Essential Role of CCR2 in Neutrophil Tissue Infiltration and Multiple Organ Dysfunction in Sepsis

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Rationale: Sepsis is defined as a systemic inflammatory response to infection, which in its severe form is associated with multiple organ dysfunction syndrome (MODS). The precise mechanisms by which MODS develops remain unclear. Neutrophils have a pivotal role in the defense against infections; however, overwhelming activation of neutrophils is known to elicit tissue damage.

Objectives: We investigated the role of the chemokine receptor CCR2 in driving neutrophil infiltration and eliciting tissue damage in remote organs during sepsis.

Methods: Sepsis was induced in wild-type mice treated with CCR2 antagonist (RS504939) or CCR2−/− mice by cecal ligation and puncture (CLP) model. Neutrophil infiltration into the organs was measured by myeloperoxidase activity and fluorescence-activated cell sorter. CCR2 expression and chemotaxis were determined in neutrophils stimulated with Toll-like receptor agonists or isolated from septic mice and patients.

Measurements and Main Results: CCR2 expression and responsiveness to its ligands was induced in circulating neutrophils during CLP-induced sepsis by a mechanism dependent on Toll-like receptor/nuclear factor-κB pathway. Genetic or pharmacologic inhibition of CCR2 protected mice from CLP-induced mortality. This protection was associated with lower infiltration of neutrophils into the lungs, heart, and kidneys and reduced serum biochemical indicators of organ injury and dysfunction. Importantly, neutrophils from septic patients express high levels of CCR2, and the severity of patient illness correlated positively with increasing neutrophil chemotaxis to CCR2 ligands.

Conclusions: Collectively, these data identify CCR2 as a key receptor that drives the inappropriate infiltration of neutrophils into remote organs during sepsis. Therefore, CCR2 blockade is a novel potential therapeutic target for treatment of sepsis-induced MODS.

Keywords: sepsis; neutrophils; CCR2; infiltration; multiple organ dysfunction syndrome

Neutrophils are highly motile phagocytic cells that constitute the first line of defense of innate immunity. The migration of neutrophils into the site of infection is mediated by chemoattractants, which are endogenously generated by the host or released by bacteria (1, 2). The chemokine receptor CXCR2 is highly expressed in neutrophils, and signaling through this receptor is essential for the maximal recruitment of this cell type into the inflammatory site (3–6). It has been demonstrated that neutrophil recruitment into the site of infection is markedly impaired during sepsis, which is associated with the severity of the disease (7). The impaired migration of neutrophils into the site of infection results in a failure to remove the invading pathogens, initiating a hyperactive inflammatory response characterized by inappropriate sequestration and infiltration of neutrophils in remote organs (8–10). In addition, the accumulation of neutrophils in remote organs results in tissue damage, which contributes to the multiple organ failure associated with severe sepsis and septic shock (11, 12). Precisely how sepsis alters this paradoxical pattern of neutrophil mobilization (impaired migration into infection focus vs. increased accumulation in remote organs) is not completely understood. In this sense, we have recently demonstrated that the failure of neutrophil migration is a consequence of the down-regulation of CXCR2 in circulating neutrophils, an event mediated by Toll-like receptor (TLR) 2 and TLR4 activation (13, 14). However, mechanisms involved in the infiltration and accumulation of neutrophils in the remote organs have not yet been addressed.

CCR2 is a chemokine receptor mainly expressed in monocytes (15–18), and it has not been detected on the surface of resting human, rat, or mouse neutrophils (19–21). In CCR2-deficient mice, monocyte recruitment to sites of inflammation is impaired, whereas neutrophil recruitment remains intact (22, 23). Interestingly, chronic inflammation has been shown to induce the expression of CCR2 on circulating neutrophils (20). Moreover, neutrophils from mice that had undergone cecal ligation and puncture (CLP), a polymicrobial model of sepsis (24), expressed mRNA for CCR2 and displayed the ability to migrate toward CCR2 chemokines (21). However, the molec-
ular mechanism of CCR2 expression and its functional implication in neutrophil migration has not yet been examined.

