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A Mechanism for the Impaired IFN-γ Production in C-C Chemokine Receptor 2 (CCR2) Knockout Mice: Role of CCR2 in Linking the Innate and Adaptive Immune Responses

Wendy Peters,*† Marc Dupuis,‡ and Israel F. Charo2*§

We have recently shown that mice with a targeted disruption of CCR2, the receptor for monocyte chemoattractant protein-1, have markedly impaired recruitment of macrophages to sites of inflammation. An unexpected finding in the CCR2−/− mice was a dramatic decrease in the production of IFN-γ after challenge with purified protein derivative of Mycobacterium bovis. In this study, we have investigated the mechanism of this cytokine production defect. In vitro, direct activation of splenocytes with CD3/CD28 Abs failed to reveal any differences in IFN-γ production between CCR2+/+ and CCR2−/− mice. However, after immunization, the number of Ag-specific, IFN-γ-producing cells in the draining lymph nodes was decreased by 70% in the CCR2−/− mice, suggesting an in vivo trafficking defect. Direct measurement of cell trafficking with fluorescently labeled CFA revealed a marked decrease in the number of monocytes/macrophages migrating to the site of immunization and to the draining lymph nodes in the CCR2−/− mice. The data suggest that impaired trafficking of APCs in the CCR2−/− mice contributes to the defect in IFN-γ production. These data support the idea that CCR2-positive monocytes/macrophages are critical in linking the innate and adaptive immune responses.


Chemokines are low m.w. chemotactic cytokines that direct the trafficking of leukocytes to areas of inflammation, aid the navigation of lymphocytes through the secondary lymphoid tissue, and modulate T cell cytokine production and proliferation (1–6). Chemokines exert their effects through activation of seven-transmembrane domain G protein-coupled receptors. Chemokine receptor 2 is the receptor for monocyte chemoattractant proteins (MCPs)3 1–5. The MCPs are potent chemoattractants for monocytes (7–9), memory T cells (10), NK cells (11, 12), and immature dendritic cells (DCs) (13). We and others have shown that deletion of CCR2 reduces the number of macrophages recruited into the peritoneum after thioglycolate administration (14–16), consistent with the possibility that CCR2 is normally present on murine monocytes/macrophages. Chemokine receptor 2 has also been found on immature murine DCs (17). Further evidence to support the idea that CCR2 plays an important role in mononuclear cell trafficking has come from recent studies showing fewer macrophages in the atherosclerotic lesions of CCR2−/− and MCP-1−/− mice than in those of wild-type mice (18–21). In addition, after immunization with the purified protein derivative (PPD) of Mycobacterium bovis, granuloma formation was reduced in the CCR2−/− mice, consistent with a monocyte/macrophage trafficking defect (14). An unexpected finding in the later study was the dramatically reduced level of IFN-γ in the draining lymph nodes (DLNs) of CCR2−/− mice (14). Decreased production of this cytokine in CCR2−/− mice infected with Leishmania donovani (22) or Cryptococcus neoformans (23) has subsequently been reported, suggesting that CCR2-dependent production of IFN-γ is critical for host defense.

Because of the importance of IFN-γ in the immune response and disease pathogenesis, we have investigated the basis of the reduced production of this cytokine in the CCR2−/− mice. We considered two possible mechanisms: first, that signaling by CCR2 acted in concert with activation of the TCR to optimize cytokine production; second, that abnormal trafficking of the T cells or APCs prevented the production of activated T cells in CCR2−/− mice. In this study, we show that the diminished IFN-γ production by the CCR2−/− mice is primarily due to a defect in the trafficking of monocytes/macrophages to the site of immunization; as a result, fewer Ag-laden APCs are present in the DLNs of the CCR2−/− mice. These studies reveal a novel role for CCR2-positive monocytes/macrophages as an important link between the innate and adaptive immune responses.

Materials and Methods

Reagents

All chemical reagents were purchased from Sigma (St. Louis, MO), unless otherwise indicated. Tissue culture reagents were from Life Technologies/BRK (Gaithersburg, MD). Cytokines and cytokine Abs were from R&D Systems (Minneapolis, MN).

Mice

CCR2−/− mice were generated as described (14) and were backcrossed nine times with C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME). Littermate CCR2+/+ and CCR2−/− mice were then bred to generate the mice used in the experiments. All mice were maintained in specific pathogen-free conditions and were used between 6 and 12 wk of age.

