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Neutrophils as regulators of macrophage-induced inflammation in a setting of allogeneic bone marrow transplantation

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SUMMARY

Clinical data reveal that patients with allogeneic hematopoietic stem cell transplantation (HSCT) are vulnerable to infection and prone to developing severe sepsis, which greatly compromises the success of transplantation, indicating a dysregulation of inflammatory immune response in this clinical setting. Here, by using a mouse model of haploidentical bone marrow transplantation (haplo-BMT), we found that uncontrolled macrophage inflammation underlies the pathogenesis of both LPS- and *E.coli*-induced sepsis in recipient animals with graft-versus-host disease (GVHD). Deficient neutrophil maturation in GVHD mice post-haplo-BMT diminished modulation of macrophage-induced inflammation, which was mechanistically dependent on MMP9-mediated activation of TGF-β1. Accordingly, adoptive transfer of mature neutrophils purified from wild-type donor mice inhibited both sterile and infectious sepsis in GVHD mice post-haplo-BMT. Together, our findings identify a novel mature neutrophil-dependent regulation of macrophage inflammatory response in a haplo-BMT setting and provide useful clues for developing clinical strategies for patients suffering from post-HSCT sepsis.

INTRODUCTION

Sepsis is defined as a dysfunction of the immune response to infection that is life-threatening due to overwhelming systemic inflammation and multiple organ dysfunctions (Singer et al., 2016). Innate immune cells recognize pathogen-associated molecule patterns (PAMPs) via the expression of pattern recognition receptors (PPRs) and are the main proinflammatory cytokine-producing cells during the process of sepsis. Macrophages are of great interest in innate immunity and play multiple roles, from initiation of inflammatory responses to resolution of inflammation (Epelman et al., 2014). Macrophages could produce a number of proinflammatory cytokines and chemokines that aggravate inflammatory responses and benefit the elimination of evading pathogens at early stages of infection (Hamidzadeh et al., 2017; Kawai and Akira, 2011; Lauvau et al., 2015). However, an unbalanced immune response in inflammation causes a remarkable production of multiple cytokines, termed a "cytokine storm," which could further lead to lethal sepsis (Delano and Ward, 2016; Nathan and Ding, 2010; Rittirsch et al., 2008). Modulation of macrophage function has been shown to have a promising effect in reducing systemic inflammation during bacterial clearance and improves survival of bacterial sepsis (Belikoff et al., 2011; Huang et al., 2009; Patoli et al., 2020).

Patients receiving hematopoietic stem cell transplantation (HSCT) are susceptible to infectious diseases, which are considered to be associated with the failure of immune reconstitution and incompetent immune regulation post-HSCT. Although the neutropenic phase remains a major risk of sepsis at early stages post-HSCT, HSCT recipients beyond the period of neutropenia are also vulnerable to bacterial infection, indicating a long-term dysfunction of donor-derived innate immunity against infection (Domingo-Gonzalez and Moore, 2013; Espinoza et al., 2018). It has been reported that severe sepsis occurs at a relatively high frequency among HSCT patients, especially for those under graft-versus-host disease (GVHD) conditions, and is associated with worse outcomes (Kumar et al., 2015). Neutrophils are well known for their essential roles in innate defense against various pathogens (Mayadas et al., 2014). Recently, neutrophils have been found to play regulatory roles in both innate and adaptive immune responses (Reber et al., 2017; Marwick et al., 2018; Germann et al., 2020). As they are the most rapidly reconstituted innate immune cells after HSCT, the number of circulating neutrophils in HSCT patients has long been used as an indicator for effective engraftment of donor cells in recipient marrows (Storek, 2008; Tecchio and Cassatella, 2021). Although neutrophils perform multifaceted functions in inflammation, studies of neutrophil functions and their roles during sepsis post-HSCT are still lacking.

Immune cells are reconstituted with different patterns post-HSCT, which provides a unique opportunity to understand how an unbalanced immune response to inflammatory stimuli may affect sepsis development. In the current study, we used a mouse model of haploidentical bone marrow transplantation (haplo-BMT) and investigated both lipopolysaccharide (LPS)- and *E. coli*-induced inflammatory responses in recipient animals under both GVHD and non-GVHD conditions. We found that, compared with non-GVHD mice, GVHD mice were markedly vulnerable to LPS challenge and *E. coli* infection and developed

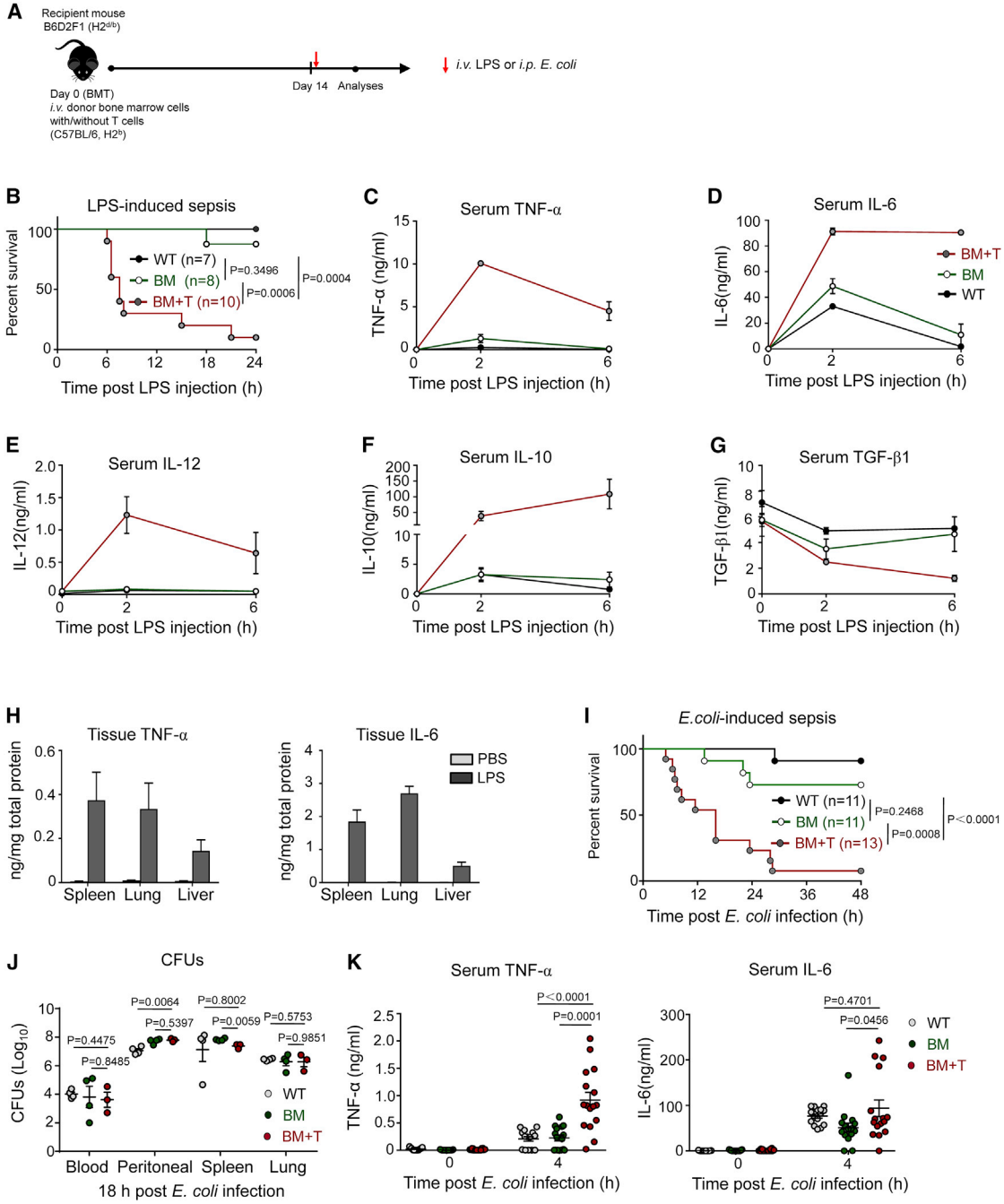

Figure 1. GVHD mice have increased susceptibility to LPS- and *E. coli*-induced sepsis

(A) A schematic of the BMT strategy and induction of sepsis after BMT. At 14 days after BMT, allogeneic recipient mice with or without GVHD were injected intravenously (i.v.) with 1 mg/kg LPS. Untransplanted WT C57BL/6 mice were injected with 1 mg/kg LPS as a control.

(B) The survival of these mice after LPS administration was monitored. Comparison of survival between each group was performed by log-rank (Mantel-Cox) test.

(C–G) Serum cytokines were measured by quantitative ELISA at 2 and 6 h after LPS administration. Serum samples before LPS injection were included as controls. Representative results of three independent experiments are shown. Data are presented as the mean ± SEM with at least three mice in each group.

(H) At 2 h after LPS administration, the mice were sacrificed, and spleens, lungs, and livers were harvested and homogenized in PBS. The content of TNF-α and IL-6 in tissues was measured by quantitative ELISA and normalized to total protein in tissues. Data are presented as the mean ± SEM with three mice in each group.

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severe sepsis that was attributable to macrophage-dominant inflammation. Impaired reconstitution and functional maturation of neutrophils post-haplo-BMT act as a mechanism underlying the dysregulation of macrophage-induced inflammation in GVHD mice. We also present evidence that mature neutrophils regulate macrophage inflammation via MMP9-mediated activation of TGF-β1.