In the present study, we show that TLR2 and TLR4 signaling up-regulates the expression of CCR2 in circulating neutrophils during sepsis, mediating their infiltration and eliciting tissue damage in remote organs. Extending our experimental observations to clinical relevance, we found that neutrophils from septic patients express high levels of CCR2 and exhibit high chemotactic responses to CCL2, which were positively correlated with clinical indicators of illness severity and organ damage. Our results collectively emphasize the importance of CCR2 as a functional marker of septic neutrophils that contributes to their tissue infiltration and organ damage. Some of the results of these studies have been previously reported in the form of an abstract (25).

METHODS

Experimental details are provided in the online supplement.

Patients

Peripheral blood samples from 30 septic patients and 25 healthy volunteers were enrolled in this study. All patients (see Table E1 in the online supplement) presented clinical or laboratory variables that fulfilled the criteria for sepsis (26). Informed consent was obtained, and the study was approved by the Human Subjects Institutional Committee of the School of Medicine of Ribeirao Preto, University of Sao Paulo (12,664/2006).

Mice

CCR2−/−, MyD88−/−, TLR2−/−, and TLR4−/− mice on the C57BL/6 background (backcrossed for over 20 generations) were bred in our animal facility. CCR2−/− mice were generated by Prof. W.A. Kuziel (22). All animal experiments were performed in accordance with the ethical guidelines of the School of Medicine of Ribeirao Preto, University of Sao Paulo, Brazil (181/2008).

Sepsis Model

Severe sepsis was induced by CLP model as described previously (13) using an 18-gauge needle. Sham-operated animals were submitted to laparotomy without CLP. In some experiments, mice were treated with CCR2 antagonist (RS 504393, 2 mg/kg, subcutaneously) (Tocris Bioscience, Ellisville, MO) (27) or ertapenem sodium (10 mg/kg, intraperitoneally) (Merck Research Laboratory, Sao Paulo, Brazil) (28) after CLP. The survival rate was determined daily for 7 days after CLP.

Air Pouch Model

Air pouches were prepared as described (29). Mice were then submitted to CLP and, simultaneously, CCL2 (30 ng per mouse) was injected into the air pouch. The pouch lavage fluid was collected for neutrophil counts 6 hours after the challenge.

Neutrophil Purification

Human neutrophils were purified from peripheral blood by Percoll density gradient as previously described (30). Mouse neutrophils were isolated from peripheral blood or bone marrow by Percoll density gradient as previously described (14).

Chemotaxis Assay

Chemokine and chemokine levels were measured by ELISA using antibodies from R&D Systems.

Myeloperoxidase Assay

Tissue myeloperoxidase (MPO) activity was used as a biochemical index of neutrophil infiltration into the lungs, heart, and kidneys as previously described (31).

TLR Stimulation In Vivo

Mice were treated systemically with TLR2 agonist (lipoteichoic acid [LTA], 15 mg/kg) and TLR4 agonist (LPS, 15 mg/kg). Two hours later, neutrophils were isolated from peripheral blood, and the chemotaxis was performed.

Statistical Analysis

Data are reported as means ± SEM of values obtained from two or three different experiments. Significance was determined by Student t test, one- or two-way analysis of variance where appropriate to determine differences within groups, with a Bonferroni post hoc analysis. Survival studies were analyzed with the log-rank test and bacterial counts by Mann-Whitney U test. P less than 0.05 was considered statistically significant.

