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M-CSF Mediates Host Defense during Bacterial Pneumonia by Promoting the Survival of Lung and Liver Mononuclear Phagocytes

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Gram-negative bacterial pneumonia is a common and dangerous infection with diminishing treatment options due to increasing antibiotic resistance among causal pathogens. The mononuclear phagocyte system is a heterogeneous group of leukocytes composed of tissue-resident macrophages, dendritic cells, and monocyte-derived cells that are critical in defense against pneumonia, but mechanisms that regulate their maintenance and function during infection are poorly defined. M-CSF has myriad effects on mononuclear phagocytes but its role in pneumonia is unknown. We therefore tested the hypothesis that M-CSF is required for mononuclear phagocyte-mediated host defenses during bacterial pneumonia in a murine model of infection. Genetic deletion or immunoneutralization of M-CSF resulted in reduced survival, increased bacterial burden, and greater lung injury. M-CSF was necessary for the expansion of lung mononuclear phagocytes during infection but did not affect the number of bone marrow or blood monocytes, proliferation of precursors, or recruitment of leukocytes to the lungs. In contrast, M-CSF was essential to survival and antimicrobial functions of both lung and liver mononuclear phagocytes during pneumonia, and its absence resulted in bacterial dissemination to the liver and hepatic necrosis. We conclude that M-CSF is critical to host defenses against bacterial pneumonia by mediating survival and antimicrobial functions of mononuclear phagocytes in the lungs and liver. The Journal of Immunology, 2016, 196: 5047–5055.

Pneumocystis jirovecii caused by aerobic Gram-negative bacilli is among the most common and dangerous nosocomial infections and an important cause of death, prolonged hospital stay, and increased healthcare costs. Gram-negative bacilli colonize the upper aerodigestive tract as part of the normal response to acute illness and cause pneumonia when introduced into the lower respiratory tract by microaspiration (1, 2). The progressive rise in antibiotic resistance among the causative organisms in recent decades has markedly diminished the available treatment options for these infections, lending new impetus to mechanistic studies that may inform the development of better therapeutic strategies.

The mononuclear phagocyte system encompasses heterogeneous populations of cells from three distinct lineages, namely tissue-resident macrophages, dendritic cells, and monocyte-derived cells that are critical in defense against pneumonia, but mechanisms that regulate their maintenance and function during infection are poorly defined. M-CSF has myriad effects on mononuclear phagocytes but its role in pneumonia is unknown. We therefore tested the hypothesis that M-CSF is required for mononuclear phagocyte-mediated host defenses during bacterial pneumonia in a murine model of infection. Genetic deletion or immunoneutralization of M-CSF resulted in reduced survival, increased bacterial burden, and greater lung injury. M-CSF was necessary for the expansion of lung mononuclear phagocytes during infection but did not affect the number of bone marrow or blood monocytes, proliferation of precursors, or recruitment of leukocytes to the lungs. In contrast, M-CSF was essential to survival and antimicrobial functions of both lung and liver mononuclear phagocytes during pneumonia, and its absence resulted in bacterial dissemination to the liver and hepatic necrosis. We conclude that M-CSF is critical to host defenses against bacterial pneumonia by mediating survival and antimicrobial functions of mononuclear phagocytes in the lungs and liver.

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Abbreviations used in this article: 7-AAD, 7-aminoactinomycin D; DC, dendritic cell.

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M-CSF and IL-34 have distinct tissue expression patterns and, at least in the development of osteoclasts, microglia, and skin Langhans cells, have nonredundant roles (13). Most recently, protein tyrosine phosphatase-ζ was described as a second receptor for IL-34 (14, 15).

