Immunology
The Journal of cells, molecules, systems and technologies

British Society for immunology

IMMUNOLOGY ORIGINAL ARTICLE

Combined treatment with a CXCL12 analogue and antibiotics improves survival and neutrophil recruitment and function in murine sepsis

Shuwen Guan,[1,2] Changrun Guo,[1,2] Basilia Zingarelli,[3] Liping Wang,[2] Perry V. Halushka,[4,5] James A. Cook[1] and Hongkuan Fan[1]

[1]*Department of Neurosciences, Medical University of South Carolina, Charleston, SC, USA,* [2]*College of Life Science, Jilin University, Changchun, China,* [3]*Division of Critical Care Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, OH,* [4]*Department of Medicine, Medical University of South Carolina, Charleston, SC, and* [5]*Department of Pharmacology, Medical University of South Carolina, Charleston, SC, USA*

doi:10.1111/imm.12382
Received 1 July 2014; revised 12 August 2014; accepted 3 September 2014.
Shuwen Guan and Changrun Guo contributed equally.
Correspondence: Hongkuan Fan, Department of Neurosciences, 173 Ashley Avenue, BSB Room 403, MSC 510, Charleston, SC 29425, USA.
Email: fanhong@musc.edu
Senior author: Hongkuan Fan

Summary

Previous studies demonstrated that the CXCL12 peptide analogue CTCE-0214 (CTCE) has beneficial effects in experimental sepsis induced by caecal ligation and puncture (CLP). We examined the hypothesis that CTCE recruits neutrophils (polymorphonuclear leucocytes; PMN) to the site of infection, enhances PMN function and improves survival of mice in CLP-induced sepsis with antibiotic treatment. Mice with sepsis ($n = 15$) were administered imipenem (25 mg/kg) and CTCE (10 mg/kg) subcutaneously versus vehicle control at designated intervals post-CLP. CTCE treatment increased PMN recruitment in CLP-induced sepsis, as evidenced by increased PMN in blood, by 2·4 ± 0·6 fold at 18 hr, 2·9 ± 0·6 fold at 24 hr, and in peritoneal fluid by 2·0 ± 0·2 fold at 24 hr versus vehicle control. CTCE treatment reduced bacterial invasion in blood [colony-forming units (CFU) decreased 77 ± 11%], peritoneal fluid (CFU decreased 78 ± 9%) and lung (CFU decreased 79 ± 8% versus CLP vehicle). The improved PMN recruitment and bacterial clearance correlated with reduced mortality with CTCE treatment (20% versus 67% vehicle controls). *In vitro* studies support the notion that CTCE augments PMN function by enhancing phagocytic activity (1·25 ± 0·02 fold), increasing intracellular production of reactive oxygen species (32 ± 4%) and improving bacterial killing (CFU decreased 27 ± 3%). These composite findings support the hypothesis that specific CXCL12 analogues with ancillary antibiotic treatment are beneficial in experimental sepsis, in part, by augmenting PMN recruitment and function.

Keywords: CTCE-0214; neutrophils; sepsis.

Introduction

Severe sepsis is a syndrome in response to an acute systemic infection characterized by dysregulated inflammation and multiple organ system failure.[1] Sepsis is a major cause of death in intensive care units and severe sepsis has emerged as a dominant and rapidly growing cause of serious illness among older Americans, with survivors suffering substantial long-term morbidity and mortality.[2] Hence there is a critical need for more effective therapeutic strategies to prevent the progression of severe sepsis.

Polymorphonuclear leucocytes (PMN) are first-responder innate immune cells essential for host defence against invasive or opportunistic bacterial pathogens and for survival of polymicrobial sepsis. Mature PMN eradicate pathogens through phagocytosis and microbial killing.[3] There is substantial evidence demonstrating that sepsis impairs immune function by inducing defects in innate

Abbreviations: ATCC, American Type Culture Collection; BM, bone marrow; CFU, colony-forming units; CM-H_2DCFDA, 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetylester; CLP, caecal ligation and puncture; CTCE, CTCE-0214; CXCR4, chemokine CXC receptor 4; LPS, lipopolysaccharide; PMN, polymorphonuclear leucocytes; ROS, reactive oxygen species; SDF, stromal cell-derived factor

immunity.[4] In particular, PMN function can become defective during severe sepsis, characterized by failure of migration to the site of infection and inadequate antimicrobial responses. These latter changes in PMN function are directly correlated with increases in sepsis-mediated morbidity and mortality.[5,6]

