Ly6C<sup>high</sup> Monocytes Protect against Kidney Damage during Sepsis via a CX3CR1-Dependent Adhesion Mechanism

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ABSTRACT
Monocytes have a crucial role in both proinflammatory and anti-inflammatory phenomena occurring during sepsis. Monocyte recruitment and activation are orchestrated by the chemokine receptors CX3CR1 and CCR2 and their cognate ligands. However, little is known about the roles of these cells and chemokines during the acute phase of inflammation in sepsis. Using intravital microscopy in a murine model of polymicrobial sepsis, we showed that inflammatory Ly6C<sup>high</sup> monocytes infiltrated kidneys, exhibited altered motility, and adhered strongly to the renal vascular wall in a chemokine receptor CX3CR1-dependent manner. Adoptive transfer of Cx3cr1-proficient monocyte-enriched bone marrow cells into septic Cx3cr1-depleted mice prevented kidney damage and promoted mouse survival. Modulation of CX3CR1 activation in septic mice controlled monocyte adhesion, regulated proinflammatory and anti-inflammatory cytokine expression, and was associated with the extent of kidney lesions such that the number of lesions decreased when CX3CR1 activity increased. Consistent with these results, the pro-adhesive I249 CX3CR1 allele in humans was associated with a lower incidence of AKI in patients with sepsis. These data show that inflammatory monocytes have a protective effect during sepsis via a CX3CR1-dependent adhesion mechanism. This receptor might be a new therapeutic target for kidney injury during sepsis.

Sepsis is defined as widespread inflammation secondary to infection. It is the major cause of admission and death in intensive care units. Its pathophysiology involves numerous components of innate immunity, especially mononuclear phagocytes.

Monocytes are believed to generate the cytokine storm that triggers a chain reaction leading to tissue damage and death. They also perform regulatory functions during inflammatory processes. They are divided into two subsets: inflammatory monocytes, which are recruited early during inflammation, and resident monocytes, which patrol the steady-state

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endothelium. Recent studies have called attention to the dual role of inflammatory monocytes in acute inflammation and especially infection. They protect tissues during infectious processes, such as pneumonia or gastrointestinal toxoplasmosis, notably via their secretion of IL-1 receptor antagonist (IL-1ra) or prostaglandin E2. Essential crosstalk among monocytes, neutrophils, and tissue (especially epithelial cells) controls the equilibrium between inflammatory and anti-inflammatory processes.

CX3CR1, the receptor of CX3CL1, may be involved in the pathophysiology of sepsis. Kidneys are subject to insult during septic episodes; AKI is a common feature of sepsis and is associated with increased mortality. AKI results, in part, from leukocyte infiltration of kidney tissue and the generation of proinflammatory and proapoptotic mediators. Studies have shown that the CX3CR1 deficiency increased renal damage and mouse mortality and was correlated with reduced monocyte margination. We further confirmed that the CX3CR1 conferred protective functions linked to inflammatory monocyte adhesiveness and reduced production of IL-1ra in Ly6C^high monocytes. The involvement of CX3CR1 in the physiopathology of sepsis was confirmed in humans, through a gene polymorphism study that showed that the 249 CX3CR1 allele is associated with both increased monocyte adhesiveness and reduced kidney damage. Our work describes the protection conferred by inflammatory monocytes against the distant kidney damage caused by septic inflammation.

RESULTS

Ly6C^high Monocytes Exhibit Increased Adhesion to the Renal Endothelium during Sepsis

To determine the role of monocytes in kidney damage during sepsis, we used a standard murine model of abdominal sepsis induced by cecal ligation and puncture (CLP). In our experimental conditions, 50% of the mice died of sepsis in 4 days (Supplemental Figure 1A). Six hours after sepsis induction, the number of CD11b^NK1.1^F4/80^Ly6C^high cells defined as Ly6C^high monocytes increased in the blood, concomitant to their release from the bone marrow, while the number of CD11b^NK1.1^F4/80^Ly6C^low cells defined as Ly6C^low monocytes remained constant. Few Ly6C^high monocytes accumulated in the kidneys, while the number of Ly6C^low monocytes and of CD11b^NK1.1^F4/80^CD11c^high cells defined as renal dendritic cells (renal DCs) did not change (Supplemental Figure 1B). Twenty-four hours after CLP, the number of Ly6C^high monocytes reverted to that in the sham (control) group. The kinetics of mobilization in the blood and infiltration into the kidneys was the same for neutrophils as for Ly6C^high monocytes (Supplemental Figure 1B).