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§ Abbreviations used in this paper: MCP, monocyte chemoattractant protein; DC, dendritic cell; Dil, 3,3′dioctadecyldihexocarbocyanine; DLN, draining lymph node; ELISPOT, enzyme-linked immunospot; KLH, keyhole limpet hemocyanin; PPD, purified protein derivative.
Immunizations

For Ag-specific cytokine analysis, mice were immunized s.c. at the base of the tail with 50 μl of keyhole limpet hemocyanin (KLH; 100 μg), emulsified with an equal volume of CFA. Ingualn DLNs were removed 4 days after immunization. For studies of cell trafficking, mice were immunized in the quadriceps with 25 μl of KLH (50 μg) emulsified with an equal volume of CFA. Cells that had taken up the Ag were then fluorescently labeled with a trinitrophenyl derivative of fluorescein isothiocyanate (FITC) or a biotinylated secondary Ab. After flow cytometry analysis, the CCR2+/− mice were positive for DiI. After flow cytometry analysis, the vast majority of cells isolated had taken up both adjuvant and Ag.

Cell culture and activation

Single-cell suspensions were prepared for Ag-specific cytokine analysis. Inguinal DLNs (2.5 × 10^6) were cultured in complete medium consisting of RPMI 1640 supplemented with FCS (10%, v/v), penicillin (100 U/ml), streptomycin (100 μg/ml), g-glutamine (2 mM), HEPES (10 mM), nonessential amino acids (100 μM), sodium pyruvate (1 mM), and 2-ME (50 μM). The cells were restimulated in vitro with KLH (100 μg/ml; PharMingen), or with Con A (2.5 × 10^5 cells/ml) in culture dish. On day 8, the nonadherent cells were collected by centrifugation. The pelleted cells were resuspended in 19 ml of fresh medium plus GM-CSF (20 ng/ml) and added back to the culture dish. On day 8, the nonadherent cells were collected and replated in fresh medium plus GM-CSF (10 ng/ml; 2 × 10^6 cells/ml). On day 9, the DCs were cultured with LPS (0111:B4; Sigma; 1 μg/ml). Supernatants were removed 24 h later and stored at −80°C until assaying for IL-12 by ELISA. The cells were also labeled with CD11c-PE (0.25 μg/ml) and MHC II-CYC (0.25 μg/ml; PharMingen) to examine DC purity by flow cytometry.

ELISA cytokine assays

 Supernatants from stimulated cells were assayed in sandwich ELISAs, as previously described (14), except that primary and secondary Abs were from R&D Systems. All Abs were used at the dilutions recommended by the manufacturer. Enzyme-linked immunospot (ELISPOT) assays for determination of IFN-γ production by individual cells were performed as previously described (24). Briefly, 96-well Immulon IV plates (Dynatech Laboratories, Chantilly, VA) were coated overnight by incubation at 4°C with the IFN-γ capture Ab. The following day, the plates were washed with PBS, and cells were added to the first well of a row in duplicate (2 × 10^5 cells). Serial dilutions were conducted across the plate, and cells were cultured overnight in complete medium plus KLH (100 μg/ml). The next day, the cells were washed away, and biotinylated anti-IFN-γ secondary Ab was added for 1 h at room temperature. The plate was washed, and streptavidin-alkaline phosphatase (Boehringer Mannheim) was added for 30 min at room temperature. The plate was washed again, and the substrate, 5-bromo-4-chloro-3-indolyl phosphate (1 mg/ml; Sigma), was dissolved in 2-amino-2-methyl-1-propanol buffer (0.1 M; Sigma) mixed with 0.6% low-melt agarose (SeaPlaque; FMC Bioproducts, Rockland, ME). After solidification, the blue spots (each spot corresponding to an IFN-γ-producing cell) were counted with an inverted microscope.