RESULTS

Uncontrollable inflammatory responses in GVHD mice after haplo-BMT

To investigate inflammatory immune responses after BMT, we first established a mouse model of haplo-BMT (B6 → B6D2F1) in which B6D2F1 mice were lethally irradiated followed by infusion with donor-derived T-cell-depleted bone marrow (TCD-BM). To induce GVHD in allogeneic recipient mice, splenic T cells purified from donor B6 mice were transferred together with TCD-BM cells. On day 14 post-haplo-BMT, allogeneic recipients that received both TCD-BM and T cells showed signs of GVHD, evident by enlarged spleens containing increased T cell infiltration (Figures S1A–S1C), as well as elevated serum IFN-γ level, compared with non-GVHD recipients (Figure S1D). To compare the inflammatory responses in haplo-BMT mice, we utilized an LPS-induced sepsis model by intravenously injecting a low dose of LPS (1 mg/kg body weight) on day 14 post-haplo-BMT (Figure 1A). As shown in Figure 1B, a low-dose LPS administration was fatal in GVHD mice (9 of 10 mice died within 24 h of LPS injection), while the majority of non-GVHD mice (7 of 8 mice) and all un-transplanted wild-type (WT) mice survived the same dose of LPS administration. We further analyzed proinflammatory cytokines in sera after LPS challenge by quantitative ELISA. As shown in Figures 1C–1E, LPS induced dramatically higher levels of TNF-α, IL-6, and IL-12 in GVHD mice, indicators of the cytokine storm, compared with non-GVHD mice and untransplanted WT mice. We also found an elevated level of serum IL-10, a classical immune-regulatory cytokine, in GVHD mice (Figure 1F), indicating a dysfunction of immune regulation in these recipient animals post-haplo-BMT. Interestingly, a remarkably decreased level of serum TGF-β1 was observed in GVHD mice after LPS administration (Figure 1G), compared with that in non-GVHD mice and untransplanted WT mice. The inflammatory response in septic GVHD mice was systemic, as increased levels of TNF-α and IL-6 production were found in spleens, lungs, and livers 2 h after LPS injection (Figure 1H).

To further extend our observation of inflammatory responses in GVHD mice, we established an *E. coli*-induced peritonitis model in recipient animals on day 14 post-haplo-BMT (Figure 1A). While the majority of untransplanted WT mice and non-GVHD mice survived a 3×10^{10} colony-forming units (CFU)/kg *E. coli* infection, GVHD mice exhibited vulnerability to *E. coli* infection, which induced 92.3% mortality in 48 h (Figure 1I). These results were not explained by differences in controlling bacteria at the primary site of infection and bacterial propagation, because comparable bacterial CFU were found in peritoneal cavity, blood, spleen, and lung between non-GVHD and GVHD mice 18 h after *E. coli* infection (Figure 1J). Notably, *E. coli* infection induced significantly increased TNF-α and IL-6 in sera of GVHD mice, compared with non-GVHD mice (Figure 1K), suggesting an inflammatory cytokine storm was associated with mortality of septic GVHD mice post-haplo-BMT. Collectively, these data indicate that GVHD mice have uncontrolled inflammatory responses in both sterile and bacterial sepsis after haplo-BMT.

Post-haplo-BMT sepsis in GVHD mice is mediated by macrophage inflammation

To investigate which cell subset contributes to the production of proinflammatory cytokines in GVHD mice, sepsis was induced by injecting LPS into recipient mice on day 14 post-haplo-BMT, and intracellular cytokine analyses of immune cells in spleens were performed 2 h later by flow cytometry. As shown in Figures 2A and 2B, splenic macrophages and monocytes, but not dendritic cells (DCs), were hyperactive cell subsets producing TNF-α and IL-6 in GVHD mice after LPS challenge. To further confirm this finding and investigate the role of macrophages in post-haplo-BMT sepsis, we used clodronate liposomes to deplete macrophages *in vivo* in GVHD mice before the induction of LPS sepsis (Figure 2C). Macrophage depletion significantly decreased levels of serum TNF-α and IL-6 after LPS injection in GVHD mice, but only slightly increased survival at early times, compared with macrophage-replete GVHD mice (Figures 2D and 2E). Consistently, macrophage depletion showed beneficial effects in *E. coli*-induced sepsis in terms of reduction of proinflammatory cytokines in sera and increase survival of GVHD mice after *E. coli* infection

(I) At 14 days after BMT, allogeneic recipient mice were injected intraperitoneally (i.p.) with 3×10^{10} CFU/kg *E. coli*. Untransplanted WT C57BL/6 mice injected with 3×10^{10} CFU/kg *E. coli* were used as controls. The survival of these mice after *E. coli* infection was monitored afterward. Comparison of survival between each group was performed by log-rank (Mantel-Cox) test.

(J) At 18 h after *E. coli* injection, the mice were sacrificed, and CFU of bacteria in blood, peritoneal cavity, spleens, and lungs were quantified by limited dilution of plating. Each dot represents one mouse in each group. Data are presented as the mean ± SEM.

(K) At 4 h after *E. coli* injection, TNF-α and IL-6 in serum were measured by quantitative ELISA. Data are presented as the mean ± SEM.

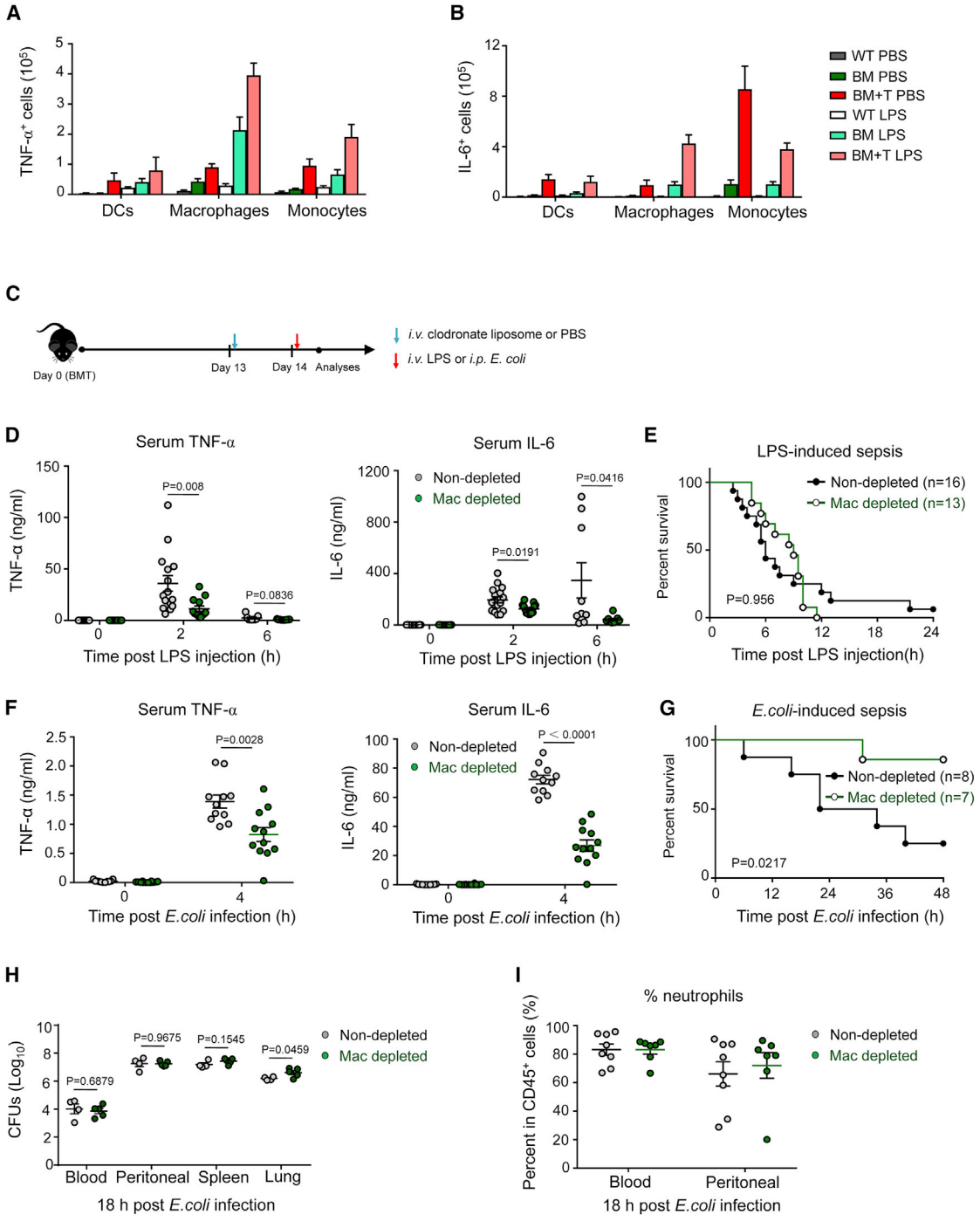

Figure 2. Macrophages are dominant inflammatory cells during sepsis post-haplo-BMT

(A and B) At 14 days after BMT, allogeneic recipient mice were injected i.v. with 1 mg/kg LPS. Untransplanted WT C57BL/6 mice were injected with 1 mg/kg LPS as control. Two hours later, mice injected with LPS or PBS control were sacrificed for spleens. Splenocytes were cultured *in vitro* in the presence of Brefeldin A for 4 h. Then cells were harvested for intracellular staining. Total numbers of TNF-α- or IL-6-positive DCs, macrophages, and monocytes in spleens are shown. Data are presented as the mean ± SEM (n = 4).

(C) A schematic of macrophage depletion and induction of sepsis after BMT. At 13 days after BMT, GVHD mice were injected with 200 μL clodronate liposome or PBS i.v., and 24 h later, these mice were injected i.v. with 1 mg/kg LPS.

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(Figures 2F and 2G). The improved survival rate of macrophage-depleted GVHD mice was not correlated with the control of infection in primary sites of infection or bacterial propagation, because the bacterial loads in the peritoneal cavity, blood, spleen, and lung between macrophage-replete and -depleted GVHD mice were not significantly different (Figure 2H). Notably, neutrophil response to infection was also unchanged after macrophage depletion in GVHD mice, evident by similar frequencies of neutrophils in both blood and peritoneal lavage 18 h after infection (Figure 2I). Together, these data indicate that infection-induced mortality of GVHD mice was mostly attributed to macrophage-derived inflammatory cytokine storm, rather than the control of bacterial proliferation and propagation.