Evidence from multiple experimental systems support a critical role for mononuclear phagocytes in host defenses but the mechanisms that control the development, homing, and survival of these cells during specific infections are incompletely defined. In the context of bacterial pneumonia, monocytes are recruited to the lungs via a CCR2-dependent mechanism, and both recruited and resident mononuclear phagocytes mediate host defenses by direct killing of bacteria and NF-κB-mediated recruitment of other leukocytes (16–20). In this context, we previously defined the dynamic changes in the various mononuclear phagocyte populations during experimental Gram-negative pneumonia and reported that CCR2 was necessary not only to the recruitment of monocyte-derived lineage cells but, unexpectedly, also controlled the number and phenotype of alveolar macrophages and all DC populations in this infection (17). Little is known about the role of the M-CSF/IL-34 biologic axis in the context of infection, and many of the published studies in the field predate current understanding of the lineages of mononuclear phagocytes (21–25). To our knowledge, this mechanism has not been investigated in pulmonary infections to date. We therefore sought to define the role of this biological axis in pneumonia by testing the hypothesis that M-CSF is required for mononuclear phagocyte-mediated host defense during Klebsiella pneumonia.

Materials and Methods

Animals and in vivo procedures

We used a previously characterized model of experimental bacterial pneumonia (26, 27). C57BL/6 and C57BL/6J mice, purchased (The Jackson Laboratory, Bar Harbor, ME), were bred and maintained under pathogen-free conditions and in compliance with institutional animal care regulations. Age- and sex-matched 6- to 8-wk-old mice were used in all experiments. Klebsiella pneumoniae strain 43816 (American Type Culture Collection, Manassas, VA) was grown overnight at 37°C to midlog phase in tryptic soy broth. Bacteria were administered intratracheally as previously described (17). Unless otherwise noted, all mice received i.p. injections of 100 μg/100 μl anti-M-CSF–neutralizing Ab (clone 5A1), anti-CSFIR–neutralizing Ab (clone AF589), IgG1 isotype control (clone HRPN), or IgG2A isotype control (clone 2A3) experiments, mice received three i.p. injections of 2 mg BrdU (BD Biosciences, San Jose, CA) and were sacrificed at the times described. Flow cytometry was performed as previously described (26, 30, 31). Cell suspensions of whole lungs, median liver lobes, bone marrow, and peripheral blood were prepared as previously described (26, 29, 32). The following reagents were used to label cells for flow cytometry (from BD Biosciences, San Jose, CA; eBioscience, San Diego, CA; or BioLegend, San Diego, CA): 7-aminoactinomycin D (7-AAD), anti-CD3–PE-Cy7 (clone 17A2), anti-CD11b–allophycocyanin-Cy7 (clone M1/70), anti-CD11c–PE-Cy7 (clone HL3), anti-CD19–PE-Cy7 (clone 1D3), anti-CD24–allophycocyanin (clone M1/69), anti-CD45–PerCP or AmCyan (clone 30-F11), and anti-CD64–BV421 (clone X54-5/71), anti-CD103–allophycocyanin (clone 2E7), anti-CD115 (clone AF589), anti-CD117–FITC (clone 2B8), anti-CD135–BV421 (clone AF210.1), anti-F4/80–Pacific Blue (clone BM8), anti-F4/80–PE (clone T45-2342), Fc blocker (anti-CD16/32), anti-I-A/I-E–FITC or AmCyan (clone M5/14.15.12), Ki-67–PE and isotype control (clones B6 and MOPC-21), anti-Ly6C–PE or Pacific Blue (clone HK1-4), anti-Ly6G–PE-Cy7 (clone M1/6), anti-NK1.1–PE-Cy7 (clone PK136), and anti–plasminogen activator inhibitor DC Ag–1–allophycocyanin (clone 129c1). In some experiments, cells were labeled, fixed, and permeabilized with a commercial kit (Cytofix/Cytoperm; BD Biosciences) before staining for intracellular Ags. Data were acquired on a FACS Canto II instrument using BD FACS Diva software (version 8.0; BD Biosciences) and analyzed using FlowJo software (version 8.8.6; Tree Star, Ashland, OR). Leukocyte subsets were identified as previously described (4, 17, 33) with minor modifications, as depicted in the figures. The absolute number of each cell type was determined as the product of the percentage of the cell type and the total number of cells in the sample, as determined on an automated cell counter (Countess; Invitrogen, Carlsbad, CA).