A large pool of mature neutrophils is retained in the bone marrow.[7] During sepsis, neutrophils are rapidly mobilized from bone marrow (BM) into peripheral blood followed by migration to sites of infection. Although multiple cellular phenotypes are recruited from BM during sepsis, most cells are mature neutrophils.[7,8] Mature PMN are recruited by chemokines to the nidus of infection.[8,9] A critical chemokine in sepsis is CXCL12, also referred to stromal cell-derived factor-1α.[10] CXCL12 belongs to the CXC chemokine family and is a multifunctional cytokine secreted by several tissues.[11] The natural receptor for CXCL12 in PMN is chemokine CXC receptor 4 (CXCR4), which was reported to have multiple biological functions when triggered, such as cell chemotaxis, proliferation, survival and cell differentiation.[12,13] Studies have demonstrated that CXCL12 can activate anti-inflammatory signalling pathways and suppress inflammation.[14] Recently Delano *et al.*[10] demonstrated that recruitment of neutrophils from BM is independent of Toll-like receptor 4, MyD88, Toll/interleukin-1 receptor domain-containing adaptor inducing interferon-β, interferon/ARα/β or CXCR2 signalling in caecal ligation and puncture (CLP) sepsis. In contrast, blocking CXCL12 in sepsis inhibited the release of neutrophils from BM. The BM retention induced by CXCL12 blockade prevented blood and peritoneal neutrophil accumulation, allowed significant bacterial invasion and increased lethality. The investigators concluded that CXCL12 signalling during sepsis is critical for neutrophil BM egress, bacterial killing and host survival.

CTCE-0214 (CTCE) is a peptide analogue of CXCL12, which has been modified to improve plasma stability.[15] Previous studies demonstrate that CTCE significantly decreased plasma tumour necrosis factor-α levels in the models of lipopolysaccharide (LPS)-induced shock and zymosan-induced multiple organ dysfunction syndromes and CTCE also suppressed the mortality in severe septic shock induced by CLP.[16] These results show that CTCE may have the beneficial effect of controlling inflammation in sepsis and the systemic inflammatory response.

In the present study, we examined the effect of CTCE on mortality and bacterial load in severe sepsis induced by CLP in mice with antibiotic therapy. As PMN are key cells in the innate immune response, and play an important role in defence against bacterial infection and sepsis, the effect of CTCE on PMN distribution and function was investigated. These composite data may in part explain the potential mechanism by which CTCE regulates PMN function in polymicrobial sepsis.

Materials and methods

Materials

CTCE-0214 (CTCE, Chemokine Therapeutics Corporation, Vancouver, BC, Canada) is a peptide analogue of CXCL12, which links the N-terminal region (residue 1–14), and the C-terminal region (residue 55–67) of CXCL12 by a four-glycine linker.[16] This analogue is cyclized between amino acid residues at positions 20 and 24. Two Cys were replaced with Ala and Phe to improve plasma stability. Cell culture reagents and fetal bovine serum were products of American Type Culture Collection (ATCC; Manassas, VA) and Invitrogen (Carlsbad, CA). The reactive oxygen species (ROS) detection reagents kit was purchased from Invitrogen. MiScript Primer Assay and miScript SYBR Green PCR kit were purchased from Qiagen (Valencia, CA).

Mice

Male CD-1 mice aged 7–8 weeks were used in all the experiments. Investigations conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and commenced with the approval of the Institutional Animal Care and Use Committee.

MPRO cell culture and differentiation to neutrophils

The mouse promyelocyte cell line (MPRO) was obtained from the ATCC and maintained in Iscove's modified Dulbecco's medium (ATCC) with 20% horse serum (Invitrogen), 2% penicillin and streptomycin, and 10 ng/ml granulocyte–macrophage colony-stimulating factor (R&D Systems, Minneapolis, MN) at 37° with 5% CO_2. To induce the differentiation of MPRO cells into neutrophils, the growth medium was supplemented with all-*trans*-retinoic acid (3 μg/ml; Sigma, St Louis, MO) for 3 days. The culture medium was replenished every day to obtain 1×10^5 cells/ml. As all-*trans*-retinoic acid is unstable, cells were treated with all-*trans*-retinoic acid daily for 3 days. For bacterial killing experiments, neutrophils were harvested on day 4.

