ORIGINAL ARTICLE

Progranulin Plays a Central Role in Host Defense during Sepsis by Promoting Macrophage Recruitment

Zhixin Song[1,2], Xuemei Zhang[2], Liping Zhang[1], Fang Xu[3], Xintong Tao[2], Hua Zhang[4], Xue Lin[1], Lihua Kang[2], Yu Xiang[1], Xaiofei Lai[1], Qun Zhang[5], Kun Huang[6], Yubing Dai[7], Yibing Yin[2]*, and Ju Cao[1]*

[1]Department of Laboratory Medicine, [3]Department of Emergency and Intensive Care Unit, [4]Department of Obstetrics and Gynecology, and [6]Department of Dermatology, The First Affiliated Hospital of Chongqing Medical University, [2]Key Laboratory of Diagnostic Medicine Designated by the Ministry of Education, Chongqing Medical University, and [5]Clinical Laboratories Center, Affiliated Children's Hospital of Chongqing Medical University, Chongqing, China; and [7]Center for Nuclear Receptors and Cell Signaling, University of Houston, Houston, Texas

Abstract

Rationale: Progranulin, a widely expressed protein, has multiple physiological functions. The functional role of progranulin in the host response to sepsis remains unknown.

Objectives: To assess the role of progranulin in the host response to sepsis.

Methods: Effects of progranulin on host response to sepsis were determined.

Measurements and Main Results: Progranulin concentrations were significantly elevated in adult (n = 74) and pediatric (n = 26) patients with sepsis relative to corresponding healthy adult (n = 36) and pediatric (n = 17) control subjects, respectively. By using a low-lethality model of nonsevere sepsis, we observed that progranulin deficiency not only increased mortality but also decreased bacterial clearance during sepsis. The decreased host defense to sepsis in progranulin-deficient mice was associated with reduced macrophage recruitment, with correspondingly impaired chemokine CC receptor ligand 2 (CCL2) production in peritoneal lavages during the early phase of sepsis. Progranulin derived from hematopoietic cells contributed to host defense in sepsis. Therapeutic administration of recombinant progranulin not only rescued impaired host defense in progranulin-deficient mice after nonsevere sepsis but also protected wild-type mice against a high-lethality model of severe sepsis. Progranulin-mediated protection against sepsis was closely linked to improved peritoneal macrophage recruitment. In addition, CCL2 treatment of progranulin-deficient mice improved survival and decreased peritoneal bacterial loads during sepsis, at least in part through promotion of peritoneal macrophage recruitment.

Conclusions: This proof-of-concept study supports a central role of progranulin-dependent macrophage recruitment in host defense to sepsis, opening new opportunities to host-directed therapeutic strategy that manipulate host immune response in the treatment of sepsis.

Keywords: sepsis; progranulin; macrophages; protection; host response

Sepsis ranks in the top 10 causes of death in the world (1, 2). Despite nearly 100 clinical trials, developing potential drugs against sepsis has been particularly frustrating (3). With the withdrawal of drotrecogin alfa (activated) (Xigris; activated protein C) from the market, there is no effective therapy to improve patient survival (4, 5). Novel treatment options for sepsis should be pursued.

Sepsis develops from an unfettered host immune response to infection (6–8). A key

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Correspondence and requests for reprints should be addressed to Ju Cao, M.D., Ph.D., Department of Laboratory Medicine, The First Affiliated Hospital of Chongqing Medical University, No. 1 Youyi Road, Yuzhong District, Chongqing 400016, China. E-mail: caoju723@163.com

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At a Glance Commentary

Scientific Knowledge on the Subject: Despite advances in clinical management, the incidence and mortality of sepsis remain high, and the need for reliable therapeutic targets is great.

What This Study Adds to the Field: This proof-of-concept study supports a novel, macrophage-dependent role for progranulin in protection against sepsis, opening new opportunities to manipulate the host response in the treatment of sepsis.

component in the successful treatment of sepsis is the removal of infection. The recruitment of phagocytes to an infection focus constitutes the first line of defense against infection (4, 9, 10). Impaired phagocyte influx has been reported to be associated with increased mortality and higher bacterial burden during sepsis, and enhancing phagocyte recruitment could attenuate sepsis (11–13).

Progranulin (PGRN), a 593-amino-acid autocrine growth factor, is widely expressed in mammalian tissues (14), where it has multiple functions involved in cell proliferation (15), wound healing (16), neurodegeneration (17), insulin resistance (18), and tumorigenesis (19). Of interest, the exact function of progranulin may vary depending on the stage and contexts involved in a variety of inflammatory diseases (15–26). The functional role of progranulin in sepsis remains unknown.

In this study, we first aimed to determine the level of circulating progranulin in patients with sepsis. Furthermore, we dissected the functional role of progranulin in the host response to sepsis.

Methods

Details of all of the methods are provided in the online supplement.

Study Population

Adult patients were recruited from the intensive care unit of The First Affiliated Hospital of Chongqing Medical University (Chongqing, China). Pediatric patients were recruited from the Children's Hospital of Chongqing Medical University. The diagnosis of sepsis was based on the criteria recommended by the American College of Chest Physicians and Society of Critical Care Medicine Consensus Conference (27). Control samples were obtained from healthy donors. This protocol was approved by the Clinical Research Ethics Committee of Chongqing Medical University, and informed consent was obtained from all participants according to the Declaration of Helsinki.

Animals

C57BL/6 mice (age, 6–8 wk) were obtained from and raised at Chongqing Medical University. Progranulin-deficient ($PGRN^{-/-}$), Toll-like receptor 2–deficient ($TLR2^{-/-}$), $TLR4^{-/-}$, and type I IFN-α/β receptor–deficient ($IFNAR^{-/-}$) mice raised on the C57BL/6 background were purchased from the Jackson Laboratory (Bar Harbor, ME). All animal experiments were done in accordance with the Chongqing Medical University Institutional Animal Care and Use Committee's guidelines.

Sepsis Model

Cecal ligation puncture (CLP) was performed to establish a model of sepsis (10, 13). Briefly, an incision was made in the abdominal cavity of mice. The cecum was exposed, ligated, and punctured with a 26-gauge needle (nonsevere CLP, death occurred in 0–10% of wild-type mice) or with a 21-gauge needle (severe CLP, death occurred in 90–100% of wild-type mice). The cecum was returned to the peritoneal cavity and incisions were closed.

Statistical Analysis

Human data were expressed as scatter dot plots with medians. Mouse data were expressed as box-and-whisker plots showing the smallest observation, lower quartile, median, upper quartile, and largest observation or as medians with interquartile ranges. Comparisons between groups were tested by Mann–Whitney *U* test. For survival studies, Kaplan–Meier analyses followed by log-rank tests were performed. All analyses were done with GraphPad Prism version 5.01 (GraphPad Software, San Diego, CA). *P* values less than 0.05 were considered statistically significant.

Results

Sepsis Results in Elevated Progranulin Production

Progranulin concentrations were markedly increased in adult patients with sepsis compared with healthy control subjects (Table E1 and Figure 1A). No difference

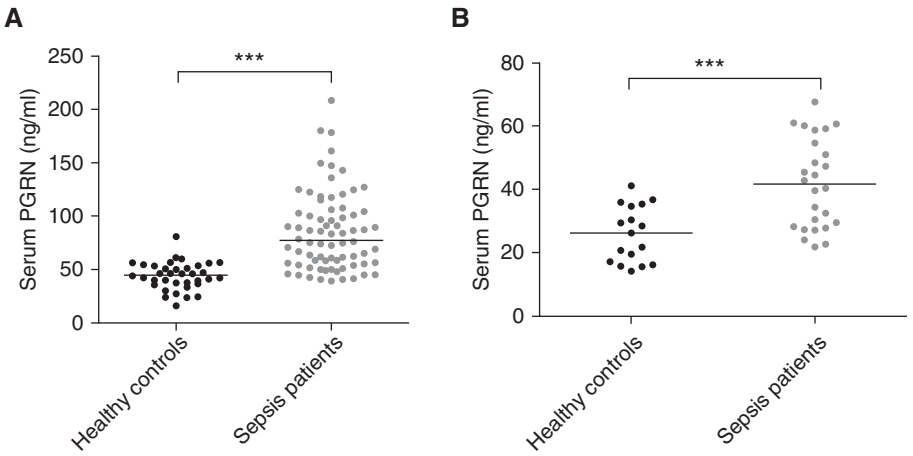

Figure 1. Sepsis results in elevated serum progranulin (PGRN) levels. (*A*) Progranulin concentrations were measured by ELISA in serum samples collected from 74 adult patients with sepsis and from 36 healthy control subjects. (*B*) Progranulin concentration was measured by ELISA in serum samples collected from 26 pediatric patients with sepsis and from 17 healthy control subjects. *Horizontal bars* represent median values, and *dots* represent individual participants. ***$P < 0.001$, compared between groups (denoted by *horizontal bracket*; Mann–Whitney *U* test).

was observed according to the site of infection or micro-organism (gram-positive vs. gram-negative). There was no apparent correlation between serum progranulin and the severity of disease: serum progranulin did not correlate with either APACHE II (Acute Physiology and Chronic Health Evaluation II) or SOFA (Sequential Organ Failure Assessment) score. No difference in progranulin was found between survivors and nonsurvivors (data not shown).