To examine the functional role of monocytes, we performed multiphoton intravital imaging on kidneys from sham- and CLP-operated MacBlue×Cx3cr1^GFP/+ mice. In the MacBlue mouse, all blood monocytes are strongly positive for enhanced cyan fluorescent protein (ECFP), whereas most tissue macrophages do not express the reporter protein. In contrast, renal DCs are CX3CR1^+ and strongly express the green fluorescent protein (GFP) reporter. In the sham-operated group, two particular subsets of myeloid cells were distinguished in the renal cortex, according to their expression of GFP and/or ECFP: A dominant population of stellate GFP^ECFP^ mice was evenly distributed throughout the tissue, while a few sparse GFP^ECFP^ cells were found (Figure 1A). GFP^ECFP^ cells expressed high levels of F4/80 and low levels of CD11b, Ly6C, and CD115 and may correspond to renal interstitial DCs. The GFP^ECFP^ cell phenotypes were stellate or round in shape, were CD11b^CD115+/F4/80^low, and expressed high or low levels of Ly6C (Figure 1A). Six hours after CLP, the number of GFP^ECFP^ cells remained constant, and small, round intravascular GFP^ECFP^ cells (likely monocytes) accumulated in large numbers (Figure 1B). Of the blood ECFP^+ cells in the sham-operated mice, 60% were CX3CR1^low/dimLy6C^high, corresponding to the phenotype of inflammatory monocytes, and 40% were CX3CR1^high/Ly6C^low, corresponding to the phenotype of patrolling monocytes. At 6 hours after CLP, >90% of the ECFP^+ cells were Ly6C^high monocytes (Figure 1C). Time-lapse imaging of the kidneys of the sham-operated mice showed that numerous ECFP^+ cells traveled through the cortex in the bloodstream, interacting very little with the endothelium, while the activity of renal DCs was strongly prospressive (Supplemental Movie 1). At 6 hours after CLP, renal DC behavior did not change, but the number of ECFP^+ cells adhering to the luminal side of the vessel increased (Supplemental Movie 2). We defined three motility patterns of ECFP^+ cells: circulating, crawling, and adhering (Figure 1D). Six hours after CLP, the proportion of circulating ECFP^+ cells had fallen by >50%, while the proportion of crawling and adhering ECFP^+ cells had increased above the levels in the sham-operated mice (Figure 1E). Mean dwell time and mean contact duration quadrupled (Figure 1F). Contact duration increased for ECFP^+, but they were mainly released without any evidence of extravasation toward the kidney tissue.

Intravital imaging on CLP-operated MacBlue×Cx3cr1^GFP/+×Ccr2^−/− mice, defective for circulating Ly6C^high monocytes, showed a near-complete abolition of the accumulation of ECFP^+ adherent cells on the renal endothelium strongly suggests that they were Ly6C^high monocytes (Supplemental Movie 3). This phenotype was further confirmed by the intravital imaging of the combined Cx3cr1^GFP/+×Ccr2^−/− mice, which showed that the adhering cells coexpressed red fluorescent protein (RFP) and GFP (Supplemental Movie 4). In Ccr2^−/− mice, Ly6C^high monocytes did not accumulate after CLP in blood or in the kidney. After CLP, Ly6C^low monocytes were more numerous in the blood of Ccr2^−/− mice than in wild-type (WT) mice, and their numbers were similar in the kidney for both groups (Supplemental Figure 2A). However, no ECFP^+ cells adhering to the renal vascular wall were imaged in Ccr2^−/− mice, in contrast to WT mice (Supplemental Movie 3). Altogether these results indicate that Ly6C^high...
monocytes are the main cells imaged by intravital imaging. Radar chart representation of the different cell dynamic measures provides a signature of the ECFP+ cell dynamic behavior (Figure 1G). Overall, CLP strongly modified the signature of ECFP+ cells compared with that of sham-operated mice. Adhesion of the ECFP+ cells increased after CLP, and their track straightness declined, as did their mean velocity. At the same time, their arrest coefficient, dwell time, and contact duration with the endothelium all increased (Figure 1G).

These findings show that within a few hours after CLP, the number of Ly6C<sup>high</sup> monocytes in the blood increased and they interacted with the renal endothelium.

