Heterogeneity of lung mononuclear phagocytes during pneumonia: contribution of chemokine receptors

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1Division of Pulmonary and Critical Care Medicine, Department of Medicine, Departments of 2Pharmacology and 3Microbiology, and 4Beirne B. Carter Center for Immunology, University of Virginia, Charlottesville, Virginia

Submitted 19 July 2013; accepted in final form 20 September 2013

Chen L, Zhang Z, Barletta KE, Burdick MD, Mehrad B. Heterogeneity of lung mononuclear phagocytes during pneumonia: contribution of chemokine receptors. Am J Physiol Lung Cell Mol Physiol 305: L702–L711, 2013. First published September 20, 2013; doi:10.1152/ajplung.00194.2013.—Bacterial pneumonia is a common and dangerous illness. Mononuclear phagocytes, which comprise monocyte, resident and recruited macrophage, and dendritic cell subsets, are critical to antimicrobial defenses, but the dynamics of their recruitment to the lungs in pneumonia is not established. We hypothesized that chemokine-mediated traffic of mononuclear phagocytes is important in defense against bacterial pneumonia. In a mouse model of Klebsiella pneumonia, circulating Ly6C<sup>hi</sup> and, to a lesser extent, Ly6C<sup>lo</sup> monocytes expanded in parallel with accumulation of inflammatory macrophages and CD11b<sup>hi</sup> dendritic cells and plasmacytoid dendritic cells in the lungs, whereas numbers of alveolar macrophages remained constant. CCR2 was expressed by Ly6C<sup>hi</sup> monocytes, recruited macrophages, and airway dendritic cells; CCR6 was prominently expressed by airway dendritic cells; and CCR6-deficient animals exhibited worse outcomes of infection. The absence of CCR2 had no detectable effect on neutrophils but resulted in reduction of all subsets of lung mononuclear phagocytes in the lungs, including alveolar macrophages and airway and plasmacytoid dendritic cells. In addition, absence of CCR2 skewed the phenotype of lung mononuclear phagocytes, abrogating the appearance of M1 macrophages and TNF-producing dendritic cells in the lungs. Taken together, these data define the dynamics of mononuclear phagocytes during pneumonia.

AEROBIC GRAM-NEGATIVE BACILLI are the most common causes of health care-associated pneumonia (45), an illness that afflicts 250,000 hospitalized patients each year in the United States (24) and carries an attributable mortality of 14% (39). Progressive emergence of antibiotic resistance among hospital-associated Gram-negative bacteria has greatly complicated the treatment of infection and to test the hypothesis that chemokine-mediated effects is incompletely defined, with evidence for both redundant and additive effects in different models (21, 38, 49). CX3CR1 is ubiquitously expressed on both populations of blood monocytes and in the resting state on lung CD11b<sup>hi</sup> DC; in some models, this receptor can contribute to migration of mononuclear phagocytes into tissue (29, 50). The sole ligand for CX3CR1 is CX3CL1. In the absence of inflammation, CCR6 is expressed by some lung DC populations and has been implicated in their traffic in several models of lung inflammation (19). The only chemokine ligand for CCR6 is CCL20; in addition, members of the β-defensin family can act as CCR6 ligands (64).

Although the monocytic phagocyte system is considered critical to defenses against many infections, the contribution of specific cells and specific chemokine receptors to host defense against bacterial pneumonia has not been defined. We sought to characterize the dynamics of the cells of the mononuclear phagocyte system in the bone marrow, blood, and lung during infection and to test the hypothesis that chemokine-mediated traffic of these cells is important to host defense against Gram-negative bacterial pneumonia.

MATERIALS AND METHODS

Animals and in vivo procedures. Mice with enhanced green fluorescent protein (eGFP) knockin at the CX3CR1 locus (22) and mice deficient in CCR2 (25, 41), CCL2 (31), and CCR6 (46) were bred in our colony, and respective wild-types were purchased (Jackson Laboratories, Bar Harbor, ME). Heterozygous CX3CR1-eGFP animals were generated by crossing homzygous and wild-type animals and were used to identify CX3CR1-expressing cells, as described (22). Animals were maintained under pathogen-free conditions and in compliance with institutional animal care regulations. Age- and sex-matched 6- to 10-wk-old mice were used in all experiments. Experimental pneumonia was induced by intratracheal inoculation of Kleb-
siella pneumoniae (K. pneumoniae) (strain 43816; American Type Culture Collection, Manassas, VA), using inocula between 100 and 400 cfu in various experiments, as previously described (15, 37). In some experiments, circulating monocytes were depleted by twice daily intravenous injection of 100 µl of liposomes containing clodronate or PBS (Encapsula NanoSciences, Nashville, TN) starting 8 h before inoculation. In other experiments, neutrophil depletion was achieved with a single i.p. injection of 200 µg of a monoclonal Ab (clone 1A8) or isotype control (clone 2A3) 1 day before an intratracheal challenge with K. pneumoniae, resulting in peripheral blood neutropenia for ~3 days (42).