To validate our findings in adults, we measured progranulin levels in an independent cohort of pediatric patients with sepsis (Table E2). Progranulin levels in pediatric patients with sepsis were also significantly elevated compared with healthy control subjects (Figure 1B).

Progranulin Production Is Up-regulated in Experimental Sepsis

In CLP-induced sepsis, a widely accepted animal model for sepsis, we detected a strong up-regulation of progranulin protein in the spleen, peritoneal lavage fluid, and serum (Figure 2A). Both TLR2$^{-/-}$ and TLR4$^{-/-}$ mice had significantly lower progranulin concentrations compared with wild-type mice after CLP (Figure 2B), suggesting that TLR2/TLR4 signaling pathways are partially required for progranulin production during sepsis. Although the IFNAR signaling pathway regulates the expression of a variety of immunomodulatory molecules (28, 29), it did not contribute to progranulin production during sepsis (Figure 2B).

Progranulin Affords Protection against Sepsis

To assess the involvement of up-regulated progranulin in sepsis, we first assessed survival after CLP-induced nonsevere sepsis (a low-lethality model) in wild-type versus progranulin-knockout (PGRN$^{-/-}$) mice. PGRN deficiency significantly decreased the survival rate, with 100% mortality observed in PGRN$^{-/-}$ mice, whereas the survival rate among wild-type mice was 100% (Figure 3A). No mortality was observed in sham-treated wild-type and PGRN$^{-/-}$ mice (data not shown). The intestinal microbiomes were not significantly different between wild-type and PGRN$^{-/-}$ mice (Figure E1A), and 16S ribosomal RNA data showed that the intestinal microbiomes were less than 5% different at the genus level (Figure E1B), suggesting that the difference in susceptibility to CLP-induced mortality was not affected by possible different intestinal microbiomes. Gram-positive *Staphylococcus aureus* is a major cause of sepsis in the clinic (30).

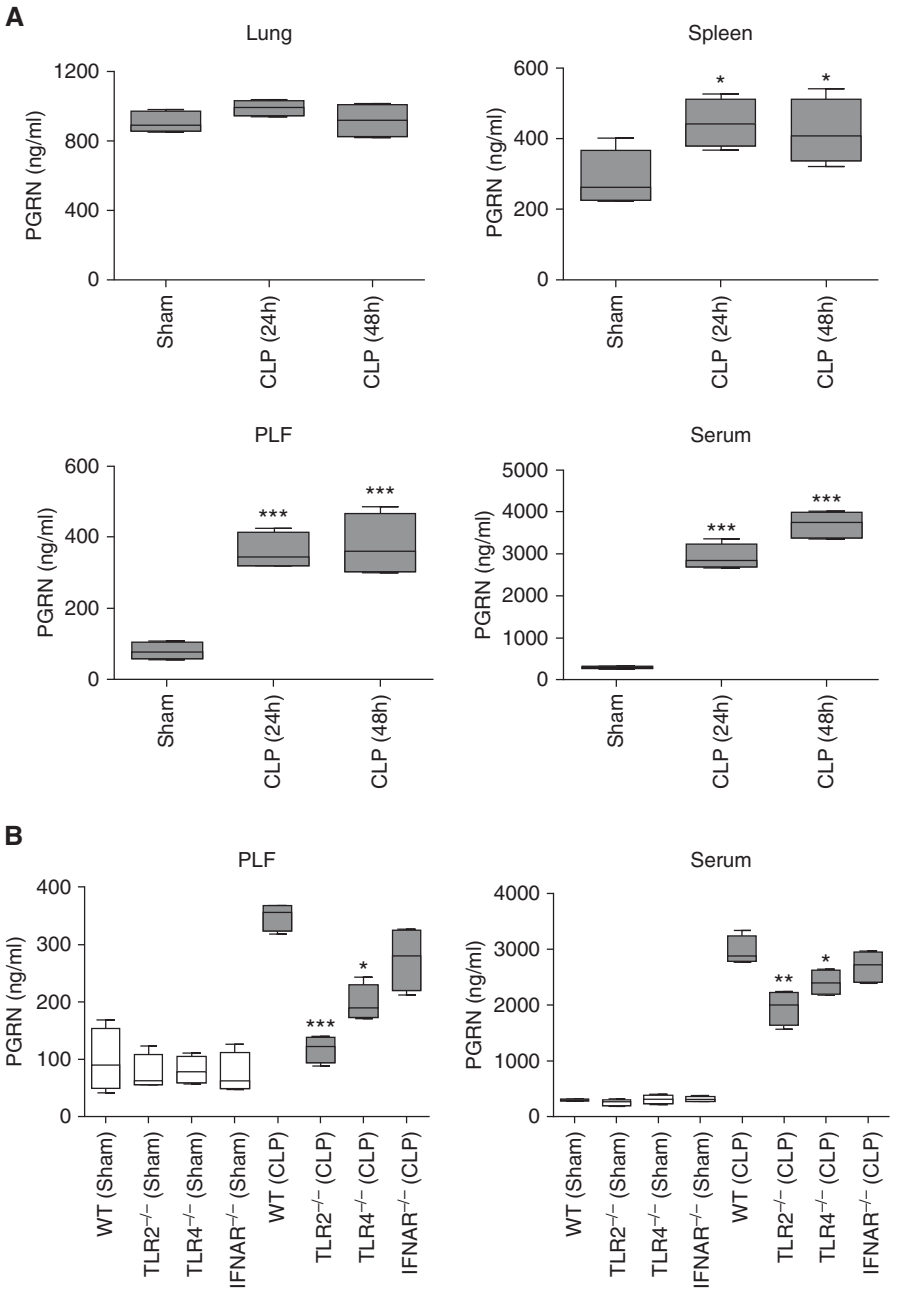

Figure 2. Local and systemic progranulin (PGRN) production in mice with cecal ligation puncture (CLP)–induced sepsis. C57BL/6 mice (n = 6 per group) were subjected to sham or nonsevere CLP with a 26-gauge needle. (*A*) Organs were removed at the indicated time points, blood was collected by cardiac puncture, and peritoneal lavage fluid (PLF) was obtained by washing the peritoneal cavity with 5 ml of sterile phosphate-buffered saline. Samples were assayed for progranulin content by specific sandwich ELISA. *$P < 0.05$, ***$P < 0.001$, compared with sham control mice (Mann–Whitney *U* test). (*B*) Progranulin concentrations in PLF and blood isolated from TLR2$^{-/-}$, TLR4$^{-/-}$, IFNAR$^{-/-}$, and wild-type mice (n = 5 per group) 24 hours after sham or nonsevere CLP. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with wild-type mice after CLP (Mann–Whitney *U* test). IFNAR = type I IFN-α/β receptor; TLR2 and TLR4 = Toll-like receptor types 2 and 4, respectively; WT = wild type.