**CX3CR1 Promotes Ly6C<sup>high</sup> Monocyte Adhesion and Prevents Renal Damage during Sepsis**

Previous reports have shown that CX3CR1 deficiency is associated with increased mortality after CLP<sup>24</sup> but the precise mechanisms involved have not been adequately defined. We sought to determine the role of CX3CR1 in the pathogenesis of organ damage during sepsis. Cx3cr1<sup>−/−</sup> mice had conspicuously more kidney histologic lesions than WT mice (Figure 2A). The proportion of damaged tubules during CLP was two times higher in the Cx3cr1<sup>−/−</sup> mice (Figure 2B), which also had substantially higher levels of markers of renal failure, such as creatinemia and uremia (Figure 2C). The kidney was not the only affected organ, but renal damages were associated with increased mortality after CLP (Supplemental Figure 3A). To determine whether this phenotype could be due to impaired bactericidal activity, as suggested<sup>25</sup> we compared mice survival in a sterile inflammation model, by injecting mice with a lethal dose of LPS. The strong reduction in survival of Cx3cr1<sup>−/−</sup> mice suggests that the phenotype observed was independent of control of the bacterial burden and emphasizes the relationship with organ damage (Supplemental Figure 3B). On the other hand, and as others have recently reported<sup>26</sup> these two types of mice did not differ in the number of Ly6C<sup>high</sup> monocytes (Figure 2D) or neutrophils (not shown) in bone marrow, kidneys, or blood. This prompted us to perform functional imaging to compare dynamic behavior of Ly6C<sup>high</sup> monocytes from CX3CR1-deficient and WT mice during sepsis.

Thus, we used MacBlue×Cx3cr1<sup>gfp/+</sup> mice 6 hours after CLP (Figure 2E). In the absence of CX3CR1, the proportion of adhering and crawling ECFP<sup>+</sup> cells fell (Figure 2F), as did ECFP<sup>+</sup> cell dwell time and contact duration (Figure 2G). The radar chart representation of the motility pattern showed distinct cell dynamic signature between septic MacBlue×Cx3cr1<sup>gfp/+</sup> and septic MacBlue×Cx3cr1<sup>gfp/gfp</sup> mice (Figure 2H). This loss of adhesion was specific to ECFP<sup>+</sup> cells: Neutrophil behavior did not differ between the two strains (Supplemental Figure 3, C–E). To further address that the reduced adhesion is intrinsic to the CX3CR1 deficiency, Cx3cr1<sup>−/−</sup> and Cx3cr1<sup>+/+</sup> ECFP<sup>+</sup> cells were transferred into septic WT mice and the proportion that adhered to the renal vascular wall was determined (Supplemental Figure 3F). Accordingly, Cx3cr1<sup>−/−</sup> ECFP<sup>+</sup> cells adhesion was much weaker than that of Cx3cr1<sup>+/+</sup> ECFP<sup>+</sup> cells. We conclude that the CX3CR1 receptor is functionally important during sepsis; its absence leads to reduced inflammatory monocyte adhesion to the renal vascular wall, more numerous kidney lesions, and increased mortality. This observation suggests that inflammatory monocytes have a potent unexpected protective effect during sepsis.

**Bone Marrow–Derived Monocytes Protect against Damage to Kidney Tissue during Sepsis**

To examine the protective role circulating Ly6C<sup>high</sup> monocytes may play, we first performed CLP in Ccr2<sup>−/−</sup> mice that display a Ly6C<sup>high</sup> monocytopenia (Supplemental Figure 2A). Consistent with our hypothesis, the number of kidney lesions was dramatically higher in Ccr2<sup>−/−</sup> than in WT mice (Figure 3A). It has also been reported that phagocyte are renoprotective during sepsis and that Cx3cr1<sup>−/−</sup> mice have a defect in renal DC