RESULTS

TLR Triggering Drives Neutrophil Chemotaxis to CCR2 Chemokine Ligands

We first examined the ability of TLRs, in particular TLR2 and TLR4, to induce neutrophil migration toward CCR2 ligands (CCL2 and CCL7). We isolated neutrophils from the bone marrow of naive mice and incubated them in the presence or absence of the TLR2-ligand LTA or the TLR4-ligand LPS. After 2 hours, neutrophil chemotaxis was assessed using a modified Boyden chamber assay. Control neutrophils exhibited strong chemotaxis to CXCL2, and no response to CCL2 or CCL7 was detected when compared with random chemotaxis toward medium alone (Figures 1A–1C). As reported (14), we observed that stimulation of neutrophils with LTA or LPS resulted in decreased chemotaxis toward CXCL2 (Figure 1A). However, LTA and LPS both induced substantial neutrophil chemotaxis in response to CCL2 (Figure 1B) and CCL7 (Figure 1C) in a time-dependent manner (Figure 1D). Chemotaxis toward CCL2 or CCL7 was specifically mediated by CCR2 in TLR-activated neutrophils because it was completely abolished in neutrophils deficient in CCR2 (CCR2−/−) or those treated with the CCR2 antagonist RS504393 (Figures 1E and E1). Nevertheless, deficiency or blockade with a CCR2 antagonist had no effect on CXCL2-induced chemotaxis (Figure E2).

To further characterize neutrophil responsiveness to CCR2 ligands after TLR activation, we analyzed the chemokine-induced cytoskeletal rearrangements by assessing F-actin staining. CXCL2 induced a marked increase in F-actin formation in control neutrophils (Figures 1F and 1G). Inhibition of F-actin formation by TLR-activated neutrophils because it was completely abolished in neutrophils deficient in CCR2 (CCR2−/−) or those treated with the CCR2 antagonist RS504393 (Figures 1E and E1). Nevertheless, deficiency or blockade with a CCR2 antagonist had no effect on CXCL2-induced chemotaxis (Figure E2).

Flow Cytometry

Human neutrophils were stained with anti-CCR2 PE (R&D Systems) and anti-CD66 fluorescein isothiocyanate (BD Biosciences) antibodies.
response to CCL2 and CCL7 was detected in LTA- or LPS-treated WT neutrophils, which was completely abrogated in CCR2−/− neutrophils (Figures 1F–1H).

To further investigate whether TLR activation induces CCR2 expression, we performed immunofluorescence experiments followed by confocal microscopy analysis. Two hours after LTA or LPS stimulation, neutrophils were stained with anti-CCR2 antibody (red) and counterstained with DAPI (4′,6-diamidino-2-phenylindole; blue) to reveal the nuclei. The staining specificity of the anti-CCR2 antibody was confirmed using CCR2−/− neutrophils (Figures 2A and 2C). As reported (20), no CCR2 expression was detectable in the resting neutrophils. However, CCR2 expression was dramatically increased on the surface of LTA- or LPS-treated neutrophils (Figures 2A and 2C).

We then determined whether protein synthesis is required for the increased expression of CCR2 using an inhibitor of protein synthesis (cycloheximide [CHX]) before stimulation with LTA or LPS. Treatment with CHX markedly decreased the expression of CCR2 on TLR-activated neutrophils (Figures 2B and 2C).

Consistent with the requirement of protein synthesis for CCR2 expression on LTA- or LPS-stimulated neutrophils, treatment with CHX prevented neutrophil chemotaxis in response to CCL2 after exposure to LTA or LPS, but did not interfere with chemotaxis to CXCL2 (Figure 3A). To further explore the link between TLR activation and CCR2 expression, we assessed the chemotaxis to CCL2 in TLR2−/−, TLR4−/−, and MyD88−/− neutrophils. As expected, neutrophil responsiveness to CCL2 was TLR2 dependent for LTA stimulation and TLR4 dependent for LPS stimulation (Figure 3B). Moreover, the adaptor molecule MyD88 was required for both stimuli (Figure 3B). We then used a pharmacologic approach to investigate the activated pathways downstream of TLR activation that are required for CCR2 expression. Neutrophils were therefore stimulated with LTA or LPS with or without pretreatment with PD98059 (MEK inhibitor); SP600125 (JNK inhibitor); SR11302 (AP-1 inhibitor); or PDTC, CAPE, or BAY 11–7082 (nuclear factor kB inhibitors). Inhibition of the MAP kinase pathway (MEK, JNK, and AP-1) had no effect on the responsiveness of TLR-activated neutrophils to CCL2 (Figure 3C). However, chemotaxis to CCL2 was completely abolished by inhibition of NF-κB (Figures 3D and E3). Consistently, the LPS-induced nuclear translocation of NF-κB p65 subunit was completely prevented by PDTC or BAY 11–7082 (Figure E4). Thus, these results suggest that TLR triggering mediates CCR2 expression via an NF-κB–dependent pathway.