Neutrophils inhibit LPS- and *E. coli*-induced sepsis in GVHD mice

Neutrophils are a heterogeneous cell population and were found to play regulatory roles in inflammatory responses (Mortaz et al., 2018; Rosales, 2018). Monoclonal antibodies against Gr-1 or Ly6G, which recognize different antigen epitopes on neutrophils, have been extensively used for *in vivo* neutrophil depletion (Coffelt et al., 2015; Engblom et al., 2017; Bansal et al., 2018; Szczerba et al., 2019). Administration of either antibody into GVHD mice could effectively deplete neutrophils in 24 h (Figures S2A and S2B), without perturbing the number of splenic macrophages (Figure S2C). To study the function of neutrophils in sepsis post-haplo-BMT, we administered anti-Gr-1 to deplete neutrophils in GVHD mice 1 day before the induction of LPS sepsis (Figure 3A). Of interest, anti-Gr-1-mediated neutrophil depletion significantly elevated levels of serum TNF-α and IL-6 in GVHD mice following LPS injection (Figure 3B) and slightly reduced the survival time of recipient mice (Figure 3C). However, WT mice with neutrophil depletion were still resistant to LPS challenge (Figure S3A), while neutrophil-depleted non-GVHD mice showed slightly, but not significantly, increased production of serum inflammatory cytokines after LPS injection (Figure S3B). These data suggested a regulatory role for neutrophils in sepsis post-haplo-BMT. To further confirm this finding, we isolated neutrophils from bone marrow of WT B6 mice by Percoll gradient centrifugation and performed an *in vivo* experiment (Figure 3D). Isolated neutrophils (over 90% purity) were identified to have a maturation phenotype by cell-surface expression of Ly6G and CXCR2, a hallmark molecule for neutrophil maturation and emigration from bone marrow (Evrard et al., 2018) (Figure S4A). Adoptive transfer of mature neutrophils into GVHD mice significantly reduced LPS-induced serum TNF-α levels (Figure 3E), although the survival did not show any significant difference between septic GVHD mice that received neutrophils or not (Figure 3F). We next performed a neutrophil adoptive transfer experiment in the *E. coli*-induced mouse sepsis model (Figure 3D). While neutrophil adoptive transfer had no impact on reducing bacterial counts in blood samples of recipient mice (data not shown), it significantly decreased proinflammatory cytokine production in sera of GVHD mice (Figure 3G) and increased their survival after *E. coli* infection (Figure 3H). Next we asked whether the regulatory effect of neutrophils in sepsis is dependent on macrophage inflammation. To this end, we used anti-Gr-1 and/or clodronate liposomes to deplete neutrophils and/or macrophages in GVHD mice before induction of LPS sepsis (Figure 3I). While depletion of macrophages abolished inflammatory responses after LPS administration, elimination of neutrophils in the absence of macrophages did not increase the production of serum TNF-α and IL-6 in GVHD mice post-haplo-BMT (Figure 3J). These data indicate that mature neutrophils could regulate macrophage-mediated inflammatory responses in sepsis post-haplo-BMT.

Abnormal granulopoiesis and deficient neutrophil maturation in haplo-BMT mice

The finding that neutrophils could mediate immune regulation on macrophage activation *in vivo* prompted us to

(D) Serum TNF-α and IL-6 were measured by quantitative ELISA 2 and 6 h after LPS administration. Serum samples before LPS injection were included as controls. Data shown are the pooled results of three independent experiments with at least four mice per group in each experiment. Data are presented as the mean ± SEM.

(E) Survival of the mice after LPS administration was monitored. Comparison of survival between two groups was performed by log-rank (Mantel-Cox) test.

(F) At 13 days after BMT, GVHD mice were injected i.v. with 200 μL clodronate liposome or PBS, and 24 h later, these mice were injected i.v. with 3×10^{10} CFU/kg *E. coli*. Serum TNF-α and IL-6 were measured by quantitative ELISA 4 h after *E. coli* infection. Data shown are the pooled results of two independent experiments with at least five mice per group in each experiment. Data are presented as the mean ± SEM.

(G) Survival of the mice after *E. coli* infection was monitored afterward. Comparison of survival between two groups was performed by log-rank (Mantel-Cox) test.

(H) At 18 h after *E. coli* injection, mice were sacrificed and CFU of bacteria in blood, peritoneal cavity, spleen, and lung were quantified by limited dilution of plating. Each dot represents one mouse in each group. Data are presented as the mean ± SEM.

(I) At 18 h after *E. coli* injection, the percentages of neutrophils in blood and peritoneal cavity were analyzed by fluorescence-activated cell sorting (FACS). Data are presented as the mean ± SEM.

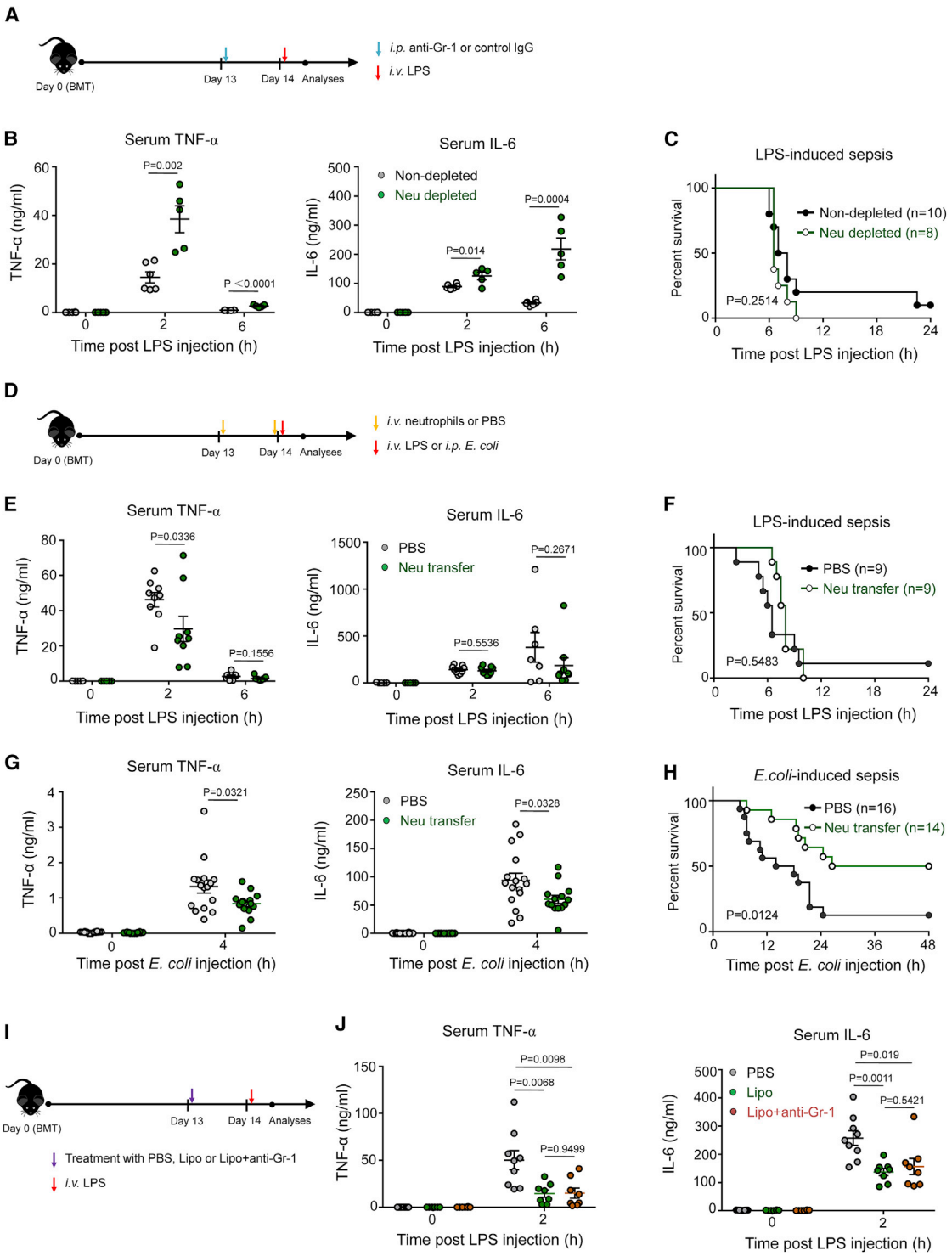

Figure 3. Neutrophils regulate macrophage-mediated sepsis in GVHD mice

(A) A schematic of neutrophil depletion and induction of sepsis after BMT. At 13 days after BMT, GVHD mice were injected i.p. with 250 μg anti-Gr-1 or isotype control antibody in 200 μL PBS, and 24 h later, these mice were injected i.v. with 1 mg/kg LPS.

(B) Serum TNF-α and IL-6 were measured by quantitative ELISA 2 and 6 h after LPS injection. Serum samples before LPS injection were included as controls. A representative result of two independent experiments is shown. Data are presented as the mean ± SEM (n = 5).