For bacterial killing, neutrophils (1×10^6 cells/ml) were serum starved and treated with LPS (ultra-pure LPS from *Escherichia coli* O111:B4, List, 1 μg/ml) or CTCE (10 μg/ml) or combined LPS and CTCE for 6 hr and incubated with *E. coli* K12.

Caecal ligation and puncture

The CLP was performed in CD-1 mice as previously described.[17] Specifically, mice were anaesthetized with isoflurane and a midline incision was made below the

diaphragm to expose the caecum. The caecum was ligated at the colon juncture with a 5-0 silk ligature suture without interrupting intestinal continuity and punctured twice with a 22-gauge needle. The caecum was returned to the abdomen, and the incision was closed in layers with a 5-0 silk ligature suture and wound clips. After the procedure, the animals were fluid resuscitated with 1·0 ml sterile saline injected subcutaneously. Sham operations were performed the same as CLP except for the ligation and puncture of the caecum.

For the survival study, CD-1 mice (n = 15/group) were subjected CLP or sham operation. The antibiotic imipenem (25 mg/kg) was administrated by subcutaneous injection at 6, 24 and 48 hr post CLP. CTCE (10 mg/kg) was administrated by injection subcutaneously at 2, 6, 24 and 48 hr post CLP. Survival was monitored for a total of 7 days.

For bacterial load and PMN numbers in BM, peripheral blood and peritoneal fluid, CD-1 mice (three to five per group) were subjected to sham treatment or CLP. Imipenem (25 mg/kg) was administrated by subcutaneous injection at 6 hr post CLP. CTCE (10 mg/kg) was administered by injection subcutaneously at 2 and 6 hr post CLP. Mice were killed at 12, 18 and 24 hr for PMN in BM and peripheral blood and at 24 hr for PMN in peritoneal fluid and bacterial load.

Bacterial load

To determine the bacterial load in the peritoneum, the peritoneal cavity was lavaged with 5 ml sterile PBS and diluted with sterile PBS. To determine the bacterial load in the blood, 100 μl blood was collected and diluted with sterile PBS. To determine the pulmonary bacterial load, the lungs were harvested and equal amounts of wet tissue were homogenized and diluted with PBS. One hundred microlitres of each dilution was then plated on chocolate agar plates (Fisher Scientific, Pittsburgh, PA) and incubated at 37° for 24 hr under aerobic conditions. Colony-forming units (CFU) were counted. Results were expressed as CFU per millilitre or gram of wet tissue.

Flow cytometry analysis of mouse PMN in vivo

PMN in BM, peritoneal cavity and peripheral blood were determined as described previously.[10] Briefly, BM cells were collected by flushing both femurs. The peritoneal cavity was lavaged with 5 ml sterile PBS and mouse blood was diluted in 10 ml PBS and cells were isolated by centrifugation. Red blood cells were removed by adding red blood cell lysis buffer (eBioscience, San Diego, CA) for 2 min at room temperature. Cells were labelled by incubation for 30 min on ice in staining buffer with the following antibodies: Phycoerythrin-conjugated anti-mouse Gr-1 antibody and FITC-conjugated anti-mouse-CD11b (eBioscience). After being washed, the samples were analysed on a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA). Data were analysed with CellQuest Pro software (BD Biosciences).

Phagocytosis measurement

Phagocytic activity of peritoneal neutrophils was analysed using fluorescein-conjugated *E. coli* followed by flow cytometry. Briefly, CD-1 mice were injected with 5 ml of 1% oyster glycogen in sterile PBS to recruit PMN to the peritoneal cavity. After 4 hr, the peritoneal cells were lavaged and harvested using 10 ml PBS. Cells (1×10^6 cells) were treated with CTCE (0·01–1000 ng/ml) for 1 hr and incubated with Alexa Fluor 488-conjugated *E. coli* (K12, approximately $1{\cdot}2 \times 10^7$ particles; Invitrogen) for 30 min. Cells were washed three times and phycoerythrin-conjugated Gr-1^+ PMN containing FITC-conjugated *E. coli* were determined by flow cytometry.