Tissue harvest, histopathology, ELISA, and myeloperoxidase assays. At designated time points, animals were euthanized by intraperitoneal administration of an overdose of heparinized ketamine and xylazine, blood was collected from the right ventricle, and the pulmonary vasculature was perfused with PBS containing 0.5 mM EDTA. In some experiments, bronchoalveolar lavage was performed; in other experiments, whole lungs and a femur were removed or tissue was processed for histology, as previously described (3, 44). Lung samples were homogenized and processed for myeloperoxidase activity and ELISA as described (37). ELISA for CCL2, CCL7, CCL12, CCL20, and CX3CL1 was performed according to manufacturer’s instructions (CCL7 was from Peprotech, Rocky Hill, NJ; all others were from R&D Systems, Minneapolis, MN; minimum detectable concentrations were 50 pg/ml for CCL2, 100 pg/ml for CCL7 and CCL12, and 5 ng/ml for CCL20 and CX3CL1).

Flow cytometry. Cell suspension of lungs, bone marrow, and peripheral blood were prepared as described (37, 42, 44, 46). The following reagents were used to label cells for flow cytometry (from BD Biosciences, San Jose, CA; eBioscience, San Diego, CA; Miltenyi, Auburn, CA; or R&D Systems): 7-aminoactinomycin D (7-AAD), annexin V-FITC, anti-B220-FITC (clone RA3–6B2), anti-CCR2-PE (clone 475301), anti-CCR6-allophycocyanin (APC, clone 140706), anti-CD3-PE (clone 145–2C11), anti-CD4-FITC (clone GK1.5), anti-CD8-PE-Cy7 (clone 53–6.7), anti-CD11b-APC-Cy7 (clone M1/70), anti-CD11c-PE-Cy7 (clone HL3), anti-CD19-PE-Cy7 (clone M1/70), anti-Ly-6C-APC-Cy7 (clone AL-21), anti-Ly-6G-FITC (clone 1A8), anti-Mac3-PE (clone M3/84), anti-PDCD1-PE (clone JF05-IC2.4.1), and anti-TNF-PE (clone MP6-XT22). In some experiments, cells were stained for intracellular antigens; after being labeled for cell surface markers, samples were fixed and permeabilized using a commercial kit (Cytofix/Cytoperm, BD Biosciences) before intracellular staining. Data was acquired on a FACS Canto II instrument using Diva software (version 5.0.3, BD Biosciences) and analyzed using FlowJo software (version 8.8.6; Tree Star, Ashland, OR), using a previously published gating strategy (4). Apoptotic cells were defined as bronchoalveolar lavage cells that were positive for Annexin-V and negative for 7-AAD; dead cells were defined as cells staining with 7-AAD. The absolute number of each leukocyte subset 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).

Statistical analysis. Data were analyzed on a Macintosh iMac computer using Prism statistical package (version 5; GraphPad Software).
ware, San Diego, CA). Survival data were compared using Fischer’s exact test. Values between two groups over multiple times were compared with two-way ANOVA; comparisons between two groups at a single time were performed with unpaired two-tailed Mann-Whitney (nonparametric) test; and comparisons between multiple groups at a single time were compared using the Kruskal-Wallis test with Bonferroni posttest. Probability values were considered statistically significant if they were <0.05.

RESULTS

Expression of chemokine ligands and receptors. We began by measuring the concentration of ligands for the chemokine receptors under study, namely the CCR2 ligands CCL2, CCL7, and CCL12, the CCR6 ligand CCL20, and the CX3CR1 ligand CX3CL1, during the course of bacterial pneumonia (Fig. 1). Consistent with prior reports (38, 46 – 48), we found notable levels of CCL20 and CX3CL1, but little or no detectable CCR2 ligands, in the lungs of uninfected animals (Fig. 1). During the early course of infection, there was marked induction of CCR2 and CCR6 ligands but a decrease in total CX3CL1 protein (consisting of both cell-bound and soluble forms) as the infection progressed.