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investigate the granulopoiesis in recipient mice haplo-BMT. We first quantified the number of total $CD11b^+Gr\text{-}1^+$ neutrophils in both bone marrow and periphery of haplo-BMT recipient mice by multiple-color flow cytometry (Figures S4B–S4D). A preconditioning regimen and GVHD resulted in neutropenia 7 days post-haplo-BMT, evidenced by reduced number of neutrophils in spleens of recipient mice receiving bone marrow plus T cell (BM + T) grafts (Figure S5), which was followed by rapid granulopoiesis, and the numbers of neutrophils in spleens and blood of both GVHD and non-GVHD mice were significantly higher by day 14 compared with those in untransplanted WT mice (Figure 4A). However, granulopoiesis in bone marrow was still deficient on day 14, evidenced by decreased numbers of neutrophils as well as reduced percentages and total numbers of $Lin^-Sca\text{-}1^+c\text{-}kit^+$ cells (LSKs) and hematopoietic stem cells (HSCs) in bone marrow of haplo-BMT recipient mice (Figures 4A–4C). Neutrophils are developmentally differentiated in bone marrow from precursors and released into the peripheral circulation after phenotypic maturation. Although the capacity of producing neutrophils in bone marrow of GVHD mice post-haplo-BMT was greatly reduced, neutrophil maturation in the bone marrow of these mice did not show obvious changes compared with untransplanted WT mice (Figure 4D). In contrast, significantly increased percentages of immature neutrophils were found in spleens of both GVHD and non-GVHD mice on day 14 post-haplo-BMT (Figure 4D). Immature neutrophils also circulated in peripheral blood, and we observed 3.93- and 1.46-fold increases in percentage of immature neutrophils in GVHD and non-GVHD mice, respectively (Figure 4D). These findings suggested abnormal granulopoiesis and deficiency of neutrophil maturation post-haplo-BMT, especially in recipient mice suffering from GVHD. Spleen has been reported to be an important organ for extramedullary hematopoiesis during bone marrow failure, tissue inflammation, injury, and other pathogenic conditions (Chiu et al., 2015; Johns and Christopher, 2012; Yamamoto et al., 2016). We found that after haplo-BMT, LSKs in spleens of GVHD mice significantly increased in both percentage and absolute number, compared with those in non-GVHD mice and untransplanted WT mice (Figures 4E and 4F). Taken together, these findings indicated that insufficient granulopoiesis in bone marrow was associated with extramedullary granulopoiesis and deficient neutrophil maturation post-haplo-BMT.

Mature neutrophils inhibit macrophage inflammation via MMP9-mediated activation of TGF-β1

The finding that neutrophils regulated macrophage inflammation *in vivo* prompted us to explore its underlying immunological mechanism. Thioglycolate-elicited peritoneal macrophages and isolated mature neutrophils from bone marrow were cultured in the presence of LPS *in vitro*. Neutrophils express relatively low levels of LPS receptor/CD14 (Sabroe et al., 2002; Murdoch et al., 2008; White et al., 2014) and were barely responsive to LPS stimulation, in contrast to macrophages, which produce high levels of TNF-α and IL-6 (Figure 5A). Interestingly, the activation of macrophages was remarkably inhibited when they were co-cultured with purified mature neutrophils *in vitro* (Figure 5A). It was reported that macrophage-mediated inflammatory responses could be regulated by CD14-dependent uptake of apoptotic neutrophils (Poon et al., 2014; Ren et al., 2008). To assess whether neutrophils regulate macrophage activation in a cell contact-dependent or independent manner, macrophages were cultured with mature neutrophils either separated by a 0.4 μm transwell

(C) Survival of the mice after LPS administration was monitored. Comparison of survival between two groups was performed by log-rank (Mantel-Cox) test.
(D) A schematic of neutrophil adoptive transfer and induction of sepsis after BMT. At 14 days after BMT, GVHD mice were injected i.v. with 1 mg/kg LPS to induce sepsis. At 24 and 1 h before LPS injection, 5×10^6 neutrophils were adoptively transferred or not into GVHD mice.
(E) Serum TNF-α and IL-6 were measured by quantitative ELISA 2 and 6 h after LPS administration. Serum samples before LPS injection were included as controls. A representative result of two independent experiments is shown. Data are presented as the mean ± SEM (n = 5).
(F) Survival of GVHD mice that received neutrophils through adoptive transfer or not was monitored after LPS injection. Comparison of survival between two groups was performed by log-rank (Mantel-Cox) test.
(G) At 14 days after BMT, GVHD mice were injected i.p. with 3×10^{10} CFU/kg *E. coli*. At 24 and 1 h before infection, 5×10^6 neutrophils were adoptively transferred or not into GVHD mice. Serum TNF-α and IL-6 were measured by quantitative ELISA 4 h after *E. coli* infection. Data shown are pooled results of two independent experiments with at least four mice per group in each experiment. Data are presented as the mean ± SEM.
(H) Survival of GVHD mice that received neutrophils through adoptive transfer or not was monitored after *E. coli* infection. Comparison of survival between two groups was performed by log-rank (Mantel-Cox) test.
(I) A schematic of macrophage and neutrophil depletion and induction of sepsis after BMT. At 13 days after BMT, GVHD mice were injected i.v. with 200 μL clodronate liposomes alone or together with 250 μg anti-Gr-1 in 200 μL PBS i.p. GVHD mice that received PBS were included as controls. Twenty-four hours later, these mice were injected i.v. with 1 mg/kg LPS to induce sepsis.
(J) Serum TNF-α and IL-6 were measured by quantitative ELISA 2 h after LPS administration. Data are presented as the mean ± SEM.

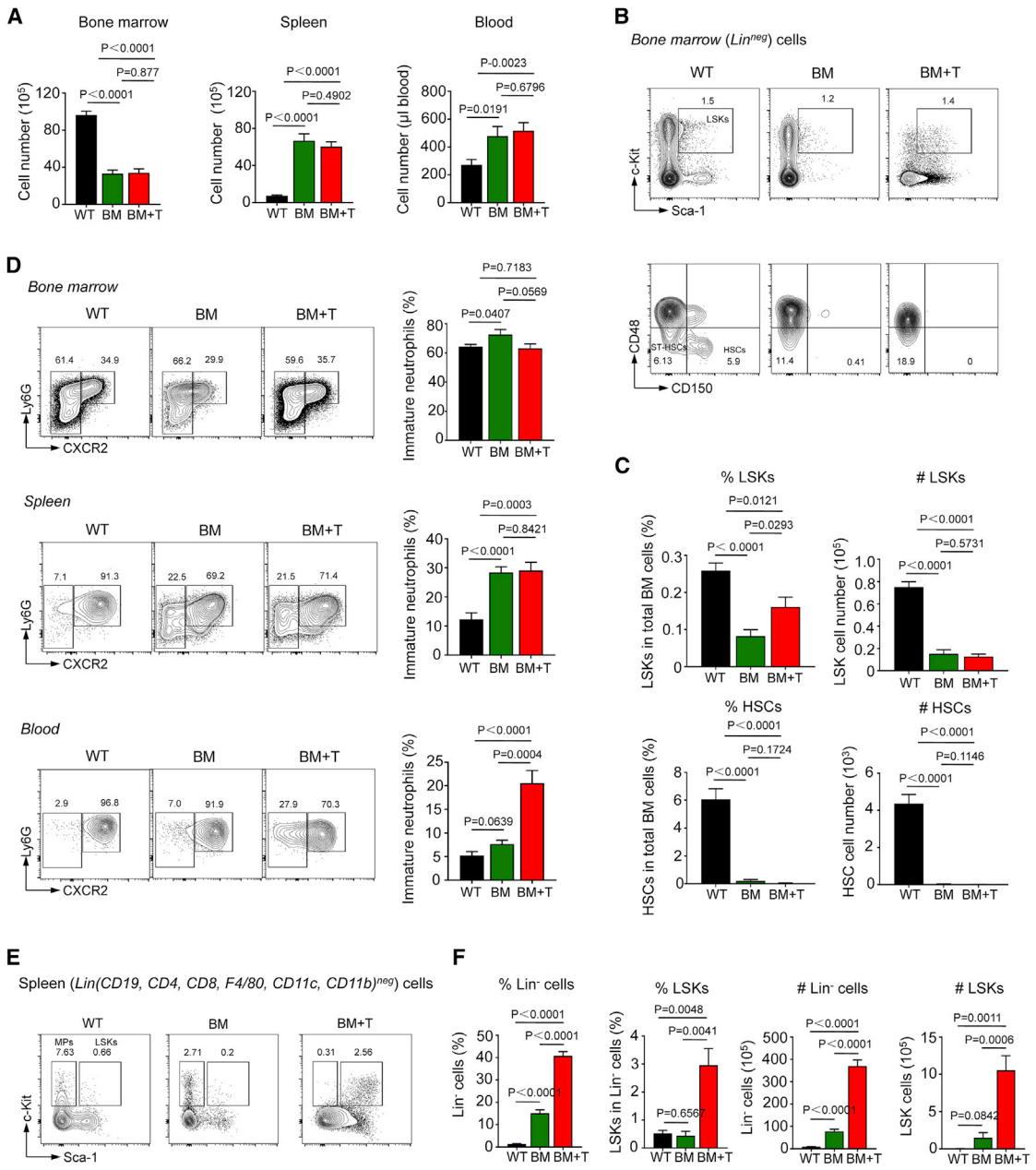

Figure 4. Abnormal granulopoiesis in GVHD mice

(A) At 14 days after BMT, $CD11b^{+}Gr\text{-}1^{+}$ neutrophils in bone marrow, spleen, and blood were enumerated in allogeneic recipient mice that received TCD-BM grafts or BM + T grafts, as well as untransplanted WT C57BL/6 mice. Data shown are pooled results of three independent experiments with four mice per group in each experiment. Data are presented as the mean ± SEM.

(B) At 14 days after BMT, bone marrow cells were analyzed for LSK cells and HSCs according to their cell-surface expression of markers.

(C) The percentages and total cell numbers of LSKs and HSCs in bone marrow are shown. Data are pooled results of two independent experiments with four mice per group in each experiment. Data are presented as the mean ± SEM.

(D) At 14 days after BMT, neutrophils ($CD11b^{+}Gr\text{-}1^{+}$) were analyzed for their cell-surface expression of Ly6G and CXCR2. The percentages of immature neutrophils ($CD11b^{+}Gr\text{-}1^{+}CXCR2^{-}$) in total neutrophils in bone marrow, spleen, and blood are shown. Data are pooled results of two independent experiments with four mice per group in each experiment.

(E) At 14 days after BMT, spleen cells were analyzed for LSKs according to their cell-surface marker expression in Lin^{-} cells.

(F) The percentages and numbers of Lin^{-} and LSK cells are shown. Data are pooled results of two independent experiments with at least three mice per group in each experiment. Data are presented as the mean ± SEM.