Measurement of reactive oxygen species

Intracellular ROS was detected using 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetylester (CM-H_2DCFDA; Invitrogen). Briefly, neutrophils (100 μl/well, 1×10^6 cells/ml) were incubated with 10 μM CM-H_2DCFDA in pre-warmed Hanks' balanced salt solution buffer for 30 min at 37°. Then, the cells were washed and incubated in the same buffer for 30 min at 37°. Trypan Blue (final concentration is 0·0025% weight/volume) was added to quench extracellularly bound dye. The increased fluorescence of cells was detected by multi-mode microplate reader (BioTek, Winooski, VT)

Bacterial killing

Escherichia coli (K12 ER2267; New England BioLabs (Ipswich, MA)) were cultured in Lysogeny broth (Fisher Scientific) overnight at 37°. The culture was centrifuged at 2000 ***g*** for 5 min to pellet the *E. coli*, then washed twice and resuspended in PBS with 10% fetal bovine serum (Invitrogen). The final concentration of the bacterial suspension was adjusted to 1×10^7 CFU/ml. The suspension was incubated for 20 min at 37° with shaking. Neutrophils were diluted to 1×10^7 cells/ml and mixed with the prepared *E. coli*. The mixture (1 ml) was added to a new tube and 1 ml ice cold PBS was added to stop killing. The mixture was centrifuged at 100 ***g*** for 5 min and marked as time 0. The rest were rotated at room temperature for 30 min. The suspension was centrifuged at 100 ***g*** for 5 min and was collected and diluted 1 : 1000. The bacteria in the supernatant were spread on an Lysogeny broth plate and counted.

Statistical analysis

Data are expressed as the mean ± SE of the mean. Statistical significance was determined by analysis of variance with Fisher's probable least-squares difference test or Student's *t*-test using GraphPad Prism software (La Jolla, CA). A $P < 0{\cdot}05$ value was considered statistically significant.

Results

CECT improves survival in CLP-induced sepsis

We have previously demonstrated that *in vivo* CTCE significantly (albeit modestly) improved survival without antibiotics in a model of severe sepsis induced by CLP.[16] To test the potential beneficial effects of CTCE in severe septic shock and treatment with antibiotics, Imipenem was administered subcutaneously at 6, 24 and 48 hr post CLP at the same time intervals as CTCE administration. CTCE significantly reduced mortality induced by CLP in mice treated with antibiotics, from 67% in CLP control to 20% in CTCE-treated mice at 7 days $P < 0{\cdot}05$ (Fig. 1).

CTCE improves bacterial clearance in peritoneal fluid, blood and lung in CLP-induced sepsis

Mortality from late sepsis is associated with bacterial overgrowth due to an impaired phagocytic capacity of macrophages and PMN. To determine whether treatment with CTCE improves survival in mice with sepsis by increasing bacterial clearance *in vivo*, bacterial CFU from different tissues were determined. Bacteria were detected in blood, peritoneal fluid and lung in CLP-induced mice with or without CTCE treatment. Treatment with CTCE significantly decreased the CFU in blood by 77 ± 11%, peritoneal fluid by 78 ± 9% and lung by 79 ± 8%, respectively (versus CLP vehicle, $P < 0{\cdot}05$, Fig. 2).

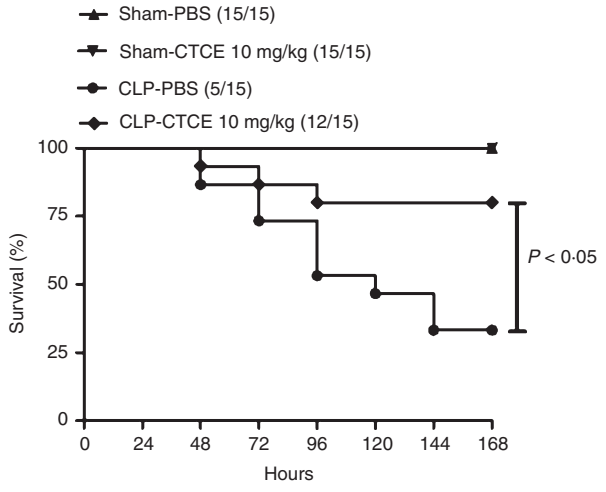

Figure 1. Effects of CTCE-0214 (CTCE) on survival in mice with sepsis induced by caecal ligation and puncture (CLP). CD-1 mice were subjected to CLP and received antibiotics as described in Materials and methods. CTCE (10 mg/kg) or vehicle PBS was administered by injection subcutaneously at 2, 6, 24 and 48 hr after CLP. Mortality was monitored every 24 hr until 168 hr; $n = 15$/group. Statistical analyses were performed using the log-rank (Mantel–Cox) test with GraphPad Prism software. $P < 0{\cdot}05$ compared with the PBS group.