ELISA and assays for alanine transaminase, aspartate aminotransferase, urea nitrogen, and lactate

ELISAs for M-CSF and IL-34 were performed according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN). Albumin ELISA was performed using 5 μg/ml goat anti-mouse albumin (A90-134A), 0.025 μg/ml goat-HRP detection (A90-134P), mouse reference serum (r10-101) (Bethyl Laboratories; Montgomery, TX), and 1-Step Ultra TMB-ELISA (Thermo Fisher Scientific). Plasma alanine transaminase, aspartate aminotransferase, and urea nitrogen were measured using commercial kits (Liquid ALT Reagent Set and Liquid AST Reagent Set; Poite Sponsored, Canton, MI; and DetectX Urea Nitrogen Detection Kit; Arbor Assays, Ann Arbor, MI). Plasma lactate was measured using a commercial device (Lactate Plus Meter; Nova Biomedical, Waltham, MA).

In vitro studies

Bone marrow cells were cultured as described to generate macrophages (34). In viability assays, macrophages were washed and resuspended in DMEM with 10% FCS without antibiotics, and 1 × 10⁶ cells were cultured overnight in opaque flat-bottom 96-well tissue culture plates (BD Falcon, Franklin Lakes, NJ). Cells were then incubated with or without 5 ng M-CSF for 2 h before the addition of K. pneumoniae. After 150 min, plates were washed with sterile HBSS, and viability was measured using a commercial kit (LIVE/DEAD viability/cytotoxicity kit for mammalian cells; Invitrogen Molecular Probes, Eugene, OR). For phagocytosis assays, bacteria were labeled with a pH-sensitive cyanine dye that acquires fluorescence in acidic environments (35, 36); 1 × 10⁴ macrophages were seeded into round-bottom tissue culture–treated plates (Costar, Corning, NY) and incubated with or without M-CSF as above. Midlog phase K. pneumoniae cells were resuspended at 1 × 10⁶/ml in sterile saline, fixed in 5 ml 70% ethanol for 5 min, washed, and resuspended in sterile HBSS; CypHer5E dye (GE Healthcare Life Sciences, Pittsburgh, PA) was added at a final concentration of 5 μmol before incubation at room temperature for 30 min. Bacteria were then washed, and 1 × 10⁵ bacteria were added to wells containing macrophages. Plates were centrifuged for 30 s to achieve cell contact and incubated at 37°C and 5% CO₂ for 30 min, then placed on ice, washed in cold HBSS, and analyzed immediately by flow cytometry. Positive gating was set using an unstained control.

Statistical analysis

Data were analyzed using Prism statistical software (version 5.0d; GraphPad Software, San Diego, CA). Data from survival experiments were analyzed using the log-rank test. Values between two groups over multiple time points were compared with two-way ANOVA. Comparisons between two groups at a single time were performed using the nonparametric Mann–Whitney U test. Comparisons between multiple groups at a single time point were performed using the Kruskal–Wallis nonparametric test with Dunn comparison posttest. Comparison of paired samples receiving different treatments was made using Wilcoxon matched-pairs signed-rank test or two-way repeated-measures ANOVA. The p values <0.05 were considered statistically significant.

Results

Role of M-CSF in pulmonary infection with K. pneumoniae

We began by measuring the concentration of M-CSF and IL-34 protein in whole-lung homogenates during experimental pneumonia induced...
by *K. pneumoniae*. Both ligands were detectable in uninfected lungs. As compared with animals challenged with intratracheal saline vehicle, the concentration of M-CSF increased ~2-fold as the infection progressed but the concentration of IL-34 did not change significantly (Fig. 1A, 1B). In contrast, the concentration of plasma M-CSF protein remained undetectable in plasma throughout the time course, suggesting that M-CSF was produced locally in the lungs during infection. Given these findings, we focused our studies on the role of M-CSF during *Klebsiella* pneumonia.