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**Figure 1.** Ly6C<sup>high</sup> monocytes exhibit increased adhesion to the renal endothelium during sepsis. Two-photon laser scanning microscopic images (left) with volume rendering (right) of ECFP<sup>+</sup> (cyan squares) and GFP<sup>+</sup> (green squares) cells in kidneys of MacBlue×Cx3cr1<sup>gfp/+</sup> mice 6 hours after (A) sham or (B) CLP operations. CFP signals are in blue, GFP signals are in green, and autofluorescent renal tubules are in red. Overlay of flow cytometric surface marker expression gated on GFP<sup>+</sup> (green histograms) and ECFP<sup>+</sup> cells (cyan histograms) in kidneys are shown for each condition. Mean percentages±SD of gated cells are indicated (n=6 mice per group out of three independent experiments). Background staining (gray histograms) gated on nonfluorescent cells is represented. (C) Dot plots represent Ly6C and CX3CR1-GFP expression, gated on blood CD11b<sup>+</sup>ECFP<sup>+</sup> cells 6 hours after sham or CLP operations. Mean percentages±SD of gated cells are indicated (n=6 mice per group out of three independent experiments). (D) Time series two-photon laser scanning microscopic images of kidney cortex of MacBlue×Cx3cr1<sup>gfp/+</sup> mice 6 hours after CLP. Examples of circulating (blue squares), crawling (green squares), and adhering monocytes (purple squares) are presented. ECFP signals are in cyan, GFP signals are in green, renal tubules are autofluorescent, and blood vessels are visualized by 2 MDa rhodamine-dextran. (E) Relative frequency of the three behaviors. Bars represent mean±SEM (n=4 sham and n=3 control from independent experiments; ANOVA with Bonferroni adjustment for multiple comparisons were used; ***P<0.001). (F) Dwell time and contact duration with renal endothelium of ECFP<sup>+</sup> monocytes. Black bars indicate means. (n=4 sham and n=4 control from independent experiments; Mann-Whitney test were used; ***P<0.001). (G) Radar chart representation shows ECFP<sup>+</sup> cell dynamic signatures in sham-operated (green) and CLP-operated (red) mice. Mean values are presented within the 95% confidence interval of the measured value scale for each parameter. Data represent a pool of cells from (n=4 sham and n=4 control from independent experiments; Mann-Whitney test were used; ***P<0.001). (See also Supplemental Figure 1 and Supplemental Movies 1–4).
Figure 2. CX3CR1 promotes Ly6C<sup>high</sup> monocyte adhesion and prevents renal damage during sepsis. (A) Photomicrographs and (B) quantification of kidney histologic lesions 24 hours after CLP in WT (blue) and Cx3cr1<sup>−/−</sup> mice (red). Bars represent mean±SD (n=10 WT, n=8 Cx3cr1<sup>−/−</sup>, from at least two repeated experiments; ANOVA with Bonferroni adjustment was used; ***P<0.001). (C) Urea and creatinine measurements in plasma of CLP-operated WT and Cx3cr1<sup>−/−</sup> mice bars represent mean±SEM (n=10 WT sham, 6 Cx3cr1<sup>−/−</sup> sham, 15 WT, and 15 Cx3cr1<sup>−/−</sup> CLP, from at least two repeated experiments; ANOVA with Bonferroni adjustment was used; *P<0.05). (D) Number of Ly6C<sup>high</sup> monocytes in bone marrow, blood, and kidney of WT (blue) and Cx3cr1<sup>−/−</sup> (red) mice. Bars represent mean±SD (n=10
number.6,27 The number of renal DCs in Ccr2−/− mice was not impaired compared with the number in WT mice, confirming that the presence of renal DCs was not sufficient to protect from renal lesion (Supplemental Figure 2B).

Adoptive transfers of CCR2-proficient monocyte-enriched bone marrow cells (MBMs) into Ccr2−/− mice drastically reduced kidney lesions during sepsis and thus further confirmed their protective effect (Figure 3A). Finally, adoptive transfer of Cx3cr1-proficient MBM into septic Cx3cr1−/− mice provided significant protection against mortality (Figure 3B) and resulted in a diminution in kidney lesions to a level similar to that of WT mice (Figure 3C), compared with the transfer of Cx3cr1-deficient MBMs. These observations confirm that bone marrow-derived monocytes have a protective role in organ damage during sepsis via a Cx3CR1-dependent mechanism.

Figure 3. Bone marrow–derived monocytes protect against damage to kidney tissue during sepsis. (A) Quantification of kidney histologic lesions 24 hours after CLP in WT, Ccr2−/− and Ccr2−/− mice with adoptive transfer of WT bone marrow monocytes before surgery. Bars represent mean±SD (n=10 WT, 11 Ccr2−/−, 9 WT in Ccr2−/−; data from at least two repeated experiments; ANOVA with Bonferroni adjustment was used; ****P<0.0001). (B) Survival of CLP-operated Cx3cr1−/− mice after adoptive transfer of WT (gray line) or Cx3cr1−/− bone marrow monocytes (black line) (n=7 per group out of three independent experiments; survival curves were compared with a log-rank test; *P<0.05). (C) Quantification of kidney histologic lesions 24 hours after CLP in Cx3cr1−/− mice with adoptive transfer of WT (gray) or Cx3cr1−/− (black) bone marrow monocytes before surgery (n=9 per group from at least two repeated experiments; ANOVA with Bonferroni adjustment was used; ****P<0.0001).