DC differentiation from bone marrow

DCs were obtained by differentiation of bone marrow precursor cells, as previously described (25), to determine whether the absence of CCR2 affected their activation efficiency. Bone marrow plugs were isolated from the femur and tibia of the hind legs, and red cells were lysed. Cells (1.2 × 10^7) were cultured in 25 ml of complete medium plus GM-CSF (20 ng/ml; R&D Systems). After 48 h, an additional 12.5 ml of complete medium containing GM-CSF (40 ng/ml) was added. On day 5, 19 ml of medium was removed, and nonadherent cells were collected by centrifugation. The pelleted cells were resuspended in 19 ml of fresh medium plus GM-CSF (20 ng/ml) and added back to the culture dish. On day 8, the nonadherent cells were collected and replated in fresh medium plus GM-CSF (10 ng/ml; 2 × 10^6 cells/ml). On day 9, the DCs were cultured with LPS (0111:B4; Sigma; 1 μg/ml). Supernatants were removed 24 h later and stored at −80°C until assaying for IL-12 by ELISA. The cells were also labeled with CD11c-PE (0.25 μg/ml) and MHC II-CYC (0.25 μg/ml; PharMingen) to examine DC purity by flow cytometry.

Statistical analysis

All statistical analyses were performed with the Mann-Whitney test. Values of p < 0.05 were considered significant.

Results

Impairment in the production of IFN-γ by CCR2+/− mice after immunization in a Th1 manner

We previously found that CCR2+/− mice challenged with PPD of M. bovis in CFA produced markedly less IFN-γ than CCR2−/− mice (14). To further investigate this finding, we immunized CCR2+/− and CCR2−/− mice with KLH emulsified in CFA, which also produces a Th1 response. Four days after immunization, cells from the DLNs were restimulated in vitro with KLH for 48 h, and supernatants were assayed for IFN-γ. As seen in Fig. 1, and consistent with the previous data obtained by challenging with PPD, the KLH/CFA immunization resulted in the production of 70–90% less IFN-γ by the CCR2−/− mice than by the wild-type mice. In contrast, production of IL-5, IL-10, or IL-13 was not reduced in the CCR2−/− mice (Fig. 1). IL-12 and IL-4 were undetectable in both CCR2 wild-type and CCR2−/− mice (data not shown). Ab isotype switching was also investigated, but no differences were observed in IgG1 (induced by IL-4) or IgG2a (induced by IFN-γ) (26–28) were found (data not shown).

![FIGURE 1. Cytokine production in immunized CCR2+/− and CCR2−/− mice.](http://www.jimmunol.org/DownloadedFrom)
Activation of T cells by CD3/CD28 and Con A

To determine whether expression of CCR2 on T cells is required for normal production of IFN-γ, we harvested naive splenocytes from CCR2−/− and CCR2+/+ mice and activated them with a combination of anti-CD3 (10 μg/ml) and anti-CD28 (10 μg/ml). No significant differences in the amounts of IFN-γ produced were found between the CCR2−/− and CCR2+/+ mice (Fig. 2A). Because the anti-CD3 and anti-CD28 were used at saturating concentrations, additional experiments were performed in which the concentration of anti-CD3 was varied between 2.5 and 0.00125 μg/ml, while anti-CD28 was kept constant at 0.1 μg/ml. Once again, at all anti-CD3 concentrations examined, we failed to detect a difference in the amount of IFN-γ produced by CCR2−/− and CCR2+/+ (data not shown). Splenocyte proliferation during this assay was also quantitated, and no significant differences were found between the CCR2−/− and wild-type mice (data not shown).

In contrast, when splenocytes from naive mice were activated by Con A, significantly lower levels of IFN-γ were produced by the CCR2−/− cells (Fig. 2B). These results suggest that, at least in vitro, signaling through CCR2 is not essential for T cells to produce optimal levels of IFN-γ in response to direct activation by anti-CD3/anti-CD28, but that optimal activation by Con A does depend upon the presence of CCR2.

Activation of DCs by LPS

Activation of T cells to produce a Th1 response requires the presence of the APC cytokine IL-12. To determine whether IL-12 function was dependent upon the presence of CCR2, DCs were derived by culturing bone marrow precursor cells of naive CCR2−/− and CCR2+/+ mice. After differentiation, the DCs were activated in vitro with LPS to stimulate cytokine production. Flow cytometry after LPS stimulation demonstrated that the cells were 90% positive for CD11c and 90% positive for MHC II (data not shown). No differences in the production of IL-12 between CCR2−/− and CCR2+/+ mice were found (Fig. 3). Thus, as was the case for T cells, activation of CCR2 was not required for cytokine production by DCs.