To assess the contribution of M-CSF during bacterial pneumonia, we next examined the outcome of animals deficient in M-CSF during infection. Mice homozygous for an inactivating mutation in the M-CSF locus (*op/op*) had notably increased mortality during experimental pneumonia as compared with littermate controls, with no animals surviving beyond 2 d; this was associated with increased incidence and severity of bacteremia on the first day of the infection (Fig. 1C, 1D). The role of M-CSF during development of the mononuclear phagocyte system results in a number of well-documented phenotypic abnormalities in *op/op* mice, including reduced number of alveolar macrophages in juvenile, but not adult, animals and spontaneous production of matrix metalloproteinases (37). To discriminate between the role of M-CSF induction during bacterial pneumonia and its role during development, we tested the effect of immunoneutralization of M-CSF starting at the onset of the infection. Wild-type mice receiving anti–M-CSF–neutralizing Ab had increased mortality, increased lung bacterial burden, and markedly increased incidence and severity of bacteremia as compared with animals receiving an isotype control Ab (Fig. 1E, 1F).

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mediates differentiation of blood Ly6C<sup>hi</sup> monocytes to their Ly6C<sup>lo</sup> counterparts (38, 39). In the context of infection, administration of anti-CSF1R Ab resulted in a similar increase in mortality and bacterial burden as noted with M-CSF neutralization (Fig. 2C, 2D). These data indicate that the M-CSF–CSF1R interaction is necessary for host defenses during bacterial pneumonia independent of their role in development.

**Mechanism of M-CSF–mediated host defense during pneumonia**

To begin to address how M-CSF mediates its beneficial effects during bacterial pneumonia, we examined the effect of M-CSF neutralization on lung mononuclear phagocyte subsets in the lungs in the context of pneumonia (Fig. 3A). M-CSF neutralization resulted in a significant reduction in the numbers of cells of monocyte lineage, namely Ly6C<sup>hi</sup> and Ly6C<sup>lo</sup> monocytes, monocyte-derived macrophages, and monocyte-derived inflammatory DC, in the lungs of mice during pneumonia (Fig. 3A–D). In addition, M-CSF neutralization caused a reduction in the number of alveolar macrophages and conventional CD11b<sup>+</sup> DC, but not other DC subsets or neutrophils (Fig. 3E–J), similar to our prior report in mice with CCR2 deficiency (17).

To understand how the M-CSF–CSF1R axis contributes to the numbers of lung mononuclear phagocytes during *Klebsiella* pneumonia, we reasoned that the observed reduction in lung monocyte-derived cells, and possibly alveolar macrophages and conventional CD11b<sup>+</sup> DC, may be attributable to impairments in monocytopoiesis, trafficking between the bone marrow, blood, and lung compartments, or survival of leukocytes that have arrived in the lungs. We found that neutralization of M-CSF had no effect on the concentration of Ly6C<sup>hi</sup> and Ly6C<sup>lo</sup> monocyte numbers in bone marrow or blood (Fig. 4A, 4B). Consistent with this, we found no difference in the proliferation of bone marrow macrophage-DC progenitor or the recently described committed-monocyte progenitor (40) after neutralization of M-CSF (Fig. 4C, 4D). These data indicate that M-CSF is dispensable for the generation of monocytes from progenitor cells in the bone marrow during bacterial pneumonia.

M-CSF can be chemotactic for mononuclear phagocytes (41, 42). To assess for this possibility, we pulsed uninfected mice with the thymidine analog, BrdU, thereby labeling populations of...
proliferating cells that were in S-phase at the time of the pulse and then quantified the number of labeled leukocytes in various compartments after the onset of infection. As expected, we found fewer BrdU-positive bone marrow neutrophils and a marked increase in labeled neutrophils in the blood and lung of infected mice compared with uninfected controls that was independent of M-CSF neutralization (Fig. 5A–C). M-CSF neutralization also did not influence the numbers of labeled Ly6C<sup>hi</sup> monocytes in the bone marrow, blood, or lungs during infection (Fig. 5A–C), suggesting that M-CSF is also dispensable for the recruitment of lung monocyte-derived cells during pneumonia.