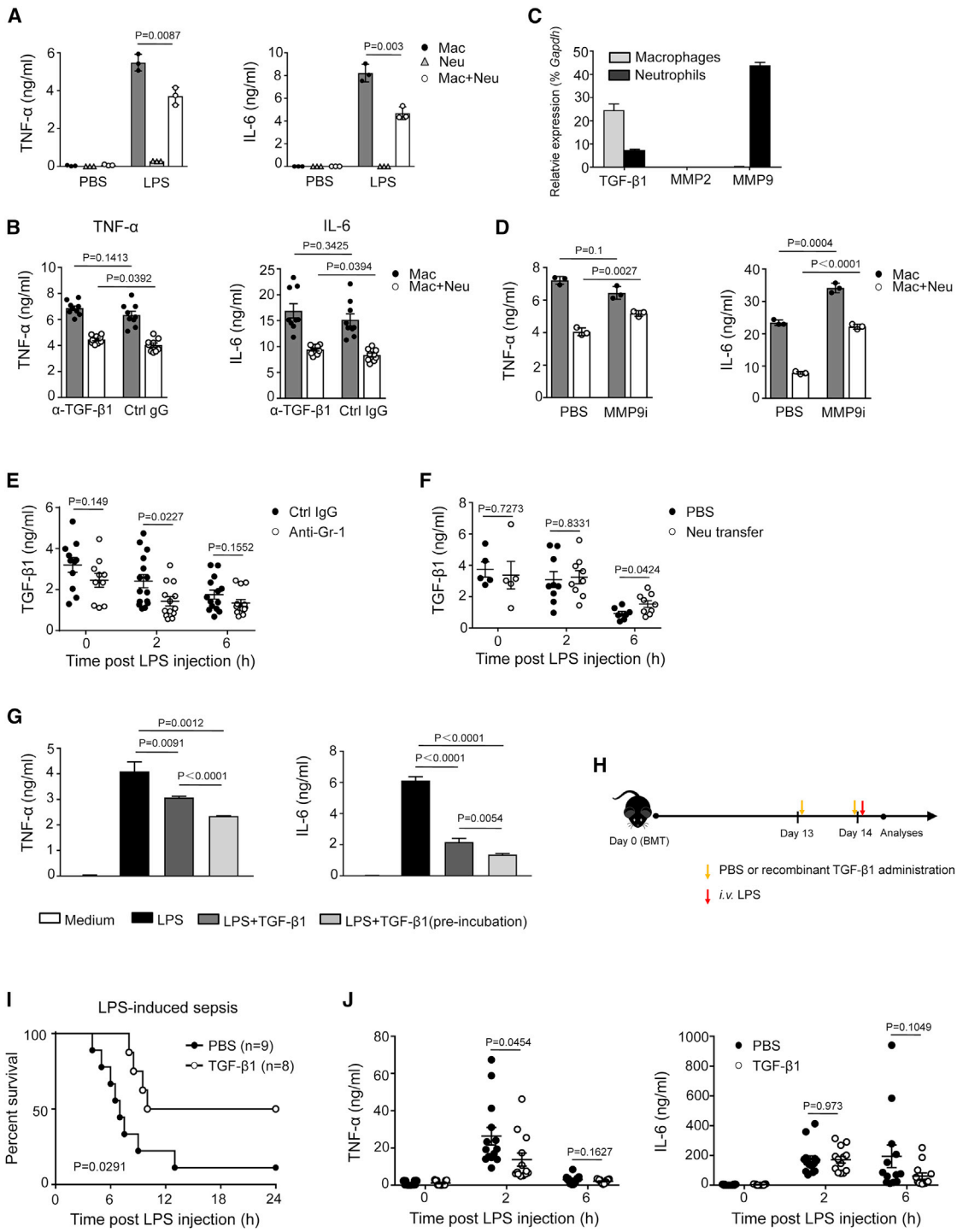

Figure 5. Mature neutrophils modulate macrophage inflammation via MMP9-mediated activation of TGF-β1

(A) Thioglycolate-elicited peritoneal macrophages (1×10^5) and mature neutrophils (2×10^5) isolated from WT C57BL/6 mice were cultured alone or together in the presence or absence of 1 μg/mL LPS in triplicate in 96-well plates for 24 h. TNF-α and IL-6 in the culture supernatant were measured by quantitative ELISA. A representative result of at least three independent experiments is shown. Data are presented as the mean ± SEM.

(B) Thioglycolate-elicited peritoneal macrophages were cultured (1×10^5) alone or together with isolated mature neutrophils (2×10^5) in the presence of 1 μg/mL anti-TGF-β or isotype control IgG. Twenty-four hours later, the culture supernatant was collected for cytokine

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membrane or not. The regulatory effect of neutrophils was probably mediated by soluble factors, but not cell contact, as separation of neutrophils and macrophages by a 0.4 μm transwell membrane did not change the inhibition of macrophage production of TNF-α, although it partially diminished the inhibition of IL-6 production (Figures S6A and S6B).

Neutrophils were found to inhibit lymphocyte activation via TGF-β1 (Germann et al., 2020; Kamenyeva et al., 2015). We therefore asked whether the inhibitory effect of neutrophils on macrophage activation was dependent on TGF-β1. Anti-TGF-β1 had no direct impact on LPS-stimulated macrophage production of TNF-α and IL-6 when they were cultured alone (Figure 5B). However, in a co-culture experiment, neutrophil-mediated inhibition of macrophage activation was partially rescued by blocking the function of TGF-β1 (Figure 5B). Interestingly, qPCR analysis revealed that macrophages, but not mature neutrophils, expressed high levels of TGF-β1 (Figure 5C). Produced by macrophages and other immune cells, the TGF-β1 precursor is first expressed to form a latent complex and cleaved into active TGF-β1 via multiple mechanisms, including MMP-mediated activation (Yu and Stamenkovic, 2000). qPCR analysis found that neutrophils expressed high levels of MMP9 but not MMP2 (Figure 5C). In addition, the expression of MMP9 was mainly restricted to mature neutrophils, rather than immature neutrophils (Figures S6C and S6D). We also found that splenic $CD11b^{+}Gr\text{-}1^{+}$ neutrophils from day 14 haplo-BMT mice showed significantly reduced MMP9 expression, compared with untransplanted WT mice (Figure S6E), consistent with their impaired maturation after haplo-BMT (Figure 4D). We next asked whether the regulatory effect of neutrophils is dependent on MMP9. MMP9 inhibitor significantly diminished neutrophil-mediated inhibition of macrophage activation in terms of TNF-α and IL-6 production in a co-culture experiment (Figure 5D).

Our above finding that serum TGF-β1 was reduced dramatically in septic GVHD mice (Figure 1G) suggested a regulatory role for TGF-β1 in LPS-induced sepsis post-BMT. To further confirm whether TGF-β1 production *in vivo* was dependent on neutrophils, sepsis was induced in GVHD mice after anti-Gr-1-mediated neutrophil depletion. As shown in Figure 5E, neutrophil depletion further reduced the serum TGF-β1 level in septic GVHD mice. Conversely, adoptive transfer of mature neutrophils isolated from WT donor mice into GVHD mice before the induction of LPS sepsis significantly increased serum TGF-β1 levels 6 h after LPS challenge (Figure 5F). These data suggested a beneficial role of TGF-β1 in GVHD mice in LPS sepsis. To test this, we first explored the effect of TGF-β1 on LPS-simulated macrophage activation *in vitro*. TGF-β1 significantly inhibited LPS-induced macrophage production of TNF-α and IL-6, while the inhibitory effect could be further enhanced by preincubation of macrophages with TGF-β1 for 2 h before LPS stimulation (Figure 5G).

analysis. Data shown are the pooled results of three independent experiments each done in triplicate. Data are presented as the mean ± SEM.

(C) qPCR analysis of TGF-β1, MMP2, and MMP9 gene expression in thioglycolate-elicited peritoneal macrophages and isolated mature neutrophils from WT C57BL/6 mice. Data are presented as the mean ± SEM.

(D) Thioglycolate-elicited peritoneal macrophages were cultured (1×10^5) alone or together with isolated mature neutrophils (2×10^5) in the presence of 5 μM MMP9 inhibitor. Twenty-four hours later, the culture supernatant was collected for cytokine analysis. A representative result of two independent experiments is shown. Data are presented as the mean ± SEM.

(E) At 13 days after BMT, GVHD mice were injected i.p. with 250 μg anti-Gr-1 or isotype control antibody in 200 μL PBS. Twenty-four hours later, these mice were injected i.v. with 1 mg/kg LPS. Serum TGF-β1 concentration was measured by quantitative ELISA 2 and 6 h after LPS injection. Serum samples before LPS injection were included as control. Data shown are the pooled results of three independent experiments with at least four mice in each experiment. Data are presented as the mean ± SEM.

(F) At 14 days after BMT, GVHD mice were injected i.v. with 1 mg/kg LPS to induce sepsis. At 24 and 1 h before LPS injection, GVHD mice received 5×10^6 mature neutrophils isolated from WT C57BL/6 mice by adoptive transfer or did not. Serum TGF-β1 concentration was measured by quantitative ELISA 2 and 6 h after LPS administration. Serum samples before LPS injection were included as control. Data shown are the pooled results of two independent experiments. Data are presented as the mean ± SEM.

(G) Thioglycolate-elicited peritoneal macrophages were stimulated with 0.1 μg/mL LPS in the presence of 50 ng/mL recombinant TGF-β1. Macrophages preincubated with 50 ng/mL recombinant TGF-β1 for 2 h before adding 0.1 μg/mL LPS were also included in this experiment. A representative result of two independent experiments done in triplicate is shown.

(H) A schematic of recombinant TGF-β1 administration and induction of LPS sepsis after BMT. At 14 days after BMT, GVHD mice were injected i.v. with 1 mg/kg LPS to induce sepsis. At 24 and 1 h before LPS injection, GVHD mice were injected with 1 μg recombinant TGF-β1 in 100 μL PBS i.v. and i.p., respectively.

(I) Survival of these mice was monitored after LPS injection. Comparison of survival between two groups was performed by log-rank (Mantel-Cox) test.