Quantitation of Ag-specific IFN-γ-producing T cells in the DLNs

We next sought to determine whether the decrease in IFN-γ production represented less cytokine being produced by each CCR2−/− T cell in the DLNs, or alternatively, whether fewer Ag-specific IFN-γ-producing T cells were present in the DLNs of the CCR2−/− mice. The ELISPOT assay was used to determine the number of Ag-specific IFN-γ-producing cells in the DLNs, after immunization with KLH/CFA. As shown in Fig. 4, there were significantly fewer Ag-specific IFN-γ-producing cells in the DLNs of CCR2−/− mice than in those of CCR2+/+ littermate controls. However, we found no differences in the total numbers of CD3-positive T cells in the DLNs after immunization. These results were consistent with a trafficking defect of either the T cell or the APC.

APC trafficking in CCR2−/− mice

Activation of T cells in vivo to produce IFN-γ requires macrophages and/or DCs to effectively present Ag to the TCR. Because fewer T cells appeared to be activated to produce IFN-γ by the ELISPOT assay and because there did not appear to be a signaling defect by the T cells lacking CCR2 (the CD3/CD28 activation experiments), we considered the possibility that APC trafficking might be abnormal in CCR2−/− mice. To test this hypothesis, the number of infiltrating monocytes/macrophages and/or DCs at the site was determined 48 h after immunization with fluorescently labeled adjuvant. Resident and infiltrating mononuclear cells were isolated from the quadriceps and quantified. As shown in Fig. 5A, there were significantly fewer cells at the site of immunization in CCR2−/− mice than in CCR2+/+ mice. Further analysis revealed that the vast majority of these cells were CD11b+/CD11c−, suggesting that they were macrophages (Fig. 5B). Peritoneal macrophages, used as control cells for Ab specificity, yielded a virtually identical staining pattern (data not shown). Using these same techniques, the number of DiI-positive cells trafficking to the DLNs after immunization was determined. Markedly fewer DiI-positive cells appeared in the DLNs of CCR2−/− mice as compared with normal production of IFN-γ.
prised to find that they produced 90% less IFN-

to signal in response to MCP-1 or other closely related

1. In 1996, Taub et al. (5) showed that RANTES, macrophage-
mice only, because too few APCs were present in the

Discussion

In our initial characterization of the CCR2−/− mice, we were sur-

We initially speculated that the decreased Ag-specific production of

by studies in which neutralizing Abs to MCP-1 were added to
cultures of CD4+ T cells, and a decrease in IL-4 production was
seen along with a concomitant increase in IFN-γ production (30).
Taken together, these in vitro studies supported the notion that
signaling through CCR2 may play an important role in IFN-γ pro-
duction in vivo.

In the current study, we tested this hypothesis but found that
CCR2−/− T cells produced as much IFN-γ as CCR2+/+ T cells after
direct activation of CD3/CD28. These results are in contrast to the
recent report of Sato et al. (22), who found that T cells derived from
CCR2−/− mice produced significantly less IFN-γ than cells from
CCR2+/+ mice in response to CD3/CD28 activation. Because Sato et
al. used lower concentrations of the CD3/CD28 Abs, we repeated the
experiments using a wide range of CD3 Ab concentrations, but again
failed to detect any differences between the CCR2+/+ and CCR2−/−
mice. Nor did we find any difference in the proliferation of CCR2+/+
and CCR2−/− splenocytes in response to CD3/CD28. The reason for
the difference between our results and those of Sato et al. (22) is not
apparent at this time.

Because in our hands the presence of CCR2 did not play a role in
IFN-γ production in response to direct activation of the TCR, we
turned our attention to the other major CCR2-positive cell type, the
APCs, and asked whether they were functioning abnormally in the
CCR2−/− mice.

