Severe reduction in leukocyte adhesion and monocyte extravasation in mice deficient in CC chemokine receptor 2

(gene targeting/monocyte chemoattractant protein 1/trafficking/peritonitis/granuloma)

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ABSTRACT CC chemokine receptor 2 (CCR2) is a prominent receptor for the monocyte chemoattractant protein (MCP) group of CC chemokines. Mice generated by gene targeting to lack CCR2 exhibit normal leukocyte rolling but have a pronounced defect in MCP-1-induced leukocyte firm adhesion to microvascular endothelium and reduced leukocyte extravasation. Constitutive macrophage trafficking into the peritoneal cavity was not significantly different between CCR2-deficient and wild-type mice. However, after intraperitoneal thioglycollate injection, the number of peritoneal macrophages in CCR2-deficient mice did not rise above basal levels, whereas in wild-type mice the number of macrophages at 36 h was ~3.5 times the basal level. The CCR2-deficient mice showed enhanced early accumulation and delayed clearance of neutrophils and eosinophils. However, by 5 days neutrophils and eosinophils in both CCR2-deficient and wild-type mice had returned to near basal levels, indicating that resolution of this inflammatory response can occur in the absence of macrophage influx and CCR2-mediated activation of the resident peritoneal macrophages. After intravenous injection with yeast β-glucan, wild-type mice formed numerous large, well-defined granulomas throughout the liver parenchyma, whereas CCR2-deficient mice had much fewer and smaller granulomas. These results demonstrate that CCR2 is a major regulator of induced macrophage trafficking in vivo.

Accumulation of leukocytes at inflammatory sites is regulated by a family of small, discrete chemotactic proteins called chemokines (1, 2). Two major chemokine subfamilies are distinguished by whether the first two conserved cysteine residues occur together (CC) or are separated by another amino acid (CXC). The monocyte chemoattractant proteins (MCPs) share about 65% amino acid identity and comprise a subgroup within the CC chemokine subfamily (3–5). MCP-1 is the most prominent and best studied of these molecules. MCP-1 is produced by a wide variety of cell types in response to pro-inflammatory stimuli (1, 6, 7), and its chemotactic and activating functions are directed at monocytes, T lymphocytes, natural killer (NK) cells and basophils (8–14).

The CC chemokine receptors (CCRs) are structurally related, seven-transmembrane-spanning proteins that signal through heterotrimeric G-protein complexes (15). In the mouse, MCP-1 appears to bind solely to CCR2 (16), although CCR2 also serves as a receptor for the other MCP subfamily members (17–20). CCR2 gene expression is highly regulated. Differentiation of monocytes to macrophages or treatment of monocytes with interferon γ, tumor necrosis factor α plus interleukin 1, or with lipopolysaccharide decreases CCR2 gene transcription whereas interleukin 2 treatment of T lymphocytes and monocytes augments CCR2 expression (21–24).

MATERIALS AND METHODS

Cloning and Targeted Disruption of the Mouse CCR2 Gene. A 748-bp PCR fragment was made from human genomic DNA using primers corresponding to unique regions of the human CCR2 gene: 5'-GCTTTTCATAGTTACTCGGCCGAAA-3' and 5'-CATAAAATTTGACGTGAAGCAA-3' (37). This fragment was radiolabeled with [α-32P]dCTP and used to screen mouse strain 129/Ola genomic libraries. A single clone was identified that contained a 2.6-kb EcoRI fragment which included 60% of the N-terminal coding sequence for mouse CCR2 (16, 18) and upstream sequences. Alignment of the mouse genomic sequence with the cDNA sequence revealed a splice site 11 bp in front of the putative translation start codon and a noncoding exon located ~2 kb upstream of the coding exon. The 2.6-kb EcoRI fragment cloned in pBlueScript served as the basis for the construction of a targeting plasmid (see Fig. 1).