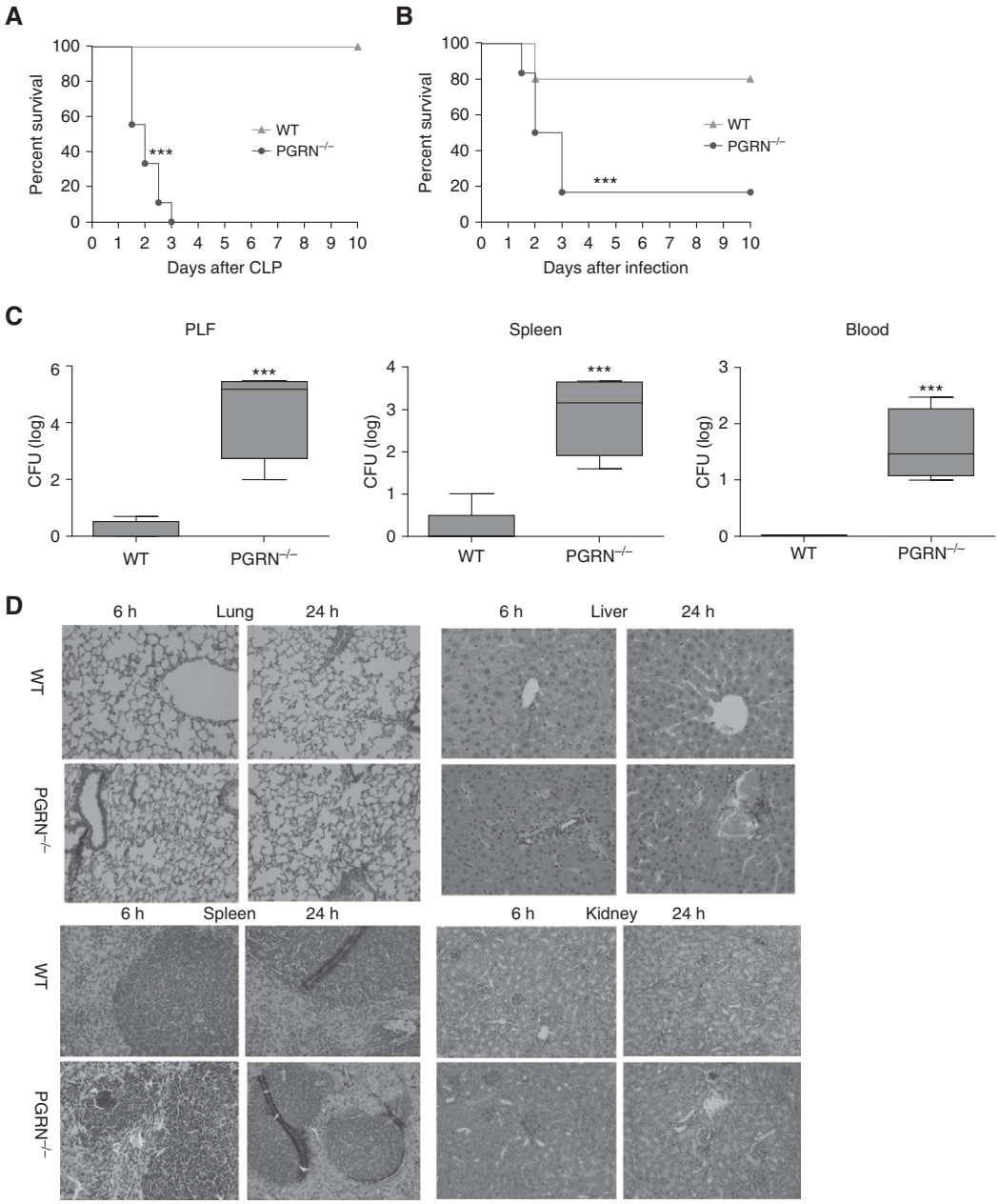

Figure 3. Progranulin is required for host defense in sepsis. (*A*) Wild-type (WT) and progranulin-deficient ($PGRN^{-/-}$) mice (n = 12 per group) underwent nonsevere cecal ligation puncture (CLP) with a 26-gauge needle, and survival was monitored. Comparison between groups was done by Kaplan–Meier analysis followed by log-rank tests. ***$P < 0.001$ when compared with wild-type mice after CLP. (*B*) Wild-type and $PGRN^{-/-}$ mice (n = 8 per group) were intraperitoneally injected with *Staphylococcus aureus* (2×10^8 cfu), and survival was monitored. Comparison between groups was done by Kaplan–Meier analysis followed by log-rank tests. ***$P < 0.001$ when compared with wild-type mice after infection. (*C*) Dilutions of peritoneal lavage fluid (PLF), spleen, and blood obtained from wild-type and $PGRN^{-/-}$ mice (n = 5 per group) 24 hours after nonsevere CLP were cultured on blood agar plates, and the number of bacterial colonies was counted. ***$P < 0.001$ when compared with wild-type mice (Mann–Whitney *U* test). (*D*) Representative examples of hematoxylin and eosin–stained lung, liver, spleen, and kidney tissues from wild-type and $PGRN^{-/-}$ mice (n = 5 per group) 6 and 24 hours after nonsevere CLP. (*E*) Histological scores for lungs, liver, spleen, and kidneys from wild-type and $PGRN^{-/-}$ mice (n = 5 per group) 6 and 24 hours after nonsevere CLP. ***$P < 0.001$ when compared with wild-type mice at the same time point (Mann–Whitney *U* test). (*F*) Serological markers of organ injury including alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatinine in wild-type or $PGRN^{-/-}$ mice (n = 5 per group) 24 hours after nonsevere CLP. **$P < 0.01$, ***$P < 0.001$ when compared with wild-type mice (Mann–Whitney *U* test). (*G*) The spleen, liver, and kidneys from wild-type and $PGRN^{-/-}$ mice (n = 5 per group) 6 and 24 hours after nonsevere CLP were subjected to DNA fragmentation analysis (terminal deoxynucleotidyltransferase dUTP nick end labeling [TUNEL]). Representative examples are shown. (*H*) TUNEL-positive cells were counted (n = 5 per group). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ when compared with wild-type mice at the same time point (Mann–Whitney *U* test). (*I*) Survival of wild-type mice following progranulin neutralization after nonsevere CLP with anti-progranulin antibodies (n = 10 per group). Comparison between groups was done by Kaplan–Meier analysis followed by log-rank tests. **$P < 0.001$ when compared with wild-type mice treated with IgG control mice.

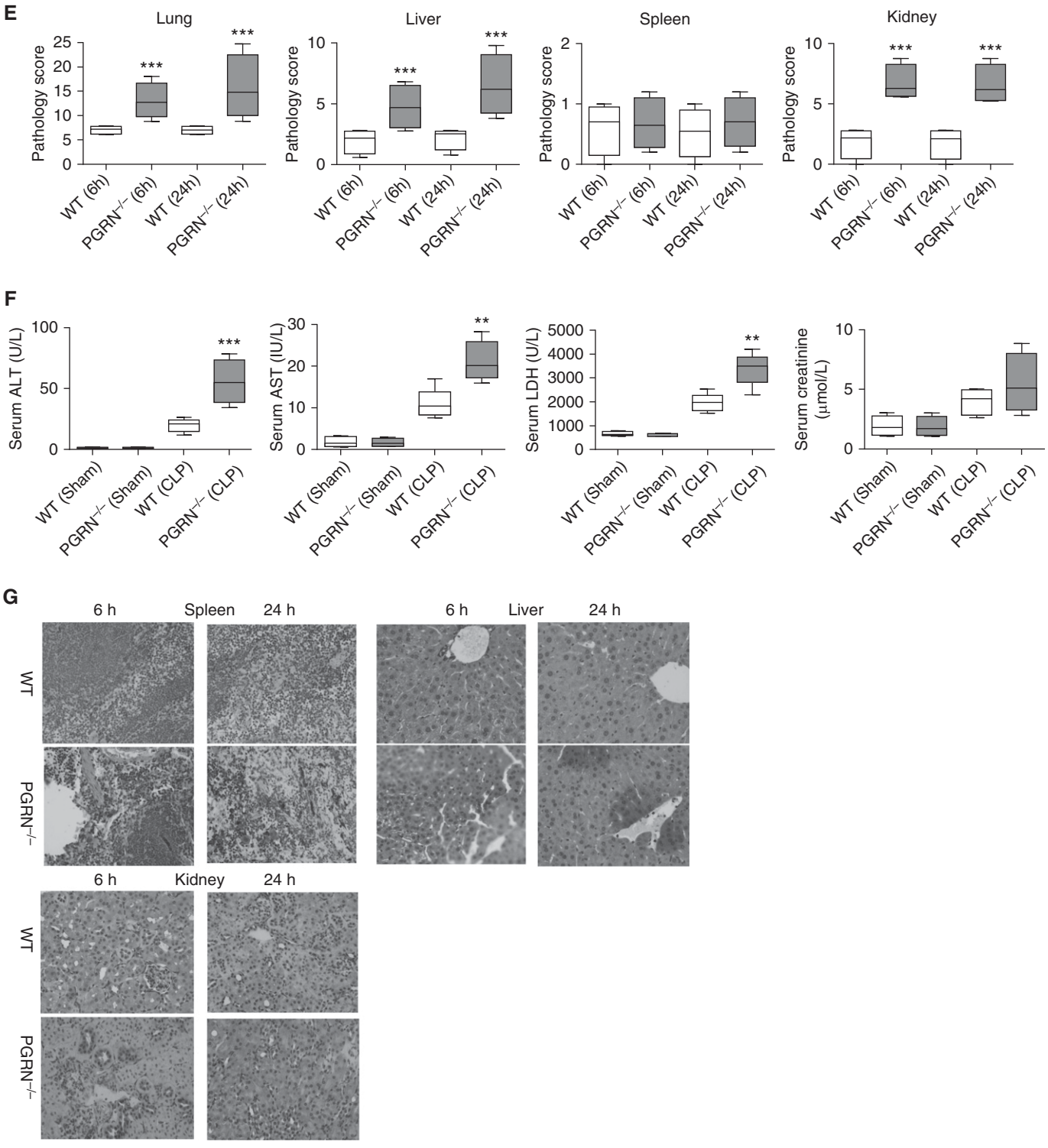

Figure 3. (Continued).

We therefore tested the impact of progranulin deficiency on survival in an *S. aureus*–induced sepsis model. Again, the mortality increased from 20% in wild-type mice to 80% in $PGRN^{-/-}$ mice (Figure 3B).