We next assessed the role of M-CSF on survival of mononuclear phagocytes during infection, because the M-CSF–CSF1R axis can mediate survival of mononuclear phagocytes under homeostatic conditions (43). We addressed this question by measuring the proportion of dead cells in the bronchoalveolar lavage fluid of infected animals to minimize the artifact of ex vivo cell death that inevitably occurs during processing of samples. M-CSF neutralization resulted in a significant increase in the proportion of dead Ly6C<sup>hi</sup> monocytes in the lungs of infected animals (Fig. 6A), potentially implicating M-CSF as a survival factor for lung monocyte-derived cells during pneumonia. To directly examine the role of M-CSF in mediating monocyte survival during infection, we also assessed the survival of bone marrow–derived macrophages coincubated with *K. pneumoniae*. As compared with cells incubated with bacteria in the presence of M-CSF, monocyte-derived macrophages incubated with bacteria in the absence of M-CSF had reduced survival (Fig. 6B). In addition, the surviving monocyte-derived macrophages had impaired phagocytosis of *K. pneumoniae* in the absence of M-CSF (Fig. 6C), suggesting that M-CSF promotes the survival of mononuclear phagocytes during infection and also contributes to the antimicrobial functions of these cells independent of its effect on the number of leukocytes.

**Role of M-CSF in controlling liver injury during bacterial pneumonia**

In the course of the above studies, we noticed that M-CSF neutralization during pneumonia was associated with gross visual evidence of liver injury. To investigate this, we assessed the effect of M-CSF neutralization on the histology of lung and liver during experimental pneumonia. The lung histology did not differ between pneumonic mice treated with anti–M-CSF and isotype controls; in contrast, M-CSF neutralization during pneumonia resulted in large areas of sharply demarcated hepatocellular necrosis, associated with prominent hepatocyte microvesicles (Fig. 7A). Consistent with this, neutralization of M-CSF resulted in elevations in plasma

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**FIGURE 4.** M-CSF is dispensable for maintenance of bone marrow and blood monocytes and progenitor proliferation during pneumonia. (A and B) Time series of mean ± SEM of indicated leukocyte populations in the blood and bone marrow. n = 10–12 from two experiments. (C) Representative flow plots of CD45<sup>+</sup> bone marrow cells, showing gating strategy of bone marrow to identify progenitor cells. (D) Time series of mean ± SEM of percent Ki67<sup>+</sup> bone marrow monocyte progenitors. n = 6–12 per group per time point from two experiments. Time 0 represents uninfected animals.

**FIGURE 5.** M-CSF is dispensable to the influx of mononuclear phagocytes during pneumonia. Mean ± SEM of BrdU-positive leukocyte populations in the bone marrow (A), blood (B), and lungs (C). n = 6–12 per group per time point from two experiments. *p < 0.05, **p < 0.001, one-way ANOVA with Dunn posttest.
FIGURE 6. M-CSF is required for the survival and function of mononuclear phagocytes during pneumonia. (A) Percentage of 7-AAD− lung Ly6Chist monocytes on day 3 of infection. n = 6 per group from two experiments (*p < 0.05, Mann–Whitney). (B) Effect of M-CSF on viability of bone marrow–derived macrophages incubated with or without Klebsiella in vitro, as measured in relative fluorescence units (RFU). Each data point represents an independent biological replicate paired to its respective control (**p < 0.01, two-way repeated-measures ANOVA). (C) Effect of M-CSF on phagocytosis of CypHer5E-labeled Klebsiella by bone marrow–derived macrophages. Each data point represents an independent biological replicate paired to its respective control (*p < 0.05, Wilcoxon matched-pairs signed-rank test).