Control Animals. Southern blot analysis of bacterial artificial chromosome (BAC) clones containing large (50–240 kb) fragments of strain 129 genomic DNA indicated that the CCR1, CCR2, and CCR5 genes are closely linked (ref. 16 and

Abbreviations: CCR, CC chemokine receptor; MCP, monocyte chemoattractant protein; ES cell, embryonic stem cell; NK, natural killer.
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our unpublished results), consistent with the clustering of human CCR genes (39). To eliminate the potential influence of strain-specific effects linked to the CCR2 gene (40), a control strain in which the CCR gene cluster is derived from the 129/Ola strain was developed by crossing wild-type F1 progeny of 129/Ola × C57BL/6J matings (40). A BglII restriction fragment length polymorphism was found that distinguishes the CCR5 allele in 129/Ola and C57BL/6J; a probe made from a 1.5-kb XhoI fragment located about 1.8 kb upstream of the CCR5 coding exons detects a 3.3-kb BglII fragment in 129/Ola and a 3.6-kb BglII fragment in C57BL/6J genomic DNA. All work with animals was done in accordance with National Institutes of Health guidelines and protocols approved by Animal Care and Use Committees at the University of North Carolina at Chapel Hill, the University of Texas at Austin, and the University of Virginia.

**Competitive Binding Assays.** Radiolabeled murine 125I MCP-1 (Genzyme, Boston, MA), a MCP family member and a ligand for CCR2, was purchased from New England Nuclear. To obtain macrophages for binding assays, mice 8–12 weeks old were injected i.p. with 1 ml of 4% Brewer's thioglycollate broth (Becton Dickinson). Three days later mice were killed by exposure to CO2 and the lungs and liver were excised intact and fixed in Bouin's solution. Sections were cut and stained with hematoxylin and eosin. Slides were examined under a light microscope at various magnifications.

**RESULTS**

**Targeted Disruption of the Mouse CCR2 Gene.** The targeting strategy used to disrupt CCR2 gene expression is shown in Fig. 1A. The neomycin-resistance cassette was inserted in front of the coding sequence for the first transmembrane domain of CCR2 in opposite transcriptional orientation (16, 18). Male chimeras generated from correctly targeted ES cells transmitted the mutant m t logo-long germ line when mated with C57BL/6J females (Fig. 1B). Heterozygote matings then produced homozygous CCR2−/− mice at the expected Mendelian frequency. The CCR2−/− mice were viable, exhibited normal growth and development and were fertile. In competitive binding assays using 125I-labeled JE, thioglycollate-elicted peritoneal exudate cells from CCR2−/− mice showed a dramatic reduction in specific binding relative to the elicited cells from control animals (Fig. 1C). The CCR2−/− mice have been observed for up to 15 months in both pathogen-free conditions and in virally-free conventional caging without observable differences with wild-type mice, suggesting that they are not severely immunocompromised.

**Defective Leukocyte Adhesion to the Microvasculature.** Current models of induced leukocyte extravasation propose a multistep process that includes rolling of leukocytes along the vessel wall, firm adhesion, and arrest at sites of high chemotactic concentration, transendothelial cell migration, and chemotaxis (47). We therefore compared leukocyte rolling in venules of MCP-1-treated cremaster muscle of CCR2-deficient and wild-type mice. The numbers of rolling cells per unit of time and the distribution of rolling velocities were not different between the two groups (Fig. 2) and are similar to those seen without MCP-1 treatment (45). This finding shows that the rolling of the majority of leukocytes is unaffected by a defi-
Genomic