Exacerbated mortality of progranulin-deficient versus wild-type mice was closely associated with decreased bacterial clearance. Twenty-four hours after CLP, $PGRN^{-/-}$ mice displayed a significant increase in bacterial loads from

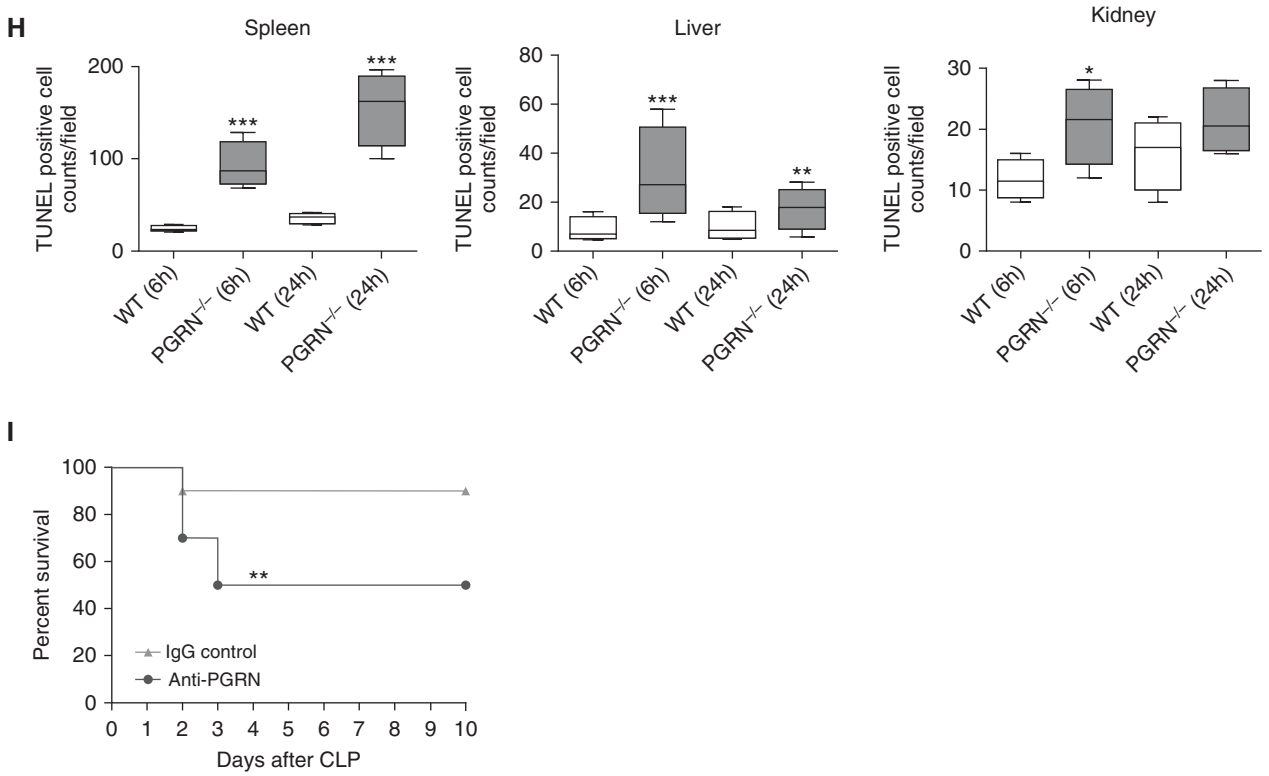

Figure 3. (Continued).

peritoneum, spleen, and blood (Figure 3C). Dysfunction of vital organs is associated with sepsis-induced lethality (1). At 6 and 24 hours after CLP, inflammation of lungs, liver, and kidneys in $PGRN^{-/-}$ mice was enhanced as compared with that in wild-type mice (Figure 3D), which was reflected by significantly higher pathology scores compared with wild-type mice (Figure 3E). Progranulin-deficient mice showed significantly increased serum levels of alanine aminotransferase and aspartate aminotransferase, markers for hepatocellular injury (Figure 3F). The serum levels of lactate dehydrogenase, a marker for general cellular injury, were also significantly elevated in $PGRN^{-/-}$ mice relative to wild-type mice 48 hours after CLP (Figure 3F). No significant difference in creatinine (Figure 3F), a marker for renal failure, was seen. In addition, progranulin deficiency strongly increased cell apoptosis in the spleen, liver, and kidneys, as measured by terminal deoxynucleotidyltransferase dUTP nick end labeling (TUNEL) histology (Figures 3G and 3H). Together, these results demonstrate that progranulin deficiency worsened tissue damage and augmented immune cell apoptosis, thereby contributing to exacerbated mortality in sepsis.

To rule out developmental abnormalities in progranulin-deficient mice as a confounding factor, we performed antibody-mediated neutralization assays. Anti-progranulin–treated mice had significantly increased mortality as compared with IgG-treated mice (Figure 3I), which is consistent with what was observed in progranulin-deficient mice.

Hematopoietic-derived Progranulin Is Required for Survival on Sepsis

Progranulin is expressed in epithelial cells and immune cells (22). To examine whether progranulin derived from the hematopoietic system is required for sepsis survival, chimeras were created to test the radiosensitive hematopoietic and the radioresistant parenchymal systems. Either wild-type or progranulin-deficient mice were lethally irradiated and reconstituted with bone marrow (BM) from either wild-type or $PGRN^{-/-}$ mice, creating (*1*) wild-type mice reconstituted with wild-type BM, (*2*) wild-type mice with a hematopoietic system that lacked progranulin expression, or (*3*) mice lacking progranulin expression in parenchymal cells but possessing intact progranulin production in their hematopoietic cells. As shown in Figure E2, $PGRN^{-/-}$ mice transplanted with wild-type BM had significantly up-regulated progranulin after CLP compared with wild-type sham control mice. However, wild-type mice receiving $PGRN^{-/-}$ BM displayed similar progranulin levels compared with wild-type sham control mice, suggesting that hematopoietic cells are the central cells producing progranulin in sepsis.

Using these chimeras, we found that mice with defective progranulin production in their hematopoietic system showed 100% mortality after nonsevere CLP, whereas wild-type mice reconstituted with a wild-type hematopoietic system showed a 100% survival rate (Figure 4). Mice that possessed a $PGRN^{-/-}$ parenchymal system ($PGRN^{-/-}$ mice) but were reconstituted with a normal hematopoietic system demonstrated a 60% survival rate, indicating the importance of progranulin from the hematopoietic system in host defense during sepsis.

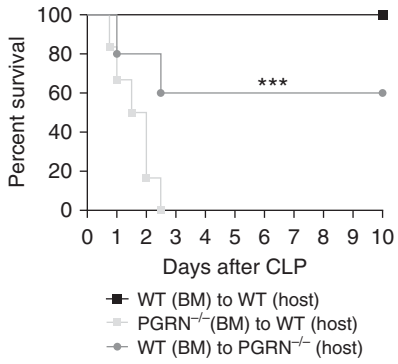

Figure 4. Progranulin derived from the hematopoietic system is needed to survive sepsis. Wild-type (WT) and progranulin-deficient ($PGRN^{-/-}$) mice irradiated and reconstituted with bone marrow (BM) from wild-type or $PGRN^{-/-}$ mice (n = 10 per group) underwent nonsevere cecal ligation puncture (CLP) with a 26-gauge needle, and survival was monitored. Comparison between groups was done by Kaplan–Meier analysis followed by log-rank tests. ***$P < 0.001$ when compared with irradiated wild-type mice reconstituted with bone marrow from $PGRN^{-/-}$ mice.

Progranulin Regulates Local Macrophage Levels and Inflammatory Responses

To determine underlying mechanisms by which progranulin-deficient mice were susceptible to sepsis-induced lethality in a low-lethality model, experiments were performed to characterize the nature of infiltrating leukocytes after CLP in septic wild-type and $PGRN^{-/-}$ animals. There was no significant difference for the total number of peritoneal leukocytes from $PGRN^{-/-}$ mice compared with wild-type mice (Figure 5A). However, at 6 and 24 hours after CLP, $PGRN^{-/-}$ mice had a significantly decreased number of peritoneal $F4/80^+$ macrophages compared with wild-type mice (Figure 5B and Figure E3A). In contrast, we did not observe a significant difference in the number of peritoneal $Ly6G^+$ neutrophils (Figure 5C and Figure E3B), $CD3^+$ T cells (Figure 5D and Figure E3C), or $CD4^+CD25^+Foxp3^+$ regulatory T cells (Figure 5E and Figure E3D).

Cytokine/chemokine responses were also analyzed over the early course of CLP. $PGRN^{-/-}$ mice produced a significantly higher level of peritoneal tumor necrosis factor (TNF)-α, 6 and 24 hours after CLP, than their wild-type counterparts (Table 1). By comparison, $PGRN^{-/-}$ mice had a significantly lower level of peritoneal CCL2 (CC receptor ligand 2), a chemokine that facilitates macrophage recruitment (11), at the early time point (6 h) after CLP. In the lungs, 6 hours after CLP, increased levels of TNF-α, IL-6, and HMGB1 (high-mobility group box 1) were detected in $PGRN^{-/-}$ mice compared with wild-type mice, which was consistent with the increased lung inflammation in $PGRN^{-/-}$ mice. In addition, $PGRN^{-/-}$ mice had a significantly higher serum TNF-α level 6 and 24 hours after CLP than did wild-type mice, and serum IL-17 and IL-10 were significantly higher in $PGRN^{-/-}$ mice 24 hours after CLP. Serum HMGB1 was also significantly released in $PGRN^{-/-}$ mice at 6 hours. The levels of other inflammatory mediators, including IFN-γ, IL-1β, CXCL1 (CXC receptor ligand 1), and CXCL10 (CXC receptor ligand 10), were comparable between $PGRN^{-/-}$ and wild-type mice. Therefore, the only mediator significantly decreased in progranulin-deficient mice was peritoneal CCL2 at the early time point after CLP, which was decreased by more than 60% compared with wild-type levels, suggesting that the preservation of local CCL2 production early in the course of sepsis is critical to effective peritoneal macrophage recruitment.