(J) Serum TNF-α and IL-6 were measured by quantitative ELISA 2 and 6 h after LPS injection. Data shown are the pooled results of three independent experiments. Data are represented as the mean ± SEM.

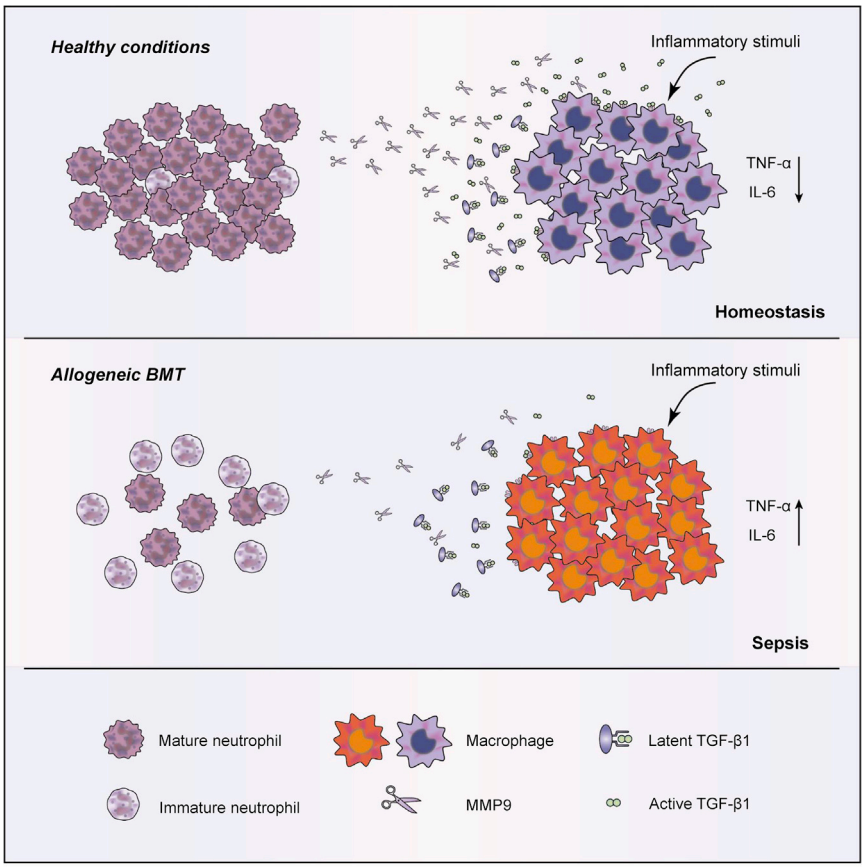

Figure 6. Schematic representation of mature neutrophil-mediated regulation of macrophage inflammation

In healthy conditions, mature neutrophil-derived MMP9 is sufficient to cleave latent TGF-β1 produced by macrophages. Activated TGF-β1 inhibits LPS-induced macrophage activation and thus reduces the inflammatory responses. After allogeneic BMT, deficient neutrophil reconstitution and maturation reduces the production of MMP9 and diminishes modulation of macrophage-induced inflammation. Dysregulation of macrophage activation contributes to the production of multiple inflammatory cytokines and development of sepsis.

To directly study the regulatory effect of TGF-β1 in septic GVHD mice, recombinant TGF-β1 was administered to GVHD mice before the induction of LPS sepsis (Figure 5H). We found that TGF-β1 administration increased the survival rate of septic GVHD mice from 11.1% to 50%, and decreased the production of serum proinflammatory cytokines (Figures 5I and 5J). Taken together, these data indicate that mature neutrophils regulate macrophage inflammation via MMP9-mediated TGF-β1 activation both *in vitro* and *in vivo*.

DISCUSSION

The prevention and treatment of sepsis are challenging due to our poor understanding of the underlying mechanism of sepsis pathogenesis. As one of the most common complications post-HSCT, infection is more likely to develop into severe sepsis, which remains a major cause of mortality for HSCT patients (Kumar et al., 2015). Inspired by these clinical findings, we utilized a mouse model of haplo-BMT to investigate both bacterial and sterile inflammation in recipient mice post-haplo-BMT. Our findings in this study indicate that neutrophils play regulatory roles in inflammation post-haplo-BMT. Neutrophil maturation deficiency associated with enhanced extramedullary granulopoiesis diminished immune modulation of macrophage-dependent inflammation, which contributed to the pathogenesis of sepsis in GVHD mice post-haplo-BMT. Mechanistically, mature, but not immature, neutrophils regulated macrophage inflammation via MMP9-mediated activation of TGF-β1 (Figure 6). Our study defines an additional risk of neutropenia in infection-induced inflammation and emphasizes the importance of neutrophil reconstitution and functional maturation in regulating inflammatory responses in sepsis post-haplo-BMT.

It was believed that neutrophils are a homogeneous population; however, in recent years, neutrophil heterogeneity has been reported in both homeostatic and pathological conditions (Ng et al., 2019). A heterogeneous cell population that expands in chronic and acute inflammation, cancer, and trauma is named as myeloid-derived suppressor cells (MDSCs), mainly defined by their immunosuppressive functions, although they share similar cell-surface markers with their counterparts in healthy conditions (Gabrilovich and Nagaraj, 2009). An inflammatory environment, induced in these pathological conditions as well as in sepsis, stimulates the emigration of immature myeloid

cells from bone marrow into the periphery, which later acquire immunosuppressive functions (Schrijver et al., 2019; Veglia et al., 2018). Although allogeneic recipient animals with GVHD showed signs of inflammation (Figure S1D), the maturation of $CD11b^{+}Gr\text{-}1^{+}$ neutrophils in bone marrow was relatively normal compared with untransplanted WT mice (Figure 4D). It has been shown that MDSCs suppress immune responses via various mechanisms, involving the production of reactive oxygen and nitrogen species, expression of arginase, and secretion of immunoregulatory cytokines like IL-10 and TGF-β (Brudecki et al., 2012). MDSCs exhibit a potent ability to regulate T cell activation and interact with regulatory T cells through a TGF-β-dependent mechanism (Huang et al., 2006; Lee et al., 2016). However, in the BMT scenario, a population of MDSCs is not likely to be induced in allogeneic recipient animals, considering the development of GVHD and disability of granulopoiesis in bone marrow. Our data also indicated that $CD11b^{+}Gr\text{-}1^{+}$ cells isolated from spleens of allogeneic recipient mice expressed low levels of TGF-β1 (Figure S6E), distinguishing them from MDSCs in inflammatory conditions. It seems that a lack of sufficient mature neutrophils, but not immature myeloid cell populations like MDSCs, diminished the regulation of macrophage activation in allogeneic recipient mice, as adoptive transfer of mature neutrophils isolated from WT donor mice inhibited sepsis in GVHD mice (Figures 3E–3H). Since cell-cell contact is dispensable for neutrophil-mediated regulation of macrophage activation, evident by our *in vitro* transwell experiment, an abundant pool of mature neutrophils and their derived soluble factors, such as MMP9, would be of significance to restrain the intensity of inflammatory macrophage activation and thus inhibit sepsis post-haplo-BMT.

During the procedure of HSCT, preconditioning regimens, such as total body irradiation and chemotherapy, as well as GVHD, could damage the bone marrow niches for supporting neutrophil development. The capacity of bone marrow to produce neutrophils is remarkably impaired in recipient mice post-haplo-BMT (Figure 4A), which might be a reason for the induction of increased extramedullary granulopoiesis in spleens of these mice. Indeed, neutrophil progenitors have been found in the spleen (Jhunjhunwala et al., 2016), a major alternative imprinting extramedullary site for neutrophil differentiation, although the underlying mechanisms of extramedullary granulopoiesis still remain elusive. In this study, we found immature neutrophils populating in the periphery of haplo-BMT mice, possibly due to augmented extramedullary granulopoiesis in the spleen. However, our findings in this study may have limitations for interpreting granulopoiesis in animal models using nonmyeloablative preparative regimens. Host-derived neutrophils could survive sublethal irradiation and might be directly targeted by donor T cells due to the major histocompatibility complex (MHC) disparity and therefore exhibit different patterns of expansion and maturation. In addition, the use of a mouse BMT model with a myeloablative preparative regimen excluded the possibility of investigating the functions of other host-derived myeloid cells, such as macrophages, in post-BMT sepsis. Since host-derived macrophages could play distinct roles versus donor-derived macrophages in GVHD (Hashimoto et al., 2011; Jardine et al., 2020; Hong et al., 2021), their functions in immune regulation and inflammation post-BMT will merit further investigation in different mouse BMT models.

GVHD is an important factor in priming inflammatory macrophages post-HSCT (Hong et al., 2020). Macrophages from GVHD mice were more sensitive to LPS stimulation and produced higher levels of TNF-α and IL-6 (Figures S6F and S6G) than their counterparts from non-GVHD mice or WT donor mice, both of which exhibited comparable low responsiveness to LPS stimulation (data not shown). This is consistent with our *in vivo* experiment showing that only GVHD mice showed increased vulnerability to infection and developed sepsis to low-dose LPS challenge, which was attributed to inflammatory macrophage activation (Figures 1B and 2D). The activation of macrophages from both GVHD mice and WT mice could be similarly inhibited by mature neutrophils *in vitro* (Figures S6F and S6G). However, our *in vivo* experiment indicated that the regulation of inflammatory macrophage activation was particularly dependent on functional neutrophils in BMT scenarios (Figures 3B and S3), and additional immunoregulatory mechanisms might exist to compensate the regulatory function of neutrophils in WT mice in response to inflammatory stimulation. Our findings demonstrate that deficiencies of neutrophil reconstitution and functional maturation aggravated the imbalance of immune regulation in macrophage-induced inflammation, which contributed to the pathogenesis of post-haplo-BMT sepsis. Further studies are needed to investigate how to reduce extramedullary granulopoiesis and promote neutrophil maturation post-BMT, which might be of interest in preventing post-HSCT sepsis.