CCR2

locus

Probe A

CCR5 probe

Targeting plasmid

Targeted allele

Genomic DNA was prepared from mouse strain 129

Bgl

(B is used and 3.1 kb when probe A is used. (Bgl of the germ-line 5.0-kb fragment to 3.7 kb when the CCR5 probe was used. A 5.0-kb BglII (Bg) fragment and a noncoding exon about 2 kb upstream of the coding exon. Other restriction enzyme sites are Xbal (X), BamHI (Bm), and EcoR1 (E). A 420-bp PCR fragment of the 3 coding region of the cross-hybridizing CCR5 gene was used as a probe to screen for correctly targeted ES clones and to detect germ-line transmission of the mutation. Probe A was used to confirm correct recombination at the 5' end. (Middle) The targeting plasmid. A 1.8-kb pgk-neo cassette was then inserted into the unique BamHI site in the CCR2 coding region. This insertion created homology arms of 2.0 kb and 0.6 kb. A pgk-thymidine kinase (TK) cassette on a KpnI fragment was inserted into the KpnI site of the pBluescript polylinker. The targeting plasmid was linearized by digestion with NotI, transfected by electroporation into E14TG2a ES cells (38), and the transfected cells were placed under double selection in 200 µg/ml G418 and 2 µM ganciclovir. (Bottom) Structure of the correctly targeted allele. There is a unique BglII site near the 3' end of the neo gene which results in a reduction of the germ-line 5.0-kb BglII fragment to 3.7 kb when the CCR5 probe is used and 3.1 kb when probe A is used. (B) Southern blot showing correct targeting and germ-line transmission of the mutation. Genomic DNA was prepared from mouse strain 129/Ola liver, the correctly targeted ES clone 20-5/17 used for blastocyst injection, and from the tails of F1 +/+ heterozygote, F2 homozygote +/+ , and F2 homozygote −/− mice. The DNA was digested with BglII and probed with the CCR5 coding region PCR fragment, detecting bands of 5.0 kb (wild-type CCR2 fragment), 3.7 kb (mutant CCR2 fragment), and 1.6 kb (wild-type CCR5 fragment). (C) Competitive binding assay using 125I-labeled murine JE on peritoneal exudate cells harvested 3 days after thioglycollate injection of CCR2+/+ (■) and CCR2−/− (▲) animals. Cells (1.7 x 10⁶ cells per well) were incubated with 0.2 nM labeled JE, and the indicated concentration of unlabeled JE for 3 h at 4°C. Each data point represents the average of duplicate determinations of bound labeled JE. In the experiment shown, the CCR2−/− cells exhibited a 93% reduction in specific binding (72% after normalization for the number of macrophages in the cell populations; see text). In three similar experiments, the mean ± SEM reduction in binding was 89.1 ± 3.5%.

Fig. 1. Strategy for targeted disruption of the mouse CCR2 gene. (A) (Top) CCR2 gene locus showing the single coding exon on a 5.0-kb BglII (Bg) fragment and a noncoding exon about 2 kb upstream of the coding exon. Other restriction enzyme sites are Xbal (X), BamHI (Bm), and EcoR1 (E). A 420-bp PCR fragment of the 3' coding region of the cross-hybridizing CCR5 gene was used as a probe to screen for correctly targeted ES clones and to detect germ-line transmission of the mutation. Probe A was used to confirm correct recombination at the 5' end. (Middle) The targeting plasmid. A 1.8-kb pgk-neo cassette was then inserted into the unique BamHI site in the CCR2 coding region. This insertion created homology arms of 2.0 kb and 0.6 kb. A pgk-thymidine kinase (TK) cassette on a KpnI fragment was inserted into the KpnI site of the pBluescript polylinker. The targeting plasmid was linearized by digestion with NotI, transfected by electroporation into E14TG2a ES cells (38), and the transfected cells were placed under double selection in 200 µg/ml G418 and 2 µM ganciclovir. (Bottom) Structure of the correctly targeted allele. There is a unique BglII site near the 3' end of the neo gene which results in a reduction of the germ-line 5.0-kb BglII fragment to 3.7 kb when the CCR5 probe is used and 3.1 kb when probe A is used. (B) Southern blot showing correct targeting and germ-line transmission of the mutation. Genomic DNA was prepared from mouse strain 129/Ola liver, the correctly targeted ES clone 20-5/17 used for blastocyst injection, and from the tails of F1 +/+ heterozygote, F2 homozygote +/+ , and F2 homozygote −/− mice. The DNA was digested with BglII and probed with the CCR5 coding region PCR fragment, detecting bands of 5.0 kb (wild-type CCR2 fragment), 3.7 kb (mutant CCR2 fragment), and 1.6 kb (wild-type CCR5 fragment). (C) Competitive binding assay using 125I-labeled murine JE on peritoneal exudate cells harvested 3 days

Fig. 2. Rolling leukocyte velocities measured in venules of the exposed cremaster muscle treated with MCP-1 for 3 h. The rolling velocities (in µm/s) of 214 leukocytes in CCR2+/+ mice (gray bars) and 178 leukocytes in CCR2−/− mice (black bars) were measured and showed a similar distribution.