Progranulin Deficiency Impairs CCL2 Production in Neutrophils and T Lymphocytes

We next determined whether progranulin participates in CCL2 production *in vitro*. With the same number of cells (1×10^5 cells), peritoneal neutrophils could produce about 10-fold higher concentrations of CCL2 (4,000–5,500 pg/ml) compared with peritoneal macrophages (450–650 pg/ml) on stimulation with heat-killed *Escherichia coli* (1×10^6 cfu). Although progranulin deficiency could modestly enhance CCL2 production in peritoneal macrophages, progranulin deficiency could impair CCL2 production in peritoneal neutrophils to nearly the basal level and significantly decrease CCL2 production by spleen T lymphocytes (Figure 6). Because both neutrophils and T lymphocytes were important effector cell types during sepsis (Figure 5), these results suggest that the low production of peritoneal CCL2 observed in progranulin-deficient mice during sepsis could be attributed to insufficient CCL2 production by progranulin-deficient neutrophils and T lymphocytes.

Administration of Recombinant Progranulin Protected against Sepsis

To determine the therapeutic effect of recombinant progranulin on experimental sepsis, we first did some preliminary experiments demonstrating that intraperitoneal administration of recombinant progranulin (5, 10, or 20 μg) 2 hours after CLP increased the survival rate in a dose-dependent manner (data not shown), and that survival was significantly improved when recombinant progranulin (20 μg) was injected 2 hours post-CLP. Therefore, our subsequent experiments were performed in CLP mice using recombinant progranulin (20 μg) beginning 2 hours after CLP. In progranulin-deficient mice after CLP-induced nonsevere sepsis, administration of recombinant progranulin could dramatically elevate peritoneal progranulin concentrations (Figure E4A). Importantly, treatment with recombinant progranulin significantly decreased mortality (Figure 7A) and increased bacterial clearance from the peritoneal cavity and blood (Figure 7B) compared with progranulin-deficient mice treated with PBS control. PGRN treatment also significantly enhanced peritoneal CCL2 production and macrophage recruitment in progranulin-deficient mice (Figure 7C).

The therapeutic effect of progranulin on experimental sepsis was further investigated in CLP-induced severe sepsis (a high-lethality model), using wild-type mice. Administration of recombinant progranulin could increase peritoneal progranulin concentrations in wild-type mice (Figure E4B). We observed 100% mortality in wild-type mice treated with PBS control after severe CLP (Figure 7D). However, administration of recombinant progranulin significantly improved the survival rate (40%) in wild-type mice. Progranulin treatment also significantly increased bacterial clearance from the peritoneal cavity and blood (Figure 7E) and enhanced peritoneal CCL2 production and macrophage recruitment (Figure 7F) compared with PBS control.

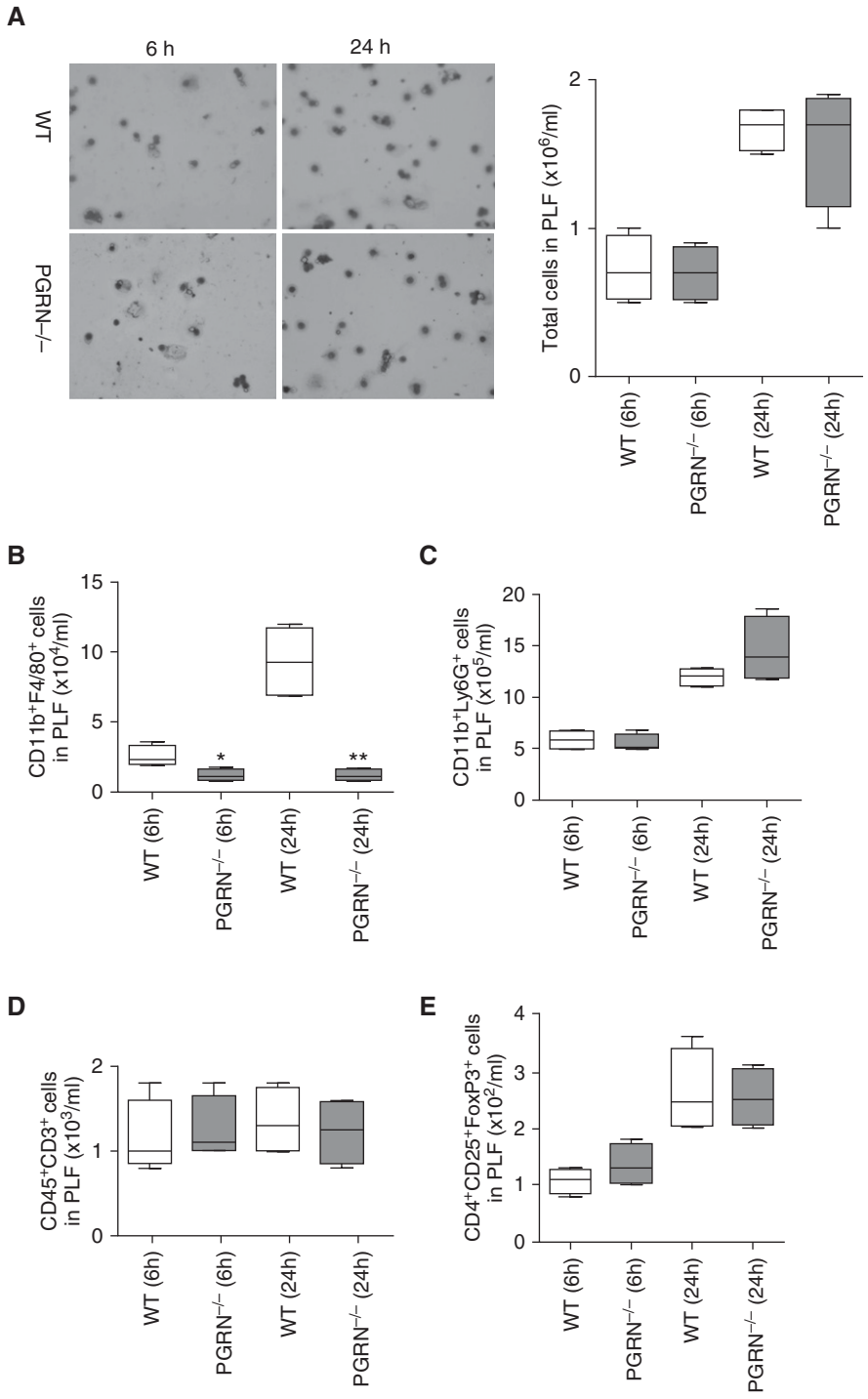

Figure 5. Progranulin regulates macrophage recruitment in sepsis. Wild-type (WT) and progranulin-deficient ($PGRN^{-/-}$) mice (n = 6 per group) were subjected to sham or nonsevere cecal ligation puncture (CLP) with a 26-gauge needle. (*A*) Cytospin centrifugation was performed for Diff-Quik staining (Baxter Dale Diagnostic AG, Dubinger, Switzerland) to assess cell counts in peritoneal lavage fluid (PLF) at the indicated time points after nonsevere CLP. (*B*) The total number of peritoneal macrophages was determined by gating on $CD11b^+F4/80^+$ cells. (*C*) The total number of peritoneal neutrophils was determined by gating on $CD11b^+Ly6G^+$ cells. (*D*) The total number of peritoneal T lymphocytes was determined by gating on $CD45^+CD3^+$ cells. (*E*) The total number of peritoneal regulatory T cells was determined by gating on $CD4^+CD25^+Foxp3^+$ cells. $^*P < 0.05$, $^{**}P < 0.01$ when compared with wild-type mice at the same time point after CLP (Mann–Whitney *U* test).

CCL2 and Macrophage Play an Important Role in Progranulin-mediated Protection against Sepsis

We next examined whether treatment with exogenous CCL2 may improve survival in progranulin-deficient mice after CLP-induced nonsevere sepsis. Treatment of progranulin-deficient mice with recombinant CCL2 significantly improved survival compared with progranulin-deficient mice treated with PBS control (Figure 8A). Similarly, CCL2 caused a significant increase in peritoneal macrophage recruitment (Figure 8B), as well as a significant decrease in peritoneal and blood bacterial counts (Figure 8C). These results suggest a recovery of CCL2-mediating macrophage recruitment to the primary site of infection and, consequently, for survival in progranulin-deficient mice during sepsis.