We next sought to assess the expression of the receptors CCR2, CCR6, and CX3CR1 on cells of the monocytic phagocyte system during the infection. We used standard surface staining for CCR2 and CCR6 using commercial monoclonal antibodies. To identify CX3CR1-expressing cells, we used mice heterozygote for eGFP transgene in the CX3CR1 locus, as previously described (22). As expected, both monocyte subsets in the bone marrow and blood (defined as Ly6Chi and Ly6Clo CD115-expressing cells) expressed CX3CR1 at all the examined time points (data not shown). We found a marked expansion of Ly6C<sup>hi</sup> monocytes in the bone marrow and an even larger expansion of these cells in the blood beginning on day 1 and continuing until day 3 after the onset of infection (Fig. 2, A and C). Approximately half of the Ly6C<sup>hi</sup> monocytes expressed CCR2 and CCR6 in the bone marrow; in contrast, in the blood compartment essentially all of the blood cells expressed CCR2, whereas very few expressed CCR6. The Ly6C<sup>lo</sup> monocytes were a small and stable population in the bone marrow throughout the infection that did not express detectable CCR2 or CCR6 (Fig. 2B).

Although there was an expansion of the pool of blood Ly6C<sup>lo</sup> monocytes during the infection, these cells constituted a small fraction of total blood monocytes given the more marked expansion of Ly6C<sup>hi</sup> monocytes. In contrast to uninfected animals, we found detectable expression of CCR2 on the blood Ly6C<sup>lo</sup> monocytes in infected animals (Fig. 2D).

We next examined the expression of chemokine receptors on major populations of monocytic phagocytes in lungs. Because the lung vascular and interstitial compartments contain substantial populations of monocytic phagocytes (4), these measurements were made in whole lung single-cell suspensions. In samples in which the vasculature had been perfused to remove circulating leukocytes, we found no detectable CD115-expressing cells. We found a population of cells with similar cell surface markers to blood and bone marrow Ly6Chi monocytes (with the exception of the expression of CD115); we identified these lung monocytes as CD45<sup>-</sup>CD11b<sup>-</sup>Ly6Chi cells that did not express Ly6G, Mac3, CD11c, or lymphocyte markers. We noted a marked increase in the number of both the lung Ly6C<sup>hi</sup> monocytes and recruited macrophages over the course of the infection (Fig. 3).
infection; in contrast, the number of alveolar macrophages remained unchanged (Fig. 3, A–C). CCR2 was expressed by most Ly6Chi monocytes and approximately half of the recruited macrophages, with a smaller proportion expressing CCR6 and CX3CR1. On the other hand, the alveolar macrophages did not express any of the receptors. Regarding the lung DC populations, there was a modest increase in the numbers of lung CD11bhi DC and plasmacytoid DC, but not airway DC, during the infection (Fig. 3, D–F). Similar to prior reports, we found most CD11bhi DC to express CX3CR1 (28), with half expressing CCR2 and smaller proportions expressing CCR6. Most airway DC, distinguished by the expression of CD103 (58), expressed CCR6 throughout the infection, whereas a minority expressed CCR2. CCR2 and CCR6 were expressed by plasmacytoid DC. These data provide descriptive evidence of the heterogeneous expression of CCR2, CCR6, and CX3CR1 on lung monocytic phagocytes during the early phase of bacterial pneumonia and, together with the expression of their ligands in the lungs, justified assessing their contribution to host defense in the infection.

Role of chemokine receptors in outcome of infection. We next examined the outcome of infection in animals deficient in CCR2, CCR6, and CX3CR1. Mice deficient in CCR2, but not CCR6 or CX3CR1, had notably increased mortality compared with respective wild-types (Fig. 4, A–C). Consistent with this, CCR2-deficient mice had 100-fold higher lung bacterial burden, higher incidence of bacteremia, and ~1,000-fold greater blood concentration of viable bacteria than wild-type animals on the third day of infection (Fig. 4E); in contrast, we found no significant difference in bacterial burden in CCR6 or CX3CR1-deficient mice compared with respective controls (Fig. 4, F–G). Interestingly, the host defense defect of CCR2-deficient mice was not replicated in CCL2-deficient animals, which exhibited similar mortality and organ bacterial burden as wild-type mice (Fig. 4, D and H), indicating redundancy between CCR2 ligands in the context of bacterial pneumonia. We therefore focused subsequent experiments on the mechanism of CCR2-mediated traffic of mononuclear phagocytes during pneumonia.