Fig. 3. Induced but not constitutive monocyte accumulation is severely reduced in CCR2-deficient mice. An i.p. injection of thioglycollate in a well-established mouse model of acute peritonitis, which is characterized by successive waves of granulocyte and macrophage accumulation. To determine whether CCR2 deficiency affects this dynamic inflammatory process, control and CCR2-deficient mice were injected with thioglycollate and the number and type of leukocytes present in the peritoneal cavity at 4, 24, 36, 72, and 120 h after injection were determined. As shown in Fig. 4, the absence of CCR2 led to multiple changes in leukocyte accumulation. In both wild-type and CCR2-deficient mice, the vast majority of resident peritoneal leukocytes were macrophages, and 4 h after thioglycollate injection, the number of macrophages in both strains was decreased by >40% (Fig. 4A). After this time point, however, the two strains differed dramatically in number of peritoneal macrophages. By 24 h, the macrophages in wild-type mice were ~2 times basal levels, peaked at 36 h at 3.5 times basal levels, and then declined at 72 h and 120 h, at which time efficiency in CCR2, although our data do not exclude the possibility that the rolling velocity of a small subpopulation of circulating leukocytes—e.g., monocytes or NK cells—might be altered in CCR2-deficient mice.

In contrast to these results with rolling, the number of firmly adherent leukocytes inside cremaster muscle venules was significantly reduced (P < 0.01) in CCR2-deficient mice after MCP-1 treatment (Fig. 3A). This reduction in leukocyte adhesion was accompanied by a diminished accumulation of leukocytes in the tissue space surrounding the venules (Fig. 3B). Whereas in wild-type mice an average of 32 cells was found next to a venule in a high power microscopic field, an average of only 20 cells was observed in CCR2-deficient animals (P < 0.01). These differences could not be attributed to differences in hemodynamic parameters between the venules in wild-type and CCR2-deficient mice as judged by our finding no significant differences in diameter, centerline velocity, and shear rate in the two types of mice (data not shown)

FIG. 1. Strategy for targeted disruption of the mouse CCR2 gene. (A) (Top) CCR2 gene locus showing the single coding exon on a 5.0-kb BglII (Bg) fragment and a noncoding exon about 2 kb upstream of the coding exon. Other restriction enzyme sites are Xbal (X), BamHI (Bm), and EcoR1 (E). A 420-bp PCR fragment of the 3' coding region of the cross-hybridizing CCR5 gene was used as a probe to screen for correctly targeted ES clones and to detect germ-line transmission of the mutation. Probe A was used to confirm correct recombination at the 5' end. (Middle) The targeting plasmid. A 1.8-kb pgk-neo cassette was then inserted into the unique BamHI site in the CCR2 coding region. This insertion created homology arms of 2.0 kb and 0.6 kb. A pgk-thymidine kinase (TK) cassette on a KpnI fragment was inserted into the KpnI site of the pBluescript polylinker. The targeting plasmid was linearized by digestion with NotI, transfected by electroporation into E14TG2a ES cells (38), and the transfected cells were placed under double selection in 200 µg/ml G418 and 2 µM ganciclovir. (Bottom) Structure of the correctly targeted allele. There is a unique BglII site near the 3' end of the neo gene which results in a reduction of the germ-line 5.0-kb BglII fragment to 3.7 kb when the CCR5 probe is used and 3.1 kb when probe A is used. (B) Southern blot showing correct targeting and germ-line transmission of the mutation. Genomic DNA was prepared from mouse strain 129/Ola liver, the correctly targeted ES clone 20-5/17 used for blastocyst injection, and from the tails of F1 +/+ heterozygote, F2 homozygote +/+ , and F2 homozygote −/− mice. The DNA was digested with BglII and probed with the CCR5 coding region PCR fragment, detecting bands of 5.0 kb (wild-type CCR2 fragment), 3.7 kb (mutant CCR2 fragment), and 1.6 kb (wild-type CCR5 fragment). (C) Competitive binding assay using 125I-labeled murine JE on peritoneal exudate cells harvested 3 days
macrophage numbers were about 1.5 times the basal level. In contrast, the peritoneal macrophages in the CCR2-deficient mice never rose above the constitutive level at any time point, and at 120 h the number of macrophages was only about 17% of the constitutive number. The differences between control and CCR2-deficient mice were significant (*P < 0.01 at 24, 36, and 120 h and *P < 0.001 at 72 h) and clearly demonstrate that CCR2 is essential for macrophage migration in this model of acute inflammation.