We further assessed the importance of macrophages in the survival of progranulin-treated septic wild-type mice after severe CLP, using cell depletion. Depleting macrophages by clodronate-encapsulated liposomes dramatically impaired the survival of septic mice receiving recombinant progranulin compared with mice treated with PBS-encapsulated liposomes as a control (Figure E5 and Figure 8D), and macrophage depletion also decreased bacterial clearance in the peritoneal cavity and in the blood (Figure 8E).

Progranulin Deficiency Does Not Influence Bacterial Phagocytosis and Killing by Phagocytes

Because phagocytes are the primary effector cells for bacterial clearance during sepsis (31), we investigated whether progranulin modulates the intrinsic antibacterial functions of peritoneal macrophages and neutrophils. There was no significant difference in bacterial phagocytosis and killing by progranulin-deficient and wild-type macrophages (Figures E6A and E6B). Also, preincubation with recombinant progranulin did not have direct effects on phagocytosis and intracellular killing of live *E. coli* by macrophages (Figures E6C and E6D). Likewise, progranulin-deficient neutrophils exhibit similar bacterial phagocytosis and killing capacities compared with wild-type neutrophils

Table 1. Cytokine and Chemokine Levels in Wild-Type and Progranulin-Deficient Mice during Cecal Ligation Puncture–induced Sepsis

	PLF		Lungs		Serum	
	Wild Type	PGRN$^{-/-}$	Wild Type	PGRN$^{-/-}$	Wild Type	PGRN$^{-/-}$
0 h						
TNF-α	5 (2–5)	5 (2–5)	30 (28–31)	32 (21–32)	5 (4–7)	6 (4–6)
IL-6	8 (5–9)	8 (5–11)	44 (36–53)	51 (31–64)	9 (6–10)	9 (8–11)
IFN-γ	26 (12–28)	25 (15–28)	112 (108–156)	121 (109–168)	66 (51–83)	67 (52–89)
IL-17	73 (66–93)	83 (61–85)	198 (131–205)	208 (176–216)	33 (28–41)	35 (33–68)
IL-1β	24 (16–30)	26 (19–28)	670 (551–734)	568 (517–712)	51 (47–62)	55 (51–60)
IL-10	228 (121–286)	221 (157–274)	843 (713–904)	811 (655–823)	111 (103–126)	116 (107–131)
CXCL1	14 (11–18)	15 (12–16)	1,931 (1,653–2,674)	1,555 (1,327–2,015)	22 (21–30)	28 (23–34)
CXCL10	3 (2–4)	3 (2–4)	12 (8–15)	11 (5–18)	28 (22–59)	33 (26–60)
CCL2	47 (34–77)	37 (31–56)	215 (201–327)	211 (195–226)	11 (5–46)	17 (5–28)
HMGB1	4.9 (4.3–5.3)	4.5 (4.1–5.1)	2.4 (2.1–3.5)	3.3 (2.1–4.2)	4.6 (3.2–5.5)	4.1 (3.3–5.5)
6 h						
TNF-α	11 (10–16)	21 (15–48)*	33 (31–34)	46 (32–86)*	11 (9–17)	17 (13–68)*
IL-6	1,057 (976–1,080)	1,017 (928–1,048)	760 (507–972)	1,250 (991–1,731)*	980 (779–982)	915 (705–988)
IFN-γ	76 (63–117)	88 (61–105)	141 (137–174)	156 (106–236)	131 (116–174)	156 (106–236)
IL-17	183 (173–199)	195 (162–217)	228 (211–263)	255 (211–276)	86 (74–90)	81 (72–87)
IL-1β	83 (78–104)	87 (77–109)	1,176 (981–1,418)	1,392 (915–2,112)	62 (51–77)	65 (58–73)
IL-10	350 (304–391)	363 (360–515)	1,101 (874–1,208)	1,065 (943–1,429)	155 (122–169)	162 (151–212)
CXCL1	756 (508–907)	891 (655–1,164)	7,255 (7,131–8,150)	7,285 (6,146–7,598)	1,440 (1,305–1,745)	1,533 (1,305–2,227)
CXCL10	288 (204–302)	219 (189–267)	112 (104–156)	87 (66–129)	135 (109–171)	135 (80–155)
CCL2	1,739 (1,289–1,816)	551 (348–625)†	1,130 (1,002–1,198)	1,171 (999–1,294)	233 (212–533)	337 (263–552)
HMGB1	6.4 (6.4–6.7)	6.2 (6.1–6.7)	3.4 (3.2–4.3)	5.1 (4.6–6.5)*	7.5 (6.5–8.2)	9.5 (9.4–9.9)*
24 h						
TNF-α	9 (6–10)	16 (11–22)*	33 (31–46)	38 (25–67)	8 (7–19)	42 (16–87)*
IL-6	934 (873–970)	881 (704–1,010)	262 (155–291)	273 (170–300)	422 (382–488)	51 (330–1,017)
IFN-γ	68 (56–112)	83 (51–228)	156 (122–183)	171 (111–255)	111 (91–117)	137 (62–225)
IL-17	126 (112–138)	131 (87–163)	228 (223–265)	231 (184–367)	66 (51–89)	89 (83–123)*
IL-1β	70 (62–98)	81 (47–83)	902 (755–1545)	997 (818–1,903)	63 (51–66)	62 (58–70)
IL-10	456 (356–512)	502 (414–677)	1,157 (963–1,265)	1,163 (961–1,555)	131 (122–151)	261 (216–299)*
CXCL1	450 (195–518)	383 (265–486)	3,611 (2,811–4,901)	3,837 (3,148–6,121)	249 (126–399)	316 (113–561)
CXCL10	163 (53–189)	89 (67–117)	67 (53–89)	63 (27–76)	143 (109–155)	126 (89–187)
CCL2	81 (51–104)	87 (67–122)	780 (763–865)	812 (661–991)	125 (5–146)	122 (5–125)
HMGB1	5.4 (5.1–6.1)	5.2 (4.5–5.7)	3.1 (2.5–3.2)	2.8 (2.3–3.1)	7.7 (6.5–7.9)	8.1 (6.1–8.8)

Definition of abbreviations: CCL2 = CC receptor ligand 2; CXCL = CXC receptor ligand; HMGB1 = high-mobility group box 1; PGRN = progranulin; PLF = peritoneal lavage fluid; TNF-α = tumor necrosis factor-α.

Data are expressed as the median (interquartile range) of n = 6 mice per group per time point. Measurements are expressed as picograms per milliliter, except for HMGB1, noted as nanograms per milliliter.

*$P < 0.05$, when compared with wild-type mice at the same time point (Mann–Whitney *U* test).

†$P < 0.001$, when compared with wild-type mice at the same time point (Mann–Whitney *U* test).

(Figures E7A and E7B). Preincubation with recombinant progranulin did not influence bacterial phagocytosis and killing in neutrophils on live *E. coli* infection (Figures E7C and E7D).

Discussion

The reported biological activities of progranulin include mainly the following: growth factor–like activities, modulation of immune responses, and neuronal effects (14). We here first found that circulating progranulin levels were highly elevated in patients with sepsis. Our present work on the levels of progranulin related to previous studies on progranulin, showing that several inflammatory disorders, such as systemic lupus erythematosus (26), rheumatoid arthritis and osteoarthritis (32), metabolic syndrome (33), dermatomyositis (34), and type 2 diabetes (35), were associated with elevated levels of progranulin. Interestingly, progranulin levels in pediatric cohorts were lower than those in adult cohorts, suggesting that progranulin production during sepsis may be, in part, age dependent.

We found that progranulin was required for protection against CLP-induced sepsis. Using bone marrow–chimeric mice, we identified that hematopoietic cells were the central cells producing progranulin during sepsis, and that hematopoietic-derived progranulin was important for host protection against sepsis. Therefore, the observed elevated systemic and local progranulin levels in patients and mice during sepsis can be explained by active release of progranulin from stimulated hematopoietic cells, including macrophages, T cells, dendritic cells, and other cells (14, 22). Likewise, one study also demonstrated that hematopoietic-derived progranulin was critical for protection against colitis (36). However, these findings should not belittle the role of nonhematopoietic progranulin,

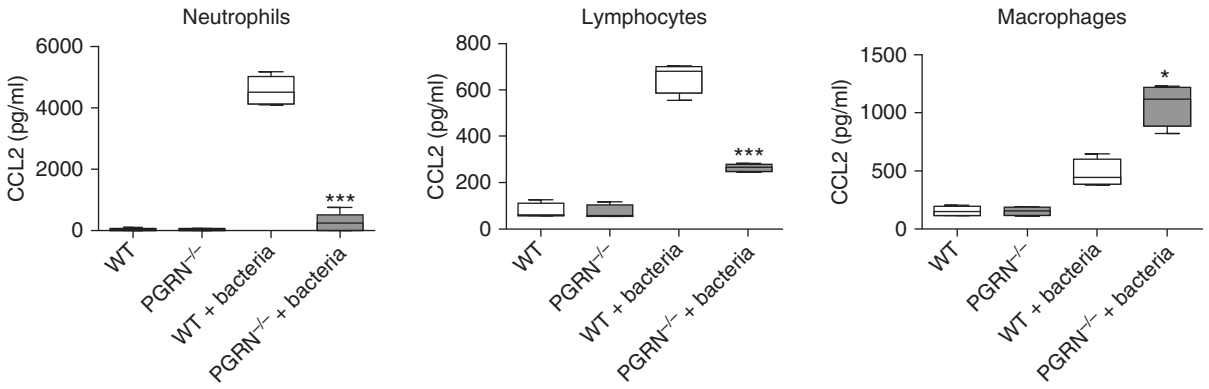

Figure 6. Dysregulated CC receptor ligand 2 (CCL2) production in progranulin-deficient ($PGRN^{-/-}$) cells on bacterial infection. Peritoneal macrophages and neutrophils, or spleen T lymphocytes (1×10^5 cells per group), were stimulated with heat-killed *Escherichia coli* (1×10^6 cfu) for 12 hours (n = 6 per group), and CCL2 levels were determined by ELISA. $^*P < 0.05$, $^{***}P < 0.001$ when compared with wild-type (WT) cells after infection (Mann–Whitney *U* test).

because epithelial cells could also express progranulin (22).