CTCE enhances blood and peritoneal PMN recruitment in CLP-induced sepsis

To investigate the effect of CTCE on PMN mobilization from bone marrow to the peripheral circulation, the numbers of PMN ($CD11b^+$ $GR\text{-}1^+$ cells) in bone marrow, blood and peritoneal fluid were counted using flow cytometry. The total number of leucocytes in bone marrow and blood were also counted. CTCE-treated septic mice were not significantly different from CLP mice (data not shown). At serial time points from 12, 18 and 24 hr after CLP, the percentage of PMN in the bone marrow was significantly decreased in CLP mice compared with sham controls. CTCE-treated septic mice were not significantly different from CLP mice (Fig. 3a). There was an increase of PMN in blood at 12 hr followed by a neutropenia at 18 and 24 hr after CLP. CTCE treatment significantly attenuated the neutropenia at 18 hr (2·4 ± 0·6 fold) and 24 hr (2·9 ± 0·6 fold) after CLP (Fig. 3b). The number of PMN in peritoneal fluid was increased after CLP. Treatment with CTCE greatly augmented peritoneal PMN recruitment 2·0 ± 0·2 fold compared with the CLP group $P < 0{\cdot}05$ (Fig. 3c).

CTCE enhances the phagocytic function of PMN

We next determined bacterial phagocytic capacity of PMN *in vitro*. Phagocytic activity of PMN isolated from peritoneal lavage was significantly sustained when the concentration of CTCE was higher than 1 ng/ml (1·25 ± 0·02 fold, $P < 0{\cdot}05$, Fig. 4). These results suggest that CTCE may promote clearance of bacteria in the peritoneum in part by enhancing the phagocytic capacity of PMN.

CTCE increases ROS production and bacterial killing activity of PMN *in vitro*

Activated PMN generate ROS to promote the capability of bacterial clearance. We examined whether treatment with CTCE could affect the production of ROS and its bacterial killing activity. PMN were differentiated from the mouse promyelocyte cell line (MPRO). CTCE (10 and 1 μg/ml) significantly increased the production of ROS (27 ± 5%

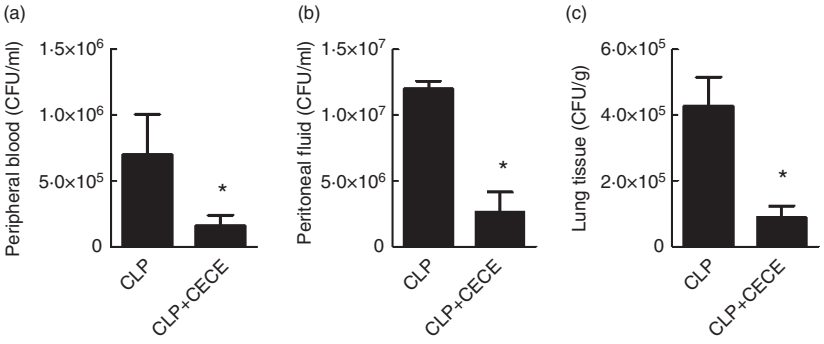

Figure 2. Effects of CTCE-0214 (CTCE) on bacterial clearance in blood, peritoneal fluid and lung in mice with sepsis induced by caecal ligation and puncture (CLP). CD-1 mice were subjected to CLP and treated with CTCE (10 mg/kg). Bacterial loads were determined in peripheral blood (a), peritoneal fluid (b) and lung (c) 24 hr post CLP. $n = 3$ to $n = 5$ mice per group. $^*P < 0{\cdot}05$ compared with CLP group.