To assess whether TLR activation promotes neutrophil responsiveness to CCR2 chemokines in vivo, mice were treated with a single intravenous injection of LTA or LPS. Three hours after injection, neutrophils were isolated from the blood and chemotactic response toward CCL2 and CCL7 was assessed. Consistent with our in vitro findings, control neutrophils isolated from saline-treated mice did not show chemotactic responsiveness to CCL2 or CCL7. In contrast, injection of LTA or LPS led to a marked increase in neutrophil chemotaxis to CCL2 and CCL7, demonstrating that TLR activation can promote neutrophil responsiveness to CCR2 chemokines in vivo.

**TLR Activation Induces CCR2 Expression in Neutrophils**

**TLRs Regulate CCR2 Responsiveness in Neutrophils Via Nuclear Factor-κB**

**TLR Ligands Modulate Neutrophil Responsiveness to CCR2 Chemokines In Vivo**
LPS into mice induced substantial neutrophil responsiveness to CCL2 and CCL7 (Figure 4A).

It has been reported that neutrophils from mice having undergone CLP express high levels of CCR2 mRNA (21). Indeed, flow cytometric analysis of circulating neutrophils from WT mice undergoing CLP showed that these cells begin to express CCR2 (Figure 4B). To address the role of TLRs in mediating CCR2 responsiveness in neutrophils during sepsis, we performed CLP in MyD88<sup>-/-</sup> mice and chemotaxis was assessed in neutrophils isolated from the blood of mice at the onset of sepsis (3 h after CLP). We found that neutrophils from WT mice showed marked chemotaxis in response to CCL2 and CCL7, whereas neutrophils from sham-operated mice only exhibit random chemotaxis (Figure 4C). Notably, MyD88 deficiency prevented neutrophil chemotaxis toward CCL2 and CCL7 after CLP (Figure 4C), but did not affect chemotaxis to CXCL2 in neutrophils from sham-operated mice (Figure 4E). These results suggest that up-regulation of CCR2 on neutrophils during sepsis is mediated by TLR signaling.

To obtain insights into the role of CLP-induced CCR2 expression in regulating neutrophil migration in vivo, we used the murine air pouch model (29). Air pouches formed by subcutaneous injection of air into the back of a mouse develop an artificial chamber with a vascularized tissue lining. The air pouch model therefore provides a suitable space to investigate the in vivo chemotaxis induced by CCR2 ligands. We performed CLP or sham laparotomy, and CCL2 or saline (control) was injected into the air pouch of mice simultaneously with surgery. The pouch lavage fluid was collected for neutrophil counts 6 hours after CCL2 injection. We found that CCL2 or saline induced similar basal migration of neutrophils in sham-operated WT and CCR2<sup>-/-</sup> mice. However, after CLP induction, CCL2 caused a 3.5-fold increase in neutrophil migration in WT mice compared with the saline group, whereas it failed to increase neutrophil migration in CCR2<sup>-/-</sup> mice (Figure 4D). Collectively, these results indicate that TLR signaling induces expression of CCR2 on neutrophils, conferring to these cells the ability to migrate in response to CCR2 ligands and that this phenomenon can be found during experimental sepsis.