We showed an important role for progranulin in macrophage recruitment during sepsis. In mice infected with *Listeria monocytogenes*, an intracellular pathogen, fewer macrophages were detected in infected spleens of progranulin-deficient mice compared with wild-type mice (37). Progranulin concentrations were closely associated with macrophage infiltration into omental adipose tissue in obesity and type 2 diabetes (35). Progranulin also increased macrophage accumulation in the wound (24). Poor recruitment of peritoneal macrophages has been observed in formyl-peptide receptor 2/3–deficient animals in sepsis, which was functionally linked to inadequate bacterial removal (11).

In accordance with impaired macrophage recruitment, we have found that progranulin deficiency was associated with decreased local CCL2 production. Our results expanded data from Matsubara and colleagues (18), who showed that CCL2 expression induced by a high-fat diet was significantly down-regulated in adipose tissue of progranulin-deficient mice, as was found in progranulin-deficient adipocytes. However, our results contrasted with those of Yin and colleagues, who showed that progranulin-deficient mice had higher levels of CCL2 but fewer macrophages in infected spleens on *L. monocytogenes* infection (37). There are major differences that may account for the differential findings. First, we found that progranulin-deficient mice displayed significantly lower CCL2 levels compared with wild-type mice at the early time point of 6 hours after CLP, before differences in bacterial burden became apparent (data not shown), whereas Yin and coworkers demonstrated enhanced CCL2 levels in progranulin-deficient mice at 24 hours on infection with *L. monocytogenes* (37), which may appear to be the result of increased bacterial loads in progranulin-deficient mice. Indeed, there were similar levels of CCL2 in progranulin-deficient and wild-type mice 24 hours after CLP-induced sepsis, when bacterial loads were significantly higher in progranulin-deficient mice at this time point. Second, these authors reported that progranulin-deficient mice had higher CCL2 in the serum, spleen, and brain than did wild-type mice (37), whereas we found that progranulin-deficient mice only had lower CCL2 levels at the primary site of infection. Therefore, differences in anatomic site, infection time, and type of infecting agent may account for the varied effects of progranulin on CCL2 production among these studies.

We further confirmed that progranulin regulated CCL2 production in a cell type–dependent manner. Depletion of PGRN could impair CCL2 production in neutrophils and T lymphocytes, but not macrophages. To the best of our knowledge, this is the first report that PGRN deficiency impairs CCL2 production by neutrophils and T lymphocytes. CCL2 treatment has been shown to protect against sepsis by recruiting macrophages (38, 39). CCL2-mediated macrophage recruitment also contributed to protection against pneumococcal infection in the lungs (40). Overall, progranulin is necessary for protection against sepsis by promoting local macrophage recruitment, which is associated with early and local CCL2 production.

Considering that the current treatment of sepsis is largely supportive, definitive therapies remain elusive (41). One promising approach for antisepsis therapy involves host-directed immunomodulatory therapies, whereby natural mechanisms in the host are exploited to enhance therapeutic benefit (42). Here, except that progranulin treatment restored protection against low-grade (low-lethality) sepsis caused by nonsevere CLP in progranulin-deficient mice, one of the most striking findings was the therapeutic efficacy of progranulin in rescuing wild-type mice from high-grade sepsis caused by severe CLP (high lethality). Because progranulin production was naturally up-regulated in human patients with sepsis and one study has suggested that the half-life of progranulin is about 40 hours (20, 43), a similar approach should translate into the treatment of severe sepsis in humans with a clinical study of progranulin administration.

There are a few important limitations to this study. First, the number of patients with sepsis in this study was relatively