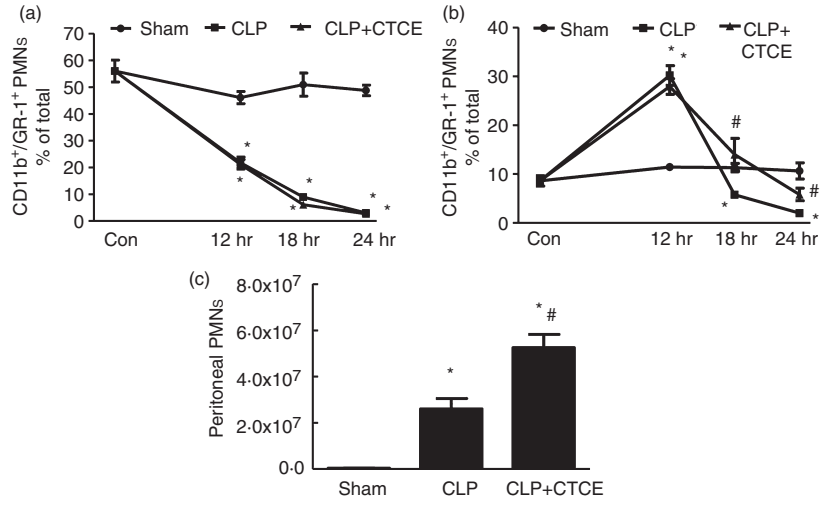

Figure 3. Effects of CTCE-0214 (CTCE) on polymorphonuclear leucocyte (PMN) migration in mice with sepsis induced by caecal ligation and puncture (CLP). CD-1 mice were subjected to CLP and treated with CTCE (10 mg/kg). $CD11b^+$ $GR\text{-}1^+$ PMN in the bone marrow (a) and peripheral blood (b) were measured at 12, 18 and 24 hr after CLP. (c) $CD11b^+$ $GR\text{-}1^+$ PMN in the peritoneal fluid were determined at 24 hr after CLP. Data represent mean ± SE. $n = 3$ to $n = 5$ mice per group. $^*P < 0{\cdot}05$ compared with sham-treated group; $\#P < 0{\cdot}05$ compared with CLP group.

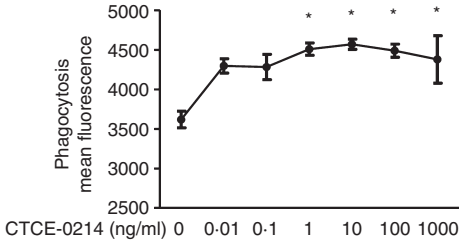

Figure 4. Effects of CTCE-0214 (CTCE) on peritoneal polymorphonuclear leucocyte (PMN) phagocytosis. Peritoneal PMN, recruited by oyster glycogen, were incubated with FITC-labelled *Escherichia coli*. Flow cytometry was used to determine the mean fluorescence ($FITC^+$) of $Gr\text{-}1^+$ PMN. All values are expressed as mean ± SE ($n = 5$). $^*P < 0{\cdot}05$ compared with basal values.

and 32 ± 4%, respectively, $P < 0{\cdot}05$, Fig. 5a) in neutrophils. Correspondingly, CTCE significantly increased PMN bacteria killing capability as the number of bacteria in CTCE (10 μg/ml) treated PMN was significantly reduced by 27 ± 3%, $P < 0{\cdot}05$ (Fig. 5b). Similar results were observed when cells were stimulated with LPS, but there were no further decreases when treated with CTCE together with LPS (Fig. 5b).

Discussion

In this study, we demonstrated that the combination of CTCE with antibiotic treatment significantly increased the survival rate of CLP-induced septic mice. CTCE increased PMN recruitment into the blood and peritoneal fluid. CLP with CTCE treatment greatly accelerated bacterial clearance in peritoneal fluid, peripheral blood and lung with a > 77% reduction in CFU compared with vehicle-treated septic mice. *In vitro* studies revealed that CTCE improved PMN function by enhancing phagocytic activity, increasing intracellular production of ROS and improving bacterial killing. The increased intracellular ROS production may contribute more to bacterial killing than enhancing phagocytic activity.

During severe sepsis, PMN function was substantially dysregulated, resulting in impaired directed migration of PMN to infectious foci and inadequate antimicrobial responses of PMN.[5] Oxidant production is one of the antimicrobial mechanisms employed by PMN to reduce infection. A recent study has demonstrated that oxidant production by PMN was temporally impaired in septic mice.[18] Likewise, microarray analysis of PMN collected form patients with sepsis within 24 hr demonstrates that