Role of CCR2 in traffic of mononuclear phagocytes. To assess the mechanism of CCR2-mediated host defense in Gram-negative pneumonia, we compared the kinetics of mononuclear phagocytes in infected CCR2-deficient mice to respective wild-types. Similar to reports in mice infected with L. monocytogenes (51), we found a paradoxical expansion of bone marrow Ly6Chi monocytes in CCR2-deficient mice, which, together with the lack of expansion in the blood pool of these cells, is consistent with failure to mobilize Ly6Chi cells from the bone marrow to the blood during infection (Fig. 5, A and C). We found no significant difference in Ly6Clo monocytes between wild-type and CCR2-deficient hosts in the bone marrow, but the concentration of blood Ly6Clo monocytes was lower in CCR2-deficient hosts compared with wild-type animals (Fig. 5, B and D).

In the lungs, we found a profound defect in accumulation of all monocyte, macrophage, and DC subsets examined, which was most notable in lung Ly6Chi monocytes and CD11bhi DC (Fig. 6, A–F). The reduced numbers of alveolar macrophages were not attributable to increased rate of cell death in CCR2-deficient mice because the proportion of early apoptotic and dead alveolar macrophages in the bronchoalveolar lavage did

![Fig. 4. Outcome of experimental Gram-negative pneumonia in mice deficient in chemokine receptors. A–D: survival studies; n = 26–29 for each group, pooled from 2 experiments. WT, wild-type. E–H: lung and blood viable bacterial content on day 3 of infection. Each data point represents 1 animal, and horizontal lines indicate medians; samples with no recoverable bacteria are depicted as containing 1 colony-forming unit (cfu) on the logarithmic scale; pooled data from 3 experiments; *P < 0.05 compared with corresponding wild-type group; NS, no significant difference (Log-rank test for A–D; Mann-Whitney for E–H).](http://ajplung.physiology.org/doi/10.1152/ajplung.00194.2013)
not differ between infected wild-type and CCR2-deficient hosts (mean ± SE, 14.2 ± 1.0% vs. 17.6 ± 3.9% early apoptotic cells and 0.60 ± 0.1% vs. 0.55 ± 0.11% dead cells on day 1 of infection; 5–6 mice per group, \( P = 0.77 \) and 0.78, respectively). We also compared the activation phenotype of cells by assessing their expression of TNF and iNOS. Although there was an increase in lung-recruited macrophages in the CCR2-deficient mice (Fig. 6B), we found very few iNOS- or TNF-producing macrophages, consistent with the M1 phenotype, in the lungs of CCR2-deficient mice (Fig. 6, G and H). Similarly, we found essentially no TNF-producing CD11b^hi DC in the lungs of CCR2-deficient mice (Fig. 6F). These data suggest that, in the context of pneumonia, the normal traffic of all examined mononuclear phagocytes is partially dependent on CCR2. Furthermore, they indicate that the absence of CCR2 results, not only in reduced number of lung mononuclear phagocytes, but also in markedly altered activation phenotype of recruited cells.

In addition to mononuclear phagocytes, CCR2 is also expressed by a subset of T cells and hematopoietic progenitor populations (33, 54), but we found no difference in the influx of activated T or B cells into the lungs of CCR2-deficient and wild-type animals with pneumonia (data not shown). To specifically test the hypothesis that the beneficial effect of CCR2 in Gram-negative pneumonia is mediated via mononuclear phagocytes, we sought to assess the effect of depletion of circulating monocytes on pathogen clearance in wild-type and CCR2-deficient animals with bacterial pneumonia. We first confirmed that, in the context of bacterial pneumonia, intravenous administration of clodronate liposomes resulted in partial depletion of circulating Ly6C^hi monocytes and more complete depletion of Ly6C^lo monocytes but had no detectable effect on the number of circulating neutrophils (Fig. 7A). Administration of clodronate resulted in increased lung bacterial burden in wild-type, but not CCR2-deficient mice (Fig. 7B), indicating that monocytes are necessary for the beneficial effect of CCR2 on bacterial clearance.