**CCR2 Deficiency Improves Survival in Experimental Sepsis**

We next sought to determine the role of CCR2 in experimental sepsis. We therefore performed CLP in WT and CCR2<sup>-/-</sup> mice.
and the survival rates were evaluated for 7 days. All WT mice died within 2 days of CLP induction; however, CCR2 deficiency resulted in a significantly higher survival rate (\(\sim 57\%\); \(P < 0.01\)) (Figure 5A). The treatment of the septic mice with CCR2 antagonist, RS 504,393 (2 mg/kg), beginning 2 hours after CLP and then every 12 hours thereafter up to Day 3, prolonged survival, but did not provide protection from CLP-induced mortality after discontinuation of the treatment (Figure 5B). These results suggest that only the sustained blockage of CCR2 provides beneficial effects in sepsis. However, the association of CCR2 blockage with antibiotic therapy (ertapenem, 10 mg/kg) conferred significant protection from CLP-induced mortality (\(\sim 71\%\); \(P < 0.01\)) (Figure 5B). Antibiotic therapy also enhanced the protective effect observed in CCR2 knockout mice (\(\sim 71\%\); \(P < 0.01\)) (Figure 5C).

As mentioned previously, neutrophils display a pivotal role in host defense against infections. We and others have demonstrated a significant association between down-regulation of CXCR2, reduction of neutrophil migration to infection focus, and sepsis mortality (5–7, 14, 30). WT and CCR2 knockout mice under severe CLP exhibited similar down-regulation of CXCR2 on neutrophils and sepsis mortality (5–7, 14, 30). WT and CCR2 knockout mice under severe CLP exhibited similar down-regulation of CXCR2 on neutrophils and sepsis mortality (5–7, 14, 30). WT and CCR2 knockout mice under severe CLP exhibited similar down-regulation of CXCR2 on neutrophils and sepsis mortality (5–7, 14, 30).

Figure 4. Lipoteichoic acid (LTA) and LPS modulates neutrophil responsiveness to CCR2 chemokines in vivo. (A) Chemotaxis of purified blood neutrophils from wild-type (WT) mice to CCL2 (1 ng/ml) and CCL7 (1 ng/ml) 2 hours after intravenous treatment with TLR2 agonist (LTA, 15 mg/kg), Toll-like receptor 4 agonist (LPS, 15 mg/kg). (B) Representative CCR2/Gr1 dot plots of blood leukocytes (gated on granulocytes) from Sham or cecal ligation and puncture (CLP) mice. (C) Chemotaxis of blood neutrophils from WT or MyD88\(^{-/-}\) mice to CCL2 and CCL7 purified 3 hours after CLP. (D) Neutrophil migration to saline or CCL2 (30 ng/cavity) in an air pouch model in WT and CCR2 knockout mice undergoing to a sham or CLP operation. \(P < 0.001\) relative to WT sham group. Results shown are representative of two to three independent experiments. Five mice per group were used in each experiment.

showed similar MPO activity (Figure E10). Similar numbers of monocytes were observed in lungs and heart of WT and CCR2 knockout mice (Figures E9A, E9D, and E9E). These findings suggest that neutrophil infiltration into multiple organs is importantly mediated by CCR2 during severe sepsis.

Subsequently, we evaluated the multiple organ dysfunction in septic mice by measuring serum levels of alanine aminotransferase, which is an indicator of liver damage; creatine kinase-MB, a indicator of heart muscle damage; and blood-urea-nitrogen, an indicator of renal dysfunction. Consistent with changes in MPO activity, WT mice showed a marked increase in serum levels of alanine aminotransferase, creatine kinase-MB, and blood-urea-nitrogen compared with sham-operated mice. Notably, treatment with antagonist or genetic deficiency in CCR2 resulted in a significant reduction of all biochemical indicators of organ injury and dysfunction (Figure 5H).