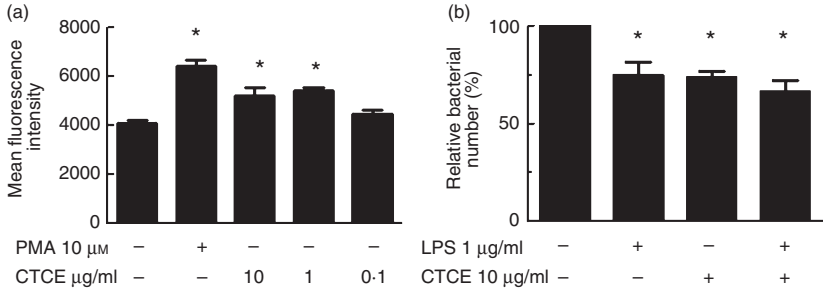

Figure 5. Effects of CTCE-0214 (CTCE) on the intracellular production of reactive oxygen species (ROS) and bacterial killing activity of polymorphonuclear leucocytes (PMN). (a) Neutrophils (1×10^6 cells/ml) were serum starved and stimulated with PMA (10 μM) or CTCE (10, 1, 0·1 μg/ml) for 6 hr in Iscove's modified Dulbecco's medium without serum. PMN (100 μl/well) were transferred in 96-well plates and loaded with H_2DCFDA for 30 min. Intracellular ROS production was determined by measuring mean fluorescence. PMA was used as a positive control. (b) Neutrophils (1×10^7 cells/ml) were treated with lipopolysaccharide (LPS; 1 μg/ml), CTCE (10 μg/ml) or a combination of LPS (1 μg/ml) and CTCE (10 μg/ml) for 6 hr. *Escherichia coli* (K12 ER2267, 1×10^7 colony-forming units/ml) were mixed with neutrophils for 30 min. The percentage of bacterial number was determined by comparing bacterial number left in the supernatant with initial bacterial number (the time 0 tube). Data are expressed as mean ± SE ($n = 5$). *$P < 0{\cdot}05$ compared with control.

the expression of genes required for oxidant production (e.g. NADPH oxidase) was suppressed.[19] Another study examining PMN function in patients with sepsis versus healthy controls found that reduced phagocytic activity of neutrophils within the first 24 hr after admission was a negative predictor for survival.[20] Hence agents such as CTCE, which improve function of PMN through enhancing recruitment, phagocytosis and bactericidal activity, may have a favourable impact on the survival of patients with severe sepsis.

Previous studies show that CTCE recruits PMN and haematopoietic progenitor cells into peripheral blood[21] and is beneficial in CLP-induced sepsis.[16] We demonstrated that CTCE promoted recruitment of PMN to the nidus of infection and improved PMN function in CLP-induced sepsis. This conclusion was supported by increased numbers of PMN in blood and peritoneal fluid and reduced bacterial load in peritoneal fluid, blood and lung in the septic mice. In addition, *in vitro* studies support the notion that CTCE enhanced PMN function by enhancing phagocytic activity, increasing ROS production and bacterial killing ability by PMN and enhancing clearance of local bacteria.

CXCL12 has been reported as a chemoattractant for lymphocytes and mononuclear cells from the bloodstream to the site of inflammation.[22] Studies also demonstrated that CXCL12 promotes natural killer cell migration into tissues.[23] A more recent study highlighted the importance of the CXCL12 axis for both neutrophil mobilization from the bone marrow and neutrophil recruitment to peripheral sites of infection in sepsis.[10] The results demonstrated that CXCL12 blockade prevents the release of neutrophils from the bone marrow during sepsis, resulting in a failure to increase blood and peritoneal neutrophil counts and a fivefold increase in peritoneal bacteria CFU during sepsis. This failure to clear bacteria led to a 40% increase in sepsis mortality in anti-CXCL12-treated mice[10] Consistent with previous reports, our present study demonstrated that, as a peptide analogue of CXCL12, CTCE enhanced neutrophil mobilization and improved survival after severe polymicrobial sepsis.

In summary, combined treatment with CTCE and antibiotics significantly reduced mortality of septic mice induced by CLP. This beneficial effect on survival may be mediated through enhancing recruitment and improving function of PMN.

Acknowledgements

HF, JAC and PVH designed the study. SG, CG, BZ, LW and HF performed the experiments. SG, HF, JAC and PVH wrote the paper. This work was supported in part by NIH GM27673 (J.A.C.), GM67202 (B.Z.) and UL1TR000062 (SCTR).

Disclosures

The authors declare that they have no competing interests.