Role of CCR2 in neutrophil-mediated host defense. Several lines of evidence indicate a potential role for CCR2 in recruitment of neutrophils to inflamed tissues and, more broadly, that monocyte recruitment is necessary for optimal neutrophil recruitment (34, 57). Given the importance of neutrophils in Klebsiella pneumonia, we assessed the kinetics of neutrophil traffic in CCR2-deficient mice with bacterial pneumonia. We found no significant difference in the number of bone marrow, peripheral blood or lung neutrophils between wild-type and CCR2-deficient animals (Fig. 8, A–C). Histologically, lungs from both groups of animals showed multifocal alveolar filling with neutrophils and, to a lesser extent, mononuclear cells (Fig. 8, D–E). Lung myeloperoxidase activity, which is primarily attributable to neutrophils in this model, was higher in CCR2-deficient mice compared with wild-types on the third day of infection (Fig. 8F), consistent with somewhat higher neutrophil numbers in the lungs at this time point (Fig. 8C) and perhaps faster turnover of neutrophils in response to a higher burden of microorganisms (Fig. 4E). Taken together, these data suggest that CCR2 does not detectably impact the traffic of neutrophils in bacterial pneumonia.

Having found no detectable effect of CCR2 to the traffic of neutrophils to the lungs, we lastly sought to assess the contribution of CCR2-mediated defense via the monocyctic phagocytes relative to neutrophil-mediated defenses in hosts with Gram-negative pneumonia. As expected, neutrophil depletion rendered hosts highly susceptible to pneumonia, resulting in three to four orders of magnitude greater lung bacterial content in wild-type animals (Fig. 9). Depletion of neutrophils also resulted in increased bacterial burden in CCR2-deficient hosts, such that, in the context of neutrophil depletion, there was no difference in bacterial burden between wild-type and CCR2-deficient animals, even when challenged with a bacterial inoculum that is sublethal for wild-type nonneutropenic mice. These data suggest that the beneficial role of CCR2 in mediating monocytic phagocyte defenses is limited to hosts with intact neutrophil function.

**DISCUSSION**

The role of mononuclear phagocytes in antimicrobial host defense was defined in the intravenous inoculation model with the opportunistic Gram-positive intracellular bacterium, *Listeria monocytogenes*, where the primary sites of infection are the spleen and liver (51). Similar to the findings in other models, we report expansion of bone marrow and blood Ly6C^hi monocytes in bacterial pneumonia and evidence for CCR2-dependent egress of these cells from the bone marrow to the blood. An unexpected finding in the present work is the broad-based reduction in the numbers of all examined cell types in the blood and lungs of CCR2-deficient hosts during infection. In this context, CCR2-deficient mice were previously reported to have
fewer recruited lung macrophages in response to LPS and bacterial pneumonia (18, 35, 63); similarly, CCR2 was necessary for influx of Ly6C<sup>hi</sup> monocytes that gave rise to CD11b<sup>hi</sup> DCs in cryptococcosis (41), recruited macrophages, and CD11b<sup>hi</sup> DC in influenza (30). Interestingly, we found that CCR2 deficiency attenuated, but did not ablate, the traffic of mononuclear phagocytes to the lungs, suggesting that CCR2-independent mechanisms of mononuclear phagocyte recruitment to the lungs are operational but profoundly influenced the phenotype of the recruited macrophages and CD11b<sup>hi</sup> DCs. Specifically, iNOS- and TNF-producing recruited M1 macrophages and TNF-producing CD11b<sup>hi</sup> DC were essentially absent from the lungs of CCR2-deficient mice. Because nitric oxide and TNF are essential to host defense in this infection (26, 62), the absence of these cells is likely a key cause of worse outcomes in CCR2-deficient animals. Regarding the DC phenotype, the CD11b<sup>hi</sup>...
DC observed in wild-type infected lungs and absent from CCR2-deficient mice are similar to those in invasive pulmonary aspergillosis (42, 43) and differ from the TNF- and iNOS-producing DC (Tip-DC) first described in the spleens of animals with listeriosis (52) in that they do not express iNOS. At the early time points that we examined, we did not find any lung macrophages expressing M2 markers in either wild-type or CCR2-deficient mice, indicating that CCR2 is either necessary for recruitment of M1 macrophage precursors or for their development in the lung from uncommitted precursors.

Alveolar macrophages are critical to host defense in Gram-negative pneumonia (6), an effect that is likely independent of other lung mononuclear phagocyte populations. Several lines of evidence indicate that tissue-resident macrophages in general, and alveolar macrophages in particular, self-renew independent of bone marrow progenitors under steady state and Th2-inflammatory conditions (16, 20). However, our observation of high rate of alveolar macrophage apoptosis during pneumonia together with the reduced numbers of alveolar macrophages in CCR2-deficient animals with pneumonia is consistent with evidence for rapid turnover of alveolar macrophages described in pneumococcal pneumonia (60), and the replacement of alveolar macrophages from a blood precursor, via lung-recruited macrophage intermediates, after challenge with LPS (27). Because alveolar macrophages can be replenished by Ly6C<sup>hi</sup> blood monocytes (28), we speculate that the observed reduction in blood Ly6C<sup>hi</sup> monocytes in CCR2-deficient mice with pneumonia impairs the ability to replace dying alveolar macrophages during pneumonia, although our data do not preclude repopulation from nonmonocyte CCR2-expressing precursors (54). Interestingly, we noted a reduction in the number of Ly6C<sup>hi</sup> monocytes in the blood but not the bone marrow of CCR2-deficient mice with pneumonia, suggesting that, in the context of the infection, circulating Ly6C<sup>hi</sup> monocytes are derived from Ly6C<sup>lo</sup> counterparts in the blood rather than the bone marrow.