**CCR2 Expression in Neutrophils from Septic Patients Correlates With Illness Severity and Organ Damage**

To assess whether these observations could be extended to humans, we first isolated neutrophils from the blood of healthy volunteers. Consistent with the results obtained with murine cells, human neutrophils exhibited strong chemotaxis to CXCL8 and only random migration in response to CCL2. Exposure of human neutrophils to LTA and LPS substantially increased neutrophil responsiveness to CCL2, which was completely blocked by treatment with the CCR2 antagonist RS504393 (Figure 6A). We then evaluated the expression of CCR2 and chemotactic responsiveness to CCL2 on neutrophils isolated from healthy volunteers (controls) and septic patients, prospected in survivors and nonsurvivors. Notably, neutrophils from nonsurvivor patients, identified as CD66-positive cells, a marker of human neutrophil activation (33), showed high expression of CCR2, determined by quantitative polymerase chain reaction (Figure 6B) and fluorescence-activated cell sorter (Figure 6C). Furthermore, this profile of expression was strongly related with chemotactic responsiveness of neutrophils from nonsurvivor patients.
septic patients to CCL2 chemokine (Figure 6D). We therefore examined the correlation of the chemotaxis of neutrophil to CCL2 with the severity of patient illness assessed by Acute Physiology and Chronic Health disease Classification System II (APACHE II) score or with organ dysfunction by SOFA Sepsis-related Organ Failure Assessment (SOFA) score. We found a significant positive correlation between increasing neutrophil chemotaxis to CCL2 and APACHE II score ($r^2 = 0.21; P < 0.04$) (Figure 6E). Interestingly, the profile of association was more expressive when it was correlated with SOFA scores ($r^2 = 0.60; P < 0.001$) (Figure 6F).

**DISCUSSION**

Neutrophils are known to play an important role in inflammatory responses by performing a series of effector functions that collectively represent a central mechanism of immunity against infections (1, 2). However, overwhelming activation of neutrophils is also known to elicit cell and tissue damage that can lead to cellular derangement and, ultimately, organ dysfunction during sepsis (11, 12). Here, we have shown that the chemokine receptor CCR2 plays a critical role in driving neutrophil infiltration and eliciting tissue damage in remote organs during sepsis. The identified harmful role of CCR2 dramatically affects a mouse’s chance of surviving sepsis and correlates with the severity of organ dysfunction in septic patients.

CCR2 is mainly expressed in monocytes and binds to a group of monocyte chemotactic protein (MCP) chemokines including MCP-1/CCL2, MCP-2/CCL8, and MCP-3/CCL7 (15–18). It has been reported that TLR2 and TLR4 agonists down-regulate the expression of CCR2 in monocytes (34–38). Although CCR2 is absent in resting neutrophils, increased expression of this receptor has been detected on the surface of circulating neutrophils from adjuvant-immunized rats (20). Moreover, an increased CCR2 mRNA expression has been found in blood neutrophils from mice with CLP-induced sepsis (21). However, the trigger signal for the induction and the functional role of CCR2 in neutrophils has not been previously investigated. In contrast to the findings in monocytes, we described here that TLR2 and TLR4 signaling up-regulates CCR2 expression in human and mouse neutrophils. Our study demonstrated that neutrophils, which are normally unresponsive to CCR2 chemo-
Failure Assessment (SOFA) scores (of neutrophil chemotaxis to CCL2 from septic patients, survivors (n = 5) to CLP-induced mortality than WT mice. Increased survival in macologic blockade of CCR2 were significantly more resistant organs during sepsis. (13, 14). Such findings suggest that other chemokine receptors migration of these cells into the primary site of infection, septic neutrophils express low levels of CXCR2, which impairs the demonstrated that systemic activation of TLR2 and TLR4 in WT mice confirmed that deficiency or blockade of TLRs protects during sepsis (40, 41). Accordingly, many groups have clearly demonstrated that systemic inflammation and multiple organ damage induced by CLP-induced sepsis triggered chemotaxis of circulating neutrophils to CCR2 chemokines, which was completely abolished in MyD88-deficient mice. Collectively, our results strongly suggest that TLR signaling up-regulates the expression and function of CCR2 in circulating neutrophils during sepsis.