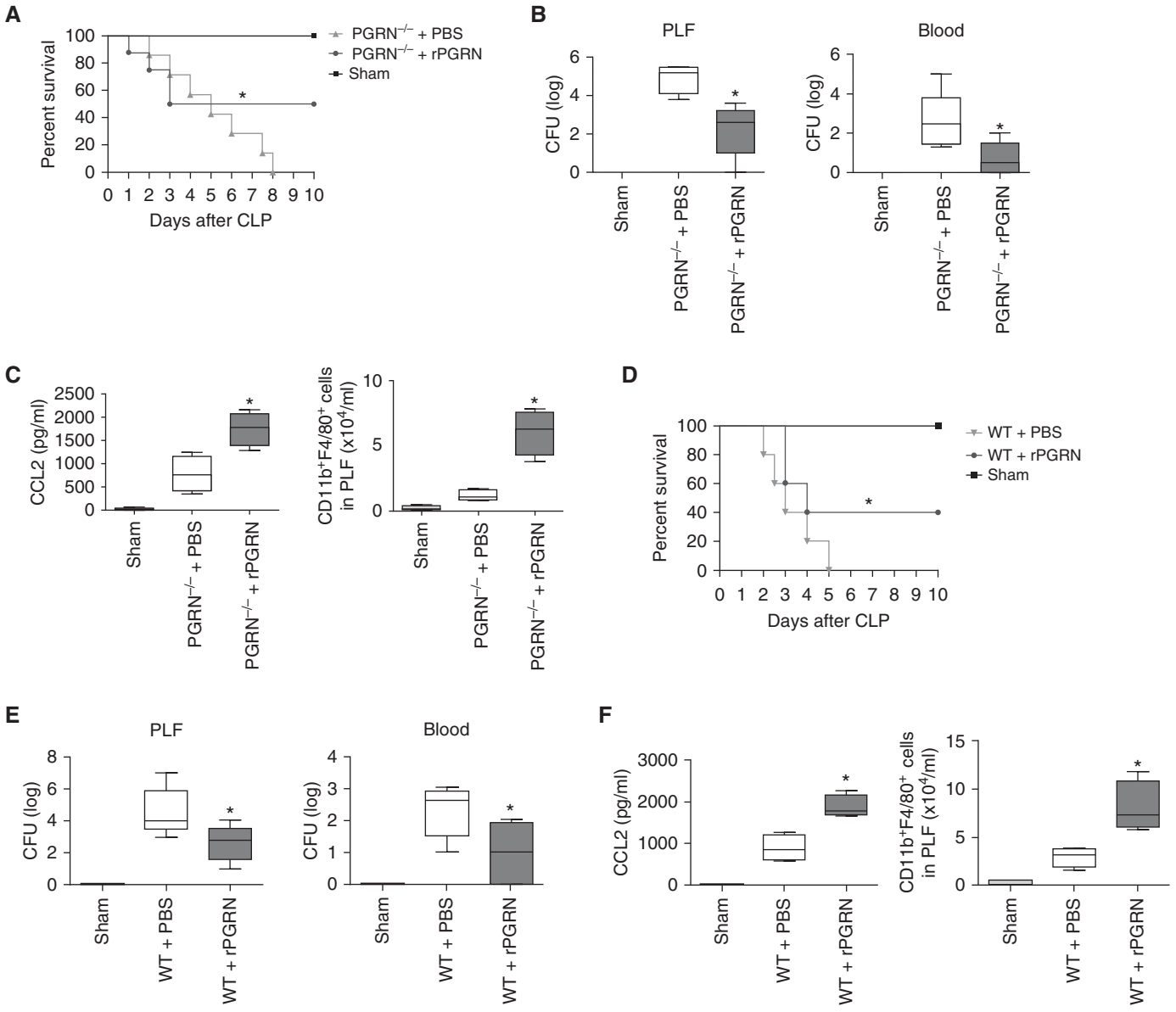

Figure 7. Progranulin treatment enhances protection against sepsis. (*A*) Progranulin-deficient ($PGRN^{-/-}$) mice (n = 12 per group) were injected intraperitoneally with 20 μg of mouse recombinant progranulin (rPGRN) or phosphate-buffered saline (PBS) control 2 hours after nonsevere cecal ligation puncture (CLP) with a 26-gauge needle, and survival was monitored. Comparison between groups was done by Kaplan–Meier analysis followed by log-rank tests. *$P < 0.05$ when compared with $PGRN^{-/-}$ mice treated with PBS control. (*B*) Bacterial counts in peritoneal lavage fluid (PLF) and blood from $PGRN^{-/-}$ mice (n = 5 per group) treated with or without recombinant progranulin 24 hours after nonsevere CLP. *$P < 0.05$ when compared with $PGRN^{-/-}$ mice treated with PBS control (Mann–Whitney *U* test). (*C*) CC receptor ligand 2 (CCL2) release and $CD11b^{+}F4/80^{+}$ macrophage counts in PLF from $PGRN^{-/-}$ mice (n = 5 per group) treated with or without PGRN 24 hours after nonsevere CLP. *$P < 0.05$ when compared with $PGRN^{-/-}$ mice treated with PBS control (Mann–Whitney *U* test). (*D*) Wild-type (WT) mice (n = 12 per group) were injected intraperitoneally with 20 μg of mouse recombinant progranulin or PBS control 2 hours after severe CLP was induced with a 21-gauge needle, and survival was monitored. Comparison between groups was done by Kaplan–Meier analysis followed by log-rank tests. *$P < 0.05$ when compared with wild-type mice treated with PBS control. (*E*) Bacterial counts in PLF and blood from wild-type mice (n = 5 per group) treated with or without recombinant progranulin 24 hours after severe CLP. *$P < 0.05$ when compared with wild-type mice treated with PBS control (Mann–Whitney *U* test). (*F*) CCL2 release and $CD11b^{+}F4/80^{+}$ macrophage counts in PLF from wild-type mice (n = 5 per group) treated with or without recombinant progranulin 24 hours after severe CLP. *$P < 0.05$ when compared with wild-type mice treated with PBS control (Mann–Whitney *U* test). CFU = colony-forming units.

small, and thus the diagnostic and prognostic value of progranulin should be further established in a larger-size clinical trial. Second, it remains to be determined whether the observed effects of progranulin deficiency in CLP-induced sepsis also apply to pneumonia-caused sepsis (44). Furthermore, the receptor(s) for progranulin and the mechanisms by which progranulin regulates CCL2 production in sepsis remain unknown. So far, there are more than 20 proteins that

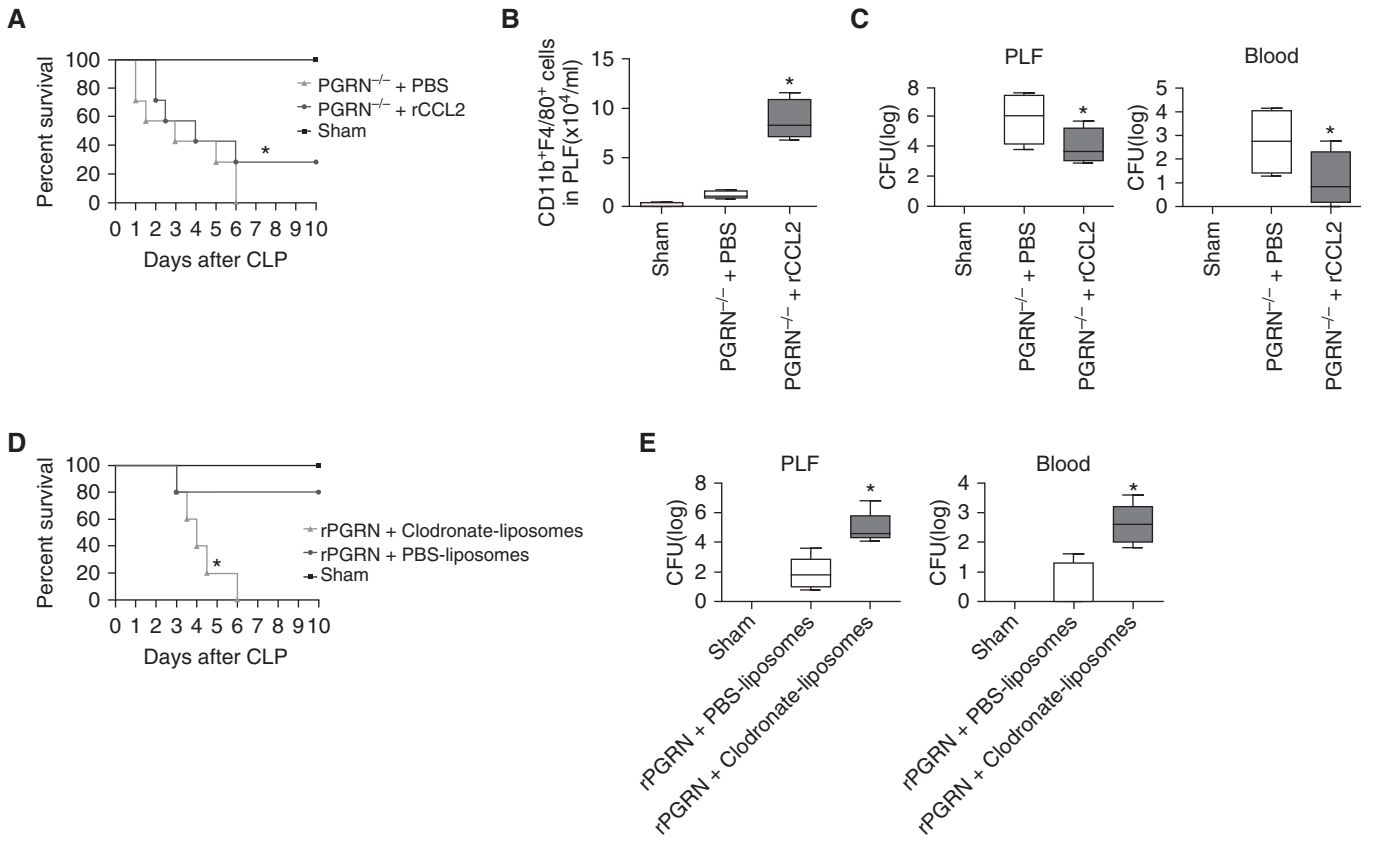

Figure 8. CC receptor ligand 2 and macrophages are involved in progranulin-mediated host defense to sepsis. (*A*) Progranulin-deficient ($PGRN^{-/-}$) mice (n = 12 per group) were injected intraperitoneally with 500 ng of mouse recombinant CC receptor ligand 2 (rCCL2) or phosphate-buffered saline (PBS) control immediately at the time of cecal ligation puncture (CLP) with a 26-gauge needle, and then intraperitoneally with 250 ng per mouse 24 and 48 hours after surgery, and survival was monitored. Comparison between groups was done by Kaplan–Meier analysis followed by log-rank tests. *$P < 0.05$ when compared with $PGRN^{-/-}$ mice treated with PBS control. (*B*) $CD11b^{+}F4/80^{+}$ macrophage counts in peritoneal lavage fluid (PLF) from $PGRN^{-/-}$ mice (n = 5 per group) treated with or without rCCL2 24 hours after nonsevere CLP. *$P < 0.05$ when compared with $PGRN^{-/-}$ mice treated with PBS control (Mann–Whitney *U* test). (*C*) Bacterial counts in PLF and blood from $PGRN^{-/-}$ mice (n = 5 per group) treated with or without rCCL2 24 hours after nonsevere CLP. *$P < 0.05$ when compared with $PGRN^{-/-}$ mice treated with PBS control (Mann–Whitney *U* test). (*D*) Wild-type mice (n = 12 per group) depleted of macrophages by clodronate liposomes were injected intraperitoneally with 20 μg of mouse recombinant progranulin (rPGRN) 2 hours after severe CLP with a 21-gauge needle, and survival was monitored. Comparison between groups was done by Kaplan–Meier analysis followed by log-rank tests. *$P < 0.05$ when compared with wild-type mice treated with PBS liposomes as a control. (*E*) Bacterial counts in PLF and blood from wild-type mice (n = 5 per group) with or without macrophage depletion 24 hours after severe CLP. *$P < 0.05$ when compared with wild-type mice treated with PBS liposomes as a control (Mann–Whitney *U* test). CFU = colony-forming units.

have been reported to bind with progranulin (14, 22). One interesting possibility is that interaction between progranulin and its associated proteins (receptors) at different levels, ranging from extracellular fluid and extracellular matrix to intracellular components, including the cytoplasm and nucleus, contributes to a fine regulation of CCL2 production in neutrophils, T lymphocytes, or macrophages. Previous studies have shown that progranulin could be digested by neutrophil proteinases, leading to the release of granulin (GRN) peptides and increased CXCL8 expression (45, 46). Furthermore, progranulin may participate in infectious diseases by enhancing TLR9 signaling as a cofactor (25, 26). However, TLR9-deficient mice had lower mortality in a CLP model (41), suggesting that progranulin and TLR9 have different roles in the pathogenesis of sepsis. In any case, the multiple functions of progranulin might be mediated by binding with different partners in a cell-specific or disease-specific manner.

In conclusion, progranulin is a protective mediator in sepsis by promoting macrophage recruitment through the enhancement of local CCL2 production. Our data provide *in vivo* proof of concept for a progranulin administration strategy that manipulates protective host defense responses and represents a feasible alternative to conventional chemotherapy in the treatment of sepsis. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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