Our study demonstrates CCR6 and CX<sub>3</sub>CR1 to be dispensable for host defense in bacterial pneumonia. Although CX<sub>3</sub>CR1 has been implicated in some inflammatory settings (36, 39), it was not prominently expressed by lung mononuclear phagocytes during infection and was not essential to host defense in this setting. Prior data show that CCR6 is expressed by CD11b<sup>hi</sup> DC but also by most B cells, regulatory, and Th<sub>17</sub> T cells and mediate their homing to epithelial surfaces expressing its high-affinity ligand, CCL20 (reviewed in Ref. 19). CCR6-mediated recruitment of CD11b<sup>hi</sup> DC to the lungs has been implicated in the pathogenesis of airway allergy (11, 32),
cigarette-induced lung injury (5, 10), and viral and fungal infections (23, 46). On the other hand, similar to our findings in bacterial pneumonia, CCR6 was not expressed by lung CD11b+ DC and did not influence the response to helminth or mycobacterial antigens (9). In the context of bacterial pneumonia, we found CCR6 to be expressed by nearly all lung plasmacytoid DC; paradoxically, however, the homing of these cells to the lungs was dependent on CCR2. Several prior reports have documented the expression of CCR6 by blood plasmacytoid DC (8, 12) that home to mucosal associated lymphoid tissue (55); the role of these cells in bacterial pneumonia remains to be established.

Several lines of evidence support a cross-talk between neutrophils and mononuclear phagocytes at sites of inflammation; these interactions include mononuclear phagocyte-mediated neutrophil recruitment, neutrophil-mediated recruitment of inflammatory mononuclear cells, and functional modifications of mononuclear phagocytes after arriving in tissue, as recently reviewed (56). In contrast to prior work that implicated CCL2 in recruitment of neutrophils to the lungs in response to LPS or Gram-negative bacteria (1, 2, 34), we found no impairment in recruitment of neutrophils to the lungs of CCR2-deficient animals with K. pneumoniae pneumonia. In fact, the much higher levels of lung myeloperoxidase in CCR2-deficient mice with pneumonia (Fig. 8F) support the contention that, in response to the higher bacterial burden, the process of recruitment, degradation, and death of neutrophils is enhanced, and certainly not diminished, in this setting. This discrepancy may be explained by the marked differences between the biology of CCL2- and CCR2-deficient animals, as noted in other models (38, 49, 61), and by the observation that CCL2-deficient animals display a greater protection against Klebsiella pneumonia compared with CCR2-deficient mice (Fig. 4). Although we found no evidence of monocyte- or CCR2-mediated neutrophil recruitment, we report that neutrophils were necessary for the beneficial role of CCR2 to be observed. This observation supports a functional cooperation between neutrophils and mononuclear phagocytes in antibacterial host defenses in the lung.

The present work has a number of implications for future research. First, although we provide evidence for the importance of the mononuclear phagocyte system in bacterial pneumonia, the contribution of individual cell types, such as Ly6C<sup>lo</sup> monocytes, CD103 DC, and plasmacytoid DC, requires further study. Second, the relative contribution of CCR2 ligands to host defense in bacterial pneumonia awaits characterization. Specifically, CCR2 binds CCL2, CCL7, and CCL12 in mice and CCL2, CCL7, CCL8, CCL13, and CCL18 in humans. Although the relative contribution of these ligands in protection against pneumonia is not known, contrary to recent reports (1), our data indicate that CCL2 is dispensable to host defense in this infection. Finally, studies aimed at therapeutic agonism of CCR2 or increasing the pool of mononuclear phagocytes available for recruitment, as a potential therapeutic target may lead to novel treatments for bacterial pneumonia.

**REFERENCES**


L710 MONONUCLEAR PHAGOCYTES IN BACTERIAL PNEUMONIA