Neutrophils and eosinophils comprise only about 2% of the resident peritoneal leukocytes in both mouse strains, but after thioglycollate injection, the two strains again showed different patterns of accumulation and clearance (Fig. 4B). In both strains there was a large influx of neutrophils and eosinophils at 4 h but the CCR2-deficient mice showed a mean of 40% more cells than control animals. Peak accumulation, ∼75 times basal levels in both strains, was reached at 24 h. Neutrophils and eosinophils declined sharply in control mice at 36 and 72 h, but they remained elevated in the CCR2-deficient mice until the 120-h time point. At both the 36- and 72-h time points the differences in cell number were significant (*P < 0.001). By 120 h both strains had returned to ∼5 times basal level. Because MCP-1 does not have a direct effect on neutrophil and eosinophil chemotaxis (1), these differences could be related to the reduced macrophage numbers or to effects on another cell type.

Lymphocytes and granulated basophils together account for <5% of peritoneal leukocytes (Fig. 4C and D). After thioglycollate injection, changes in these cell populations followed clearly distinct trends in wild-type and CCR2−/− mice. Control mice showed a substantial early increase in lymphocytes at 4 h, reaching a peak at 24 h of 2.6 times the basal level (Fig. 4C). CCR2-deficient animals did not have this early increase but showed a 4.5-fold increase at 36 h. In both cases lymphocytes returned to approximately basal levels by 120 h. Both strains had similar constitutive numbers of basophils (Fig. 4D). After thioglycollate injection, control animals showed a gradual reduction in basophilic cells at each time point and by 120 h only about 15% of the constitutive number remained. CCR2-deficient mice showed a reduction in basophils of 55% at 4 h, but no further decline through 120 h. The results summarized in Fig. 4 demonstrate that CCR2 deficiency, either directly or indirectly, alters the accumulation of multiple leukocyte subtypes in this acute inflammatory process.

Reduced Liver Granuloma Formation in CCR2−/− Mice. Previous studies by others have shown that 10 days after i.v. injection of yeast glucan, about 10% of the total liver parenchymal volume becomes granulomatous, with the typical lesion consisting predominantly of macrophage-derived cells (48, 49). Because MCP-1 has been implicated in hepatic and pulmonary granuloma formation (50, 51), we assessed granuloma formation in the livers of control and CCR2-deficient mice after intravenous injection of the β-glucan extract Zymocel. The livers of uninjected mice showed no evidence of granuloma formation. Ten days after glucan injection of control mice, numerous (>50/×100 microscope field) large, well-circumscribed granulomas had developed throughout the liver parenchyma (data not shown). These were composed mainly of epithelioid histiocytes with occasional polymorphonuclear cells (Fig. 5A). In contrast, the livers of CCR2-deficient mice did not show these large granulomas, but instead had fewer (<10/×100 microscopic field) small cellular foci consisting of only a few histiocytes and polymorphonuclear cells. In addition to these small foci, the CCR2-deficient mice also had occasional (1–2/×100 microscopic field), larger necrotising granulomas.

![Fig. 3.](image1.png)  
**Fig. 3.** (A) Leukocyte adhesion, measured as firmly adherent cells per 200-μm segment of venule. A total of 368 cells in 16 venules adhered in CCR2+/+ mice (gray bar) and 246 cells in 19 venules adhered in CCR2−/− mice (black bar). In mice receiving saline only, fewer than two adherent leukocytes were observed per venule. (B) Leukocyte emigration, expressed as number of cells emigrated per venule. A total of 493 cells were observed next to 31 venules of CCR2+/+ mice (gray bar) and 323 cells were observed next to 31 venules of CCR2−/− mice (black bar). No emigrated leukocytes were seen in mice receiving saline only. Data are presented as mean ± SEM. Significant difference: *P < 0.01.

![Fig. 4.](image2.png)  
**Fig. 4.** Peritoneal leukocyte accumulation in CCR2+/+ mice (gray bars) and CCR2−/− mice (black bars) following thioglycollate injection. The number of each leukocyte type was determined on cytopsin slides at the times indicated after thioglycollate injection: (A) Mac, macrophages; (B) N+E, neutrophils and eosinophils; (C) Lym, lymphocytes; (D) Bas, granulated basophils (n ≥ 5 CCR2+/+ and CCR2−/− animals at each time point. Data are presented as mean ± SEM; significant difference: †, P < 0.01; ††, P < 0.001. Differences in C and D did not reach statistical significance.