CX3CR1 Activation Controls Ly6C^high Monocyte Adherence and Outcome of CLP-Mediated Sepsis

In further considering the role of CX3CR1-dependent adhesion of Ly6C^high monocytes during sepsis, we treated septic mice with the Cx3cr1 ligand (Cx3CL1) or the antagonist (F1) of Cx3CR1 that we have previously shown to inhibit monocyte adhesion to Cx3CL1.28 The proportion of adherent monocytes increased in the presence of the agonist and diminished in the presence of the antagonist (Figure 4, A and B). In addition, dwell time and contact duration of monocytes increased after treatment with Cx3CL1 and decreased after treatment with F1 (Figure 4C). The cell dynamic signature was inversely affected by treatment with Cx3CL1 and F1 compared with control CLP-operated mice (Figure 4D). These different signatures were associated with a different level of organ failure. In particular, injection of the antagonist, F1, led to increased lesions (Figure 4E) and mortality (data not shown) without altering renal DC numbers (Supplemental Figure 2B). In contrast, Cx3CL1 injection diminished the number of kidney lesions (Figure 4E). These findings show that pharmacologic modulation of CX3CR1 activation strongly correlates with Ly6C^high monocyte margination and kidney damage, thereby demonstrating CX3CR1 as a potential therapeutic target.

CX3CR1 Blockade Increases Kidney Inflammation and Reduces IL-1ra Production by Ly6C^high Monocyte during Sepsis

To further investigate the molecular mechanism involved in protective effect by Ly6C^high monocytes, we measured by flow cytometry the intracellular production of IL-1ra that was previously shown to attenuate lung injury after LPS treatment9 (Figure 5A). IL-1ra mean fluorescence intensities were nearly doubled in Ly6C^high monocytes 6 hours after CLP compared with sham-operated mice. F1 treatment efficiently reduced IL-1ra mean fluorescence intensities in Ly6C^high monocytes (Figure 5B) and leads to increased TNF-α (Figure 5C), decreased TGF-β (Figure 5D), and IL1ra (Figure 5E) transcripts in the whole kidney 6
hours after CLP. In conclusion, CX3CR1 blockade is associated with reduced production of IL-1ra by monocytes and with a higher proinflammatory environment in the kidney.

The Proadhesive CX3CR1 I249 Allele Is Associated with a Reduced Incidence of AKI in Septic Patients

To evaluate whether the mechanism we described in this murine model might be relevant to the pathophysiology of human disease, we studied the effect of a frequent CX3CR1 polymorphism, I249. This polymorphism was shown to increase monocyte adhesion to CX3CL1 and could modulate the course of coronary artery disease, atherosclerosis, age-related macular degeneration, glioblastoma, or obesity.29–33 We tested the effects of this polymorphism in vitro in cell adhesion assays and in a cohort of patient admitted to intensive care for sepsis. PBMCs from individuals with CX3CR1 VV genotype or VI genotype were assayed for adhesion (Figure 6, A and B). PBMCs from donors heterozygous for the I249 allele adhere significantly more than those homozygous for V249 allele, confirming our previous results.34 We then studied the effect of the I249 allele on the occurrence of AKI in a cohort of patients with sepsis. This study included 239 patients without chronic renal failure. In most cases, sepsis origins were in primary or secondary peritonitis and respiratory tract infections, with no difference between groups. At least one I249 allele was present in nearly 50% of the patients, with a distribution of the VV, VI, and II genotype of 52%, 41%, and 6%, respectively. The I249 allele (VI or II genotypes) was associated with a lower incidence of AKI (Figure 6C), with no differences between the groups in terms of age, disease severity, or comorbidities. Patients carrying the I249 allele had fewer coagulation disorders but the same rate of circulatory or respiratory failures and the same 28-day mortality (Supplemental Table 1). In the multivariate analysis, CX3CR1 was still significantly associated with a reduced occurrence of AKI after adjustment for age, Simplified Acute Physiology Score II, IL-6 levels, and number of comorbidities. The odds ratio for AKI in a patient with the I249 allele was 0.43 (95% confidence interval, 0.27 to 0.93) (Table 1). Thus, we

![Figure 4](https://www.jasn.org)
showed that the I249 allele of CX3CR1 is associated with reduced AKI incidence in septic patients.