A. Mice were immunized in the quadriceps with KLH/CFA that
was labeled with the fluorescent dye, DiI. After 48 h, the APCs were
isolated and purified on a metrizamide gradient and counted. Shown are the
results from pooling six mice of each genotype, in three independent ex-
periments. B. Flow cytometry was used to identify cell types within the
population of APCs that took up the labeled adjuvant. CD11b-CYC was
used to stain macrophages, and CD11c-CYC to stain DCs. Shown are data
for CCR2+/+ only, because too few APCs were present in the

B. FACS analysis revealed that DiI-positive cells

Determination of the number of APCs at the site of immu-
nization. A. Mice were immunized in the quadriceps with KLH/CFA that
was labeled with the fluorescent dye, DiI. After 48 h, the APCs were
isolated and purified on a metrizamide gradient and counted. Shown are the
results from pooling six mice of each genotype, in three independent ex-
periments. B. Flow cytometry was used to identify cell types within the
population of APCs that took up the labeled adjuvant. CD11b-CYC was
used to stain macrophages, and CD11c-CYC to stain DCs. Shown are data
for CCR2+/+ only, because too few APCs were present in the

CCR2−/− mice (Fig. 6a), and the vast majority of these cells were
CD11b positive (Fig. 6B).

Determination of APCs in the DLNs after immunization. A. Mice were immunized as in Fig. 5. After 48 h, the DLN cells were dis-
persed by treatment with collagenase, and the APCs were purified on a
metrizamide gradient. Flow cytometry was used to quantify the number of
DLN cells that took up the labeled adjuvant. Shown are the results of three
independent experiments. B. FACS analysis revealed that DiI-positive cells
were CD11b+. Gates were set to exclude autofluorescent cells.
The lack of detectable cytokine defects in isolated cells led us to suspect that impaired trafficking of leukocytes to the site of immunization and/or to the DLNs was the underlying abnormality in the CCR2−/− mice. Indeed, macrophage trafficking defects in the CCR2−/− mice have been well documented. Fewer macrophages enter the peritoneal cavity after thioglycolate administration (14–16), and fewer macrophages are recruited to the aortic wall (18, 21) in CCR2−/− than in CCR2+/+ mice. In addition, Kurihara et al. (15) found that CCR2−/− mice were susceptible to lethal infection with Listeria monocytogenes, which they attributed to a lack of macrophage trafficking into infected tissues. None of these studies, however, considered macrophage recruitment in the context of Ag presentation. In mice, CCR2 is expressed on both immature DCs (17) and monocytes/macrophages. We therefore hypothesized that trafficking of these cells to the site of immunization, and subsequently to the DLNs, might be impaired.

We began the analysis of cell trafficking by determining the number of Ag-specific IFN-γ-producing cells in the DLNs after immunization, using the ELISPOT assay. These studies revealed that there were indeed fewer Ag-specific IFN-γ-producing cells in the DLNs of the CCR2−/− mice, even though the total number of CD3+ cells was equivalent in the CCR2−/− and CCR2+/+ mice. It was still not clear, however, whether the primary defect was at the level of the T cell or the APC, because both are required for efficient cytokine production. A clue came from a result in our earlier studies (14), and confirmed in this study, that splenocytes from CCR2−/− mice produced significantly less IFN-γ than cells from CCR2+/+ mice after activation with Con A. Unlike anti-CD3/anti-CD28, optimal activation of T cells by Con A requires the presence of APCs. It is of interest in this regard that we found decreased Con A-induced IFN-γ production in naive mice as well as in KLH/ CFA-immunized CCR2−/− mice, implying that CCR2 may play a role in the constitutive trafficking of APCs as well as in activated APC trafficking, even though the total number of CD11b-positive cells is the same in the CCR2+/+ and CCR2−/− mice (14). Consistent with this hypothesis are the reports of constitutive MCP-5 expression in naive LNs (32) and the recent findings of Gu et al. (33), who have noted the presence of MCP-1 in both the spleen and lymph nodes of naive mice. It is also possible that MCP-1 production by APCs serves to increase the efficiency of Ag presentation by attracting CCR2-positive T cells within the LN. In this regard, Sallusto et al. (34) have found that mature DCs express MCP-1 and MCP-2.