References

1 Lever A, Mackenzie I. Sepsis: definition, epidemiology, and diagnosis. *BMJ* 2007; **335**:879–83.
2 Iwashyna TJ, Cooke CR, Wunsch H *et al.* Population burden of long-term survivorship after severe sepsis in older Americans. *J Am Geriatr Soc* 2012; **60**:1070–7.
3 Kobayashi SD, Voyich JM, Burlak C *et al.* Neutrophils in the innate immune response. *Arch Immunol Ther Exp* 2005; **53**:505–17.
4 Cohen J. The immunopathogenesis of sepsis. *Nature* 2002; **420**:885–91.
5 Alves-Filho JC, Spiller F, Cunha FQ. Neutrophil paralysis in sepsis. *Shock* 2010; **34**:15–21.
6 Kovach MA, Standiford TJ. The function of neutrophils in sepsis. *Curr Opin Infect Dis* 2012; **25**:321–7.
7 Borregaard N. Neutrophils, from marrow to microbes. *Immunity* 2010; **33**:657–70.

8 Christopher MJ, Link DC. Regulation of neutrophil homeostasis. *Curr Opin Hematol* 2007; **14**:3–8.

9 Petit I, Szyper-Kravitz M, Nagler A *et al.* G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. *Nat Immunol* 2002; **3**: 687–94.

10 Delano MJ, Kelly-Scumpia KM, Thayer TC *et al.* Neutrophil mobilization from the bone marrow during polymicrobial sepsis is dependent on CXCL12 signaling. *J Immunol* 2011; **187**:911–8.

11 Kryczek I, Wei S, Keller E *et al.* Stroma-derived factor (SDF-1/CXCL12) and human tumor pathogenesis. *Am J Physiol Cell Physiol* 2007; **292**:C987–95.

12 Tilton B, Ho L, Oberlin E *et al.* Signal transduction by Cxc chemokine receptor 4 stromal cell-derived factor 1 stimulates prolonged protein kinase B and extracellular signal-regulated kinase 2 activation in T lymphocytes. *J Exp Med* 2000; **192**:313–24.

13 Cencioni C, Capogrossi MC, Napolitano M. The SDF-1/CXCR4 axis in stem cell preconditioning. *Cardiovasc Res* 2012; **94**:400–7.

14 Hu X, Dai S, Wu W-J *et al.* Stromal cell-derived factor-1α confers protection against myocardial ischemia/reperfusion injury role of the cardiac stromal cell-derived factor-1α–CXCR4 axis. *Circulation* 2007; **116**:654–63.

15 Li K, Chuen CKY, Lee SM *et al.* Small peptide analogue of SDF-1α supports survival of cord blood $CD34^+$ cells in synergy with other cytokines and enhances their *ex vivo* expansion and engraftment into nonobese diabetic/severe combined immunodeficient mice. *Stem Cells* 2006; **24**:55–64.

16 Fan H, Wong D, Ashton SH *et al.* Beneficial effect of a CXCR4 agonist in murine models of systemic inflammation. *Inflammation* 2012; **35**:130–7.

17 Fan H, Bitto A, Zingarelli B *et al.* β-arrestin 2 negatively regulates sepsis-induced inflammation. *Immunology* 2010; **130**:344–51.

18 Delano MJ, Thayer T, Gabrilovich S *et al.* Sepsis induces early alterations in innate immunity that impact mortality to secondary infection. *J Immunol* 2011; **186**:195–202.

19 Tang BM, McLean AS, Dawes IW *et al.* The use of gene-expression profiling to identify candidate genes in human sepsis. *Am J Respir Crit Care Med* 2007; **176**:676–84.

20 Danikas D, Karakantza M, Theodorou G *et al.* Prognostic value of phagocytic activity of neutrophils and monocytes in sepsis. Correlation to CD64 and CD14 antigen expression. *Clin Exp Immunol* 2008; **154**:87–97.

21 Pelus LM, Bian H, Fukuda S *et al.* The CXCR4 agonist peptide, CTCE-0021, rapidly mobilizes polymorphonuclear neutrophils and hematopoietic progenitor cells into peripheral blood and synergizes with granulocyte colony-stimulating factor. *Exp Hematol* 2005; **33**:295–307.

22 Okabe S, Tauchi T, Ohyashiki K *et al.* Stromal-cell-derived factor-1/CXCL12-induced chemotaxis of a T cell line involves intracellular signaling through Cbl and Cbl-b and their regulation by Src kinases and CD45. *Blood Cells Mol Dis* 2006; **36**:308–14.

23 Goda S, Inoue H, Umehara H *et al.* Matrix metalloproteinase-1 produced by human CXCL12-stimulated natural killer cells. *Am J Pathol* 2006; **169**:445–58.