DISCUSSION

Inflammatory monocytes play crucial roles during sepsis through cytokine secretion and direct cell contact.5,35–37 Recent studies have identified a regulatory protective role for inflammatory monocytes in infectious diseases as they synthetize and secrete anti-inflammatory mediators, such as IL-1ra and prostaglandin E2.8,9 However, the specific contribution of inflammatory monocytes to the regulation of inflammation in early phases of sepsis remains unclear. Our results have unveiled a key role these monocytes play in renal tissue protection via a CX3CR1-dependent adhesion of inflammatory monocytes to the renal vascular endothelium.

Previous studies have shown that inflammatory monocytes are involved in controlling inflammation in gram-negative pneumonia and abdominal infections.8,9,38–42 A lower number of inflammatory monocytes has been associated with increased lesions in the lung and in the intestinal lamina propria.8,9,38,41 Other studies have shown that the CX3CR1/CX3CL1 axis is involved in the pathogenesis of sepsis. Genetic disruption of the Cx3cr1 gene has been associated with increased mortality without any effect on neutrophil or monocyte recruitment.24 In that study, the indirect interaction of monocytes with neutrophils reduced the ability of Cx3cr1−/− mice to eradicate bacteria. Recent findings by Hochheiser et al.26 showed that CX3CR1 deficiency was associated with a reduced entry of DC precursors in the inflamed kidney. Additionally, this work showed that in a model of infectious GN, the absence of CX3CR1 was not associated with an increased bacterial burden in the kidney.26 Antibacterial role associated with Cx3cr1 was unlikely to be the main role for this gene during sepsis. Indeed, we showed that mortality increased in Cx3cr1−/− mice subjected to sterile lethal inflammation induced by LPS injection. Regarding our result during CLP, the reduced number

Figure 5. CX3CR1 blockade increases kidney inflammation and reduces IL-1ra production by Ly6C<sup>high</sup> monocyte during sepsis. (A) Intracellular production of IL-1ra was evaluated by intracellular cytokine staining gated on CD11b<sup>+</sup> Ly6G<sup>−</sup> NK1.1<sup>−</sup> Ly6C<sup>high</sup> cells from sham-operated (green) and CLP-operated mice (red) 6 hours after surgery. Gray histogram represents isotype staining. (B) Mean fluorescence intensity of IL-1ra intracellular staining was compared between sham-operated (green) and CLP-operated mice (red) treated or not treated with F1 (purple), 6 hours after surgery (n=6 sham, F1, and 12 WT CLP from two independent experiments; ANOVA t test was used; *P<0.05; ***P<0.001). Kidneys from CLP-operated mice treated with PBS (control) or F1 were extracted 6 hours after CLP and were evaluated by quantitative PCR for (C) TNF-α production, (D) TGF-β production, and (E) IL1ra production. Results are represented as fold increase of CLP-operated mice (n=6 in each groups from two independent experiments; t test was performed; P value are indicated).

Figure 6. The proadhesive CX3CR1 I249 allele is associated with a reduced incidence of AKI in septic patients. PBMCs from individuals with CX3CR1 VV (black) or VI (gray) genotypes were assayed for adhesion with (A) CX3CL1-expressing HEK (n=9–10 individuals) or (B) with adherent smooth muscle cells (SMCs) treated with TNF-α and IFN-γ (n=5 individuals in each group). Bars indicate mean±SEM. A t test was used; *P<0.05. (C) V249I polymorphism distribution and incidence of AKI in patients with VV<sup>−</sup> (black bar) or VI/II<sup>−</sup> (gray bar) polymorphisms (n=239; chi-squared test was used; and 95% confidence intervals of the estimate are presented).
of renal DCs in Cx3cr1−/− mice is unlikely the only cause of the adverse phenotype because in both bone marrow–derived cell-adaptive transfer that rescued CX3CR1-deficient mice from renal lesions and treatment of the antagonist F1 that mimicked the adoptive transfer that rescued CX3CR1-deadverse phenotype because in both bone marrow

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Data are presented as mean±SEM. Comparison of incidence of AKI was made with a two-tailed t test. See also Supplemental Table 1. SAPS, Simplified Acute Physiology Score; OR, odds ratio; 95% CI, 95% confidence interval.