Taken together, these data implicated the APC as the culprit and suggested that a defect in monocyte/macrophage and/or DC trafficking might be the primary cause of the IFN-γ production defect in CCR2−/− mice. That equivalent levels of IFN-γ were produced by CCR2+/+ and CCR2−/− splenocytes in response to CD3/CD28 further suggested that T cell trafficking was not the problem. We therefore sought to directly quantitate the number of cells that entered the site of immunization and to track them to the DLNs in CCR2−/− and CCR2+/+ mice. To directly visualize cells that interacted with the Ag, we immunized mice with KLH and fluorescently labeled CFA (35). Thus, any cell that phagocytosed the Ag could be detected by fluorescence analysis. Using this technique, Dupuis et al. (35) showed that DI-labeled APCs trafficked to the T cell areas of the DLNs, where they could present Ags. In our studies, ~70% fewer fluorescently labeled cells were found at the site of immunization in the quadriceps in the CCR2−/− mice than in the wild-type mice. Analysis of these cells for expression of CD11b and CD11c revealed that greater than 80% of the fluorescent cells were macrophages, and we failed to detect any cells that were strongly positive for CD11c, suggesting the absence of DCs. This result is also consistent with recent work by Randolph et al. (36), who found that after s.c. injection with FITC-latex beads, the majority of cells that arrived at the site were CD11b+ monocytes/macrophages. We further found a marked reduction in the number of fluorescent CD11b+ cells in the DLNs of CCR2−/− mice, as compared with the CCR2+/+ mice.

These findings suggest that impaired trafficking of the monocytes/macrophages to the site results in fewer Ag-bearing APCs in the DLNs and contributes to reduced production of IFN-γ in CCR2−/− mice after immunization. However, we have not ruled out a trafficking defect in the T cells themselves, although this would have to be limited to Ag-specific T cells because we did not detect differences in the number of total CD3-positive cells in LNs from CCR2−/+ vs CCR2−/− mice. Adoptive transfer experiments will be required to address this question directly.

Our results immediately raise the question of why wild-type levels of IL-5, IL-10, and IL-13 are produced in the CCR2−/− mice. One possibility is that CCR2 may be expressed on only a subpopulation of APCs that tend to induce Th1 responses. In this regard, recent reports suggest the presence of at least two populations of DCs that can differentially polarize T cells to produce either Th1 or Th2 cytokines (37, 38). There have also been reports suggesting that distinct populations of macrophages exist. Stein et al. (39) suggested the presence of two separate populations of macrophages: APC1s that promoted inflammatory responses and APC2s that were designated noninflammatory inducers. It is possible, therefore, that CCR2 is preferentially expressed on the APC1-type macrophages that predominate at sites of inflammation. This would be consistent with the presence of MCP-1 at sites of inflammation (40–43) and would extend to the APC the recent paradigm that different chemokine receptors are present on Th1 vs Th2 lymphocytes. A second possibility is that we did not detect a defect in Th2 cytokines because the genetic background of the CCR2−/− mice (C57BL/6) and the immunization protocol (KLH/ CFA) favored the production of Th1 cytokines. In this regard, Warmington et al. (44) found reduced levels of both Th1 and Th2 cytokines when CCR2−/− mice were immunized in a more Th2-like manner with Schistosoma mansoni egg Ags. To provide a more rigorous test of the role of the genetic background, we are crossing the CCR2 null mice onto a pure BALB/c background, and we will then test their ability to synthesize and secrete Th2 cytokines after immunization in a Th2 manner.

Gu et al. (33) have recently reported that MCP-1−/− mice have a defect in the production of Th2 cytokines, but make normal levels of IFN-γ. The disparity in cytokine production between the ligand (MCP-1) and receptor (CCR2) knockouts is intriguing, and suggests several plausible explanations for reconciling the data. First, it is possible that there are additional functional receptors for MCP-1. Thus, Nibbs et al. (45) have described a murine chemokine receptor, known as D6, which binds MCPs and other chemokines, but does not signal. More recently, Schweickart et al. (46) cloned a human chemokine receptor (CCR11) that binds and signals to MCPs, although at relatively high concentrations. It is not yet known if the murine form of CCR11 is activated by murine MCPs. Second, we know that in the mouse, MCP-3 and MCP-5 are also potent CCR2 agonists (32, 47). CCR11 is activated by murine MCPs. Second, we know that in the mouse, MCP-3 and MCP-5 are also potent CCR2 agonists (32, 47).
In summary, we have found that CCR2−/− mice have a profound and selective defect in IFN-γ production when immunized in a Th1 manner and that this defect is due, at least in part, to impaired trafficking of monocytes/macrophages to the immunization site and APCs to the DLNs. These studies reveal an important function for CCR2 in the modulation of the immune response. It is interesting to speculate that the importance of CCR2 in this regard might depend upon whether or not MCP-1 (or other CCR2 agonists) are generated at the site of inflammation as part of an immune response.

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References