![Fig. 5.](image3.png)  
**Fig. 5.** Differences in glucan-induced hepatic granuloma formation in wild-type and CCR2-deficient mice. (A) A typical large, well-defined granuloma consisting predominantly of epithelioid histiocytes and a few polymorphonuclear cells found in the livers of wild-type but not CCR2−/− mice after intravenous glucan injection. (B) One of the small number of well-formed necrotising granulomas consisting of polymorphonuclear cells found in the livers of glucan-injected CCR2-deficient mice. (×330.)
DISCUSSION

Blocking MCP-1 binding to CCR2 has been proposed as a possible intervention to reduce the pathological sequelae caused by the accumulation of macrophages and T cells that occurs during the course of some diseases. Mice deficient in CCR2 provide a means of determining whether this strategy can provide therapeutic benefits and can help ascertain the potential negative effects of this strategy on normal immune processes. We find that CCR2 deficiency has no observable effects on growth, development, or fertility, and that it does not result in significant reductions in constitutive accumulation of peritoneal macrophages (Fig. 4), Kupffer cells, and alveolar macrophages (R. Alam and W.A.K., unpublished observations). However, the biological consequences of CCR2 deficiency in mice become clear when the animals are challenged with pro-inflammatory stimuli.

Our results with intravital microscopy indicate that CCR2 is not required for the rolling of leukocytes in the presence of MCP-1. However, it is important for firm adhesion to the endothelium and subsequent diapedesis. The finding that both processes are similarly reduced suggests that the reduction in leukocyte passage through the endothelium is only a reflection of the reduced firm adhesion and that the ability of CCR2-negative adherent cells to transmigrate is not impaired.

The dramatic reductions in macrophage accumulation in the peritonitis model and hepatic granuloma assay clearly demonstrate that CCR2 is important for induced macrophage trafficking, although we cannot exclude the presence of a low level of induced macrophage migration into the peritoneal cavity or liver that is CCR2 independent. For example, the small, histiocyte-containing cell foci in the livers of our glucan-injected CCR2-deficient mice might have arisen from a small subpopulation of CCR2-independent macrophages.

Which of the MCPs is the major stimulus for CCR2-dependent macrophage accumulation in the peritonitis model and granuloma assay is not yet established, but our current data make MCP-1 a strong candidate. Neutrophils and eosinophils have the ability to produce MCP-1 (52–54), and they accumulate in the peritoneal cavity of normal animals treated with thioglycollate prior to macrophage recruitment. High peritoneal MCP-1 concentrations generated by these cells would likely lead to increased basophil degranulation (9–11), contributing to the observed steady reduction in basophilic cells in control animals. The absence of this reduction in basophils in the CCR2–/– animals would then be understood.

Phagocytosis of intact neutrophils and eosinophils that have accumulated at inflammatory sites and the efficient resolution of inflammatory responses are important macrophage functions (55). The prolonged or excessive presence of activated neutrophils and eosinophils can contribute to the damage of normal tissues through the release of oxygen intermediates, proteolytic enzymes and pro-inflammatory cytokines (56–58). Several modes of inflammatory cell clearance have been identified that would avoid the release of these substances, including apoptosis followed by macrophage engulfment (59, 60), phagocytosis without apoptosis (61), and emigration out of the inflammatory site (62). We observed the characteristic features of apoptotic granulocytes (63, 64) in the cytospins of freshly harvested peritoneal exudate cells from both wild-type and CCR2-deficient mice at all time points after thioglycollate injection (data not shown), although the proportion of cells showing these features was low. The time course data in Fig. 4B show that most of the accumulated neutrophils and eosinophils have disappeared by 5 days irrespective of the strain, yet the CCR2-deficient mice have relatively few resident peritoneal macrophages and do not recruit large numbers of new macrophages. Possibly, therefore, emigration may be largely responsible for the sharp drop in granulocytes that occurs between 36 and 72 h in the mutant animals. However, our data do not exclude the contribution of multiple clearance mechanisms contributing to resolving the peritonitis.

In conclusion, we have found that CCR2-deficient mice have no obvious phenotypic defects and are not severely immune-compromised. The mutant mice are not significantly different from wild-type animals in leukocyte rolling and constitutive macrophage accumulation, but they do exhibit dramatic reductions in induced leukocyte firm adhesion, extravasation, and macrophage accumulation in vivo. Our results support the idea that specific blockers of CCR2 might provide significant therapeutic benefit without severe side effects in pathological conditions characterized by MCP-1 production and subsequent eosinophilic granuloma formation. The CCR2-deficient mouse should provide a valuable tool for studying the pathogenesis of inflammatory diseases and for determining which conditions might improve or be exacerbated by antagonists of CCR2 ligands.

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