EXPERIMENTAL PROCEDURES

Mice

B6D2F1 mice ($H\text{-}2^{b/d}$) were bred at the Soochow University Animal facility from female C57BL/6 ($H\text{-}2^{b}$) and male DBA/2 ($H\text{-}2^{d}$) mice or purchased from the Model Animal Research Center of Nanjing University (Nanjing, China) and used for experiments. C57BL/6 and DBA/2 mice were purchased from the Beijing Vital River Laboratory Animal Technology Company. Mice were kept in a specific-pathogen-free facility in microisolator cages, and experiments

were performed with mice at 8–10 weeks of age. All animal protocols were approved by the Institutional Laboratory Animal Care and Use Committee of Soochow University.

Haploidentical bone marrow transfer models

Bone marrow cells from female C57BL/6 mice were removed aseptically from femurs and tibias by flushing with ice-cold PBS. After red blood cell lysis, T cells were depleted from bone marrow cells by incubation with anti-Thy 1.2 antibody (BioLegend) for 30 min at 4°C, followed by another incubation with Low-TOX-M rabbit complement (Cedarlane) for 40 min at 37°C. Splenic T cells were purified by positive selection using anti-CD5 antibody-conjugated magnetic beads (Miltenyi, Auburn, CA). TCD-BM cells (5×10^6) were washed and resuspended in 100 μL PBS and injected into lethally irradiated recipient female B6D2F1 mice through the lateral tail vein. To induce GVHD, 1×10^6 splenic T cells were injected together with 5×10^6 TCD-BM cells into recipient mice. B6D2F1 mice received twice total body irradiation (5.5 Gy each) with a 3 h interval between each irradiation to minimize the gastrointestinal toxicity.

Sepsis models

Sepsis was induced in recipient mice by a single i.v. injection of LPS (1 mg/kg, O26:B6, Sigma-Aldrich) on day 14 after BMT. To establish a mouse model of bacteria-induced septic peritonitis, *E. coli* (BL21) was grown overnight in LB broth with shaking at 37°C. Bacteria were harvested, pelleted by centrifugation, and resuspended in PBS (CFU were estimated by OD 600 nm and confirmed by quantitative culture). Haplo-BMT recipient mice were infected with 3×10^{10} CFU/kg of *E. coli* i.p. on day 14 post-BMT. Recipient mice were monitored for survival and bled at indicated time points for serum cytokine analysis after LPS injection or *E. coli* infection.

Quantitative real-time PCR

Total mRNA was isolated using the EZNA HP Total RNA Kit (Omega) according to the manufacturer's instructions. cDNA was generated with SuperScript reverse transcriptase (Invitrogen). The SYBR Green Master Mix (Applied Biosystems), commercially ordered primes, and an ABI7500 real-time PCR system (Applied Biosystems) were used for quantitative real-time PCR amplification of cDNA. Results were presented as relative expression normalized to *Gapdh* expression. Relative expression was calculated as $(2^{(\text{CT test} - \text{CT ref})}) \times 100\%$. Primer sequences are listed in Table S1.

Cell staining and flow cytometry

For cell surface staining, single-cell suspensions were treated with CD16/CD32 FcR blockers (BioLegend) followed by staining with fluorescence-conjugated monoclonal antibodies (mAbs) (mAbs used in this study are listed in Table S2). After staining and thorough washes, samples were acquired on an Attune NxT cytometer (Thermo Fisher Scientific) and data were analyzed by FlowJo software. See supplemental information for the detailed protocol.

Cytokine assay

The TNF-α, IL-6, IFN-γ, IL-12, IL-10, and TGF-β1 concentrations in serum samples or cell culture supernatants were measured by mouse ELISA kits according to the manufacturer's instructions. A standard curve was generated to calculate the concentration of each cytokine in serum samples. See supplemental information for details.

Co-culture experiment

For macrophages and neutrophils co-culture experiments, purified peritoneal macrophages (1×10^5) and isolated bone marrow mature neutrophils (2×10^5) from WT C57BL/6 mice were cultured alone or together in RPMI with 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 U/mL) (Gibco) for 1 h in 96 well plates. Then, 1 μg/mL LPS was added and the cells were cultured for another 20 h in the presence of 1 μg/mL anti-mouse TGF-β1 (clone 1D11.16.8) or isotype control IgG (Bio X Cell), 5 μM MMP9 inhibitor (Sigma-Aldrich), or vehicle control. Culture supernatant was collected for cytokine analysis by ELISA.

Statistical analysis

Data are expressed as the mean ± SEM. Comparison of the average values between two sample groups was made with unpaired Student's t test. For *in vivo* sepsis experiments, log-rank test (Mantel-Cox) was used to compare the differences in survival rate between two groups of mice. All statistical analyses were performed with Prism software (GraphPad Software).

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.stemcr.2022.05.021.

AUTHOR CONTRIBUTIONS

C.H. conceived the study, performed experiments, analyzed data, interpreted results, and wrote the manuscript. H.L., X.H., M.C., and R.J. performed experiments. X.D. assisted in data analysis and generated the schematic interpretation. F.G., H.D., and H.W. assisted in experimental design and critically discussed the results. X.G. critically reviewed the manuscript and secured funding.

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CONFLICT OF INTERESTS

The authors declare no competing interests.

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REFERENCES

Bansal, S., Yajjala, V.K., Bauer, C., and Sun, K. (2018). IL-1 signaling prevents alveolar macrophage depletion during influenza and

streptococcus pneumoniae coinfection. J. Immunol. *200*, 1425–1433. https://doi.org/10.4049/jimmunol.1700210.

Belikoff, B.G., Hatfield, S., Georgiev, P., Ohta, A., Lukashev, D., Buras, J.A., Remick, D.G., and Sitkovsky, M. (2011). A2B adenosine receptor blockade enhances macrophage-mediated bacterial phagocytosis and improves polymicrobial sepsis survival in mice. J. Immunol. *186*, 2444–2453. https://doi.org/10.4049/jimmunol.1001567.

Brudecki, L., Ferguson, D.A., McCall, C.E., and El Gazzar, M. (2012). Myeloid-derived suppressor cells evolve during sepsis and can enhance or attenuate the systemic inflammatory response. Infect. Immun. *80*, 2026–2034. https://doi.org/10.1128/iai.00239-12.

Chiu, S.C., Liu, H.H., Chen, C.L., Chen, P.R., Liu, M.C., Lin, S.Z., and Chang, K.T. (2015). Extramedullary hematopoiesis (EMH) in laboratory animals: offering an insight into stem cell research. Cell Transplant. *24*, 349–366. https://doi.org/10.3727/096368915x686850.

Coffelt, S.B., Kersten, K., Doornebal, C.W., Weiden, J., Vrijland, K., Hau, C.S., Verstegen, N.J.M., Ciampricotti, M., Hawinkels, L.J.A.C., Jonkers, J., and de Visser, K.E. (2015). IL-17-producing γδ T cells and neutrophils conspire to promote breast cancer metastasis. Nature *522*, 345–348. https://doi.org/10.1038/nature14282.

Delano, M.J., and Ward, P.A. (2016). The immune system's role in sepsis progression, resolution, and long-term outcome. Immunol. Rev. *274*, 330–353. https://doi.org/10.1111/imr.12499.

Domingo-Gonzalez, R., and Moore, B.B. (2013). Defective pulmonary innate immune responses post-stem cell transplantation; review and results from one model system. Front. Immunol. *4*, 126. https://doi.org/10.3389/fimmu.2013.00126.

Engblom, C., Pfirschke, C., Zilionis, R., Da Silva Martins, J., Bos, S.A., Courties, G., Rickelt, S., Severe, N., Baryawno, N., Faget, J., et al. (2017). Osteoblasts remotely supply lung tumors with cancer-promoting SiglecF(high) neutrophils. Science *358*, eaal5081. https://doi.org/10.1126/science.aal5081.

Epelman, S., Lavine, K.J., and Randolph, G.J. (2014). Origin and functions of tissue macrophages. Immunity *41*, 21–35. https://doi.org/10.1016/j.immuni.2014.06.013.

Espinoza, J.L., Wadasaki, Y., and Takami, A. (2018). Infection complications in hematopoietic stem cells transplant recipients: do genetics really matter? Front. Microbiol. *9*, 2317. https://doi.org/10.3389/fmicb.2018.02317.

Evrard, M., Kwok, I.W.H., Chong, S.Z., Teng, K.W.W., Becht, E., Chen, J., Sieow, J.L., Penny, H.L., Ching, G.C., Devi, S., et al. (2018). Developmental analysis of bone marrow neutrophils reveals populations specialized in expansion, trafficking, and effector functions. Immunity *48*, 364–379.e8. https://doi.org/10.1016/j.immuni.2018.02.002.

Gabrilovich, D.I., and Nagaraj, S. (2009). Myeloid-derived suppressor cells as regulators of the immune system. Nat. Rev. Immunol. *9*, 162–174. https://doi.org/10.1038/nri2506.

Germann, M., Zangger, N., Sauvain, M.O., Sempoux, C., Bowler, A.D., Wirapati, P., Kandalaft, L.E., Delorenzi, M., Tejpar, S., Coukos, G., and Radtke, F. (2020). Neutrophils suppress tumor-infiltrating T cells in colon cancer via matrix metalloproteinase-mediated activation of TGF β. EMBO Mol. Med. *12*, e10681. https://doi.org/10.15252/emmm.201910681.

Hamidzadeh, K., Christensen, S.M., Dalby, E., Chandrasekaran, P., and Mosser, D.M. (2017). Macrophages and the recovery from acute and chronic inflammation. Annu. Rev. Physiol. *79*, 567–592. https://doi.org/10.1146/annurev-physiol-022516-034348.

Hashimoto, D., Chow, A., Greter, M., Saenger, Y., Kwan, W.H., Leboeuf, M., Ginhoux, F., Ochando, J.C., Kunisaki, Y., van Rooijen, N., et al. (2011). Pretransplant CSF-1 therapy expands recipient macrophages and ameliorates GVHD after allogeneic hematopoietic cell transplantation. J. Exp. Med. *208*, 1069–1082. https://doi.org/10.1084/jem.20101709.

