cells migrating into the site of inflammation normally become activated and secrete additional chemokines, including CCL3, thus recruiting additional cells. Once this cascade is disrupted through CCR1 inhibition, it results in long-term anti-inflammatory effects, as suggested by this study. In fact, in clinical trials conducted in rheumatoid arthritis patients, CP-481,715 was able to significantly decrease cell infiltration (25) at plasma trough levels comparable to those achieved in these studies. Consequently, hCCR1 KI animals are useful to help predict the efficacious dose levels necessary to see activity in clinic.
that have equipotent activity on murine CCR1 and hCCR1 (31). Although these antagonists have demonstrated activity in a murine arthritis model, again selectivity vs other murine receptors was not reported. Nonetheless, our studies do support a role for CCR1 on neutrophil- and lymphocyte-mediated inflammatory responses in mice, suggesting that CCR1 may play a role in models of transplant rejection and multiple sclerosis, as suggested with BX-471. Our studies do raise questions, however, on any effects observed with these agents on monocyte infiltration, as this is unlikely to be directly CCR1 mediated.

Generation of human chemokine receptor KI animals represents a viable strategy to assess the in vivo activity of human specific chemokine receptor antagonists. Leukocytes from KI animals express hCCR1 and migrate to CCR1 ligands. Studies in hCCR1 KI animals demonstrate the potent activity of CP-481,715 to decrease CCL3-induced cell infiltration, prevent inflammatory responses (delayed-type hypersensitivity), and alter cytokine responses in sensitized animals. These studies underscore the importance of CCR1 in inflammation and the role of chemokines in these responses, and raise the possibility that inhibiting CCR1 will modulate inflammatory responses in clinic. In addition, the ability of CP-481,715 to inhibit cell infiltration at dose levels and plasma concentrations achievable in clinic suggests the potential clinical utility of this agent in human inflammatory diseases.

Disclosures
All of the authors are employees of Pfizer Global Research and Development.

References