The escape of bacteria and their products from the infectious site to the circulation and endogenous TLR ligands released by injured tissue contribute significantly to the development of the systemic inflammatory response and multiple organ damage during sepsis (40, 41). Accordingly, many groups have clearly demonstrated that deficiency or blockade of TLRs protects mice from experimental models of sepsis (13, 14, 42, 43). Investigating the harmful role of TLRs during sepsis, we demonstrated that systemic activation of TLR2 and TLR4 in WT mice impaired the migration of neutrophils into the site of infection by down-regulating the expression of CXCR2 in circulating neutrophils (13, 14). However, despite the fact that circulating neutrophils express low levels of CXCR2, which impairs the migration of these cells into the primary site of infection, septic mice still showed increased neutrophil infiltration in the lung (13, 14). Such findings suggest that other chemokine receptors are required for maximal infiltration of neutrophils into remote organs during sepsis.

Notably, we found here that mice with deficiency or pharmacologic blockade of CCR2 were significantly more resistant to CLP-induced mortality than WT mice. Increased survival in CCR2−/− mice was not attributable to improved migration of neutrophils to infection focus or enhancement of bacterial clearance. However, the absence of CCR2 markedly reduced neutrophil infiltration in the lungs, kidneys, and hearts of mice undergoing CLP, despite the increased levels of neutrophil-attracting chemokines (CXCL1 and CXCL2) found in tissue homogenates. This suggests that, although CCR2 is not involved in the recruitment of neutrophil to infection focus, which is mainly mediated by CXCR2 (13, 14), it plays an important role in driving activated neutrophils into remote organs during sepsis. This is consistent with the elevated levels of CCL2 found in tissue homogenates and may explain why the blockade of CCR2 reduces organ damage and protects mice from CLP-induced mortality. Accordingly, it has been shown that the blockade of CCL2 synthesis reduces tissue damage in the lungs and livers of mice undergoing CLP (44).

CCR2 is constitutively expressed in monocytes and plays a key role in their migration in different experimental models and diseases (15–18, 22, 23). However, the improved survival conferred by CCR2 blockade is unlikely to be correlated with alteration of monocyte recruitment, because WT and CCR2−/− mice showed comparable numbers of monocytes in the infection focus and in remote organs under CLP. These results also suggest that, at least in the early phase of CLP-induced sepsis, CCR2 does not mediate monocytes migration. One possible explanation is that, during severe sepsis, CCR2 is down-modulated in monocytes by TLR activation (34–38, 45) and the recruitment of these cells is mediated by other chemokine receptors, such as CCR1, CCR5, or BLT1 (16–18, 46).

Serum concentrations of CCL2 are predictive of mortality and strongly correlate with organ dysfunction in septic patients (47, 48). We then investigated and found that blood neutrophils from septic patients expressed high levels of CCR2 and
exhibited significantly higher chemotaxis to CCL2 than blood neutrophils from healthy volunteers. However, the clinical relevance of our findings is underscored by the fact that the severity of patient illness, judged by their APACHE II or SOFA scores, was strongly correlated to the increasing chemotaxis of circulating neutrophils to CCL2.

In conclusion, we have identified the harmful role of CCR2 signaling in the pathophysiology of sepsis by driving neutrophils into remote organs. Our findings do not question the well-recognized contributions of the chemokine receptor CXC2R in the recruitment of neutrophils into the site of inflammation (3–5, 49), but introduce a TLR/NF-κB-regulated, CCR2-dependent mechanism for amplifying neutrophil infiltration and tissue damage in remote organs during sepsis. Furthermore, our studies in mice raise the possibility that inhibiting CCR2 signaling combined with antibiotic therapy may confer a benefit in sepsis-induced organ dysfunction, a syndrome that has considerable unmet clinical needs.

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