concentrations of aspartate transaminase and alanine transaminase during pneumonia (Fig. 7B). Because M-CSF neutralization resulted in increased incidence and extent of bacteremia (Fig. 1), we next assessed whether the observed hepatocellular injury was associated with increased hepatic bacterial content after the liver had been perfused to displace its blood content. We found that, in mice with pneumonia, M-CSF neutralization resulted in increased liver bacterial burden (Fig. 7C), potentially explaining the increased liver injury. To assess whether the hepatocellular injury is merely a reflection of multiorgan dysfunction in the context of sepsis, we also quantified other parameters of end-organ dysfunction. As expected, M-CSF neutralization resulted in increased lung injury, as measured by albumin concentration in the bronchoalveolar lavage fluid (Fig. 8A). In contrast to its effect on the liver, however, M-CSF neutralization did not affect renal function, as measured by the concentration of blood urea nitrogen, nor cause significant end-organ hypoperfusion, as quantified by plasma lactate concentration (Fig. 8B, 8C), suggesting that M-CSF neutralization results in specific injury to the liver in addition to the lungs.

In order to investigate the contribution of M-CSF in the liver during bacterial pneumonia, we assessed the liver levels of M-CSF protein, because the plasma concentrations of M-CSF were undetectable during the infection. The liver contained high concentrations of M-CSF protein at baseline that did not change significantly during the infection (Fig. 9A). We therefore tested the hypothesis that, similar to our findings in the lungs, liver M-CSF mediates the survival of liver mononuclear phagocytes during the infection, leading to better clearance of bacteria from the liver. Consistent with this and analogous to the lungs, we found that liver monocyte-derived macrophages and F4/80+CD11b+ resident Kupffer cells were reduced in number with M-CSF neutralization during pneumonia (Fig. 9B, 9C). Also similar to our findings in the lungs, M-CSF neutralization resulted in increased death of liver Ly6Chist monocytes during pneumonia (Fig. 9D). Taken together, these data indicate that M-CSF is also required for mononuclear phagocyte-mediated defense in the liver during bacterial pneumonia.

Discussion

The mononuclear phagocyte system has been implicated in host defense against bacterial pneumonia (16, 17, 44, 45), but the mechanisms that generate and maintain these cells during the infection have not been defined. In the current study, we report that M-CSF, but not IL-34, is induced in the lung during the infection. M-CSF was essential to the survival of the host as a critical survival signal for mononuclear phagocytes in infected tissues, but appeared to be dispensable for the generation, recruitment,
and differentiation of mononuclear phagocytes during the infection. The absence of M-CSF thus resulted in reduced numbers of both monocyte-derived cells and resident macrophage populations in the lungs and the liver, resulting in increased bacterial burden and injury to these organs and increased incidence and extent of bacteremia.

M-CSF is produced by many cell types, including mononuclear phagocytes themselves, and has been documented to have a multitude of effects on mononuclear phagocytes in different experimental conditions, including development during embryogenesis, monocytopoiesis in adulthood, proliferation, differentiation, chemotaxis, and survival (as reviewed in Ref. 11). M-CSF has not been studied extensively in the context of infections, but has been associated with host defense in intracellular infections caused by Listeria monocytogenes (22, 46) and influences macrophage phenotype in experimental tuberculosis and schistosomiasis (47, 48). We report that M-CSF appeared to be dispensable for the proliferation and recruitment of monocytes and their progenitors during pneumonia, similar to recent findings in sterile peritonitis (39). Our studies did not directly examine the role of M-CSF in the differentiation of mononuclear phagocytes in the lungs in vivo, but the intact recruitment of monocytes to the lungs, together with the role of M-CSF in maintaining the numbers of both lung monocytes and monocyte-derived cells during infection indicates that the differentiation of monocytes into other mononuclear phagocytes during pneumonia remained intact despite M-CSF neutralization. In contrast, M-CSF was essential in maintaining lung and liver monocyte phagocyte populations by promoting their survival. M-CSF has been shown to be critical to survival of mononuclear phagocytes during development and under homeostatic conditions (49–52), but, to our knowledge, has not been shown to mediate mononuclear phagocyte survival in the setting of infection or inflammation. In this context, macrophage survival is a known regulatory factor in the context of infection: alveolar macrophage apoptosis, for example, is both antimicrobial and anti-inflammatory during pneumococcal pneumonia (53, 54), whereas macrophage necroptosis induces lung damage during Gram-negative and S. aureus pneumonia (55, 56).