Hong, Y.Q., Wan, B., and Li, X.F. (2020). Macrophage regulation of graft-vs-host disease. World J. Clin. Cases *8*, 1793–1805. https://doi.org/10.12998/wjcc.v8.i10.1793.

Hong, C., Jin, R., Dai, X., and Gao, X. (2021). Functional contributions of antigen presenting cells in chronic graft-versus-host disease. Front. Immunol. *12*, 614183. https://doi.org/10.3389/fimmu.2021.614183.

Huang, B., Pan, P.Y., Li, Q., Sato, A.I., Levy, D.E., Bromberg, J., Divino, C.M., and Chen, S.H. (2006). Gr-1+CD115+ immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host. Cancer Res. *66*, 1123–1131. https://doi.org/10.1158/0008-5472.can-05-1299.

Huang, X., Venet, F., Wang, Y.L., Lepape, A., Yuan, Z., Chen, Y., Swan, R., Kherouf, H., Monneret, G., Chung, C.S., and Ayala, A. (2009). PD-1 expression by macrophages plays a pathologic role in altering microbial clearance and the innate inflammatory response to sepsis. Proc. Natl. Acad. Sci. U S A *106*, 6303–6308. https://doi.org/10.1073/pnas.0809422106.

Jardine, L., Cytlak, U., Gunawan, M., Reynolds, G., Green, K., Wang, X.N., Pagan, S., Paramitha, M., Lamb, C.A., Long, A.K., et al. (2020). Donor monocyte-derived macrophages promote human acute graft-versus-host disease. J. Clin. Invest. *130*, 4574–4586. https://doi.org/10.1172/jci133909.

Jhunjhunwala, S., Alvarez, D., Aresta-DaSilva, S., Tang, K., Tang, B.C., Greiner, D.L., Newburger, P.E., von Andrian, U.H., Langer, R., and Anderson, D.G. (2016). Frontline Science: splenic progenitors aid in maintaining high neutrophil numbers at sites of sterile chronic inflammation. J. Leukoc. Biol. *100*, 253–260. https://doi.org/10.1189/jlb.1hi0615-248rr.

Johns, J.L., and Christopher, M.M. (2012). Extramedullary hematopoiesis: a new look at the underlying stem cell niche, theories of development, and occurrence in animals. Vet. Pathol. *49*, 508–523. https://doi.org/10.1177/0300985811432344.

Kamenyeva, O., Boularan, C., Kabat, J., Cheung, G.Y., Cicala, C., Yeh, A.J., Chan, J.L., Periasamy, S., Otto, M., and Kehrl, J.H. (2015). Neutrophil recruitment to lymph nodes limits local humoral response to Staphylococcus aureus. PLoS Pathog. *11*, e1004827. https://doi.org/10.1371/journal.ppat.1004827.

Kawai, T., and Akira, S. (2011). Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity *34*, 637–650. https://doi.org/10.1016/j.immuni.2011.05.006.

Kumar, G., Ahmad, S., Taneja, A., Patel, J., Guddati, A.K., and Nanchal, R.; Milwaukee Initiative in Critical Care Outcomes Research Group of, I (2015). Severe sepsis in hematopoietic stem cell transplant recipients. Crit. Care Med. *43*, 411–421. https://doi.org/10.1097/ccm.0000000000000714.

Lauvau, G., Loke, P., and Hohl, T.M. (2015). Monocyte-mediated defense against bacteria, fungi, and parasites. Semin. Immunol. *27*, 397–409. https://doi.org/10.1016/j.smim.2016.03.014.

Lee, C.R., Kwak, Y., Yang, T., Han, J.H., Park, S.H., Ye, M.B., Lee, W., Sim, K.Y., Kang, J.A., Kim, Y.C., and Mazmanian, S.K. (2016). Myeloid-derived suppressor cells are controlled by regulatory T cells via TGF-beta during murine colitis. Cell Rep. *17*, 3219–3232. https://doi.org/10.1016/j.celrep.2016.11.062.

Marwick, J.A., Mills, R., Kay, O., Michail, K., Stephen, J., Rossi, A.G., Dransfield, I., and Hirani, N. (2018). Neutrophils induce macrophage anti-inflammatory reprogramming by suppressing NF-κB activation. Cell Death Dis. *9*, 665. https://doi.org/10.1038/s41419-018-0710-y.

Mayadas, T.N., Cullere, X., and Lowell, C.A. (2014). The multifaceted functions of neutrophils. Annu. Rev. Pathol. *9*, 181–218. https://doi.org/10.1146/annurev-pathol-020712-164023.

Mortaz, E., Alipoor, S.D., Adcock, I.M., Mumby, S., and Koenderman, L. (2018). Update on neutrophil function in severe inflammation. Front. Immunol. *9*, 2171. https://doi.org/10.3389/fimmu.2018.02171.

Murdoch, C., Muthana, M., Coffelt, S.B., and Lewis, C.E. (2008). The role of myeloid cells in the promotion of tumour angiogenesis. Nat. Rev. Cancer *8*, 618–631. https://doi.org/10.1038/nrc2444.

Nathan, C., and Ding, A. (2010). Nonresolving inflammation. Cell *140*, 871–882. https://doi.org/10.1016/j.cell.2010.02.029.

Ng, L.G., Ostuni, R., and Hidalgo, A. (2019). Heterogeneity of neutrophils. Nat. Rev. Immunol. *19*, 255–265. https://doi.org/10.1038/s41577-019-0141-8.

Patoli, D., Mignotte, F., Deckert, V., Dusuel, A., Dumont, A., Rieu, A., Jalil, A., Van Dongen, K., Bourgeois, T., Gautier, T., et al. (2020). Inhibition of mitophagy drives macrophage activation and antibacterial defense during sepsis. J. Clin. Invest. *130*, 5858–5874. https://doi.org/10.1172/jci130996.

Poon, I.K.H., Lucas, C.D., Rossi, A.G., and Ravichandran, K.S. (2014). Apoptotic cell clearance: basic biology and therapeutic potential. Nat. Rev. Immunol. *14*, 166–180. https://doi.org/10.1038/nri3607.

Reber, L.L., Gillis, C.M., Starkl, P., Jonsson, F., Sibilano, R., Marichal, T., Gaudenzio, N., Berard, M., Rogalla, S., Contag, C.H., et al. (2017). Neutrophil myeloperoxidase diminishes the toxic effects and mortality induced by lipopolysaccharide. J. Exp. Med. *214*, 1249–1258. https://doi.org/10.1084/jem.20161238.

Ren, Y., Xie, Y., Jiang, G., Fan, J., Yeung, J., Li, W., Tam, P.K.H., and Savill, J. (2008). Apoptotic cells protect mice against lipopolysaccharide-induced shock. J. Immunol. *180*, 4978–4985. https://doi.org/10.4049/jimmunol.180.7.4978.

Rittirsch, D., Flierl, M.A., and Ward, P.A. (2008). Harmful molecular mechanisms in sepsis. Nat. Rev. Immunol. *8*, 776–787. https://doi.org/10.1038/nri2402.

Rosales, C. (2018). Neutrophil: a cell with many roles in inflammation or several cell types? Front. Physiol. *9*, 113. https://doi.org/10.3389/fphys.2018.00113.

Sabroe, I., Jones, E.C., Usher, L.R., Whyte, M.K.B., and Dower, S.K. (2002). Toll-like receptor (TLR)2 and TLR4 in human peripheral blood granulocytes: a critical role for monocytes in leukocyte lipopolysaccharide responses. J. Immunol. *168*, 4701–4710. https://doi.org/10.4049/jimmunol.168.9.4701.

Schrijver, I.T., Theroude, C., and Roger, T. (2019). Myeloid-derived suppressor cells in sepsis. Front. Immunol. *10*, 327. https://doi.org/10.3389/fimmu.2019.00327.

Singer, M., Deutschman, C.S., Seymour, C.W., Shankar-Hari, M., Annane, D., Bauer, M., Bellomo, R., Bernard, G.R., Chiche, J.D., Coopersmith, C.M., et al. (2016). The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA *315*, 801–810. https://doi.org/10.1001/jama.2016.0287.

Storek, J. (2008). Immunological reconstitution after hematopoietic cell transplantation - its relation to the contents of the graft. Expert. Opin. Biol. Ther. *8*, 583–597. https://doi.org/10.1517/14712598.8.5.583.

Szczerba, B.M., Castro-Giner, F., Vetter, M., Krol, I., Gkountela, S., Landin, J., Scheidmann, M.C., Donato, C., Scherrer, R., Singer, J., et al. (2019). Neutrophils escort circulating tumour cells to enable cell cycle progression. Nature *566*, 553–557. https://doi.org/10.1038/s41586-019-0915-y.

Tecchio, C., and Cassatella, M.A. (2021). Uncovering the multifaceted roles played by neutrophils in allogeneic hematopoietic stem cell transplantation. Cell. Mol. Immunol. *18*, 905–918. https://doi.org/10.1038/s41423-020-00581-9.

Veglia, F., Perego, M., and Gabrilovich, D. (2018). Myeloid-derived suppressor cells coming of age. Nat. Immunol. *19*, 108–119. https://doi.org/10.1038/s41590-017-0022-x.

White, M., Webster, G., O'Sullivan, D., Stone, S., and La Flamme, A.C. (2014). Targeting innate receptors with MIS416 reshapes Th responses and suppresses CNS disease in a mouse model of multiple sclerosis. PLoS One *9*, e87712. https://doi.org/10.1371/journal.pone.0087712.

Yamamoto, K., Miwa, Y., Abe-Suzuki, S., Abe, S., Kirimura, S., Onishi, I., Kitagawa, M., and Kurata, M. (2016). Extramedullary hematopoiesis: elucidating the function of the hematopoietic stem cell niche (Review). Mol. Med. Rep. *13*, 587–591. https://doi.org/10.3892/mmr.2015.4621.

Yu, Q., and Stamenkovic, I. (2000). Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. Genes Dev. *14*, 163–176. https://doi.org/10.1101/gad.14.2.163.