CONCISE METHODS

Experimental details are provided in the Supplemental Material.

Mice

Male C57BL/6 mice were purchased from Elevage Janvier (Saint Isle, France). Cx3cr1P20/−, Csf1r−/−, C57BL/6 mice were kindly provided by Israel Charo (Gladstone Institute, San Francisco).51 Ccr2−/− and Cx3cr1−/− C57BL/6 mice, Cx3cr1P20/−, Csf1r−/− C57BL/6 mice, MacBlue,20 MacBlue×Cx3cr1P20/−, MacBlue×Csf1r−/−, and MacBlue×Cx3cr1P20/− mice were bred in our animal facility. All experiments and protocols were approved by the local animal experimentation ethics committee.

Human Polymorphism Study

We retrospectively studied DNA from patients included in previous studies.55 Patients meeting the criteria for severe sepsis/septic shock and who had at least two organ failures defined by the Sepsis-related Organ Failure Assessment55 were included. Patients with chronic renal failure were excluded. The Cx3cr1 V249I polymorphism (rs3732379) was detected as previously described.52 The AP-HP Cochin Hospital Ethics Committee approved the study. The patient or the patient's next-of-kin provided written informed consent.

Polymicrobial Sepsis Induction

Polymicrobial sepsis was generated after a CLP procedure as described using a 21-gauge needle.24 In the control animals, the cecum was exteriorized and reinserted in the abdomen. For some experiments, 3 μg of fractalkine (full-length fractalkine/CX3CL1; R&D Systems) was injected...
intraperitoneally 30 minutes before surgery. For F1 (kindly provided by A. Proudfoot), 50 μg was injected intraperitoneally 30 minutes before and 6 hours after surgery.

**LPS Injection**

LPS (Escherichia coli O111:B4) was injected at a dose of 15 mg/kg intraperitoneally.

**Histologic Analysis**

Quantification was performed on periodic acid-Schiff–stained, 3- to 5-μm kidney sections and on at least three mice per condition. For each mouse, the percentage of damaged tubules was calculated on three to five different randomly chosen fields comprising 200–300 tubules.

**Flow Cytometry**

Cell staining for flow cytometry and antibodies used are described in the Supplemental Material. Flow cytometry was performed with FACScanto (BD Biosciences) flow cytometer. Analysis was performed with FlowJo software (TreeStar, Inc.).

**Adaptive Transfer Experiments**

Bone marrow cells were isolated from MacBlue-Cx3cr1gfp/+ and MacBlue-Cx3cr1fl/+ mice; cells were co-transferred at a 1:1 ratio 30 minutes before the CLP procedure. Analyses were done 6 hours after CLP. Bone marrow monocytes were extracted after negative selection removal of other cell types (see Supplemental Material). Before sorting, Ly6Chi monocytes represented nearly 16% of myeloid cells and were enriched to nearly 60% after sorting, while the neutrophils population was drastically reduced. In all experimental conditions, mice were injected with 4–5×10⁶ monocytes just before the CLP procedure.

**Multiphoton Imaging**

Mice were anesthetized with isoflurane. Their temperatures were maintained at 37°C. An incision was made in the flank, and the kidney was exposed. In some experiments, 2×10⁶ molecular weight tetramethylrhodamine-dextran (Invitrogen) was injected to stain the vasculature. Cell motility was measured every 30 seconds. Cells were tracked for 5 minutes with three-dimensional automatic tracking and manual correction. Cell staining for flow cytometry and antibodies used are described in the Supplemental Material. Flow cytometry was performed with FACScanto (BD Biosciences) flow cytometer. Analysis was performed with FlowJo software (TreeStar, Inc.).

**Statistical Analyses**

Data are reported as mean±SD or mean±SEM +/− SD/SEM as indicated. Groups were compared using a two-tailed unpaired t test and adjusted for multiple comparison analysis. Nonparametric Mann-Whitney test or ANOVA with adjustments was performed according to Gaussian distribution of each sample. Survival curves were compared using a log-rank test. Multivariate analysis of the human cohort was performed with JMP (SAS Institute, Inc., Cary, NC).

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**DISCLOSURES**

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