In addition, we found that M-CSF mediates enhanced phagocytosis of bacteria by surviving mononuclear phagocytes. In this context, macrophages from op/op animals have been shown to have impaired phagocytosis against bacteria but not parasites (57, 58), but it is difficult to differentiate whether any effect in op/op macrophages is attributable to developmental abnormalities of the cells or lack of exposure to M-CSF during acute infection. Similar to our findings, however, M-CSF results in enhanced phagocytosis of fungal conidia by human monocyte-derived macrophages in vitro (59). Taken together, these data suggest that locally produced M-CSF can enhance antimicrobial properties of mononuclear phagocytes in target organs.

An unexpected finding in our study was the role of M-CSF in protecting the liver from injury during experimental pneumonia. The propensity of Gram-negative bacteria, specifically Klebsiella, to infect the liver is recognized clinically (60). In the context of experimental pneumonia, the liver is responsible for a protective acute-phase response, but is also susceptible to TNF-mediated injury (61–63). M-CSF has recently been shown to mediate proliferation of liver macrophages and recruitment of monocytes to the injured liver (64). Our data add to this literature, indicating that endogenous production of M-CSF during pneumonia mediates the accumulation of both monocyte-derived macrophages and Kupffer cells and protects the liver from disseminated infection.

The present work has several implications for future research. First, our data suggest that M-CSF is required not only for maintaining the number of monocyte-derived cells, but also alveolar macrophages and CD24+ [and thus pre-DC–derived (4)] CD11b+ DC in the lungs as well as Kupffer cells in the liver. This finding is reminiscent of our prior report in CCR2-deficient mice (17) and suggests either that Ly6Chi monocytes repopulate other lineages of

![FIGURE 8. M-CSF neutralization causes lung injury but not renal failure or tissue hypoperfusion during pneumonia. Time series of mean ± SEM of bronchoalveolar lavage (BAL) albumin concentration (A), blood urea nitrogen (BUN; B), and plasma lactate (C) in mice with pneumonia, treated with anti–M-CSF or isotype Ab. n = 8–12 per group per time point from two experiments. Time 0 represents uninfected animals. *p < 0.05, two-way ANOVA.](http://www.jimmunol.org/)

![FIGURE 9. M-CSF is required for the maintenance of mononuclear phagocytes in the liver during pneumonia. (A) Time series of mean ± SEM of M-CSF protein in the left liver lobe in mice with pneumonia. n = 6–16 per time point per group from two experiments. (B) Representative flow cytometry plots showing gating strategy for Kupffer cells. (C) Time series of mean ± SEM of indicated mononuclear phagocytes in the median liver lobe. Monocyte-derived macrophages were identified as depicted in Fig. 3A. n = 10–12 per group per time point from two independent experiments. ***p < 0.001, two-way ANOVA. (D) 7-AAD–positive Ly6Chi liver monocytes on day 3 of infection; data from two independent experiments (*p < 0.05, Mann–Whitney). Time 0 represents uninfected animals. Mφ, macrophages; SSC, side scatter.)
mononuclear phagocytes in the acute setting, as has been shown previously (9, 65), or that M-CSF is necessary to the maintenance of all three lineages of mononuclear phagocytes in the setting of acute injury. Second, we found M-CSF to have a marked role in the accumulation of Ly6Chigh monocytes in the lungs, but any role of these cells in the setting of acute infection remains undefined. Third, our work documents the critical role of M-CSF during bacterial pneumonia but does not preclude an additional contribution for IL-34 or its second receptor, protein tyrosine phosphatase-ζ, in the context of pneumonia. Finally, our findings may be relevant to strategies that may be expected to predispose patients to more severe pneumonia.

Disclosures

The authors have no financial conflicts of interest.

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